Original article

Traditional Sour Dough Bread (*Difo Dabbo*) Making: II. effects on the HCL-extractability of minerals

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Abstract: Traditional sour dough bread (*Difo dabbo*) was prepared from wholemeal wheat flour, soy-fortified wheat flour (*Dubbie* flour) and white flour. Yeast-raised bread was prepared from *Dubbie* flour by the straight-dough process. Sour dough fermentation of bread significantly reduced phytic acid content and increased the HCl-extarctability of calcium, iron, zinc and phosphorus. The extractability increased with an increase in the period of fermentation. Higher extractability of the minerals was obtained in white flour sour dough bread. Wholemeal wheat flour sour dough bread exhibited relatively lower extractability of the minerals compared to the other two sour dough breads. Significantly ($p<0.05$) lower values for HCl-extractability of minerals were observed in bread prepared by the straight dough process. The sour dough fermentation is an effective method for improving HCl-extractability and possibly the bioavailability of minerals which helps to prevent and ameliorate mineral deficiencies and improving the nutritional status of people consuming such food. *Ethiop. J. Health Dev.* 1998;12(3):175-181]

Introduction

Grains and products made from milled grains supply considerable percentages of the nutritionally important proteins, B vitamins, and minerals and trace minerals. Bread, and particularly bread from high extraction flours and whole grain, contains many materials. The higher the degree of extraction of the flour, that is, the greater the yield of flour for a given weight of grain, the greater the amount of seed coat and of the outer layer in the flour. Such flours are darker. Short extraction flour (or white flour) contains almost exclusively portions of the endosperm (1).

The major portion of the minerals is found in the outer layers of the grain, where high extraction flour contains higher concentration of minerals. However, absorption studies in humans indicate that higher extraction flour substantially reduces the availability of minerals due to the presence of high concentration of phytic acid in the bran (2).

Phytic acid (the hexaphosphate ester of *myo*- inositol, present in considerable amounts in cereals, has been considered as an antinutrient due to its inhibitory effect on mineral bioavailability. The most striking chemical impact of phytic acid is its chelating ability with multivalent cations, especially divalent and trivalent cations to form cation-phytic acid complexes. The complexes are usually soluble at acidic pH, but they have limited solubility at neutral pH, a pH near to that in the small intestine. The insolubility of the complexes is regarded as a major reason for the reduced bioavailability of phytic acid-mineral complexes (3).

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The amount of phytate in the diet is, therefore, of practical importance in relation to mineral nutrition in diets based on cereals. The loss of phytate during normal food preparation, therefore, deserves further investigation. Fermentation is known to bring about several desirable nutritional changes on the fermented products. Previous studies have shown that sour dough and yeast fermentation of bread

Table 1: **Phytic acid (%) and HCl-extractability (%) of calcium, iron, zinc, and phosphorus in soy-fortified wheat flour sour dough bread***

Time (hr)	Calcium	Iron	Zinc	Phosphorus	Phytic acid
Phase I					
0	26.13±1.12[a]	32.31±3.06[a]	48.57±0.95[a]	38.21±0.24[a]	0.52±0.04[a]
6	32.42±3.71[b]	41.26±2.85[b]	61.72±3.41[b]	44.41±0.51[b]	0.35±0.03[b]
12	40.76±2.34[c]	49.57±1.30[c]	65.46±4.41[c]	51.54±1.19[c]	0.23±0.03[c]
18	45.61±4.23[d]	54.37±2.15[d]	70.78±5.95[d]	9.81±0.98[d]	0.13±0.02[d]
Phase II					
0	39.67±5.76[c]	49.63±4.58[c]	73.39±1.41[d]	64.45±1.87[e]	0.64±0.08[e]
3	47.59±1.64[d]	64.51±3.17[e]	78.63±2.58[e]	71.15±1.42[d]	0.25±0.11[c]
6	66.41±1.82[e]	77.12±4.92[f]	85.71±6.27[f]	78.18±4.51[f]	0.12±0.03[d]
Bread	73.63±7.21[f]	79.36±5.14[f]	88.26±2.41[f]	86.41±4.31[g]	0.06±0.02[e]

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

resulted in a significant reduction in phytic acid content (4-6).

Besides reducing the level of phytic acid in the fermented products, fermentation has also been reported to convert bound form of minerals to free forms which is responsible for increased HClextractability (an index of their bioavailability to humans) of minerals of the fermented product (7). Solubility of minerals in foodstuffs under simulated gastric conditions has also been reported to be indicative of their bioavailability from those foodstuffs (8).

The effect of natural fermentation on the HCl-extractability of minerals from tef (*Eragrostis tef*) has been reported previously (9). However, no systematic studies have been carried out so far to evaluate the effect of sour dough and yeast fermentation on the extractability of minerals. This study reports the effect of the traditional Ethiopian sour dough bread (*Difo dabbo*) and yeast-raised bread making on the HCl-extractability of phosphorus, calcium, iron and zinc in 0.03N HCl, the concentration of HCl in the human stomach.

Methods

Ingredients: Soy-fortified wheat flour (soy flour, 5%), locally known as *Dubbie* flour, obtained from Faffa Foods Factory, Addis Ababa, Ethiopia, was transported to India and stored at 4°C until used. Commercial whole wheat flour, 72% extraction, and white flour, 62% extraction, was kindly supplied by the Department of Milling and Baking Technology, Central Food Technological Research Institute, Mysore, India.

Bread making: Sour dough breads (*Difo dabbo*) were prepared from soy-fortified wheat flour, whole wheat flour, and white flour as described previously (10). Yeast-raised bread was baked as pup loaves following the straight-dough procedures as described in the AACC (11). The dough contained the following ingredients: soy-fortified wheat flour, 300 g; double distilled water, 186 g; sugar, 7.5 g; fat, six g; compressed yeast, six g; and barley malt flour, 1.5g. The doughs were mixed to optimum in a Hobart mixer, fermented (90% rh) for 175 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 constant weight and ground in an electric grinder (M/S Milone, Rajkot, India) using 0.5mm sieve.

Analytical methods: Phytic acid contents of the cereal flours and their food products were determined using a spectrophotometer method (12). 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).

Mineral analysis: The samples were acid-digested using a nitric acid-perchloric acid mixture

[HNO_3: $HClO_4$, 5:1 (v/v)]. The amounts of iron and zinc in the digested samples were determined by atomic absorption spectrometry (Perkin-Elmer, Model 3110, Norwalk, CT, USA) according to the method of Lindsey and Norwell (14). Phosphorus in the digested samples was estimated colorimetrically (13), whereas calcium was determined by the titration method (15).

Hcl-extractablity of minerals: The minerals in the fermented and unfermented samples were extracted with 0.03 N HCl by shaking (Environ Shaker, Model 3597-I, LabLine Instruments, Melrose Park, Ill., USA) the contents at 37°C for three hr. The clear extract obtained after filtration with Whatman #42 filter paper was oven-dried at 100°C and wet-digested with diacid mixture. The amounts of extractable phosphorus, iron, calcium, and zinc in the digested samples were determined by the methods described earlier.

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0.03N HCl}}{\text{Total mineral}} \times 100$$

Samples from different fermentation periods were statistically compared using analysis of variance to estimate the level of significance and correlation coefficients according to standard methods (16). Differences were considered significant at <0.05.

Results

In the initial phase of fermentation, the phytic acid contents of soy-fortified wheat flour decreased by 75% (Table 1). Phytic acid in wholemeal wheat flour (Table 2) and white flour (Table 3) decreased by 75 and 90%, respectively. Addition of fresh flour to the fermented sour doughs increased the phytic acid content which was subsequently decreased to 0.06 and 0.11%, respectively, in soyfortified wheat flour (Table 1) and wholemeal wheat flour (Table 2) following second phase of fermentation and then baking of bread. Phytic acid in white flour was completely hydrolysed (Table 3) during the second phase of fermentation and baking of bread. In the straight dough process, the overall phytic acid reduction was 39% following fermentation and bread making (Table 4).

Sour dough fermentation significantly ($p<0.05$) improved the HCl-extractability of phosphorus, calcium, iron, and zinc; the longer the period of fermentation, the higher was the HCl-extractability. HCl-extractability of phosphorus in soy-fortified wheat flour sour dough increased gradually with an increase in the period of fermentation, i.e, from 0-18 hr (Table 1). Similarly HCl-extractability of, phosphorus increased significantly ($p<0.05$) in wholemeal wheat flour (Table 2) and white flour (Table 3) following initial phase of fermentation. At the start of the second phase of fermentation, the percentage extractability of phosphorus was decreased by about 5%, 8% and 7% in soy-fortified wheat flour (Table 1), wholemeal wheat flour (Table 2), and white flour (Table 3), respectively. This percentage was increased to about 86, 78 and 87% in soy-fortified wheat flour, whole wheat flour, and white flour sour dough bread, respectively. A significantly ($p<0.05$) negative correlation occurred between the phytic acid and extractable phosphorus. The extractable phosphorus in the straight-dough bread, however, was low (73%) (Table 4).

Table 2: **Phytic acid (%) and HCl-extractability (%) of calcium, iron, zinc, and phosphorus in wholemeal wheat flour sour dough bread***

Time (hr)	Calcium	Iron	Zinc	Phosphorus	Phytic acid
Phase I					
0	21.47±0.82[a]	28.62±1.47[a]	44.93±0.14[a]	34.64±1.19[a]	0.68±0.07[a]
6	25.62±0.63[a]	30.19±2.71[a]	48.94±0.28[b]	48.89±0.44[b]	0.43±0.07[b]
12	32.71±0.41[b]	43.24±1.42[b]	54.15±0.70[c]	56.11±0.37[c]	0.28±0.08[c]
18	46.38±0.87[c]	46.17±5.78[b]	61.78±0.68[d]	62.66±0.88[d]	0.17±0.06[d]
Pahse II					

0	41.18±0.46[d]	51.14±1.17[c]	66.34±1.62[e]	54.93±0.93[c]	0.85±0.08[e]
3	58.67±3.81[e]	56.84±2.44[d]	66.51±2.58[e]	65.27±6.72[d]	0.38±0.05[f]
6	61.65±1.62[f]	66.92±3.27[e]	70.48±3.94[f]	75.36±1.45[e]	0.15±0.05[d]
Bread	66.72±1.78[g]	68.11±2.43[e]	72.76±6.17[f]	78.37±2.87[f]	0.11±0.05[g]

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

Table 3: **Phytic acid (%) and HCl-extractability (%) of calcium, iron, zinc, and phosphorus in white flour sour dough bread***

Time (hr)	Calcium	Iron	Zinc	Phosphorus	Phytic acid
Phase I					
0	37.62±2.43[a]	45.78±0.49[a]	43.65±2.65[a]	51.33±1.20[a]	0.20±0.01[a]
6	45.17±1.71[b]	51.89±0.89[b]	49.63±3.88[b]	54.41±2.48[a]	0.17±0.01[b]
12	51.86±0.85[c]	57.36±0.45[c]	54.60±3.52[c]	62.46±1.21[b]	0.06±0.01[c]
18	59.78±0.93[d]	64.21±0.61[d]	59.54±1.69[d]	75.28±4.24[c]	0.02±0.01[d]
Phase II					
0	55.82±2.19[c]	54.58±0.93[b]	64.64±2.14[d]	68.65±5.18[d]	0.23±0.05[a]
3	60.65±5.36[d]	69.62±1.16[e]	68.56±4.64[e]	79.31±3.45[c]	0.09±0.01[c]
6	72.71±4.46[e]	77.73±4.34[f]	72.46±7.03[f]	85.17±2.69[e]	0.02±0.01[d]
Bread	74.15±5.09[e]	83.69±3.27[g]	86.16±3.59[f]	87.49±6.54[e]	-----

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

The calcium extactability increased to varying extent depending upon the period and type of fermentation. Following initial phase of fermentation, calcium extractability increased by 45, 46 and 59% in soy-fortified wheat flour (Table 1), wholemeal wheat flour (Table 2), and white flour (Table 3), respectively. The extractable calcium was lower in wholemeal wheat flour bread (Table 2) compared with the breads prepared from soy-fortified wheat flour (Table 1) and white flour (Table 3). In the straight-dough process, the extarctability of calcium was much lower (only 62%) compared to the sour dough process bread.

The unfermented soy-fortified wheat flour (Table 1), wholemeal wheat flour (Table 2) and white flour (Table 3) water mixtures contained 32, 28 and 46% extractable iron, respectively. Fermentation of the mixture resulted in a significant ($p<0.05$) increase in the extractability of iron. The extractability increased by about 69% in wholemeal wheat flour (Table 2),72% in soy-fortified wheat flour (Table 1) and 46% in white flour (Table 3) following the initial phase of fermentation. The extractability of iron reached a maximum after six hr of second phase of fermentation and baking; iron extractability in white flour sour dough breads was significantly higher than in soyfortified wheat flour and white flour. Bread prepared by the straight dough process has only 56% extractable iron (Table 4).

The HCl-extractability of zinc from the soy-fortified wheat flour (Table 1), wholemeal wheat flour (Table 2), and white flour (Table 3) was 49, 45 and 43%, respectively, at zero hour which increased to 71%, 62% and 60%, respectively, following fermentation for 18 hr (initial phase of fermentation). Addition of flour to the sour doughs and fermentation for sixhr and baking further improved the extractability of zinc. The highest extractability of zinc (88%) was observed in sour dough bread prepared from white flour (Table 3). The extractable zinc in bread prepared by the straight-dough process was low and only 55% (Table 4).

Discussion

Sour dough fermentation of bread resulted in a significant reduction in phytic acid content compared with the yeast-raised bread. The presence of ingredients of high phytase activity (sour dough) in the fermenting mixtures may have significantly contributed to achieve effective phytate reduction.

With regard to availability, phosphorus in plant materials is much less available than in animal materials as a substantial proportion is originally bound in the form of phytate phosphorus. In the present study, the improvement in the phosphorus extractability corresponded with a proportional decrease in phytic acid at all periods of fermentation in both the sour dough and straight-dough processes. This showed that hydrolytic reduction of phytic acid during sour dough fermentation and yeast fermentation may contribute towards increase in the extractable-phosphorus. Correlation coefficients showed a significant ($p<0.05$) negative correlation

Table 4: **Phytic acid (%) and HCl-extractability (%) of calcium, iron, zinc and phosphorus in soy-fortified wheat flour yeast-raised bread***

Time (min)	Calcium	Iron	Zinc	Phosphorus	Phytic acid
90	35.84±4.38[b]	36.48±1.28[a]	36.41±1.32[a]	44.23±0.29[b]	0.42±0.07[b]
145	44.73±2.81[c]	40.60±3.84[b]	43.55±2.05[b]	55.91±0.84[c]	0.36±0.02[c]
170	53.47±3.12[d]	44.19±0.97[b]	50.12±3.14[c]	63.85±1.04[d]	0.34±0.05[c]
225	58.20±1.95[e]	52.65±4.15[c]	52.35±1.68[c]	67.02±0.76[d]	0.34±0.03[c]
Bread	62.72±5.12[f]	55.54±1.93[c]	55.25±1.76[d]	73.66±1.19[e]	0.33±0.06[c]

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

between phytic acid and extractable phosphorus. Thus, the lower the phytic acid, the greater was the extractable phosphorus in the breads.

The reduction in phytic acid during sour dough and yeast fermentations may be attributed to the hydrolysis of phytic acid by phytase. Phytase, a normal constituent of wheat grain, becomes active in appropriate pH conditions (17). Phytase may also be produced by the fermenting microflora (18). Phytase from these two sources hydrolyses the phytic acid in the fermenting mixtures and liberates inorganic phosphorus (values not shown) which may increase the HCl-extractability of phosphorus in the fermented product. A decrease in phytic acid and simultaneous increase in phosphorus extractability of pearl millet has been reported previously during natural fermentation (19).

The effect of reduction of phytate from the yeast used in yeast-fermented bread can be regarded as minor since, according to previous investigators, it does not contain any or very little phytase (4). It seems likely that the main phytate reduction in yeast-raised bread was as a result of endogenous phytase activity of the soy-fortified wheat flour.

Sour dough fermentation processes improved the extractability of minerals, an index of their bioavailability to the human system. The low extractability of minerals during the first six hours of initial phase of fermentation may be ascribed to the high phytic acid content and pH of the fermenting mixtures. Champagne and Phillipy (1989) observed that the solubility of minerals decreases, and the binding of minerals and phytic acid increases, with increasing pH (20); therefore, it is expected that pH influences the extractability of minerals.

Addition of flour to the fermented dough led to a significant increase in phytic acid content. This again led to a decrease in the extractability of calcium, iron, and phosphorus at the start of the second phase of fermentation. Hallberg *et al.*(1987) reported that the removal of phytic acid in bran by endogenous phytase significantly increased iron absorption, and the inhibition could be restored to a marked extent by restitution of the phytate content (21). However, despite the rise in phytic acid content, extractable zinc in the sour doughs remained unaffected at the start of the second phase of fermentation.

Highest extractability of calcium, iron, and zinc was obtained in white flour sour dough bread; that of phosphorus in soy-fortified wheat flour, and white flour sour dough breads. The HClextractability of calcium, iron, zinc, and phosphorus in the sour dough bread prepared from wholemeal wheat bread was relatively lower compared to the other two sour dough breads. McCance and Widdowson (1942) found that bread from high extraction flour reduced the retention of minerals in humans, and that phytic acid destruction improved their retention (2). In the prsent study, lower values for HCl-extractability minerals were observed in bread prepared by the straightdough process.

Higher HCl-extractabilities of calcium, iron, and zinc from the breads prepared from soy-fortified wheat flour, whole wheat flour, and white flour may be partly ascribed to the decreased content of phytic acid, which had a significant negative correlation with the minerals extractability. Decrease in phytic acid content possibly through hydrolysis by flour phytase and phytase of the fermentative microflora (18) may indicate that the divalent cations are freed from the phytate-mineral complex, which may account for the increased HCl-extractability in the sour dough and yeast-raised breads. Fermentation has also been reported to increase the HCl-extractability of minerals in corn and soybean (22-23).

Fermented foods such as bread, contain considerable quantities of inosotol phosphates with less than six phosphates. The dephosphorylation of phytate to lower inosotol phosphates (which might eliminate the negative effect on mineral absorption) may be achieved by fermentation during dough making. In the present study, the decrease in phytic acid appeared to parallel the increase in extractable phosphorous indicating that at no time during fermentation of the sour doughs and baking do any intermediary hydrolysis products accumulate from phytate and perhaps all intermediate inositol phosphates were dephosphorylated.

Lonnerdal *et al*. (1989) found that the inhibitory effect of phytate was dependent on the degree of phosphorylation of inosotol (24). At higher degrees of phosphorylation, calcium and zinc absorption was significantly inhibited, whereas no effect was observed at lesser degrees of phosphorylation.

Consumption of low extraction flours in Ethiopian households is limited due to cost and ease of availability. The traditional diets including sour dough bread (*Difo dabbo*) are prepared mainly from high extraction flours which are usually rich in phytic acid and minerals. Effective reduction of phytic acid in foods prepared from such high extraction flours can be obtained through sour dough fermentation. Sour dough fermentation thus appears to make the wholemeal foods a good source of calcium, iron, zinc, and phosphorus.

The traditional sour dough bread (*Difo dabbo*) prepared by the sour dough fermentation of wheat is, therefore, an effective method for improving HCl-extractability and possibly the bioavailability of minerals, thus improving the nutritional quality of wholemeal flours.

The availability of minerals from plant foods such as cereals and legumes is limited due to the presence of antinutrients. Increased extractability of minerals in wholemeal foods, especially sour dough bread, is particularly important from a nutritional view point. Consumption of these indigenous fermented products may be useful in preventing and ameliorating mineral deficiencies caused by their limited bioavailability and improving the nutritional status of populations consuming such foods.

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References

1. Spicher G. Baked goods. Biotechnol 1893;5:1-80.
2. McCance R and Widdowson E. Mineral metabolism of dephytinized bread. J Physiol 1942;101:304-309.
3. Erdman JW. Oilseed phytates: Nutritional implications. J Am Oil Chem Soc 1979;56:736-741.
4. Tangkongchitr U, Sieb P A, and Hosney R C. Phytic acid. II. Its fate during breadmaking. Cereal Chem 1981;58:229- 234.
5. Van Lonkhuijsen HJ and van Gelderen AW. Changes in the phytic acid contents during bread making with yeast and with sour-dough. Voeding 1985;:46:98-101.
6. Fretzdorff B and Brummer J-M. Reduction of phytic acid during breadmaking of whole meal breads. Cereal Chem 1992;69:266-270.
7. Mahajan S and Chauhan BM. Effect of natural fermentation on the HCl-extractability of minerals from pearl millet flour. J Food Sci 1988;53:1576-1577.
8. Lock S and Bender AE. Measurement of chemically available iron in foods by incubation with human gastric juice *in vitro*. Br J Nutr 1980;43:413-415.

9. Kelbessa U and Narasimha HV. Effect of natural fermentation on the HCl-extractability of minerals from tef. Bull Chem Soc Ethiop 1997;11:3-10.
10. Kelbessa U. Bioavailability of Iron and Zinc in Some Traditional Diets of Ethiopia. Report to the UNU, Tokyo, Japan, 1996.
11. American Association of Cereal Chemists. Approved Methods of Analysis of the AACC. The Association, St .Paul, MN, 1969.
12. Haug W and Latzsch HJ. A sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agric 1983;34:1423-1426.
13. Fiske CH and Subbarrow Y. The colorimetric determination of phosphorus. J. Biol. Chem 1925;66:375-400.
14. Lindsey WL and Norwell MA. A new DPTA-TEA soil test for zinc and iron. Agron Absts 1969;61:84.

15. Association of Official Analytical Chemists (AOAC). 16th edn. Official Methods of Analysis, Washington DC, 1995.
16. Snedcor GW and Cochran WG. Statistical methods, 8th edn. Ames: Iowa State University Press, 1989.
17. Lim PE and Tate ME. The phytases. I. Lysolecithin-activated phytase from wheat bran. Biochem Biophys Acta 1971;250:155-158.
18. Lopez Y, Gordon DT and Fields ML. Release of phosphorus by natural lactic acid fermentation. J Food Sci 1983;48:953-954.
19. Mahajan S and Chauhan BM. Phytic acid and extractable phosphorus of pearl millet as affected by natural lactic acid fermentation. J Sci Food Agric 1987;41:381-386.
20. Champagne ET and Phillipy BQ. Effects of pH on calcium, zinc and phytic acid solubilities and complexes following *in vitro* digestions of soy protein isolate. J Food Sci 1989;54:587-592.
21. Hallberg L, Rossander L and Skanberg AB. Phytates and the chelating effect of bran on iron absorption in man. Am J Clin Nutr 1987;45:988-996.
22. Chompreeda PT and Fields ML. Effect of heat treatment and fermentation on the extractability of minerals from soybean meal and corn meal blends. J Food Sci 1984;49:566-568.
23. Goyal R and Khetarpaul N. Effect of fermentation on HCl- extractability of minerals from rice-defatted soy flour blend. Food Chem 1994;50:419-422.
24. Lonnerdal B, Sandberg A-S, Sandstom B and Kunz C. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. J Nutr 1989;119:211-214.