Original article

Traditional sour dough bread (*Difo Dabbo*) making: I. Effects on phytic acid destruction

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Abstract: The quantitative changes of phytate during the preparation of the traditional sour dough bread (*Difo dabbo*) and yeast-raised bread were investigated. Raw materials chosen for investigation were flour of high extraction, soy-fortified wheat flour (*Dubbie flour*), and white flour. The content of phytic acid was determined in all components (raw materials), intermediate products (doughs), and bread. It was found that pH was the most important factor in reducing phytic acid content. The most marked phytate reduction of 96%-100% occurred in bread made with soy-fortified wheat and white flour sour doughs. Reduction of phytate content in bread made from wholemeal wheat flour sour dough was relatively low. The phytate content in yeast-raised bread was reduced at most to 39% of the initial amount. The study results showed that it should be possible to bake traditional sour dough bread (*Difo dabbo*) from wholemeals with a low phytic acid content by using the sour dough procedure. Such traditional sour dough bread with very low levels of phytate may be a good source of iron, calcium, and zinc since phytate is known to interfere with the absorption of these minerals. [*Ethiop. J. Health Dev.* 1998;12(3):167-173]

Introduction

The preparation of sour dough is one of the oldest biochemical processes used for producing food. Traditionally, sour doughs have been used to produce many types of bread. Although the primary purpose of the sour dough is leavening by yeasts, a simultaneous souring action takes place due to the activities of the lactic acid bacteria present (1). The acidification by sour dough lactic acid bacteria results in bread with a good grain, an elastic crumb and, usually, the characteristic sensory quality of sour dough bread (2). Sour dough fermentation involves a considerable number of heterogeneous metabolic and fermentation reactions which constitute a complicated biological system.

Sour dough bread (locally known as *Difo dabbo*) occupies a prominent place in the Ethiopian diet and continues to play an important socio-economic role and Ethiopian families would not like to pass a single holiday without it. The exact origin of sour dough in Ethiopia is, however, unknown.

The main raw materials employed are wheat supplemented with tef (*Eragrostis tef*) flour or barley flour. The bread may also be made of dark wheat flour, or from a mixture of various types of flour of consistently higher extraction rate. The method used to prepare a sour dough bread in Ethiopia is largely of empirical origin evolved over a long period.

Traditionally, sour dough bread is produced in Ethiopia by a spontaneous and largely uncontrolled fermentation process. The method of production of Ethiopian sour dough bread resembles the "Sur levain" method of French bread preparation (3). It differs in the secondary fermentation stage where the sour dough is freshened by additional flour and water and left to ferment for another 3 - 6 hr before baking. The Ethiopian sour dough bread like "Levain" bread is characterised by a marked acidic taste and characteristic aroma. Wheat sour dough is used in France and Italy (4).

Cereals (including wheat) are among the main dietary sources of phytate (*myo*-inositol hexaphosphate) which have inhibitory influence on mineral availability in the gut, notably calcium,

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magnesium, iron, and zinc (4). The amount of phytate in the diet is, therefore, of practical importance in relation to minerals nutrition on diets based on cereals. The loss of phytate during normal food preparation therefore deserves investigation.

Previous studies on the loss of phytate during bread making have been reported with reference to yeasted and rye sour dough breads (5-7). However, phytate reduction during Ethiopian sour dough bread (*Difo dabbo*) preparation and the biochemical changes during the fermentation process have not been studied earlier.

This article reports results of a study of the effect of sour dough fermentation on phytic acid degradation in soy-fortified wheat flour, wholemeal wheat flour and white flour breads. We also report on the reduction of phytic acid content in yeast-fermented bread.

Methods

Ingredients: Soy-fortified wheat flour obtained from Faffa Foods Factory, Addis Ababa, Ethiopia, was transported to India and stored at 4°C until used. Commercial wholemeal wheat flour (72% extraction) and white flour (62% extraction) were kindly supplied by the Department of Milling and Baking Technology, Central Food Tech-nological Research Institute, Mysore, India.

Ersho (starter) preparation: *Ersho* was prepared by incubating the flour and double distilled water (40:60, w/w) at 30°C for two days at the start of each fresh ersho preparation. The fermentation culture was kept viable by recycling 10% of the fresh dough with incubation at 30°C for 24 hr. Traditionally, this type of fermentation technology is widely practised in households in Ethiopia. The recycled *ersho* had a pH of 3.6 to 3.8 and this is referred to as starter culture.

Breadmaking: Sour dough breads were prepared from soy-fortified wheat flour, whole wheat flour, and white flour. The processing steps in sour dough bread production are illustrated in Figure 1. The sour doughs were

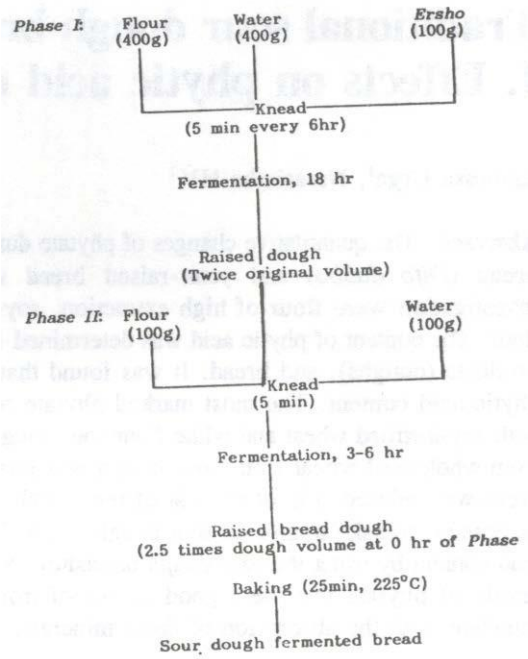

Figure 1: **Flow diagram of Ethiopian sour dough fermented bread *(Difo dabbo)* production**

fermented at 30°C for 18 hr during the initial phase of fermentation and then six hr in the second phase of fermentation prior to baking with occasional kneading every six hr for five min. The final dough was baked 25 min at 225°C in the Department of Milling and Baking Technology. Straightdough bread was baked as pup loaves according to the procedure of AACC (10). The dough contained the following ingredients: soy-fortified flour, 300 g; double distilled water, 186 g; sugar, 7.5 g; fat, 6 g; compressed yeast, 6 g; and barley malt flour, 1.5g. The doughs were mixed to optimum in a Hobart mixer, fermented (90% rh) for 170 min at 30°C with 55 min proofing at 30°C, and baked for 25 min at 220°C as described earlier. At the end of each fermentation and baking, the samples were oven-dried at 65°C to a constant weight and ground in an electric grinder (M/S Milone, Rajkot, India) using 0.5mm sieve.

Analytical methods: For phytic acid estimation, the samples were extracted with 0.2N HCl for three hr with continuous shaking in a mechanical shaker at room temperature. Phytic acid in the extract was estimated colorimetrically (11). Phytate phosphorus was derived by using the following formula (12):

$$\text{Phytate phosphorus (mg)} = \frac{A \times 28.18}{100}$$

where

A= phytate content.

Inorganic phosphorus in the sample was extracted in double distilled water by shaking at room temperature for three hr. Inorganic phosphorus in the extract was determined colorimetrically (13). Total phosphorus was determined colorimetrically (13) after digesting the samples in diacid mixture (HNO_3:$HClO_4$, 5:1, v/v). The pH of doughs and breads was measured with a pH meter in a mixture of ground dry sample (5.0 g) in double distilled water (100 ml). Titratable acidity was determined by titration of 50 ml of filtrate used for pH estimation against 0.1 N NaOH with 1% phenolphthalein indicator. Titratable acidity was expressed as percent lactic acid.

Results were analysed by analysis of variance and means compared by the use of Duncan's multiple range test at the 5% level of probability (14).

Results

Table 1 shows fermentation characteristics of soy-fortified wheat flour sour dough and bread. In the first phase of fermentation, the pH of the dough decreased significantly ($p<0.05$) compared to the unfermented flour; it dropped to 3.76 after 18 hr fermentation. Addition of flour and water to the fermented dough increased the pH of the dough due to the buffering action of the flour. The pH, however, decreased significantly ($p<0.05$) during the second phase of fermentation and baking of bread. With the drop in pH, a concomitant rise in titratable acidity was also observed in both phases of fermentation. Wholemeal wheat flour fermented for 18 hr had slightly higher pH and titratable acidity (Table 2) compared to the soy-fortified wheat flour (Table 1) and white flour (Table 3) doughs fermented for similar period. White flour and soy-fortified wheat flour doughs, however, had similar pH and titratable acidity values following initial and second phase of fermentation.

In the straight dough process, the pH and titratable values of the dough at 0 hr fermentation were 6.1 and 0.41%, respectively (Table 4). The pH of the dough after declining to 5.76 during 90 min fermentation time remained essentially constant throughout the remaining fermentation period while the titratable acidity of the final dough increased by 1.6-fold. The baking process has slightly increased the pH and decreased titratable acidity of the final loaf.

The total, inorganic and phytate phosphorus content of the sour dough and bread prepared from soy-fortified wheat flour are shown in Table 1. The phytate and inorganic phosphorus amounted to

about 53% and 19%, respectively, of the total phosphorus of the flour. The concentration of phytic acid (expressed as phytate phosphorus) in wholemeal wheat flour (Table 2) and white

Table 1: **pH, titratable acidity (TA), inorganic, phytate and total phosphorus in soy-fortified flour sour dough and bread***

Time(hr)	pH	TA%	Total P mg/100g	Inorganic P mg/100g	Phytate P mg/100g
Phase I					
0	5.91±0.17[a]	0.41±0.01[a]	275.13±5.21[a]	50.91±6.14[a]	150.05±1.27[a]
6	4.20±0.02[b]	0.65±0.04[b]	275.42±3.34[a]	95.17±2.31[b]	102.22±0.98[b]
12	3.95±0.03[c]	0.77±0.06[c]	274.67±1.43[a]	200.74±5.24[c]	66.11±0.81[c]
18	3.76±0.04[d]	1.12±0.08[d]	276.09±2.87[a]	233.62±7.42[d]	37.78±1.03[d]
Phase II					
0	3.91±0.03[b]	0.93±0.05[e]	551.93±7.21[b]	284.13±3.51[e]	183.81±2.23[f]
3	3.83±0.02[d]	1.13±0.04[d]	552.87±6.31[b]	393.36±8.24[f]	73.43±3.27[g]
6	3.76±0.06[d]	1.15±0.04[d]	551.28±9.17[b]	430.76±1.84[g]	36.11±0.72[d]
Bread	4.17±0.07[b]	0.95±0.02[e]	551.89±6.32[b]	449.82±5.15[h]	17.78±0.46[e]

*Mean values ± SD of three determinations. Values within the same column followed by different siperscript letters are significantly different (p<0.05).

Table 2: **pH, titratable acidity (TA), inorganic, phytate and total phosphorus in wholemeal wheat flour sour dough and bread***

Time (hr)	pH	TA	Total P	Inorgani P	phytate P mg/100g
Phase I					
0	5.13±0.01[a]	0.52±0.05[a]	317.80±7.18[a]	57.16±1.23[a]	195.38±2.15[a]
6	4.12±0.04[b]	0.57±0.02[a]	319.12±1.32[a]	86.12±1.23[b]	12.31±5.04[b]
12	3.92±0.06[c]	1.38±0.03[b]	317.54±4.35[a]	186.21±4.01[c]	81.12±2.21[c]
18	3.83±0.05[d]	1.80±0.07[c]	318.87±3.67[a]	235.51±1.93[d]	91.02±7.30[d]
Phase II					
0	3.92±0.07[c]	1.41±0.07[b]	632.97±5.63[b]	333.51±1.93[e]	244.38±2.21[e]
3	3.83±0.05[d]	1.75±0.08[c]	635.13±9.23[b]	470.57±2.75[f]	108.73±4.76[f]
6	3.83±0.06[d]	1.81±0.05[c]	643.25±7.57[a]	535.71±4.45[g]	43.34±5.27[g]
Bread	4.02±0.02[f]	1.79±0.07[c]	634.34±7.23[a]	538.11±1.07[e]	30.42±5.22[f]
Phase I	5.13±0.01[a]	0.52±0.05[a]	317.80±7.18[a]	57.16±1.23[a]	195.38±2.15[a]
0	4.12±0.04[b]	0.57±0.02[a]	319.12±1.32[a]	86.12±1.23[b]	12.31±5.04[b]

*Mean values ± SD of three determinations. Values within the same column followed by different superscript letters are significantly different (p<0.05).

flour (Table 3) were 61.5 and 70%,and 18 and 28%, respectively, of the total phosphorus.

In the fermentation process, the phytate phosphorus content of soy-fortified wheat flour dough was reduced by 81% within 18 hr (Table 1). However, addition of flour to the fermented dough has increased the phytate phosphorus content which later decreased to 91% during the second phase of fermentation (Table 2). The major reduction in phytate phosphorus occurred during the first 12 hr of the initial phase of fermentation in the pH range of 5.91 to 3.95. The rate of phytate degradation was, however, higher in the second phase of fermentation.

In the initial phase of fermentation for wholemeal wheat flour dough, there was a 75% decrease of phytate (Table 2). In the second phase of fermentation this value increased to about 85%. Baking the bread also further reduced the phytic acid by about 3%. Phytic acid in white flour dough was reduced by 88% during the initial phase of fermentation (Table 3). However, no phytate was detected following second phase of fermentation and baking into bread.

In the straight-dough process, the phytate phosphorus was lost linearly with time during the first 145 min of fermentation period. After this when loss of phytate had reached 35%, the rate of loss of phytate had declined, so that loss of phytate phosphorus after 225 min of fermentation was only 39%. The rate of loss of the latter was most rapid between 90 and 145 min fermentation period and pH of 5.76 to 5.75 (Table 4).

Losses of phytate phosphorus were followed by increases in inorganic phosphorus. During the first 6 hr of initial phase of fermentation, the soy-fortified wheat flour dough piece gained 45mg of inorganic phosphorus per 100g dough (Table 1). The inorganic phosphorus in the dough increased

by about 4.6-fold following 18 hr fermentation. Increase in inorganic phosphorus was 1.6-fold at the end of the second phase of fermentation and baking of bread.

Table 3: **pH, titratable acidity (TA), inorganic, phytate and total phosphorus in white flour sour dough and bread***

Time (hr)	pH	TA mg/100g	Total P%	Inorganic P mg/100g	Phytate P mg/100g
Phase I					
0	5.84±0.11^{a}	0.37±0.07^{a}	83.40±4.56^{a}	23.11±2.17^{a}	58.13±0.28^{a}
6	5.72±0.09^{b}	0.39±0.10^{a}	83.12±2.54^{a}	30.26±1.71^{b}	50.06±0.19^{b}
12	3.91±0.07^{c}	0.95±0.30^{b}	82.97±2.31^{a}	61.31±1.15^{c}	17.13±1.21^{c}
18	4.12±0.03^{d}	0.94±0.09^{b}	83.21±6.13^{a}	63.93±1.32^{c}	25.63±4.53^{d}
Phase II					
0	3.98±0.05^{c}	0.94±0.09^{b}	167.31±3.59^{a}	92.14±1.82^{d}	67.33±5.27^{a}
3	3.80±0.03^{e}	1.10±0.04^{c}	168.71±4.17^{a}	139.26±2.24^{e}	24.81±5.43^{d}
6	3.77±0.04^{e}	1.25±0.03^{d}	168.37±3.42^{a}	161.74±1.14^{f}	4.35±1.21^{e}
Bread	4.34±0.04^{d}	1.12±0.07^{d}	169.51±7.15^{a}	163.78±0.43^{f}	----

*Mean values ± SD of three determinations. Values within the same column followed by different superscript letters are significantly different ($p<0.05$).

Table 4: **pH, titratabl e acidity (TA), inorganic, phytate and total phosphorus in straight-dough bread***

Time (hr)	pH	TA mg/100g	Total P%	Inorganic P mg/100g	Phytate P mg/100g
0	6.06±0.02^{a}	0.41±0.02^{a}	275.13±5.23^{a}	65.71±2.51^{a}	158.51±5.72^{a}
90	5.76±0.03^{b}	0.54±0.01^{b}	275.98±2.31^{a}	69.53±2.30^{a}	120.34±2.27^{b}
145	5.75±0.01^{b}	0.643±0.03^{c}	275.78±5.43^{a}	74.48±4.13^{b}	102.72±3.58^{c}
170	5.78±0.04^{b}	0.67±0.04^{c}	274.76±1.35^{a}	78.86±3.18^{c}	98.75±1.53^{c}
225	5.77±0.03^{b}	0.68±0.02^{c}	2.75.18±8.31^{a}	81.35±2.15^{c}	97.22±0.83^{c}
Bread	5.83±0.03^{b}	0.58±0.05^{b}	274.98±2.24^{a}	84.84±4.39^{d}	96.78±1.68^{c}

*Mean values ± SD of three determinations. Values within the same column followed by different superscript letters are significantly different ($p<0.05$).

In wholemeal wheat flour fermentation, the inorganic phosphorus was increased by about 4.6fold during the initial phase of fermentation (Table 2). During the second phase of fermentation and baking of the bread, the inorganic phosphorus content was increased by 1.6-fold. In white flour the percentage increase in inorganic phosphorus was about 2.8-fold, after 18 hr fermentation (Table 3). The inorganic phosphorus in the final dough and bread was increased by 1.8-fold. In the straight-dough process, there was only a 24 % increase in inorganic phosphorus following 225 min of fermentation (Table 4). The inorganic phosphorus content of the bread was 31% of the total phosphorus.

Discussion

Ethiopian sour dough bread preparation is a two-phase process in which the mother dough obtained during the initial phase of fermentation is freshened by additional flour and water followed by a second phase of fermentation which usually lasts 6 hr before baking. A number of biochemical changes take place as a result of such fermentation processes. During the initial phase of fermentation of sour doughs, the amount of acid produced increased with concomitant drop in the pH. The low pH and higher titratable acidity in the fermented doughs may be due to the production of organic acids by the fermenting microflora. Rapid drop in pH with a corresponding increase in titratable acidity have been reported during the preparation of sour wheat bread (15).

In the initial phase of fermentation for soy-fortified wheat flour dough, there was about 75% decrease of phytic acid. The decrease of phytate was higher (83%) only in the case of white flour. This phenomenon might be caused by the use of low phytate raw material for this phase.

In spite of long fermentation time (18 hr) and acidity favourable for phytase activity (pH) the high amount of phytate in wholemeal wheat and soy-fortified flour could not be destroyed as it was the case with white flour. The slow hydrolysis of phytic acid in wholemeal wheat flour fermentation observed in the present study may be due to presence of high concentration of phytate phosphorus in the medium which inhibits phytase activity. This confirms earlier observations made for several sorts of Polish bread and wholemeal wheat flour (9, 16).

As compared with the initial phase, hydrolysis of phytate in doughs in the second phase of fermentation was faster. Addition of the flour to the sour doughs as the pH lowered was the possible reason for increased rate of phytate hydrolysis during the second phase of fermentation.

In the straight-dough process, when breads made with soy-fortified flour, the percentage of phytate hydrolysed in the bread is much lower than in the bread prepared from the sour dough. The major reduction of phytate occurred in the breads during the first 145 min of rising followed by little additional loss up to 225 min of rising. Harland and Harland (1980) observed similar trends in breads prepared from rye flour, white flour, and wholemeal wheat flour (17). In contrast, Ranhotra *et al*. (1974) observed that more than three-fourth of the phytate was hydrolysed when yeasted breads were made with soy-fortified wheat flour (18).

Ter-Sarkisian *et al* (1974) compared fermentative loss of phytate in yeasted and sour doughs made from a sample of 75% extraction flour. In two hr at 23°C, flour doughs lost 37-50% of phytate, whereas the yeasted doughs lost only 12-37% (19). Chhabra and Sidhu (1988) in bread made with 1.5% yeast using 85% extraction flour, reported 42-46% destruction of phytate after 3-6 hr of fermentation (20).

The complete hydrolysis of phytate in white flour sour dough observed in the present study confirms earlier observations that all of the phytate in wheat bread was hydrolysed during the process of bread making apparently due to phytase in wheat and/or yeast (17, 21). However, Tangkongchitr *et al*.(1982) reported that no phytase enzyme is associated with yeast cells as proposed by Harland and Harland (17, 22).

The main controlling factor in phytate hydrolysis appears to be the pH of the dough. A maximum hydrolysis of about 91% and 100%, respectively, of phytate was noted in bread prepared from soy-fortified wheat flour and white flour. The pH of the dough was 3.76. The hydrolysis of wholemeal wheat flour dough bread having a pH value of 3.83 was 88%. This confirms previous observations that hydrolysis of phytate depends mostly on the acidity and is very low in higher pH values (22). The importance of acidity in doughs was reported by Meuser *et al* and Bartnik *et al* (16, 23).

The comparison of the phytate content in the dough after fermentation and in bread indicates that some destruction of phytate takes place during the baking process, when the temperature is still below the inactivating point of phytase. Hydrolysis of phytate in the first stage of the baking process has also been reported previously (16).

The decrease in phytate phosphorus as accompanied by an increase in inorganic phosphorus content in the sour dough fermentation may be attributed to the activity of phytase present in the flour and the fermenting microorganisms as reported in previous studies (6, 17).

The increase in inorganic phosphorus appeared to parallel the breakdown of phytate in sour dough fermentation; an almost quantitative conversion of phytate phosphorus to inorganic phosphorus in dough was noted. All the phytate phosphorus lost during fermentation ended up as inorganic phosphorus in the breads prepared from soy-fortified wheat flour, wholemeal wheat flour, and white flour sour doughs equalled to 73, 90 and 96%, respectively, of total phosphorus.

Thus, it can tentatively be concluded that at no time during fermentation of the sour doughs and baking of bread do any intermediary hydrolysis products accumulate from phytate. Harland and Harland (1980) and Tangkongchitr *et al*. (1981) observed the same phenomenon which indicates that perhaps all intermediate inositol phosphates in the traditional sour dough breads were dephosphrylated (7, 17).

However, in the straight-dough process, the loss of phytate phosphorus was not equal to the gain in inorganic phosphorus; this indicates that intermediate phosphate esters of inositol accumulated in the dough or bread. Fermentation and baking of yeast-raised bread cause a partial degradation of

phytate to simple phosphates and to inositol phosphates with fewer phosphate groups (24). It is possible that these latter phosphates may have similar iron binding properties compared with those of hexaphosphates (25).

This study showed that phytic acid in low extraction flours was more easily hydrolysed than in wholemeal wheat flour. Accordingly, phytic acid levels in breads made from whole wheat meals would be higher than in breads made from white flour. Our results showed that it should be possible to bake traditional sour dough bread with a low phytic acid content by using the sour dough procedure.

This reduction of phytate content to very low levels would make the wholemeal breads a good source of iron, phosphorus, calcium and zinc, since phytate is known to interfere with absorption of these minerals. The presence of ingredients of high phytase activity (e.g. sour dough), particularly in bread containing wholemeal flour is important to achieve effective phytate reduction.

Acknowledgement

This study was financially supported by the United Nations University, Tokyo, Japan and Central Food Technological Research Institute, Mysore, India. The excellent technical assistance of Sasikala BV is gratefully acknowledged.

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