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The Effect of Traditional Sourdough Starter Culture and Involved Microorganisms on Sensory and Nutritional Quality of Whole Wheat Bread

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Abstract: Consumers wish to have a wide range of foods that are nutritious and flavorful with long shelf life without added preservatives. The objective of this research work was to study the effect produced on whole wheat bread by using natural sourdough starter culture. PH and acidity of natural sourdough starter culture were measured after 72 hrs of fermentation and sourdough after 24 hrs of fermentation. pH and acidity of sourdough bread and commercial yeast bread were analyzed. Sensory characteristics and nutritional value of whole wheat sourdough bread and commercial yeast bread were analyzed. Microfilora of natural sourdough starter culture and sourdough throughout fermentation were also analyzed. Proximate composition of whole wheat flour was analyzed. Traditional sourdough and commercial yeast bread were evaluated for their acceptability by semi-trained panelists using 9- point hedonic scale. Decreased pH of sourdough to /4.0/ leads to phytate degradation and as a result phytate content in whole wheat flour which is 20.1% Phytic acid in mg/100g was reduced by 14.1% Phytic acid in mg/100g sourdough bread. The traditional sourdough bread did not show mould growth after four days storage at room temperature while commercial yeast bread do. Among the sensory attributes tested by panelists, aroma is significantly ($p<0.05$) differed positively from commercial yeast bread. Lastly the present study concluded that the use of natural sourdough starter culture and fermentation had a number of beneficial effects including prolonged shelf life, improved bread flavor and good nutritional value (bioavailability of minerals). Sourdough also improves sensory characteristics such as color, aroma, taste, and texture of breads.

Keywords: Whole Wheat, Bread, Fermentation, Traditional Sourdough Starter, Microflora, Sensory Characteristics

1. Introduction

Cereals are grown over 73% of the total world harvested area and contribute over 60% to the world food production providing dietary fiber, proteins, energy, minerals and vitamins required for human health. However, the nutritional quality of cereals and the sensorial properties of their products are sometimes inferior or poor in comparison with milk and dairy products. The reasons behind these are the lower protein content, deficiency of certain essential amino acids, the presence of antinutrients (phytic acid, tannins and polyphenols) and the coarse nature [1].

Due to increasing consumer demand for more natural, tastier and healthier food, the traditional process of sourdough bread production has enjoyed renewed success in recent years. Sourdough is employed in the manufacture of a number of baked products, such as breads, cakes and crackers. It is reported that the application of sourdough to wheat breads produced several effects, including leavening, acidification, improvement of the dough properties, and flavor of the bread, texture, delayed firmness and staling, increased resistance to microbial spoilage and improved nutrient availability of cereals. It was suggested that all these benefits were attributable to the lactic acid bacteria (LAB) and yeasts naturally present in sourdough [2]. Moreover, it has been noted that when sourdough is added, there are changes in the fundamental rheological properties of wheat dough, making it soft, less elastic and therefore easily extendable [3]. The impact of changes in the rheology of dough must be considered in order to choose an appropriate

fermentation time and obtain good quality bread. The dough must contain a large volume of gas and also reserve gas retention for oven rise [4]. However, there are other factors to be taken into account, such as the type of flour, the sourdough fermentation conditions (pH and temperature) and the selection of starter cultures with specific and desirable metabolic properties [2].

Bread baking using sourdough is a common practice and has the advantage of improving the nutritional value and sensory qualities of breads, achieving the baking ability of dough for wheat bread production, and increasing the shelf life of breads by delaying the germination of bacterial and mould spores. The microbiota of sourdoughs consists of specifically adapted lactic acid bacteria (LAB), mostly lactobacilli, as well as yeasts [5]. Its composition is affected by the endogenous ecological factors which in turn are determined by the flour and process (exogenous) factors.

1.1. Statement of the Problem

Fermented plant products are among the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for many people in the developing world. However, due to the lower protein content and deficiency of certain essential amino acids, the nutritional and sensory qualities of these products are considered poor in comparison with foods of animal origin. Attempts to improve nutritional qualities of cereal products include genetic improvement and amino acid supplementation with protein concentrates or other protein-rich sources such as grain legumes. Additionally, several processing technologies, which include cooking, sprouting, milling and fermentation, have been put into practice to improve the nutritional properties of cereals. Fermenting microorganisms can synthesize certain amino acids and improve protein quality and availability of B group vitamins. Fermentation also results in reduction in phytate, which may increase the amount of soluble iron, zinc and calcium several folds [1]. Even though all the above described advantages are mostly related to fermentation and byproducts of involved microorganisms it is less known about the microbial effect during fermentation stage of wheat sourdough and how to use traditional sourdough starter for production of good quality bread with improved shelf-life and enhanced nutritional quality compared to commercial yeast wheat bread.

1.2. Hypothesis of the Study

raditional sourdough starter culture has positive effect on sensory characteristics, microflora, nutritional and sensory quality of whole wheat bread.

1.3. Objectives of the Study

1.3.1. General Objective

The objective of this research work is to investigate the effect of traditional sourdough starter culture on the sensory acceptance, microflora, nutritional quality and shelf life of whole wheat bread.

1.3.2. Specific Objectives

1) To analyze the proximate analysis of kekeba variety wheat flour.
2) To analyze mineral content and phytate mineral molar ratio of whole wheat flour, sourdough bread and commercial yeast bread for prediction of mineral bioavailability.
3) To determine the pH change and titratable acidity of sourdough starter culture, sourdough and bread and.
4) To enumerate involved fungi and lactic acid bacteria in the process of traditional sourdough fermentation.
5) To evaluate sensory quality/acceptance of both the commercial yeast bread and traditional sourdough bread.

2. Materials and Methods

2.1. Sample Collection and Flour Preparation

Kekeba variety wheat was obtained from Melkassa Agricultural Research Center Eastern Oromia, Ethiopia. The grain was made into flour using a flour miller and sieved through 0.5 mm sieve and stored in air tight plastic container for further analysis.

2.2. Study Site

Practical activities were done in the laboratories of Food Science and Nutrition Center (FDSN) of Addis Ababa University, Entoto Poly Technique College, baking technique room and the Ethiopian Public Health Institute (EPHI). Entoto Poly Technique College was preferred for baking activity since they have baking technique practical room and all necessary facilities.

2.3. Methods

2.3.1. Homemade Starter Culture Preparation

Sourdough starter culture was prepared by mixing 150g of wheat flour with proportional luke warm tap water and left for 72 hrs until it become ready to be used as a starter.

2.3.2. Bread Making Processes

Two types of breads were baked; one with traditionally prepared sourdough starter culture and the other with commercial baker's yeast as control. For traditional sourdough bread, 1.5kg wheat flour, 10g salt, 150g traditional sourdough starter culture which was spontaneously fermented and 200 ml oil were added and mixed well, while for the bread using commercial yeast all recipes were the same except leavening agent which were commercial baker's yeast. In the mixing step the formula (recipe) were incorporated into a homogenous mixture. This was done in two stage processes. First: flour, water, salt, oil and leavening were mixed together and water was added gradually. After 60% (900 ml) of water on flour base was added, the second stage of gluten formation (kneading) begins. The resulting dough was allowed to rest for 10 minutes in a cabinet at room temperature (inter-proofing) and afterwards, the dough was molded and put into baking tins

and proofed at 30°C for another 10 minutes the loaves were baked at 150°C /200°C in an electric oven for 30 minutes and then cooled at room temperature and ready for further sensory and proximate analysis. Both breads were baked at similar conditions. However, the fermentation period for both types of breads was different. The traditional sourdough was ready for baking after 48 hrs of fermentation time but the yeasted dough was fermented only for 1 hr before baking.

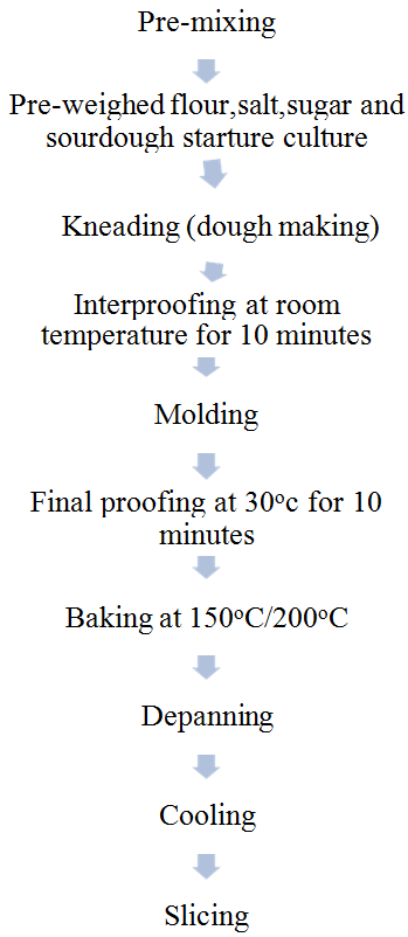

Figure 1. *Process flow diagram of traditional sourdough making and bread making.*

2.4. Proximate Composition Analysis

Moisture, total ash, crude fat, crude protein, crude fiber and total carbohydrate of wheat flour were determined according to AOAC (2000).

2.4.1. Determination of the Moisture Content of Flour

Empty drying dishes (made of porcelain) were cleaned and dried using a drying oven for 1 hour at 105°C. The dishes were cooled for 30 minutes in a desiccator with granular silica gel and weighed using a digital analytical balance to the nearest milligram (W_1). About 5 g of fresh sample was weighed (W_2) in dried and pre-weighed drying dishes. The dishes and their contents were then placed in drying oven and dried for 3 hours at 105°C. The dishes and their contents were cooled in desiccators to room temperature and weighed (W_3). The procedure was repeated until a constant weight was attained (AOAC, 2000).

$$\text{Moisture content (\%)} = \frac{w2-w3}{w2-w1} \times 100\%$$

W_1= weight of the crucible, W_2= Weight of crucible and fresh sample, W_3= Weight of the crucible and dry sample

2.4.2. Determination of Total Ash Content of Flour

Ash was quantified by (AOAC 923.03) as the inorganic residue present after incineration at 550°C for 5 hours until loss of organic matter. Porcelain crucible were cleaned and dried in a muffle furnace for 30 min at 105°C. Crucibles were cooled in a desiccator (with granular silica gel) for about 30 minutes or more at room temperature and weighed as (W_1). About 2.5 g of fresh sample was weighed to an accuracy of 2 decimal places in the dish and weighed as (W_2). The sample was burned on a hot plate under a fume hood and slowly increased the temperature until smoking becomes over. Then, ashed in the muffle furnace at 550°C for 5 hrs. The ash looked clean and has white appearance. Finally, the crucibles were cooled to room temperature and reweighed (W_3) each crucible with ash.

$$\text{Total ash (\%)} = \frac{w3-w1}{w2} \times 100\%$$

Where, W_1= weight in grams of the dried dish, W_2= weight in grams of the dish and the sample, W_3= weight in grams of the dish and ash

2.4.3. Determination of Crude Fat of Flour

Crude fat was determined by AOAC 45.01, following appropriate procedure. Extraction cylinders were washed with hot water and impurities were removed and then dried in drying oven at 105°C for 1 hr and cooled in a desiccator. The masses of the cooled extraction cylinders were measured by analytical balance and recorded as W_1. The bottom of the extraction thimbles were covered with the layer of fat free cotton. About 2 g of the powdered sample was weighed and added into each thimble lined with cotton at their bottom and covered with layer of fat free cotton and thimbles were putted into extraction chamber. The thimbles with their samples content were placed into the Soxhlet extraction apparatus. Then 50 ml of diethyl ether was added in to the extraction cylinder and moved in to the heating plank and the extraction last for four hours. Then, the extraction cylinder was disconnected and putted in the drying oven at 70°C for 30 minute. Finally, the extraction cylinders were cooled in the desiccators and weighed W_2.

$$\text{Crude fat\% by weight} = \frac{w2 - w1}{w} \times 100\%$$

Where: W_1=weight of dried extraction flask, W_2= weight of extraction flask and dried crude fat, W= weight of the sample

2.4.4. Determination of Crude Protein of Flour

Crude protein of the flour was determined by the Kjeldahl

method (AOAC979.09) in a Kjeltec system with acid (sulfuric acid) digestion of the sample and then an alkaline (sodium hydroxide) distillation using a nitrogen-to-protein conversion factor of 6.25.

Wheat flour sample; 0.5 g was measured in a tecator tube and placed in the tecator rack. A 6 ml of concentrated sulphuric acid was added from glass pipette and immediately the sample and the acid was carefully shaked. A 3 g of the catalyst mixture was added and step by step 3.5 ml of hydrogen peroxide was added. Violent reaction was observed and the tube was shaked a few times manually and placed back to the rack.

(i). Digestion

The temperature of the digester was adjusted to 370°C and lowered the tubes in the rack into the digester. The digestion was continued until a clear solution was obtained which takes about 3-4 hrs. Then, the tube was transferred in the rack into the fume hood for cooling.

(ii). Distillation

Distillation was done by adding 25 ml of 40% sodium hydroxide solution into the digested and diluted solution. A 250 ml conical flask containing 25 ml of boric acid, 25 ml of distilled water and indicator solution was placed under the condenser of the distiller with its tip immersed into the solution. The distillation was continued until a total volume become between 200 and 250 ml. The tip was rinsed with a few ml of distilled water before the receiver was removed.

(iii). Titration

Titrate was done with 0.1 N hydrochloric acids to a reddish color

Calculation:

$$\text{Nitrogen (\%)} = \frac{\text{VHCl in L} \times \text{N HCl (N 0.1)} \times 14.00 \times 100}{wo}$$

Where, V- volume of HCl in L consumed to the end point of titration

N- The normality of HCL (0.1 N) W_O- weight of sample

14.00- the molar weight of nitrogen Conversion:

$$\text{Protein (\%)} = 6.25 \times \text{\% nitrogen}$$

2.4.5. Determination of Crude Fiber of Flours

Crude fiber was determined by the method of AOAC 962.09, as the combustible and insoluble organic residue was obtained after the samples were subjected to acid digestion and then alkaline distillation. Clean crucible was dried with 1g celite in the oven at 105°C for 1 hour and placed in the desiccator to cool. A 1gram of wheat flour sample was measured in the dried crucible using analytical balance. A 200 ml of 1.25% (R1) H_2SO_4 solution was added to each beaker and allowed to boil for 37 minutes. The temperature was set between 6-8. After 37 minutes, the acid was drained using vacuum pump and the sample was cooled for 5 minutes and then, the sample was washed three times using distilled water. For the second step, 1.25%NaOH solution (R_2) was added to each column and the same step was followed as previous. Crucibles containing residue was dried at 130°C for 2 hrs by drying oven and cooled in the desiccator and crucibles were weighted (W_2). Crucibles were transferred to muffle furnace for 3 hrs at 525°C and cooled down to 250°C before removing them from the furnace. Finally, crucibles were cooled in the desiccator and weighed (W3).

Calculation was:

$$\text{Crude fiber (g/100g)} = \frac{w2 - w3}{w1} \times 100\%$$

Where: W2= mass of the crucible, W3= mass of the crucible and the sand, W1 = Weight of sample

Carbohydrate content was determined by difference which will be calculated as:

% Carbohydrates = 100 – (% moist +% ashes +% Proteins +% fats +% total dietary fiber)

2.5. Mineral Analysis

2.5.1. Calcium, Iron and Zinc Determination

Calcium, iron and zinc content of kekeba variety wheat flour, traditional sourdough bread and commercial yeast bread were determined according to the standard method of AOAC (2005) using atomic absorption spectrophotometer (AAS). Ashing of the samples was followed by digestion and absorption. Crucibles and glass wares were washed with 10% nitric acid and placed in the oven at 105°C for 1 hour. Crucibles were cooled in the desiccator for 30 minutes and accurately 2.5 g samples were taken and charred on the hot plate under the hood until the smoke was completed. Then, the samples were ashed in the muffle furnace at 550°C for 5 hours and taken out from the furnace and cooled in the desiccator and the total ash weight was measured. Some drops of deionized water was added to moisten it and evaporated on the hot plate and some drops of concentrated nitric acid were added and evaporated again on the hot plate and ashed once more for 30 minutes to ensure its complete ashing.

Dissolution of the ash was started by treating the ash with 7 ml of 6N HCl to wet it completely and carefully taken to the dryness on a lower temperature hot plate. Then, 15 ml of 3N HCl was added on each dish and heated on the hot plate until the solution just boils. Then the solution was cooled and filtered through the filter paper into a 50 ml graduated flask. Again, 10 ml of 3N HCl was added to the crucible and heated until the solution just boiled. Then the solution was cooled and filtered into the graduated flask. Crucibles were washed with de-ionized water three times and the washing was filtered into the flask. The filter paper was washed thoroughly with de- ionized water and the washing was collected in to the flask. A

2.5 ml of lanthanum chloride solution was added per 50 ml of solution. Finally, the contents of the flask was diluted and marked to 50 ml with de-ionized water. The sample solutions were transferred to the urine cap bottle. The blank was prepared by taking the same amount of the reagents following the same instruction used for the sample.

Calculation:

$$\text{mg/100g of metal content} = \frac{(Cs-Cb)\times V}{10\times w}$$

Where, C_s: concentration of sample in ppm C_b: Concentration of blank in ppm, V: Volume (ml) of the extract, W: weight (g) of sample

2.5.2. Standard Reagent Preparation

10pp 1m Zn: 1ml of zinc was added from 1000ppm in 100ml of volumetric flask with de-ionized water. For a series of standard of Zn prepared: 0.5, 1.0, 1.5, 2.0 and 2.5 ppm was taken respectively in 10 ml flask containing 2.5 3N HCl and marked up with de-ionized water.

20ppm Fe: 2ml of Fe was added from 1000 ppm in 100ml of de-ionized water. For a series of standard of Fe prepared 0.5, 1.0, 1.5 and 2 ppm was taken respectively in 10 ml flask containing 2.5 3N HCl and marked up with de-ionized water.

10ppm Ca: 1ml of Ca was added from 1000 ppm in 100ml of de-ionized water. For a series of standard of Ca prepared: 2, 4, 6, 8 and 10 3ppm was taken respectively in 10 ml flask containing 2.5 3N Hcl, 0.5ml 10% $LaCl_3$ and marked up with de-ionized water.

2.5.3. Phytate Mineral Molar Ratio Calculation

The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals [6].

Phytate content was determined using calometric method [7]. About 0.1gram of each samples were weighed separately in centrifuging test tube and the samples were extracted with 10 ml of 0.2 N HCl for 1hr at room temperature and centrifuged at 3000 rpm for 30minutes. Clear supernatant was used for the phytate determination. A 2 ml of wade reagent was added to 3 ml of the supernatant sample solution. Then homogenized and centrifuged at 3000 rpm for 10 seconds. The absorbance at 500 nm was measured using UV-Vis spectrophotometer. The phytate concentration was calculated from the difference between the absorbance of the blank (3 ml of 0.2 N HCl + 2 ml of wade reagent) and that of assayed sample. The amount of phytic acid was calculated using phytate acid standard curve and the result was expressed as phytate in mg/100 g fresh weight.

$$\text{Phytic acid in mg/100 g} = \frac{[(Ab-As)-intercept)]x10}{slopexwx3}$$

Where As = sample absorbance
Ab = blank absorbance
W = weigh of sample

2.5.4. Standard Solution Preparation

A series of standard solution was prepared containing 40 mg/100 g phytic acid in 0.2N HCl. A 3 ml of each standard was pipetted into 15 ml centrifuge tubes and 3ml of 0.2 N HCl to the blank. A 2ml of wade reagent was added to each tube and the solution was mixed on vortex mixer for seconds. The supernatant and the standard absorbance were read at 500 nm by using distilled water to make the spectrometer zero. Using x-cell the calibration curve was plotted and the slope and intercept was found.

2.6. pH and Titratable Acidity Determination

2.6.1. Determination of pH

Starter culture, sourdough and bread samples; a 10 g of each placed in a beaker containing 90 ml of distilled water, mixed homogeneously, and then left for 30 min at room temperature. The resultant supernatant was measured with a pH meter (Hanna Instrument-pH 301) after calibrating the instrument using pH 4.0 and 6.86 buffer [8].

2.6.2. Determination of Titratable Acidity

Starter, sourdough and bread; 10 g of each samples were mixed with 30 ml distilled water as one part of the flour to three parts of the water (w/v) ratio in beaker and left for 30 minutes. A 10 ml of distilled water was used for further dilution in order to hydrolyze all the acids in the samples. Before the titration of the sample the water that had been used for dilution purpose was titrated and used as blank. Three drops of 1% alcoholic phenolphthalein indicator was added into water extract of the sample (dispersion). Then the dispersion was titrated with standard base (0.1N NaOH) to phenolphthalein end point. The result of determination was reported as percentage acid consuming definite volume of 0.1N NaOH. The end point of the titration was reached after the white dispersion changed from a clear white solution to a faint violet colored turbid solution. Duplicate determinations were made in all cases [8].

The amount of the acid in the samples was determined by the relation:

$$\%\text{acid (wt/wt)} = \frac{100}{1000}$$

Where: N=normality of titrat, usually NaOH
(mEq/ml) V=volume of titrant (ml)
Eq.wt. =Equevalent weight of predominant acid (mg/mEq)
W=mass of samples (g)
1000=factor relating mg to grams (mg/g)

2.7. Microbial Analysis

2.7.1. Enumeration of Lab

One g of each samples of traditional sourdough and commercial yeast dough were mixed separately with 9 ml sterile distilled water in a sterile flask. One ml of the mixture was taken and serially diluted in test tubes each containing 9 ml sterile distilled water and aliquots of 0.1 ml from appropriate dilutions (10^{-1}-10^{-5}) were spread-plated in duplicate on pre-dried agar plates of MRS, Colonies of LAB were counted on MRS agar plates after anaerobic incubation in Gas Pak jars (GasPak System,) incubated at 32°C for 48 hrs. [9].

2.7.2. Dominant Yeast Enumeration

(i). Media Preparation

Yeast extract peptone dextrose (YPD) agarwas prepared by combining (yeast extract, 10g; peptone, 20 g; glucose, 20 g; agar 20 g; tap water 1,000 ml) containing 0.01g chloramphenicol/ml to inhibit the growth of bacteria. These

ingredients were mixed in conical flask, boiled and autoclaved for 15 minutes. Then after the media was poured into Petri dish.

(ii). Making Dilution

One g of each samples traditional sourdough and yeasted dough were mixed separately with 9ml sterile distilled water in a sterile flask. One ml of the mixture was taken and serially diluted in test tubes each containing 9ml sterile distilled water. This is followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1}-10^{-5}) on YPD agar plates which were prepared in the presence chloramphenicol of. All the plates were incubated at 28°C for 5 days. The colonies were counted by colony counter and recorded accordingly [10].

2.8. Shelf Life of Traditional Sourdough and Commercial Yeast Bread

Both breads were stored at room temperature in the same condition and visual observation was done at 12 hrs interval for physical change and mold growth and the change was recorded.

2.9. Sensory Evaluation

Ten panelists (2 females and 8 male, ranging in age between 24 and 40), who were M.Sc student at Addis Ababa University, Center for Food Science and Nutrition were selected. Hedonic evaluation of the sensory attributes of traditional sourdough and yeast bread samples were done using a nine-point hedonic scale valued as (1 = extremely dislike, 9 = extremely like). In the test, participants were provided with the two different breads coded with three random digit numbers and were asked about their liking or disliking concerning experimental traditional sourdough and yeasted bread. The samples were randomly presented to the panelists.

The panelists were given their informed consent to participate in the study and the scorecard sheets were prepared based on five attributes. Descriptive terms (color/appearance, taste /flavor, texture/consistency, aroma/smell and overall acceptability) were provided to the panelists and they were asked to rank all products on nine hedonic scale. Mean values of the scores from all panelists for each of the attributes was computed and analyzed.

2.10. Statistical Analysis

The raw data were analyzed using SPSS version 20 and. The mean comparison was done by least significant difference (LSD). One way ANOVA was used to find the mean difference between the groups. A $p< 0.05$ was considered as significant.

3. Results and Discussions

3.1. Proximate Composition of the Kekeba Variety Wheat Flour

Proximate composition of the sample; Kekeba wheat variety was determined before using for experimental activities and the results were presented in Table 1.

Table 1. *Proximate composition of Kekeba variety wheat flour.*

Moisture (%)		Protein (%)		Fat (%)		Ash (%)		Fiber (%)		Total Carbohydrate (%)	
2.00	0.00	10.15	0.25	1.25	0.35	1.35	0.22	3.91	0.00	81.34	0.00

Values are expressed as mean standard deviation

The moisture content shows very less result followed by protein but high ash, fat and fiber compared to other research [11]. This high ash content indicate that the wheat flour contain high mineral.

3.2. Mineral and Phytate Content Analysis of Kekeba Variety Wheat Flour, Sourdough Bread and Commercial Yeast Bread

Table 2 shows the Fe, Ca, Zn and phytate content of whole- wheat flour, sourdough bread and yeasted bread. The phytate content of the flour (20 mg/100g) was the highest followed by yeasted bread; bread baked with traditional sourdough had the lowest level (6 mg/100g). This is due to long time fermentation and phytase enzyme in cereals and some LAB. The values for Iron, calcium and zinc content in whole wheat flour, sourdough bread and commercial yeast bread did not show significant ($p>0.05$) difference. Lopez *et al.* (2001) found that the phytate content was more efficiently reduced in wheat sourdough bread (62%) as compared to yeast fermented bread (38%) and the results of this study shows better improvement in phytate reduction since phytate content in natural sourdough and commercial yeast dough bread were reduced by 70% and 30% respectively. The values for Iron, Calcium and Zinc content in wheat flour, sourdough and commercial yeast bread did not show significant ($p>0.05$) difference.

Table 2. *Mineral and phytate composition (mg/100g) of whole wheat flour, sourdough bread and commercial yeast bread.*

Samples	Phytate (mg/100g)		Iron		Calcium		Zinc	
WWF	20.1	0.46[a]	2.78	0.00 [a]	48	0.00	1.95	0.00 [a]
SDB	6	1.13[c]	2.77	0.37 [a]	48.49	2.04 [a]	1.987	0.08 [a]
YB	14.6	0.45	2.75	0.16 [a]	46.89	3.65 [a]	1.90	0.03 [a]

Values are reported as mean standard deviation SDB= sourdough bread, YB=yeast bread, WWF= whole wheat flour; Means with different superscripts in the same column are significantly different ($p<0.05$)

The calculated [phytate]: [Fe], [Ca]: [phytate], [phytate]: [Zn] and [Ca]: [phytate]/[Zn] molar ratios are presented in Table 3. The [Phytate]: [Fe] ratio was highest in whole wheat flour (0.6) followed by the commercial yeast bread. The sourdough bread had the lowest level (0.185) of.

Table 3. *Calculated phytate/Fe, Ca/phytate and (Ca): (phytate)/(Zn) molar ratios of whole-wheat flour, sourdough bread and commercial yeast bread (mg/100g).*

Samples	[Phytate]: [Fe]		[Phytate]: [Ca]		[Phytate]: [Zn]		[Phytate]: [Ca]/[Zn]	
WWF	.60	0.00[a]	0.025	0.00[a]	1.02	0.00[a]	0.011	0.00 [a]
SDB	0.185	0.003	0.0075	0.00[c]	0.30	0.01[c]	0.00	0.00 [b]
YB	0.45	0.002[b]	0.019	0.00[b]	0.754	0.01[b]	0.00	0.00 [b]

Values are reported as mean standard deviation SDB= sourdough bread, YB=yeast bread, WWF= whole wheat flour; Means with different superscripts in the same column are significantly different ($p<0.05$)

The mean values of phytate: Iron, molar ratio in present the study was 0.60, 0.45 and 0.185, for whole wheat flour, yeast and sourdough bread, respectively Table 3. For all samples phytate: Fe, phytate: Zn and phytate: Ca molar ratios were calculated and their results were found to be lower than their reported critical values This indicates that absorption of iron, zinc and calcium were not inhibited by phytate and as a result these minerals in all samples are bioavailabile. The [Phytate]: [Ca]/ [Zn] molar ratios for all samples also show the result below the 0.5 mol/kg critical value, thus predicting the bioavailability of Zn. Diets with a phytate-zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate-zinc molar ratio between 5 and 15 have medium zinc bioavailability and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability [12].

3.3. Change in pH During Fermentation and After Baking

3.3.1. pH and TA of Starter Culture Before Used for Dough Leavening

At the beginning of fermentation time; 0 hr, the pH of sourdough starter culture shows the value 6.475 and after 72 hrs fermentation the pH had decreased to 5.253 (Table 4). Following a decrease in pH, TA value increased significantly ($p<0.05$) from 0.199 at 0 hr to 0.355 after three days fermentation and this shows that there was production of organic acids by naturally occurring LAB and yeasts in spontaneously fermented starter culture.

Table 4. *Changes in pH and titratable acidity of Kekeba variety wheat sourdough starter culture during fermentation.*

Titratable acidity Fermentation time	starter culture		pH change	
0 hrs	6.475	0.007[a]	0.199	0.015[b]
72 hrs	5.253	0.0.007[b]	0.355	0.007[a]

Reported values are mean standard deviation. Means with different superscripts in the same column are significantly different ($p<0.05$)

Decrease in pH of starter culture is less compared to sourdough fermented for 24 hrs which might be due to the absence of sugar in preparation of starter since yeast need sugar as a source of glucose to produce acetic acid as by product and scarcity of nutrients due to long fermentation time.

3.3.2. Change in pH During Fermentation of Traditional Sourdough and Commercial Yeast Dough

The changes in pH during fermentation of wheat dough treated with traditional sourdough and baker's yeast were presented in Table 5. At initial point of fermentation, pHvalue of traditionally fermented dough and baker's yeast fermented dough were 6.2 and 5.8, respectively. During the fermentation period (from 0 hr to 48 hrs) of traditional sourdough and (from 0 hr to 1 hr) yeast dough acidification increased and resulted in a pH drop from an initial value of 6.2 to a final value of 4.00 and from 5.8 to 5.8, respectively.

Table 5. *Changes in pH during fermentation of Kekeba variety wheat dough treated with traditional starter culture and baker's yeast.*

Fermentation time	TSD		Fermentation time	YD	
0 hrs	6.2	0.014 [a]	0 hr	5.865	0.007 [a]
24hrs	4.33	0.02	30 min	5.860	0.0
48hrs	4.000	0.144[c]	1hr	5.830	0.0

Reported values are the mean standard deviation (n=2). Means with different superscripts in the same column are significantly different ($p<0.05$ TSD: - traditional sourdough YD: - yeast dough

The drop in pH was high in the traditionally treated sourdough than in yeast dough as indicated in Table 5. The lowest value of pH for traditionally fermented sourdough is 4.00 while 5.830 is for yeasted dough. Long fermentation decreased the pH of the traditional sourdough significantly ($p<0.05$) with time but not in yeast dough.

The results agreed with the fact that effect of fermentation on pH was significantly influenced by the presence of LAB in

traditional sourdough since they can produce many organic acids such as lactic, acetic and propionic acids as end products of fermentation and provide an acidic environment unfavorable for the growth of many pathogenic and spoilage microorganisms [13]. The lowest pH /4.00/ recorded in traditional sourdough after 48hrs fermentation was therefore due to the production of the organic acids by lactic acid bacteria.

3.3.3. Change in pH and TA of Sourdough Bread and Commercial Yeast Bread

The pH of fermented products was 5.2 and 5.48, respectively; Table 6. Sourdough bread scored less pH compared to yeasted bread and this value is probably due to long fermentation time and activities of LAB in cooperation with yeasts in traditional sourdough bread. The TA for both breads showed significant ($p<0.05$) difference, but less increase was observed as pH decrease.

3.4. Change in Titratable Acidity of Sourdough and Commercial Yeast Dough

The changes in titratable acidity (TA) during fermentation of traditional sourdough and yeasted dough were presented in Table 6. At the beginning of fermentation (0 hr.) TA values ranged from /0.274%/ to /0.360% /for traditional sourdough (TSD) and yeasted dough (YD), respectively. At the end of fermentation time (48hrs), the TA for traditional sourdough (TSD) increased significantly ($p<0.05$) from 0.274% to 0.783%. But there was a drop in TA from 0.360% to 0.324% during fermentation (from 0 hr to 1 hr.) for yeasted dough that might be because of the absence of LAB metabolic activities to produce lactic acid as a result of short fermentation time.

Table 6. *The pH and titratable acidity of sourdough bread and commercial yeast bread.*

Titratable acidity		
Sample types	**pH value**	
SDB	5.2	0.014^{b}
YB	5.48	$0.0.00^{a}$

Reported values are the mean standard deviation (n=2). Means with different superscripts in the same column are significantly different ($p<0.05$), TSDB=traditional sourdough bread, YB=yeast bread.

Table 7. *The changes in titratable acidity during fermentation of wheat dough treated with traditional starter culture and baker's yeast.*

Titratable acidity (% of lactic acid)					
Fermentation time	**TSD**		**Fermentation time**	**YD**	
0hrs.	0.274	0.00^{c}	0 hr	0.360	0.0^{b}
24hrs	0.590	0.0^{b}	30 min	0.337	0.006^{ab}
48 hrs.	0.738	0.006^{a}	1 hr.	0.324	0.00^{a}

Reported values are the mean standard deviatin (n=2). Means with different superscripts in the same column are significantly different ($p<0.05$). Sample codes are as stated in Table 4.

From the results of the pH and TA of traditional sourdough, it could be observed that as pH decrease the TA of the dough increased. This can hinder or inhibit the growth of food borne pathogens [13].

3.5. Microbial Analysis

3.5.1. Lactic Acid Bacterial Counts

The data on the lactic acid bacterial colony counts in traditional sourdough and yeasted dough at different fermentation times are given in Table 9. Lactic acid bacteria counts on MRS agar shows an exponential phase significantly ($p<0.05$) from 5.990 Log cfu/ml to 6.454 Log cfu/ml at 0 hr and 24hrs fermentation time but decreased to 6.005 Log cfu/ml at 48 hrs fermentation time. This result is in agreement with the finding of Emils et al., (2008). These authors reported that at the first stage of fermentation time which is from 0 hr to 24 hrs, LAB showed high counts although at the end of first stage sourdough fermentation LAB count started to decrease caused by limitations of nutrients.

Table 8. *Lactic acid bacterial count during fermentation of traditional sourdough and commercial yeast dough.*

LAB count (Log cfu/ml)					
Fermentation time		**TSD**	**Fermentation time**	**YD**	
0 hr	5.990	0.224^{b}	0 hr	ND	
24 hr	6.454	0.021^{a}	30 min	ND	
48 hrs	6.005	0.057^{ab}	1 hr	4.770	0.0^{a}

Reported values are the mean standard deviatin (n=2). Means with different superscripts in the same column are significantly different ($p<0.05$). Sample codes are as stated in Table 4.
ND=Not detected

The colony counts of LAB in baker's yeast dough shows no growth at both 0 hrs and 30 min fermentation time but 4.770 Log cfu/ml were recorded after 1hr fermentation time and this result show that LAB needs long fermentation time to be in exponential phase (growth phase).

3.5.2. Yeast Counts

The results of yeast colony count for traditional sourdough and yeasted bread were as indicated in Table 10. Yeast growth in traditional sourdough at 0 hr fermentation time was 3.776 Log cfu/ml but not detectable for 24 hrs and 48 hrs

sourdough fermentation time that is due to dominance of LAB which is naturally present in traditional sourdough starter which was used to start fermentation instead of baker's yeast.

Table 9. *Yeast count during fermentation of traditional sourdough and yeast dough.*

Yeast count (Log cfu/ml)					
Fermentation time	**TSD**		**Fermentation time**	**YD**	
0hr	3.776	0.509[a]	0 hr.	6.332	0.011 [a]
24 hrs.	ND		30 min	6.389	0.013 [a]
48 hrs.	ND		1 hr.	6.283	0.123 [a]

Reported values are the mean standard deviation. Sample codes are as stated in Table 4. ND=Not detected

Similarly as that of LAB in traditional sourdough which shows different growth patterns, yeasts colony count also show an increase in number from 6.332 Log cfu/ml to 6.389 Log cfu/ml at o hr and 30 min fermentation time but decreased to 6.283 Log cfu/ml at 1hr fermentation time that is due to limited nutrients.

3.6. Shelf Life Determination

After four days storage at room temperature in the same environmental conditions, mold growth appeared highly on the yeast bread, while the traditional sourdough bread showed a little growth of molds. The softness of the bread with traditional sourdough is also better than yeast bread. This observation is due to use of sourdough in the preparation of bread as it has a positive effect on mold-free shelf life [14].

a. Sourdough bread b. yeasted bread

Figure 2. *Shelf life of sourdough bread and yeast bread.*

3.7. Sensory Analysis of the Traditional Sourdough and Baker'S Yeast Bread

Results of sensory evaluation by the panelists are presented in Table 10. Sensory evaluation of sourdough and yeast bread was done using the attributes of taste, color, aroma, texture and overall acceptability.

3.7.1. Taste

Taste is an important criterion for every product to be accepted by the users and the same is true for traditional sourdough bread. The recorded mean score of taste for both traditional and yeast bread is presented in Table 10 and are not significantly ($p>0.05$) different but traditional sourdough bread scores 7.10 while 6.40 is scored for yeast bread which indicates less taste acceptance of yeast bread.

3.7.2. Aroma

The mean score of aroma for traditional sourdough and yeasted bread were 7.60 and 6.7, respectively. Even though both products show score above acceptable range, traditional bread had significantly ($p<0.05$) higher mean score in aroma 7.60 while the yeasted bread shows less mean score 6.70. These results are in agreement with results of studies of Rehman et al. [11]. These authors stated that the sourdough fermentation is central to acceptability in flavor, as chemically acidified breads prepared with pure commercial starter cultures are not well scored in sensory preference assessments. Sourdough fermentation makes the food palatable by enhancing its aroma and flavor [16].

3.7.3. COLOR

The mean score for the color of traditional sourdough and yeasted bread were 6.8 and 6.5 respectively. These results show that the color of both product were liked by panelists and no significant ($p>0.05$) difference were observed among the two types of breads.

Table 10. *Sensory characteristics of traditional sourdough bread and baker's yeast bread.*

Code	Taste		Color		Aroma		Texture		Acceptability	
TSB	7.1	1.4 [a]	6.8	0.9 [a]	7.6	1.0[a]	6.8	1.4 [a]	7.1	0.1.2 [a]
BYB	6.4	1.1 [a]	6.5	1.0 [a]	6.7	1.0 [b]	6.5	1.3[a]	6.5	1.1[a]

Reported values are the mean standard deviatin (n=2). Means with different superscripts in the same column are significantly different ($p<0.05$). TSB:-Traditional sourdough bread BYB:-Baker's yeast bread.

3.7.4. Texture

Texture is another important sensory attributes of food. The outcome of sensory evaluation of texture showed the same mean scores as scored in color for both traditional and yeast bread with no significant ($p>0.05$) difference between the two products but the traditional one show larger mean score as compared to yeast bread. This result is in agreement with that exopolysaccharides produced by LAB enhances water retention and plays the role of hydrocolloids [15]. Therefore, the inside of the bread keeps its sensory quality

over a longer period of time; in particular, it is more elastic compared with yeast bread.

3.7.5. Over All Acceptability

Generally, the average over all acceptability of traditional sourdough bread and yeast bread mean score were lasted with the acceptance of both products by the panelists in spite of their difference in degree of acceptance in which traditionally baked bread showed higher liking by panelist with mean score of 7.1 for traditional bread and 6.5 for yeast bread.

4. Conclusion and Recommendation

4.1. Conclusion

The phytate mineral molar ratio results show the bioavailability of Ca, Zn and Fe which was as a result of phytate degradation during fermentation. This study suggests that baking bread with spontaneously fermented starter culture and longtime fermentation further reduced phytate compared to baker's yeast. Hence using sourdough improves Zn and Ca bioavailability which is nutritionally beneficial.

Traditional sourdough increased the nutritional quality of the product by significantly decreasing phytic acid, enhances mineral bioavailability and improved nutritional status of the products. The use of sourdough is useful for making bread products with an increased level of flavor compounds, ultimately increasing consumer satisfaction. Overall sensory acceptance of traditional sourdough bread for this study showed higher mean score than control bread and was highly accepted by panelists compared to control bread. Thus sourdough could be useful in serving mankind with wholesome, tasty, and convenient foods.

4.2. Recommendation

Since traditional way of preparing food products using natural starter culture and longtime fermentation had effect on acceptance of sensory characteristics, improving nutritional quality, decreasing anti nutritional factors (phytate), more emphasis should be given to advocate / advertise the importance of baking bread using traditional sourdough at home to mothers of Ethiopia and to everyone concerned.

Stakeholders and government should create awareness to use fermented product and additional investigation should be encouraged on exact processing methods, specific LAB strains for use and appropriate ingredients for best bread.

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