Original Article
DOI: https://doi.org/10.70749/ijbr.v3i5.1269

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Crossref

OPEN ACCESS

The Impacts of Fermentation and Varied Leavening Agents on Phytic Acid Degradation and Mineral Nutritional Quality of Conventional Pakistani Flat Wheat Breads

Fazia Ghaffar[1]

[1]Department of Food & Nutrition Sciences, College of Home Economics, University of Peshawar, Pakistan

ARTICLE INFO

Keywords
Phytic Acid, Mineral Solubility, Phytate Degradation, Fermentation, Conventional Flat Breads

Corresponding Author: Fazia Ghaffar
Department of Food & Nutrition Sciences, College of Home Economics, University of Peshawar,Pakistan
Email: faziaghaffar@uop.edu.pk

Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 08-02-2025 Revised: 31-03-2025
Accepted: 28-04-2025 Published: 09-05-2025

ABSTRACT

Introduction: Wheat bread is a staple Pakistani food, and whole grain is generally thought of as being healthier than white bread due to its higher dietary fiber, vitamins, and many important minerals. However, wholegrain bread also contains high levels of phytate, which may bind desirable nutrients, preventing their absorption in the gut and thereby reducing the efficacy of the end product. Fermentation has been shown to decrease the amount of phytate in wholegrain cereals. The indigenous methods of leavening may be a potential biofriendly way of enhancing the nutritional quality of local wheat breads. **Methods**: The study aimed to evaluate the impact of heat processing on phytic acid, mineral content, and mineral solubility of conventional wheat breads. Six types of wheat flours were selected, including fine wheat flour, chakki wheat flour, maida, whole wheat flour, and granulated wheat flour. Initially, the raw flour was analyzed for percent phytate, phosphorus, and iron concentrations. Three types of breads: chapatti, flat bread made on an iron skillet, and tandoori roti (conventional hot oven), after preparing doughs as unleavened, fermented with bicarbonate soda, commercial yeast, and sourdough culture. All types of breads were analyzed for percent phytate, percent phytate degradation, mineral content, and mineral solubility as per standard procedures. **Results:** The results showed that raw fine wheat flour had the highest phytic content, while unleavened categories had the highest phytic acid content. The sourdough cultured leavened flour, the highest levels were observed as tawa Roti at 0.67% ± 3.30, chapatti at 0.99% ± 2.44, and tandoori Roti at 1.15% ± 2.29. The percentage degradation of phytate in fine wheat flour was highest in sourdough cultured tandoori roti at 61.8 %, followed by sourdough tawa roti at 61.0% and yeast tandoori roti at 60.47. The per cent degradation of phytate in Chakki wheat flour breads was highest in sourdough cultured tandoori roti (75.3), tawa Roti (70.1%), chapatti (57.5) and for the yeast fermented roti ithe higher degradation occurred in chapatti (55.4), tandoori roti (51.8) and tawa roti (48.2). soda tawa Roti (67.6 %), and yeast chapatti (0.3 %). Doughs leavened with soda also showed appreciable degradations. The percent degradation of phytate in maida wheat flour was highest in sourdough cultured tandoori roti (79.8%), tawa roti (69.5%), and chappati (58.5). The percent degradation of phytate in whole wheat flour was highest in sourdough cultured tandoori roti 57.7%, followed by tawa roti 47.6%, and chapatti 45.3%. Fermentation with the addition of bicarbonate soda also resulted in degradation. The unleavened breads showed the least reduction. The effects of fermentation were positive on both mineral content and mineral solubility. **Conclusion:** The current study concludes that the conventional method of making fermented wheat breads degrades phytate significantly while improving the mineral nutrition of these breads.

INTRODUCTION

Wheat (*Triticum aestivum L.)* belongs is a member the most substantial significant *Poaceae* family and serves as the principal cereal crop for the majority of the world's population. This cereal is polyploid nature and domestically is cultivated globally. The name for wheat, i.e., Triticum, derived from the Latin word 'tero' (I 'tero,' meaning 'I thresh.' name, *Triticum aestivum*, represents hexaploid bread refers to with genomes wheat, which possesses the D, differentiating it D genomes, distinguishing macaroni wheat, which is Triticum durum, comprising known as and B, which comprises the consumed predominantly B genomes the production primarily used Nowadays, pasta production. Today, the most extensively grown wheat. It is widely cultivated type of type of free-threshing wheat (genome variety According to Nesbitt with the genome configuration AABBDD. it stemmed from the recent hybridization originated the diploid (DD) *Aegilops tauschii var. strangulata and* an allotetraploid wheat

strangulata no longer than 8,000 years ago [1].

Triticum aestivum and *Triticum durum* have seven pairs of chromosomes (2n = 14). Wheat is grown as either spring or winter crops. In very cold regions, spring wheat varieties are planted in the spring to ensure they grow and mature quickly, allowing for harvest before autumn snow arrives. In more temperate climates, winter wheat is sown before winter snowfall, which covers the seedlings and promotes vernalization, enabling rapid growth when the snow melts in spring. In warmer climates, the distinction between spring and winter wheat is less relevant, with the main difference being the timing of maturity. [2]. Types of wheat have been frequently differentiated according to endosperm texture, seed coat, dough strength, color, and planting season. [3].

Wheat is one of the most important domesticated crops grown around the world. Bread wheat plays a major role among the few crop species being extensively grown as food sources, and was likely a central point to the beginning of agriculture [4]. Global wheat production is concentrated mainly in Australia, Canada, China, European Union, India, Pakistan, Russia, Turkey, Ukraine and the United States, accounting for over 80% of world wheat production. Pakistan is the 8th largest wheat producer, contributing about 3.17% of the world's wheat production from 3.72% of the wheat growing area. Wheat in Pakistan is a leading food grain and occupies a central position in agriculture and its economy [5]. An estimated 35 % of the world's population depends on wheat as their main crop. More than two-thirds of the world's wheat is consumed for human consumption, while just a fifth is used for animal feed [6]. Pakistani wheat varieties are grown over a wide agro-climatic range and, as such, are expected to exhibit yield and quality differences [7].

The nutritional value of wheat mostly composed of 75–80% carbohydrates, 9–18% protein, fiber, several vitamins (particularly B vitamins), calcium, iron, and a variety of macro- and micronutrients [8]. Also, the germ part is composed of almost 50%g/100 g dry matter total carbohydrates and various micronutrients such as phosphorus, magnesium, zinc, iron, manganese, thiamin (B1), riboflavin (B2) and pyridoxine (B6) [9-11]. Moreover, the outer layer of the seed (bran) contains 67.5%g/100 g dry matter carbohydrates and 48.2%g/100 g dry matter [12]. This exceeds the number of all other grain crops (including rice, maize, etc) in terms of acreage, application, usability, consumption and industrial production, so it could be considered as world's most important cereal grain crop [13].

Phytic acid is the main storage form of phosphate in cereals, amounting up to 70% of total seed phosphate in cereals [14]. In addition, minerals such as Fe, Zn and Se are partially bound by phytic acid in a complex [14-16]. Monogastric animals and humans have only very low intrinsic phytase activity in their digestive tracts [14]. Thus, these minerals remain bound to phytic acid without absorption from the intestine and are largely excreted undigested, which in the case of phosphate is also of importance for the environment. Thus, the availability of minerals is a nutritional issue for humans and animals as well as an issue of environmental and resource protection. The latter issue has long been the subject of scientific research [17]. Apart from being a mineral chelating molecule, Phytic acid can also impact amino-acid and carbohydrate metabolism (by binding of amino acids, peptides and digestion enzymes), can act as a strong antioxidant (e.g., reducing iron induced lipid peroxidation) and possess anticancer properties (e.g., by binding the strong pro-oxidant free iron and reinforcing apoptosis in cancerous cells). Sodium phytate is registered as a generally recognized safe substance that can be used for food stability in a wide variety of food production processes [18]

Phytic acid (PA) is a six-fold dihydrogen phosphate ester of inositol, also called as myo-inositol 1,2,3,4,5,6 hexakisphosphate. The numbers 1–6 stand for the presence of 6 potential binding sites in the molecule. Phytic acid serves as a phosphate storage (Ins P6) molecule in cereals, which, due to its affinity for metal ions, also strongly chelates the cations Zn^{2+} and Fe^{2+}, as well as Mg^{2+}, Ca^{2+}, K^{+}, Mn^{2+} and Cu^{2+}. During seed development, phytic acid accumulates as mixed salts containing P, Mg and K and to a lesser extent Ca, Zn and Mn [19, 20].

Efforts have been made to reduce the concentrations of phytic acid in a variety of cereals and grains and some practical and relatively economical procedures such as soaking, germination, and fermentation are reported to reduce the phytic acid content of legumes at the domestic levels. Over the last decade, the baking industry has performed intensified efforts to reduce phytic acid in whole grain bread, using two potential approaches: (1) inducing acidity during dough fermentation in bread making, which increases endogenous phytase activity, and by (2) adding exogenous phytases to the dough making process. Many studies have shown that the type of grain (wheat, rye, oat, maize, etc) have different phytic acid and endogenous phytase contents and the type of flour used (whole meal, white flour) and the fermentation conditions (yeast or sourdough (SD), their specific microbiota composition, fermentation duration and temperature, level of pH reduction are mutual determinants of phytase activity and phytate degradation in the dough [21-24]. Due to the high content of phytic acid and phytates in the bran fraction of whole grain, compared to isolated white flour, sourdough fermentation of whole grain flours, resulted in significant reduced dough pH, the highest phytate reductions, and a related increase in free Ca^{2+}, Zn^{2+}, Fe^{2+}, Mg^{2+} [25]. Long-term sourdough fermentation, with a

mutual effect of acidification, by stimulating flour endogenous phytases, and the presence of phytates expressing microbiota, results in significantly higher phytate degradation compared to short-term yeast fermentation. The latter has been attributed to the less acid formation and the low phytase activity of yeast [26, 27]. Conventional heat treatments, such as those used in domestic cooking or industrialized processing, have generally been reported to cause more moderate losses of phytic acid. The current study aimed at analyzing the effects of various fermentation procedures commonly used in Pakistan on phytate degradation and mineral quality in wheat breads commonly consumed in Pakistan.

MATERIALS AND METHODS

Sampling

The sample comprises of wheat (raw wheat, unleavened and leavened chapatti, roti with soda, sourdough culture, and yeast) commonly used in Pakistan. The wheat samples were procured t from the agricultural field of NIFA and Research Institute Tarnab, Peshawar and were mixed thoroughly for homogeneity. Two processing techniques (cooking without fermenting/leavening and with fermenting/leavening) were applied.

Table 1: Sampling Procedures

SAMPLE	PROCESSING	REPETITION (MEAN)	TOTAL SAMPLE
1. Wheat flour 2. Chapatti, 3. Overheat roti, 4. tawa roti, 5. tandoori roti	1. Raw 2. Unfermented/Unleavened Cooking	The whole process was repeated three times for the mean	6 wheat samples x 2 processing methods x 3 (mean) Total = 36 wheat samples
1. Chapatti, 2. Overheat roti 3. Tawa roti 4. Tandoori roti	Fermented/Leavened 1. Bicarbonate soda 2. Yeast 3. sourdough culture		

Determination of Phytic Acid

The phytic acid content of the samples was determined by the method of Haung W & Lantzch H (2006) under the principle of spectrophotometry, which was an instrument used for the determination of small quantities of substances when light passed through a medium.

Sample Preparation

A Ground sample of 0.06 gram each was taken in separate conical flasks & 10 ml of 0.2 N HCL was added to each flask. The flask was shaken for 39 minutes in a shaking water bath at 37ºC. After shaking samples were filtered. Filtrate was taken in a test tube. The same procedure as applied for reference solutions. Percent phytate content of the sample was calculated from the standard curve. The phytic acid content of the sample was estimated by the following equation.

Phytic acid = (phytate phosphorous × 4.97) / (1000 × 0.06)

The following solutions were necessary:

Phytate Reference Solution

The sodium salt of phytic acid type V (97% purity), containing approximately 15% water, obtained from Sigma (NO. P-5756), was used without further purification. The actual content of phytate must be determined for each new purchase of phytic acid using a direct method. A stock solution was prepared by dissolving 0.15 g of sodium phytate in 100 mL of distilled water. Since phytase was absent, this stock solution remained stable. The reference solutions were prepared by diluting the stock solution with HCl, resulting in phytate phosphorus concentrations ranging from 3 to 30 µg/ml (requiring about 1.2 to 11.7 mL of stock solution in 100 mL). The final concentration of HCl in the reference solutions was 0.2 N.

Preparation of a Series of Reference Solutions

3µg/ml 6µg/ml 9µg/ml
12µg/ml 15µg/ml 18µg/ml
21µg/ml 24µg/ml 27µg/ml
30µg/ml

Calculations

Molecular weight of phytic acid ($C_6H_{18}O_{24}P_6$) = 660
12Na atoms replace 12-H atoms.
So, 12 x 23 = 276 - 12 (H) = 264.
So, the molecular weight of sodium phytate = 660 + 264 = 924g.
Now there were 6 phosphorus atoms in sodium phytate.
P = 31 x 6 = 186.
So, 924 of Na-phytate contains 186 grams of phosphorus.
0.15g of Na phytate contains 186/925 x 0.15 = 0.0302g/100ml.
Or 30.2mg/100ml or 0.302mg/100ml or 302µg/ml.
The concentration of phosphorus in 0.15g/100 ml of Na phytate solution was 302mg/ml.

Ferric Solution

Dissolve 0.2 g-ammonium iron (III) sulphate 12 H_2O (Merck Art. 3776) in 100 ml 2N HCL & make up to 1000 ml with distilled water.

2, 2'-Bipyridine Solution

Dissolved was a 10g 2, 2'-bipyridine (Merck art. 3098)

& 10 ml thioglycolic acid (Merck art. 700) in distilled water & make up to 1000 ml. These solutions were stable at room temperature for several months.

Procedure for Developing Standard Curve

From each standard 0.5 ml was taken in a test tube & 1 ml of ferric solution was added. Heat in boiling water for 30 minutes (make sure that tubes remain covered with stopper for the first 5 minutes). Tubes were cooled under tap water & then ice water for 15 minutes. Upon wheat flour to room temperature, add 2 ml of 2, 2' bipyridine solution. The optical density was measured within 0.5–1 minute at 519nm with the help of a spectrophotometer. The optical density against concentration plots the standard curve. It was linear between 0 & 35mg/g concentration of phytic acid.

Spectrophotometry

The Shimadzu UV-1280 spectrophotometer was used to determine small quantities of substances by analyzing how light passes through a medium, with some light reflected, some absorbed, and the rest transmitted. LO = la + H + Ir (It can be minimized to zero by the use of a comparison cell)

Determination of Mineral Content & Mineral Solubility

Determination of Phosphorus

Phosphorus was determined colourimetrically using the vanadate molybdate method as described by the AOAC (2001) method.

Barton's Reagent

25 mg of ammonium molybdate was dissolved in 400 ml of distilled water. 1.5 g of ammonium metavanadate was dissolved in 200 ml of boiling water and cooled. Then, 250 ml (65%) concentrated nitric acid was added to the Meta vanadate solution. Molybdate solution was poured into the vanadate solution, and the volume was made to 1000 ml with distilled water.

Standard Phosphorus Solution

A stock solution of phosphorus 500 ppm was prepared by dissolving 2.1 g of K H2PO4 into distilled water, and the volume was made to 1000 ml. From the stock solution, a series of solutions was prepared to contain 1 to 100 ppm of phosphorus. Colour was developed by adding a few drops of NH_3, a few drops of HNO_3. HOCL (1: 1) mixture and 12.5 ml of Barton's reagent. The Volume was made to 50 ml with distilled water, and the absorbance was read at 470 nm against a blank. A curve was drawn from the results. Wet distilled samples, 5 ml, were taken in a 50 ml volumetric flask. A few drops of NH3, HOCl mixture and 12.5 ml of Barton's reagent were added to it, and the volume was made up with distilled water. The absorbance was noted after 10 minutes at 470nm against a blank, and the amount of phosphorus in the sample was determined using the standard curve.

P (M1/100, pulp) = ppm from Graph X/ - Weight

Determination of Iron by Spectrophotometric Method

This method determines iron content by the reaction with Ortho-phenanthroline and spectrophotometric measurement. It applied to cereals and cereal-based products.

Reagents

(i) Ortho-phenanthroline solution:

About 0.1 g of Ortho-phenanthroline was dissolved in about 20 ml of water at 80°C, cooled, and dilute to 100 ml. It was stored in an amber bottle in the refrigerator. *(Stable for up to several weeks.)*

(ii) Iron standard solution, 10 mg Fe/ml:

About 3.512 g Fe $(NH_4)_2$ $(SO_4)_2 \cdot 6H_2O$ was dissolved in water, and 2 drops of HCl, and diluted to 500 ml. Dilute 10 ml of this solution to 1 litre.

(iii) Hydroxylamine Hydrochloride solution:

About 10 g of $NH_2OH \cdot HCl$ was dissolved in water and diluted to 100 ml. It was stored in an amber bottle in the refrigerator. *(Stable for up to several weeks.)*

(iv) Acetate buffer solution:

About 8.3 g of anhydrous sodium acetate (previously dried at 100°C) was dissolved in water and 12 ml of acetic acid was added and diluted to 100 ml.

(v) Working standards solution:

Place aliquots of the 10 mg/ml standard solution according to the table below into 100 ml volumetric flasks. Add 2 ml concentrated HCl to each dilution and make up to volume.

Aliquots of 10 mg/ml solution taken (ml)	Final Concentration (mg/ml)
0	0.0
5	0.5
10	1.0
15	1.5
20	2.0
25	2.5
30	3.0
35	3.5
40	4.0
45	4.5
50	5.0

Procedure

To prepare the sample, 2 to 10 grams (depending on the expected concentration of iron) were weighed into a clean crucible and heated on a hot plate. The sample was then ashed overnight in a muffle furnace at a temperature below 550°C. Afterwards, the crucible was removed and allowed to cool to room temperature. Next, 5 ml of concentrated hydrochloric acid (HCl) was carefully added to the crucible, ensuring the acid rinsed the upper portion. The mixture was then evaporated to dryness.

Once dry, the residue was dissolved by adding 2 ml of concentrated HCl, which was accurately measured. A watch glass was placed over the crucible, and the mixture was heated for 5 minutes. After heating, the watch glass was rinsed with water, and the solution was quantitatively filtered into a 100 ml volumetric flask. The flask was filled to the mark with distilled water and mixed thoroughly. Subsequently, a 10 ml aliquot was pipetted into a 25 ml volumetric flask. To this, 1 ml of Hydroxylamine-HCl solution was added and mixed thoroughly. After 5 minutes, 5 ml of buffer solution and 1 ml of ortho-phenanthroline solution were added, and the mixture was again diluted to volume and mixed thoroughly. The solution was left to stand for 30 minutes, after which the absorbance of the sample was measured against standard and blank solutions in a spectrophotometer at 510 nm. The results were calculated using a standard curve and the provided Excel spreadsheet.

Calculation of Mineral Solubility

The mineral solubility is calculated as the percentage of soluble minerals relative to the total minerals in the sample.

Statistical Analysis

Data was statistically analyzed through IBM SPSS version 19**.** Data was analyzed for mean, standard deviation. while a paired sample t-test was used to test the significance of the differences in phytate contents in the raw and cooked samples.

RESULTS AND DISCUSSION

Phytate Content and Percent Degradation Wheat Breads

Phytate Degradation Content and Percent Degradation Breads from Fine Wheat Flour

The % age of phytate degradation in fine wheat flour breads is shown in Table 2. The data categorized the samples into three groups: raw, leavened, and unleavened fine wheat flour. Leavened fine wheat flour was further divided into three types: yeast, soda, and sourdough cultured wheat flour. Each of these types underwent three methods of processing: overheated Roti, tawa Roti, and tandoori Roti. According to the table, the phytic acid content in raw fine wheat flour was 0.67% ± 2.10. In the unleavened category, the highest phytic acid content was found in tawa Roti at 1.01% ± 2.48, followed closely by tandoori Roti at 1.00% ± 2.69, and chapatti at 0.96% ± 2.70. For soda leavened flour, the highest phytic acid content was in tandoori Roti at 1.60% ± 3.18, followed by chapatti at 1.21% ± 2.68 and tawa Roti at 0.82% ± 3.00. In the yeast leavened category, chapatti had the highest phytic acid content at 0.82% ± 3.14, followed by tandoori Roti at 0.66% ± 2.27 and tawa Roti at 0.59% ± 2.01. For sourdough cultured leavened flour, the highest levels were observed as tawa Roti at 0.67% ± 3.30, chapatti at 0.99% ± 2.44, and tandoori Roti at 1.15% ± 2.29. The percentage degradation of phytate in fine wheat flour was highest in sourdough cultured tandoori roti at 61.8 %, followed by sourdough tawa roti at 61.0% and yeast tandoori roti at 60.47. These findings are in strong agreement with the findings of other such studies [28-30].

Table 1: Phytate Degradation Content and Percent Degradation of Breads from Fine Wheat Flour

S. No	Sample	Processing	Mean PA %+ ± S.D	%Deg
A) Raw Flour				
1.	Raw	Fine Wheat Flour	1.67% ± 2.10	0.0
B) Unleavened				
1	Unleavened	Chapatti	0.96% ± 2.70*	43.3
2		Tawa Roti	1.01% ± 2.48*	50.7
3		Tandoori Roti	1.00% ± 2.69*	49.3
C) **Fermented/Leavened**				
1	Yeast	Chapatti	0.82% ± 3.14*	50.8
2		Tawa Roti	0.69% ± 2.01**	58.6
3		Tandoori Roti	0.66% ± 2.27	60.47
D) **Fermented/Leavened**				
1	Bicarbonate Soda	Chapatti	1.21% ± 2.68	27.5
2		Tawa Roti	0.82% ± 3.00	50.8
3		Tandoori Roti	1.06% ± 3.18	36.5
E) **Fermented/Leavened**				
1	Sourdough Culture	Chapatti	0.98% ± 2.44	47.8
2		Tawa Roti	0.67% ± 3.30	61.0
3		Tandoori Roti	0.64% ± 2.29	61.8

Phytate Content and Percent Degradation Chakki Wheat Flour Breads

Table 3 shows that flour and bread are categorized into three parts such as raw, leavened, & unleavened Chakki wheat flour while leavened Chakki wheat flour has been categorized as yeast, soda & sourdough cultured wheat flour which three types of processing apply on it such as overheat Roti, tawa Roti & Tandoori Roti. In above table, shows that in raw Chakki wheat flour, phytic content was 0.93% ± 3.27, In the unleavened category, phytic acid content was highest in tawa Roti 1.35% ± 3.83, chapatti 1.46% ± 3.37, tandoori Roti 1.01% ± 3.13. In soda leavening phytic acid content was highest in tawa Roti 1.41% ± 3.57, chapatti 1.96% ± 3.40, and tandoori Roti 1.40% ± 3.01. In yeast leavening, the phytic acid content was highest at 1.96% ± 3.40, tawa roti at 1.00% ± 3.30, and tandoori Roti at 0.93% ± 3.04. In sourdough cultured leavening, the phytic acid content was highest in tawa Roti was 1.47% ± 2.84, chapatti at 1.34% ± 2.54,

and tandoori Roti at 1.31% ± 3.12. The per cent degradation of phytate in Chakki wheat flour breads was highest in sourdough cultured tandoori roti (75.3), tawa Roti (70.1%), chapatti (57.5) and for the yeast fermented roti ithe higher degradation occurred in chapatti (55.4), tandoori rotie (51.8) and tawa roti (48.2). soda tawa Roti (67.6 %), and yeast chapatti (0.3 %). Doughs leavened with soda also showed appreciable degradations. The percent degradation in the current study are similar to other such studies on the effect of fermentation on phytate reduction in wheat-based products [31-33].

Table 2: Phytate Content and Percent Degradation Chakki Wheat Flour Breads

S.No	Sample	Processing	Mean PA %+ ± S.D	%Deg
A) **Raw Flour**				
	Raw:	Raw	1.93% ± 3.27	0.0
B) Unleavened				
1.	Unleavened	Chapatti	1.46% ± 3.37	24.3
2.		Tawa Roti	1.35% ± 3.83	30.05
3.		Tandoori Roti	1.01% ± 3.13	47.7
C) **Fermented/Leavened**				
1.	Yeast	Chapatti	0.86% ± 3.70	55.4
2.		Tawa Roti	1.00% ± 3.30	48.2
3.		Tandoori Roti	0.93% ± 3.04	51.8
D) **Fermented/Leavened**				
1.	Bicarbonate Soda	Chapatti	1.22% ± 3.40	36.7
2.		Tawa Roti	1.22% ± 3.57	36.7
3.		Tandoori Roti	0.96% ± 3.0	50.2
E) **Fermented/Leavened**				
1.	Sourdough Culture	Chapatti	0.86% ± 2.54	57.7
2.		Tawa Roti	0.56% ± 2.84	70.1
3.		Tandoori Roti	0.48% ± 3.12	75.3

Phytate Content and Percent Degradation Maida Wheat Flour Breads

Table 4 exhibits the bread samples against raw flour. The samples were categorized into three parts, such as raw, leavened, & unleavened maida wheat flour while leavened maida wheat flour has been categorized as yeast, soda & sourdough cultured wheat flour, on which three types of processing apply on it, such as overheat Roti, tawa Roti & Tandoori Roti. In above table shows that in raw maida wheat flour phytate content was 0.54% ± 1.70. In unleavened, phytic acid content was highest in tandoori Roti 1.09% ± 2.62, chapatti 1.28% ± 2.20, tawa Roti 1.05% ± 2.32. In soda leavening, phytic acid content was highest in tandoori Roti 1.74% ± 3.72, tawa Roti 1.75% ± 3.68, and chapatti 1.89% ± 3.69. In yeast leavening, phytic acid content was highest in tawa Roti was 0.96% ± 2.24, chapatti, 0.93% ± 2.13, and tandoori Roti 1.22% ± 3.14. In sourdough cultured leavening, phytic acid content was highest in tawa Roti was 1.47% ± 2.84, chapatti 1.34% ± 2.54, and in tandoori Roti was 1.31% ± 3.12. The percent degradation of phytate in maida wheat flour was highest in sourdough cultured tandoori roti (79.8%), tawa roti (69.5%) and chappati (58.5). These findings are in strong agreement with other studies who reported cultured mediums for fermentation and their subsequent effects on phytate degradation [34-35]

Table 3: Phytate Content and Percent Degradation of Maida Wheat Flour Breads

S.No	Sample	Processing	Mean PA % ± S.D	%Deg
A) **Raw**				
	Raw	Raw	1.54% ± 1.70	0.0
B). Unleavened				
1	Unleavened	Chapatti	1.28% ± 2.20	14.6
2		Tawa Roti	1.05% ± 2.32	31.8
3		Tandoori Roti	1.09% ± 2.62	29.2
C) **Fermented/Leavened**				
1	Yeast	Chapatti	0.93% ± 2.13	39.6
2		Tawa Roti	0.96% ± 2.24	37.7
3		Tandoori Roti	0.72% ± 3.14	53.2
D) **Fermented/Leavened**				
1	Soda:	Chapatti	0.89% ± 3.69	42.2
2		Tawa Roti	0.75% ± 3.68	51.2
3		Tandoori Rtti	0.74% ± 3.72	50.6
E) **Fermented/Leavened**				
1	Sourdough Culture	Chapatti	0.64% ± 2.54	58.5
2		Tawa Roti	0.47% ± 2.84	69.5
3		Tandoori Roti	0.31% ± 3.12	79.8

Phytate Content and Percent Degradation of Whole Wheat Flour Breads

Table 5 shows that samples being prepared from whole wheat flour and cooked as leavened, & unleavened against raw flour being control. While leavened whole wheat flour has been categorized as yeast, soda & sourdough cultured wheat flour on which three types of processing apply on it such as overheat Roti, tawa Roti & Tandoori Roti. In above table shows that in raw whole wheat flour phytic acid content were 0.84% ± 2.98. In unleavened, phytic acid content was highest in tandoori Roti was 1.41% ± 3.36, chapatti 0.94% ± 2.50, tawa Roti 1.24% ± 3.21. In soda leavening, phytic acid content was highest in tawa Roti was 1.21% ± 2.99, chapatti, 0.95% ± 2.00, and tandoori Roti, 1.51% ± 2.85. In yeast leavening, phytic acid content was highest in tawa Roti was 1.16% ± 3.08, tandoori Roti, 0.99% ± 2.52, chapatti Roti, 1.20% ± 2.88. In sourdough cultured leavening, phytic acid content was highest in tawa Roti was 1.74% ± 3.41, chapatti 0.96% ± 3.30, and tandoori Roti was

1.67% ± 3.24. The percent degradation of phytate in whole wheat flour was highest in sourdough cultured tandoori roti 57.7%, followed by tawa roti 47.6%, and chapatti 45.3%. fermentation with the adiition of bicarbonate soda also resulted degradation/ the unleavened breads showed least reduction. The percent reduction in the phytate reduction after fermenataion after similar to other other studies which attributed the reduction to the nature of bacterial and yeast strains used for leavening [36, 37].

Table 4: Phytate Content and Percent Degradation of Whole Wheat Flour Breads

S.No	Sample	Processing	Mean PA % ± S.D	%Deg
A) **Raw Flour**				
	Raw	Raw	0.84% ± 2.98	0.0
B) **Unleavened**				
1	Unleavened	Chapatti	0.79% ± 2.50	14.1
2		Tawa Roti	0.74% ± 3.21	26.3
3		Tandoori Roti	0.64% ± 3.36	23.8
C) **Fermented/Leavened**				
1	Yeast	Chapatti	0.59% ± 2.88	29.8
2		Tawa Roti	0.56% ± 3.08	33.3
3		Tandoori Roti	0.59% ± 2.52	29.8
D) **Fermented/Leavened**				
1	Bicarbonate Soda	Chapatti	0.65% ± 2.00	22.5
2		Tawa Roti	0.57% ± 2.99	32.4
3		Tandoori Roti	0.51% ± 2.85	39.3
E) **Fermented/Leavened**				
1	Sourdough Culture	Chapatti	0.46% ± 3.30	45.3
2		Tawa Roti	0.44% ± 3.41	47.6
3		Tandoori Roti	0.38% ± 3.24	57.7

Phytate Content and Degradation in Breads from Granulated Wheat Flour

The data regarding the phytate content and percent degradation in the phytate in breads prepared from granulated fine wheat flour (Table 6) showed in raw granulated wheat flour phytic acid content was 0.92% ± 2.36, In unleavened, phytic acid content was highest in tandoori Roti was 1.10% ± 3.68, in tawa Roti was 1.55% ± 3.62, over heat was 1.70% ± 3.40. In soda leavening, phytic acid content was highest in chapatti was 1.55% ± 2.68, in tawa Roti 1.71% ± 3.09, tandoori Roti 1.99% ± 3.40. In the yeast leavening, phytic acid content was highest in tandoori Roti was 1.10% ± 3.68, chapatti 1.33% ± 2.47, tawa Roti 1.23% ± 2.48. In sourdough cultured leavening, phytic acid content was highest in chapatti Roti was 1.08% ± 2.19, tawa Roti 1.38% ± 2.15, tandoori Roti 0.49% ± 1.82. The percent degradation of phytate in granulated wheat flour was highest in sourdough cultured tandoori roti (58.6), tawa roti (48.8), and chapatti (44.6). the same results were found in yeast fermented tawa roti (53.7), tandoori roti (53.7), and chapatti (45.4). the higher percent degradation in the refned wheat flour used mostly in bakeries are in agreement with other such studies [38,39].

Table 5: Phytate Content and Degradation in Breads from Granulated Wheat Flour

S.No	Sample	Processing	Mean PA % ± S.D	%Deg
A) **Raw Flours**				
	Raw	Raw	0.93% ± 2.36	0.0
B) Unleavened				
1	Unleavened	Chapatti	0.70% ± 3.40	24.7
2		Tawa Roti	0.55% ± 3.62	40.8
3		Tandoori Roti	0.59% ± 3.68	40.8
C). Fermented/Leavened				
a) 1	Yeast	Chapatti	0.53% ± 2.47	45.4
2		Tawa Roti	0.43% ± 2.48	53.7
3		Tandoori Roti	0.45% ± 2.71	51.6
D) Fermented/Leavened				
b) 1	Bicarbonate Soda	Chapatti	0.55% ± 2.68	40.8
2		Tawa Roti	0.51% ± 3.09	45.2
3		Tandoori Roti	0.59% ± 3.40	36.6
E) Fermented/Leavened				
1	Sourdough Culture	Chapatti	0.52% ± 2.19	44.6
2		Tawa Roti	0.48% ± 2.15	48.8
3		Tandoori Roti	0.39% ± 1.82	58.6

Mineral Content & Mineral Solubility of Wheat Flour and Breads

Phosphorus Content & Phosphorus Solubility of Wheat Flours and Breads

The phosphorus content & phosphorus solubility of different wheat flour and breads are shown in Table 7. The samples include fine wheat flour, Chakki wheat flour, and whole-wheat flour treated by different heat processing, i.e., cooking, leavening with yeast & sourdough cultured atta (sourdough) techniques. the percent solubility of phosphorus was highest in raw fine wheat flour (119.1%), in yeast (114.4%), and in sourdough culture was (112.5%). In raw whole wheat flour, it was (53.6%), in yeast (51.7%), and in sourdough cultured (49.2%). In raw Chakki wheat flour, it was (55.9%), in yeast (39.3%), and in sourdough cultured (43.0%). While phosphorus content was more in fine wheat flour with sourdough cultured leavening was best

Table 6 : Phosphorus Content & Phosphorus Solubility of Wheat Flours and Breads

S.No	Sample	Processing	Total P	Soluble P. (ppm)	% Solubility
1	Fine wheat flour	Raw	1336	1591	119.1
		Yeast	1223	1399	114.4
		Sourdough cultured	1364	1535	112.5
2	Chakki wheat flour	Raw	3164	1767	55.9
		Yeast	3512	1379	39.3
		Sourdough cultured	3224	1386	43.0
3	Whole wheat flour	Raw	2662	1426	53.6
		Sourdough cultured	2568	1328	51.7
		Yeast	2501	1230	49.2

Iron Content and Iron Solubility of Wheat Flours and Breads

The iron content & iron solubility of different wheat flours and breads is given in Table 7. The samples include fine wheat flour, Chakkee wheat flour & Whole-wheat flour were treated by different fermentation processes. i.e., leavening with yeast & sourdough cultured atta techniques. The data table showed that the percent solubility of iron in the raw fine wheat flour was (54.8 %), in yeast (60.3 %), and in sourdough cultured (78.6 %). In raw whole wheat flour (64.7 %), in yeast (50.3 %), & in sourdough cultured (40.6 %). In raw chakki wheat flour was (32 %), in yeast was (56.0 %), and in sourdough cultured (42.7 %). According to a nutritional point of view, chakki wheat flour with yeast leavening was good due to its high iron content maximum reduction in phytates after fermentation with yeast and sourdough. The effects of different processes and their subsequent effects on mineral content and mineral solubility, specifically the iron, align with other such studies which reported the positive effects of fermentation on phytate reduction and enhancement of mineral availability [40-42].

Table 6: Iron Content & Iron Solubility of Wheat Flour and Breads: -

S.No.	Sample	Processing	Total iron	Soluble iron. ppm	% Solubility
1.	Fine wheat flour	Raw	44	24	54.8
		Yeast	24	14	60.3
		Sourdough cultured	31	24	78.6
2.	Chakki wheat flour	Raw	76	25	32.8
		Yeast	44	25	56.0
		Sourdough cultured	57	24	42.7
3.	Whole wheat flour	Raw	38	25	64.7
		Yeast	47	24	50.3
		Sourdough cultured	37	15	40.6

CONCLUSION

The current study investigated phytate degradation in conventional wheat breads from different types of wheat flours. The doughs were assessed as unleavened and fermented with bicarbonate soda, commercial yeast, and sourdough culture. Both fermentation methods were found to be useful techniques to influence phytate levels and hence mineral bioavailability. During breadmaking, significant degradation of phytate occurred, being higher for those with inherently high in initial phytate content. This study emphasizes and proposes fermentation as a preferred method of phytate degradation in wheat flour types rich in minerals

REFERENCES

1. Haudry A, Cenci A, Ravel C, Bataillon T, Brunel D, Poncet C, et al. Grinding up Wheat: a massive loss of nucleotide diversity since domestication. Mol Biol Evol. (2007) 24:1506–17. doi: 10.1093/molbev/msm077.
2. Wrigley C. Wheat: a unique grain for the world In: Khan K, Shewry PR, editors. Wheat: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists; (2009). 1–17.
3. *Gooding M, Khan K, Shewry P. The Wheat crop. Wheat: Chemistry and Technology. (2009). 4th Edn: Amsterdam: Elsevier, 19–49.* [
4. Harlan JR. The Early History of Wheat. In: Evans LT, Peacock WJ, editors. Wheat Science Today and Tomorrow. Cambridge, UK: Cambridge University Press; 1981. pp. 1–9
5. Shuaib M, Zeb A, Ali Z, Ali W, Ahmad T, Khan I. Characterization of wheat varieties by seed storage protein electrophoresis. Afr J Biotechnol. 2007;6(5):497–500
6. Grote U, Fasse A, Nguyen TT, Erenstein O (2021) Food security and the dynamics of wheat and maize value chains in Africa and Asia. Frontiers in Sustainable Food Systems. https://doi.org/10.3389/fsufs.2020.617009
7. Chowdhry MA, Ramzan M, Alam K, Khaliq I. Correlation analysis for grain yield and

quality traits in wheat. J Agric Res. 1995;33(2):71–74.
8. Igrejas G, Branlard G (2020) Wheat quality for improving processing and human health. Springer, Cham. https://doi.org/10.1007/978-3-030-34163-3
9. Bálint A, Szira F, Galiba G, Rakszegi M, Monostori I (2014) Micronutrient contents and nutritional values of commercial wheat flours and flours of field-grown wheat varieties—a survey in hungary. Cereal Res Commun Prepr Version. https://doi.org/10.1556/CRC.2013.0059
10. Central, U.S. Agricultural Research Service Food Data. (2021). Wheat germ nutritional value and analysis. https://nutrientoptimiser.com/nutritional-value-wheat-germ-crude/
11. Mahmoud A, Mohdaly A, Elneairy N (2015) Wheat germ: an overview on nutritional value antioxidant potential and antibacterial characteristics. J Food Nutr Sci 06:265–277. https://doi.org/10.4236/fns.2015.62027
12. Onipe OO, Jideani AIO, Beswa D (2015) Composition and Functionality of wheat bran and its application in some cereal food products. J Food Sci Technol 50:2509–2518. https://doi.org/10.1111/ijfs.12935
13. Hussain SS, Qamar R (2007) Wheat genomics: challenges and alternative strategies. Proc Pak Acad Sci 44:305–327
14. Brinch-Pedersen, H.; Madsen, C.K.; Holme, I.B.; Dionisio, G. Increased understanding of the cereal phytase complement for better mineral bio-availability and resource management. *J. Cereal Sci.* 2014, *59*, 373–381.
15. Zimmermann, B.; Lantzsch, H.-J.; Langbein, U.; Drochner, W. Determination of phytase activity in cereal grains by direct incubation. *J. Anim. Physiol. Anim. Nutr.* 2002, *86*, 347–352
16. Brouns, F. Phytic acid and whole grains for health controversy. *Nutrients* 2022, *14*, 25.
17. Rodehutscord, M.; Sommerfeld, V.; Kühn, I.; Bedford, M.R. Phytases: Potential and limits of phytate destruction in the digestive tract of pigs and poultry. In *Enzymes in Farm Animal Nutrition*, 3rd ed.; Bedford, M.R., Partridge, G., Hruby, M., Walk, C.L., Eds.; CABI: Wallingford, UK, 2022; pp. 124–152. ISBN 9781789241563
18. Wang R., Guo S. Phytic acid and its interactions: Contributions to protein functionality, food processing, and safety. Compr Rev. Food Sci. Food Saf. 2021;20:2081–2105. doi: 10.1111/1541-4337.12714.
19. Raboy V. Low phytic acid Crops: Observations Based On Four Decades of Research. Plants. 2020;9:140. doi: 10.3390/plants9020140.
20. Raboy V. myo-Inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry. 2003;64:1033–1043. doi: 10.1016/S0031-9422(03)00446-1
21. Brune M., Rossander-Hulten L., Hallberg L., Gleerup A., Sandberg A.S. Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J. Nutr. 1992;122:442–449. doi: 10.1093/jn/122.3.442
22. Lopez H.W., Krespine V., Guy C., Messager A., Demigne C., Remesy C. Prolonged fermentation of whole wheat sourdough reduces phytate level and increases soluble magnesium. J. Agric. Food Chem. 2001;49:2657–2662. doi: 10.1021/jf001255z.
23. *Buddrick O., Jones O.A., Cornell H.J., Small D.M. The influence of fermentation processes and cereal grains in wholegrain bread on reducing phytate content. J. Cereal Sci. 2014;59:3–8. doi: 10.1016/j.jcs.2013.11.006.*
24. Yildirim R.M., Arici M. Effect of the fermentation temperature on the degradation of phytic acid in whole-wheat sourdough bread. LWT Food. Sci. Technol. 2019;112:108224. doi: 10.1016/j.lwt.2019.05.122
25. De Angelis M., Minervini F., Siragusa S., Rizzello C.G., Gobbetti M. Wholemeal wheat flours drive the microbiome and functional features of wheat sourdoughs. Int. J. Food Microbiol. 2019;302:35–46. doi: 10.1016/j.ijfoodmicro.2018.08.009.
26. Rodriguez-Ramiro I., Brearley C.A., Bruggraber S.F., Perfecto A., Shewry P., Fairweather-Tait S. Assessment of iron

bioavailability from different bread making processes using an in vitro intestinal cell model. Food Chem. 2017;228:91–98. doi: 10.1016/j.foodchem.2017.01.130
27. Bottani M., Brasca M., Ferraretto A., Cardone G., Silvetti T. Chemical and nutritional properties of white bread leavened by lactic acid bacteria. J. Funct. Foods. 2018;45:330–338. doi: 10.1016/j.jff.2018.04.030
28. Zhao PH, Hou YC, Wang Z, Liao AM, Pan L, Zhang J, Dong YQ, Hu ZY, Huang JH, Ou XQ. Effect of fermentation on structural properties and antioxidant activity of wheat gluten by *Bacillus subtilis*. Front Nutr. 2023 Feb 23;10:1116982. doi: 10.3389/fnut.2023.1116982
29. Verni M, Rizzello CG, Coda R. Fermentation biotechnology applied to cereal industry by-products: nutritional and functional insights. Front Nutr. (2019) 6:42. doi: 10.3389/fnut.2019.00042
30. Tomassi, E., Arouna, N., Brasca, M., Silvetti, T., de Pascale, S., Troise, A. D., Scaloni, A., & Pucci, L. (2025). Fermentation of Whole-Wheat Using Different Combinations of Lactic Acid Bacteria and Yeast: Impact on In Vitro and Ex Vivo Antioxidant Activity. *Foods*, *14*(3), 421. https://doi.org/10.3390/foods14030421
31. Sakandar, H.A.; Hussain, R.; Kubow, S.; Sadiq, F.A.; Huang, W.; Imran, M. Sourdough Bread: A Contemporary Cereal Fermented Product. *J. Food Process. Preserv.* 2019, *43*, e13883.
32. Liu, J.; Yu, L.L.; Wu, Y. Bioactive Components and Health Beneficial Properties of Whole Wheat Foods. *J. Agric. Food Chem.* 2020, *68*, 12904–12915.
33. Sharma, R.; Garg, P.; Kumar, P.; Bhatia, S.K.; Kulshrestha, S. Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods. *Fermentation* 2020, *6*, 106.
34. Garrido-Galand, S.; Asensio-Grau, A.; Calvo-Lerma, J.; Heredia, A.; Andrés, A. The Potential of Fermentation on Nutritional and Technological Improvement of Cereal and Legume Flours: A Review. *Food Res. Int.* 2021, *145*, 110398
35. De Vuyst, L.; Neysens, P. The Sourdough Microflora: Biodiversity and Metabolic Interactions. *Trends Food Sci. Technol.* 2005, *16*, 43–56
36. Kurtzman, C.P.; Robnett, C.J. Identification and Phylogeny of Ascomycetous Yeasts from Analysis of Nuclear Large Subunit (26S) Ribosomal DNA Partial Sequences. *Antonie Van Leeuwenhoek* 1998, *73*, 331–371
37. Pérez-Alvarado, O.; Zepeda-Hernández, A.; Garcia-Amezquita, L.E.; Requena, T.; Vinderola, G.; García-Cayuela, T. Role of Lactic Acid Bacteria and Yeasts in Sourdough Fermentation during Breadmaking: Evaluation of Postbiotic-like Components and Health Benefits. *Front. Microbiol.* 2022, *13*, 969460.
38. Mosharraf L., Effect of processing on the decrease in phytic acid and pesticide residue in wheat bran, *Journal of Agricultural Engineering Research*. (2015) 16, no. 2, 71–82.
39. Arya S. S. and Sonawane S. K., Impact of fiber mixture on dough and chapatti quality using D-optimal response surface methodology, *Journal of Microbiology, Biotechnology and Food Sciences*. (2016) 5, no. 5, 424–433, https://doi.org/10.15414/jmbfs.2016.5.5.424-433.
40. Nkhata SG, Ayua E, Kamau EH, Shingiro JB. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci Nutr. 2018 Oct 16;6(8):2446-2458. doi: 10.1002/fsn3.846.
41. haven, J. K. , & Kadam, S. S. (1989). Nutritional improvement of cereals by fermentation. Critical Reviews in Food Science Technology, 28, 349–400. 10.1080/10408398909527507
42. Hassan, G. F. , Yusuf, L. , Adebolu, T. T. , & Onifade, A. K. (2015). Effect of fermentation on mineral and anti-nutritional composition of cocoyam (*Colocasia esculenta* linn). Sky Journal of Food Science, 4, 042–049