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FOOD PROCESSING AND FERMENTATION STUDIES ON REDUCTION OF PHYTIC ACID IN *TRITICUM AESTIVUM* AND *SORGHUM BICOLOR* (L.)

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ABSTRACT

Wheat and sorghum are the main dieter cereals for millions of people in Africa and Asia. Their nutritive value in wheat and sorghum is limited due to presence of several anti-nutritional substances such as tannins, phytic acid, proteinase inhibitors and cyanogenic glycosides. Phytic acid (PA) or myo-inositol hexakisphosphate (IP6) in salt form known as phytate is the principal storage form of phosphorus in plant tissues, typically accounting for 60-90% of total seed phosphorous. Phytase enzyme catalyses the hydrolysis of phytate and releases the phosphate for absorption. This study explains the use of different methods for the reduction of PA content in wheat and sorghum such as soaking, cooking, roasting and fermentation using lactic acid bacteria (LAB). Among all the methods, fermentation using LAB was found to be effective in reduction of PA. LAB was isolated from the curd sample and fermentation of wheat and sorghum flour was carried out to reduce the PA content. The effect of different fermentation conditions such as pH, temperature and incubation time was also carried out. The results have shown that the optimal fermentation conditions for reduction of PA in wheat and sorghum flour using LAB were; pH- 6 (wheat) and 6.5 (sorghum), temperature 32 ° C (wheat) and 37 ° C (sorghum) and fermentation time of 4 to 6 h for both wheat and sorghum. The percentage of reduction of PA using fermentation process was compared with the other methods such as soaking, cooking and roasting process.

Keywords: Phytic acid, Antinutrients, Fermentation, Lactic Acid Bacteria, Wheat, Sorghum

INTRODUCTION

In contrast to severe malnutrition, from the past 20 years World Health Organization and Research programs were focused on the management of nutritional properties of food with different food processing methods **(Konietzny & Greiner, 2004)**. Malnutrition in poor communities is not only because of insufficient amount of food but also because of poor nutritional quality of the available food **(Brown, 2008)**. Insufficient and poor quality food intake is also adverse effect on adult health and responsible for many diseases like Anaemia, osteoporosis, rickets and immune diseases due to lack of availability of minerals **(Stein, 2010)**. Micro–nutrients such as Phosphorus, Iron, Calcium and Zinc are essential for all organisms and ideal to act as a catalytic molecule in numerous biochemical reactions **(Dangour *et al.*, 2017; Minihane & Rimbach, 2002)**. Plant based food products such as wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor (L.) Moench*) are the main cereal crops used for human consumption and they are world's most important crop in terms of production and consumption **(Dykes & Rooney, 2006)**. They constitute an important dietary source of fibers, complex carbohydrates, proteins, vitamins, and minerals. But their nutritive value is limited due to the presence of several anti-nutritional substances such as phytate, proteinase inhibitors, cyanogenic glycosides and tannins **(O'Callaghan *et al.*, 2019; Soetan *et al.*, 2010)**.

PA has a strong ability to chelate multivalent metal ions, especially Zn^{2+}, Mg^{2+}, Ca^{2+} and Fe^{2+} to form phytate-mineral complexes which are insoluble and therefore unavailable for absorption in the intestinal tract **(Coulibaly *et al.*, 2011;Lopez *et al.*, 1983; Palacios *et al.*, 2008)**. Its negative charge makes it to bind with positively charged minerals leads to unavailability of minerals for biological activities. Monogastric animals like poultry, swine and human beings do not contain mechanism to hydrolyse PA **(Hajati & Rezaei, 2010)** because they have very low or zero phytase activity in their gastrointestinal tracts **(Kumar *et al.*, 2010)**. Hence processed food is required for complete bio-accessible of minerals with lower level of phytic acid. Phytase (myo-inositol hexakis phosphate phophohydrolase) a specific group of phosphatise enzyme, hydrolyses phytic acid to myo-inositol and phosphoric acid. The Phytase enzyme is widely distributed in microorganisms, plants and animals. Among microorganisms Lactic acid bacteria were found to have more phytase activity **(Leroy & De Vuyst, 2004; Palacios *et al.*, 2008; Tamang *et al.*, 2009)**.

Traditional processes such as soaking, germination and fermentation can activate native grain phytases which degrade inositol-6-phosphate to its lower forms **(Liu *et al.*, 2019).** On the other hand fermentation is more effective since the organic acids produced by the micro-organisms reduce the pH of the aqueous cereal mixture close to the optimum pH required for phytase activity. Phytate degradation can be extensive or complete in products such as lactobacilli-fermented sorghum **(Kumar *et al.*, 2010)**. The industrially processed; fermented and enzymatically treated foods are in practice but these are expensive to meet the needs of poor communities **(Lai *et al.*, 2013; Leroy & De Vuyst, 2004)**. Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. Probiotic supplementation to wheat and sorghum has a promising role to play in the bioavailability of essential nutrients **(Ajao *et al.*, 2018)**. Fermentation of wheat and sorghum with LAB reduces the PA content and helps in improved availability of nutritional factors by degrading the IP6 **(Grajek et al., 2005)**. This study reports the use of traditional process and fermentation on reduction of phytic acid in wheat and sorghum flours to enhance the bioavailability of micronutrients in plant based diets.

MATERIAL AND METHODS

Isolation and identification of Lactic Acid Bacteria (LAB)

The samples of fresh curd were collected from commercial market. The De Man, Rogasa and Sharpe (MRS) broth medium (Hi-media, India) was prepared according to the manufactures instruction. The test tubes containing media were sterilized by autoclaving at 121°C with 15 Pounds/Inch^2 pressure. 1mL of 1: 10 diluted curd sample prepared was inoculated and incubated at 37 °C for 24 hours. The sterilized MRS agar medium (15 ml) was dispensed into sterile petri plates and a loop full of inoculums from enriched MRS broth was streaked on MRS Agar plates. Plates were incubated for 24 h at 37 °C and examined for development of bacterial colonies. The isolated LAB was identified based on

their morphological and biochemical characteristics such as Gram Staining, Methyl Red Test, Catalase test and Sugar fermentation test.

Phytate degradation during food processing

Soaking: The seeds of wheat and sorghum were taken separately and were surface sterilized by treating with 0.1% mercuric chloride and were rinsed and soaked in distilled water (1:10; w/v) at different time intervals of 1, 5, 7, 10 and 15 hours at room temperature (28±2°C). The soaked seeds were rinsed with distilled water, dried and ground powder was used for PA determination.

Cooking: The flours of wheat and sorghum samples were taken separately mixed with distilled water (1:20 w/v) and cooked with continuous stirring at different time intervals of 10, 15, 30, and 60 min. The cooked samples were centrifuged and the supernatant was taken for PA estimation.

Roasting: The flours of wheat and sorghum samples were roasted separately at different time intervals of 1, 2, 3, 4, 5 min. The roasted samples were taken for PA estimation using Modified WADE reagent method.

Fermentation optimization studies

Preparation of Inoculum

10 mL of MRS broth was prepared in 8 test tubes and autoclaved at 121°C for 15 min and cooled at room temperature. A loop full of LAB from slant was inoculated to the above test tubes and incubated at 37° C for 24 h in a shaker incubator at 220 rpm.

Fermentation optimization study of pH

Ten grams of ground samples of sorghum and wheat were separately autoclaved at 121ºC for 20 min the samples were mixed uniformly with 100 mL of sterile distilled water in different conical flasks in an aseptic condition. The pH of the medium was adjusted to 3, 4, 5.5, 6, 6.5, 7.4 and 9 using 0.1N HCl and NaOH. 10 mL of the inoculum was added to the medium. Fermentation was carried out in an incubator shaker at 220 rpm at optimum temperature 37ºC. Aliquots of the fermenting slurries were drawn at 24, 48 and 72 h intervals under aseptic conditions and analyzed for phytic acid using colorimetric WADE reagent method.

Fermentation optimization study of Temperature

10 g of ground samples of sorghum and Wheat were autoclaved separately at 121ºC for 20 min. Samples were mixed uniformly with 100 mL of sterile distilled water in different conical flasks in an aseptic condition. 10 mL of the inoculum was added to the medium. Fermentation was carried out in an incubator shaker at 220 rpm at different temperature 27, 32 and 37ºC. Aliquots of the fermenting slurries were drawn at 24, 48 and 72 h. Intervals of time under aseptic conditions analyzed for phytic acid using colorimetric WADE reagent method.

Fermentation time optimization study

Powdered samples of wheat and sorghum were autoclaved at 121ºC for 20 min. 10 g of Samples were mixed uniformly with 100 mL of sterile distilled water in different conical flasks in an aseptic condition. The pH of the medium was adjusted to 6.5 using 0.1N HCl and NaOH. 10 mL of the inoculum was added to the medium. Fermentation of wheat and sorghum sample was carried out in an incubator shaker at 220 rpm at optimum temperatures of 32ºC (wheat) and 37ºC (sorghum). Aliquots of the fermenting slurries were drawn at 1, 2, 3, 4, 5, 6, 24, 48 and 72 h intervals under aseptic conditions. Analysis of phytic acid in samples was carried out using colorimetric WADE reagent method.

Determination of PA content in raw and fermented samples

The standard graph of sodium phytate was prepared as described in previous reports **(Newsome, 1980)** and the determination of phytic acid in the raw samples of wheat and Sorghum flour were carried out according to **Kwanyuen *et al.* (2007)**. The Fermented samples drawn at different interval of time were centrifuged at 4800 rpm for 20 min at 10° C. 1mL of supernatant was pipetted out into two test tubes with 2 mL of distilled water. Each tube was added with 1 mL of the Wade reagent (0.03% $FeC1_3$-$6H_20$ and 0.3% sulfosalicylic acid in distilled water), and the solution was mixed on a vortex mixer for 5 second. The mixture was centrifuged for 10 min and the absorbance of supernatant was measured at 490 nm using colorimeter.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel (2010). The values are represented as mean ± standard deviation (SD). Probability levels (P values) < 0.05 were considered significant.

RESULTS AND DISCUSSION

Isolation and identification of LAB

Lactic acid bacteria are commonly found in fermented dairy products. *Lactobacillus* is a genus of lactic acid bacteria and described as a heterogeneous group of regular, non-spore forming, gram-positive, rod shaped non-motile bacteria with catalase negative activity. After the incubation for 24 h, colour of the colonies was found to be creamy to white on the surface of MRS agar plate as shown in the figure 1A. The morphological characteristics were further resolved on the basis of microscopic examination. LAB being gram positive bacteria appears deep purple indicating that they have thick outer layer peptidoglycan. Gram's staining of the bacterial culture showed that they were gram positive, rod shaped and some of them were coccid shaped (Figure 1A-1D). The results of catalase test showed that the isolates were not able to produce air bubbles when mixed with 3% H_2O_2 which confirms that isolates were catalase negative and belong to LAB. The results of sugar fermentation showed that isolates fermented glucose to acid which was evident by changing color of medium from red to yellow. It also showed that LAB was homo fermentative because in case of homo fermentation, there will be production of acid along with the change in color of the medium from red to yellow. Whereas in hetero fermentation, there will be gas production in Durham tube alongside the change in the color. The results of Methyl red test showed that isolates were mixed acid fermenting bacteria that yield a stable acid end product which was evident by changing color of the medium from yellow to red. Similar results were obtained by **(Kale, 2014).**

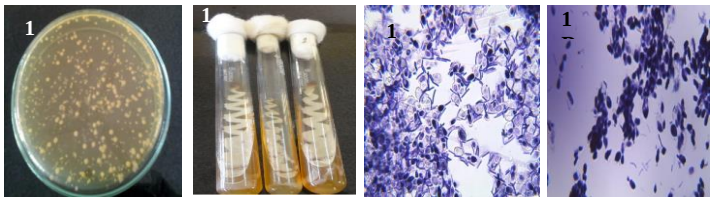

Figure 1 Isolation and identification of Lactic acid bacteria: 1A) Morphology of LAB; 1B) Pure Culture of LAB; 1C) & 1D) Gram Staining for LAB; 1E) Catalase test; 1F) Sugar fermentation test

Study on effect of food processing methods on Phytic acid degradation

The estimation of PA was found to be 1.5% in whole wheat, 2% in wheat bran, 1.3 % in Fine wheat and 2.1% in sorghum (Table 1). The estimation was carried out using sodium phytate as a standard (figure 2). There are different methods for PA degradation such as soaking, cooking, roasting and fermentation **(Kumar *et al.*, 2010)**. Soaking of legume grains and cereal seeds are done as a pre-treatment to facilitate processing, which lasts for 15–20 min, or even longer, depending on further, processing steps. Generally, cereals and legumes are soaked in water overnight; phytate is water-soluble, so a considerable amount of phytate is removed to the water. In addition, this process also enhances the action of naturally occurring phytase in cereals and legumes. It has been shown that phytate hydrolysis, during soaking, is greatly influenced by temperature and pH **(Greiner & Konietzny, 1999)**. In the present study reduction of PA in soaked samples was found to be 45% in whole wheat and 27.5% in sorghum after soaking for15 min. Reduction of PA content in cooked samples was found to be 53% in whole wheat, and 47% in sorghum at 60 min of cooking. PA reduction in roasted samples was found to be 26% in whole wheat, and 18.57% in sorghum at 5 min of roasting. From the figure 3 it is evident that as the time of soaking, cooking or roasting increases the reduction in PA increased. Phytate, being a heat stable component in plant foodstuffs, is not easily degraded while cooking. However, the intrinsic plant phytase is thermo labile; prolonged exposure to high temperature may lead to the inactivation of endogenous enzyme. Therefore, to improve phytate dephosphorylation during cooking, plants with heat-stable Phytases or addition of exogenous heat-stable Phytases have been recommended **(Hotz & Gibson, 2007).**

Table 1 PA content in raw samples

Raw Samples	% Weight (w/w)
Wheat Bran	2
Fine Wheat	1.3
Whole Wheat	1.5
sorghum	2.1

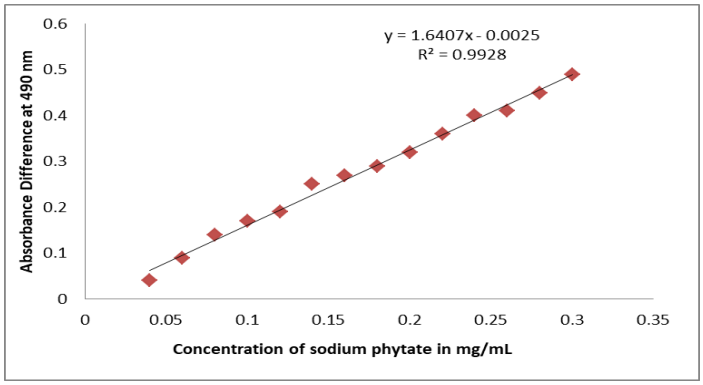

Figure 2 Standard graph of sodium phytate assay

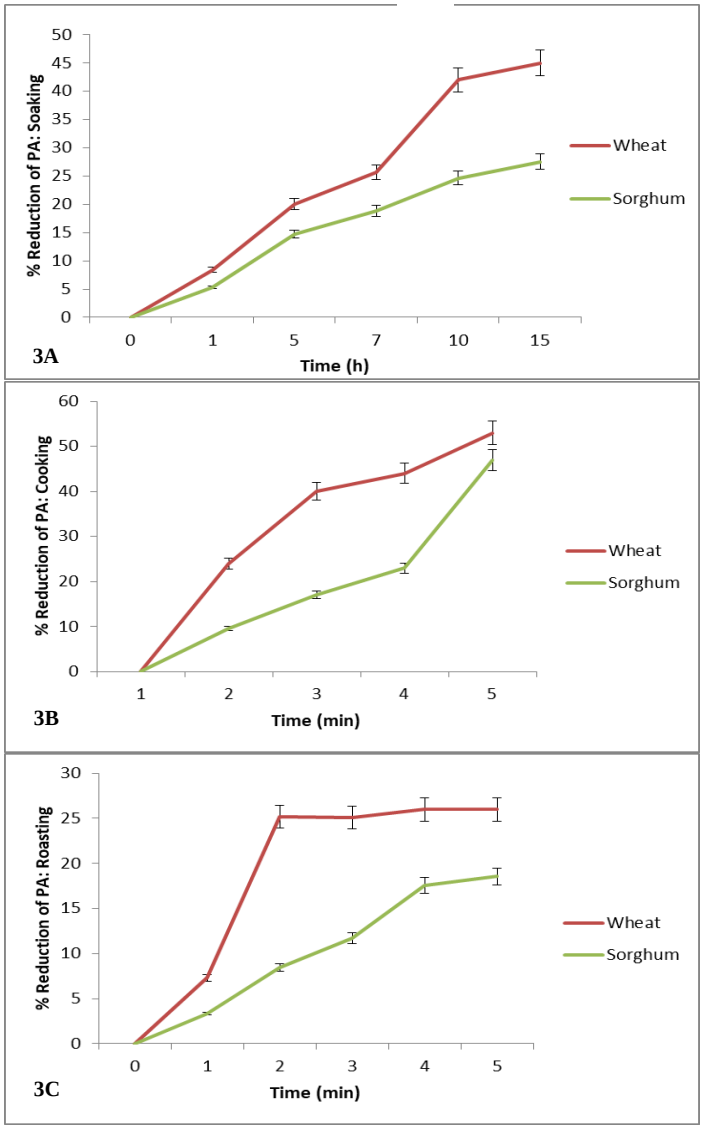

Figure 3 Phytic acid reduction during food processing: 3A) effect of soaking on PA content of wheat & sorghum; 3B) effect of cooking on PA content of wheat & Sorghum; 3C) effect of roasting on PA content of wheat & Sorghum

Study on fermentation optimization for reduction of PA

LAB from milk and curd were considered to be the possible source of phytase in fermentation. Phytase is also present in wheat and sorghum. As the fermentation continues, it resulted in increased acidity of wheat sample; this activates the phytase enzyme in wheat and sorghum samples **(Elyas *et al.*, 2002).** The scientific data are interpreted as supporting the hypothesis that either lactic acid bacterial phytase is significantly involved in phytate degradation during sourdough fermentation **(Eale, Onietzny *et al.*, 2007; Lopez *et al.*, 2000; Rezaei *et al.*, 2019)** or the intrinsic cereal phytases are responsible for phytate degradation after being activated by a fall in pH due to lactic acid production by the lactic acid **(Gupta *et al.*, 2013)**.

In wheat, it was found that 100% reduction of PA occurred in pH 6, temperature 32 °C & time 4 to 6 h of fermentation and there was a change in pH from 6 changed to 3.96 at 6 h of fermentation. In sorghum, it was found that 100% reduction of PA occurred in pH-6.5 temperature 37 °C & fermentation time of 4 to 6 h. With prolong of fermentation time the PA content increases due to inhibition of phytase enzyme by phosphates enzyme released from lactic acid bacteria. During fermentation, constant agitation also plays an important role on reduction of PA. After 24 h of fermentation, PA content remains almost constant (Figure 4-5).

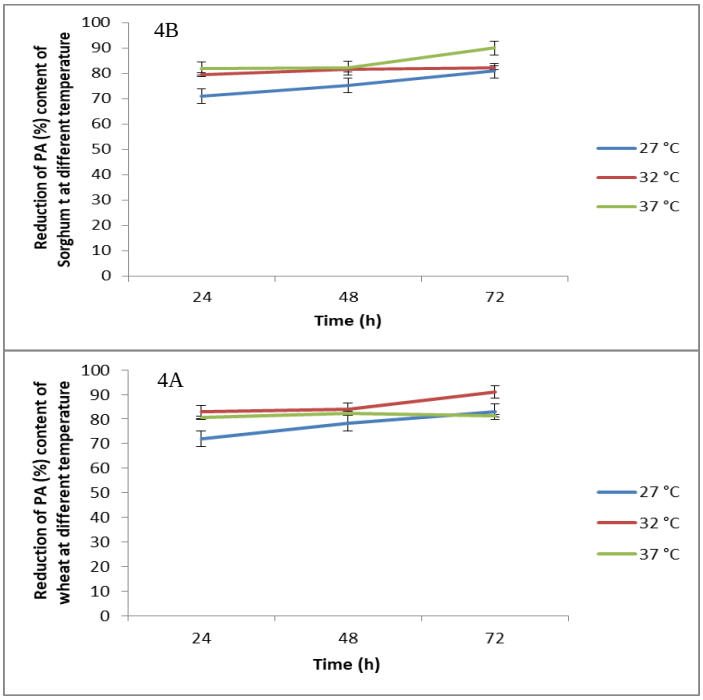

Figure 4 Reduction of PA (%) after 24, 48 and 72 h of fermentation at different temperature in wheat (Fig 4A) & sorghum (Fig 4B).

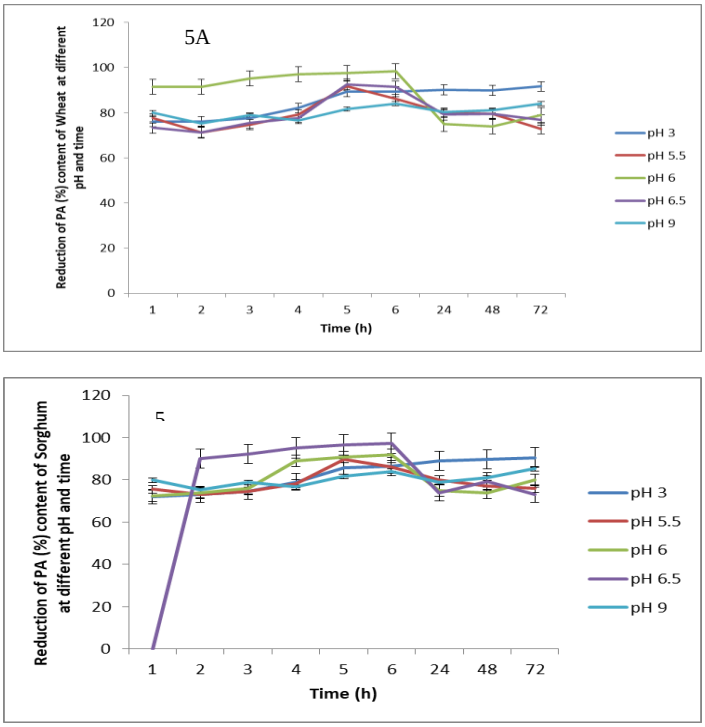

Figure 5 Optimization of pH and fermentation time on reduction of PA in wheat (fig 5A) & sorghum (fig 5B)

PA is present in wheat and sorghum is suspected of impairing mineral absorption of Zn, Fe and Ca **(Dykes & Rooney, 2006)**. The binding of PA alters the solubility, functionality, digestibility and absorption of the food components. PA binds with proteins and mineral nutrient, thereby reducing their bioavailability. The release of phosphate from phytate is initiated by a class of enzymes called phytases which hydrolyse phosphate motilities from PA to release myo inositol

and inorganic phosphate thereby resulting in the loss of ability of PA to chelate metal ions. LAB Phytase enhance the availability of phosphate from phytate and they also have the potential to be used as a probiotics **(Fischer *et al.*, 2014; Greiner & Konietzny, 1999; H. W. Lopez *et al.*, 2001)**. Probiotic supplementation to wheat and sorghum has a promising role to play in to increase the bioavailability of essential nutrients **(Grajek et al., 2005)**. Even though there are different methods for degradation of PA in wheat and sorghum such as soaking, cooking and roasting the fermentation process provides optimum conditions for enzymatic degradation of PA present in complex form with polyvalent cations like Fe, Zn, Ca, Mg and proteins **(Minihane & Rimbach, 2002)**. The lactic acid fermentation showed greater significance in reduction of PA content when compared to soaking, cooking and roasting. In this study a modified colorimetric WADE reagent method was used for the identification of PA content in raw wheat and sorghum and WADE colorimetric method was used for PA content estimation in fermented samples (H. W. Lopez et al., 2001). The determination of PA content with these colorimetric methods is based on decolouration of the pink $Fe3^{+}$- sulfosalicylate complex (WADE reagent). Increase in absorbance reading (490nm) after fermentation showed that reduction of PA content of wheat and sorghum.

CONCLUSION

In the present study describes the isolation of phytate degrading LAB and optimization of fermentation conditions for degradation of phytate in wheat and sorghum flour. Further, this study can be extended for isolation of other probiotic bacteria such as *bifidobacterium* for the reduction of PA content in wheat and sorghum. This will help in formulations of LAB with varieties of plant based foods to decrease the phytic acid content and to increase availability of phosphorus.

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REFERENCES

Ajao, O., Banwo, K., Ogunremi, O., & Sanni, A. (2018). Antimicrobial Properties and Probiotic Potentials of Lactic Acid Bacteria Isolated From Raw Beef in Ibadan, Nigeria. *Journal of Microbiology, Biotechnology and Food Sciences*, 8(2), 770–773. https://doi.org/10.15414/jmbfs.2018.8.2.770-773.

BROWN, K. H. (2008). Appropriate Diets for the Rehabilitation of Malnourished Children in the Community Setting. *Acta Paediatrica*, 80(s374), 151–159. https://doi.org/10.1111/j.1651-2227.1991.tb12018.x.

Coulibaly Abdoulaye, Kouakou Brau, C. J. (2011). Phytic Acid in Cereal Grains: Structure, Healthy or Harmful Ways to Reduce Phytic Acid in Cereal Grains and Their Effects on Nutritional Quality. *American Journal of Plant Nutrition and Fertilization Technology*, 1(1), 1–22. https://doi.org/10.3923/ajpnft.2011.1.22.

Dangour, A. D., Mace, G., & Shankar, B. (2017). Food systems, nutrition, health and the environment. *The Lancet Planetary Health*, 1(1), e8–e9. https://doi.org/10.1016/s2542-5196(17)30004-9.

Dykes, L., & Rooney, L. W. (2006). Sorghum and millet phenols and antioxidants. *Journal of Cereal Science*, 44(3), 236–251. https://doi.org/10.1016/j.jcs.2006.06.007.

Reale, A., Konietzny, U., Coppola, R., Sorrentino, E., & Greiner, R. (2007). The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. *Journal of agricultural and food chemistry*, 55(8), 2993-2997. https://doi.org/10.1021/jf063507n.

Elyas, S. H., El Tinay, A. H., Yousif, N. E., & Elsheikh, E. A. (2002). Effect of natural fermentation on nutritive value and in vitro protein digestibility of pearl millet. *Food chemistry*, 78(1), 75-79 https://doi.org/10.1016/S0308-8146(01)00386-7.

Fischer, M. M., Egli, I. M., Aeberli, I., Hurrell, R. F., & Meile, L. (2014). Phytic acid degrading lactic acid bacteria in tef-injera fermentation. *International Journal of Food Microbiology*, 190, 54–60. https://doi.org/10.1016/j.ijfoodmicro.2014.08.018.

Grajek, W., Olejnik, A., & Sip, A. (2005). Probiotics, prebiotics and antioxidants as functional foods. *Acta biochimica polonica-english edition-*, 52(3), 665. https://doi.org/10.1038/sj.bmt.1705084.

GREINER, R., & KONIETZNY, U. (1999). Improving enzymatic reduction of myo-inositol phosphates with inhibitory effects on mineral absorption in black beans (Phaseolus vulgaris var. preto). *Journal of food processing and preservation*, 23(3), 249-261 https://doi.org/10.1111/j.1745-4549.1999.tb00383.x.

Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2013). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, 52(2), 676–684. https://doi.org/10.1007/s13197-013-0978-y.

Hajati, H., & Rezaei, M. (2010). The application of prebiotics in poultry production. *International Journal of Poultry Science*, 9(3), 298–304. https://doi.org/10.3923/ijps.2010.298.304.

Hotz, C., & Gibson, R. S. (2007). Traditional Food-Processing and Preparation Practices to Enhance the Bioavailability of Micronutrients in Plant-Based Diets. *The Journal of Nutrition*, 137(4), 1097–1100. https://doi.org/10.1093/jn/137.4.1097.

Kale, P. S. (2014). Isolation and identification of bacteria from curd and its application in probiotic chocolate. *Pelagia Research Library European Journal of Experimental Biology*, 4(6), 95–97. Retrieved from www.pelagiaresearchlibrary.com.

Konietzny, U., & Greiner, R. (2004). Bacterial phytase: Potential application, in vivo function and regulation of its synthesis. *Brazilian Journal of Microbiology*, 35(1–2), 11–18. http://dx.doi.org/10.1590/S1517-83822004000100002.

Kumar, V., Sinha, A. K., Makkar, H. P. S., & Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition : A review. *Food Chemistry*, 120(4), 945–959. https://doi.org/10.1016/j.foodchem.2009.11.052.

Gao, Y., Shang, C., Maroof, M. A., Biyashev, R. M., Grabau, E. A., Kwanyuen, P., ... & Buss, G. R. (2007). A modified colorimetric method for phytic acid analysis in soybean. *Crop Science*, *47*(5), 1797-1803. https://doi.org/10.2135/cropsci2007.03.0122.

Lai, L. R., Hsieh, S. C., Huang, H. Y., & Chou, C. C. (2013). Effect of lactic fermentation on the total phenolic, saponin and phytic acid contents as well as anti-colon cancer cell proliferation activity of soymilk. *Journal of Bioscience and Bioengineering*, 115(5), 552–556. https://doi.org/10.1016/j.jbiosc.2012.11.022.

Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*, 15(2), 67–78. https://doi.org/10.1016/j.tifs.2003.09.004.

Liu, K., Zheng, J., Wang, X., & Chen, F. (2019). Effects of household cooking processes on mineral, vitamin B, and phytic acid contents and mineral bioaccessibility in rice. *Food Chemistry*, 280, 59–64. https://doi.org/10.1016/j.foodchem.2018.12.053.

Lopez, H. W., Krespine, V., Guy, G., Messager, A., Demigne, C., & Remesy, C. (2001). Prolonged fermentation of whole wheat sourdough reduces phytate level and increases soluble magnesium. *Journal of Agricultural and Food Chemistry*, 49(5), 2657–2662. https://doi.org/10.1021/jf001255z.

Lopez, H. W., Ouvry, A., Bervas, E., Guy, C., Messager, A., Demigne, C., & Remesy, C. (2000). Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. *Journal of Agricultural and Food Chemistry*, 48(6), 2281-2285. https://doi.org/10.1021/jf000061g.

LOPEZ, Y., GORDON, D. T., & FIELDS, M. L. (1983). Release of Phosphorus from Phytate by Natural Lactic Acid Fermentation. *Journal of Food Science*, 48(3), 953–954. https://doi.org/10.1111/j.1365-2621.1983.tb14938.x.

Marie Minihane, A., & Rimbach, G. (2002). Iron absorption and the iron binding and anti-oxidant properties of phytic acid. *International journal of food science & technology*, 37(7), 741-748. https://doi.org/10.1046/j.1365-2621.2002.00619.x.

Latta, M., & Eskin, M. (1980). A simple and rapid colorimetric method for phytate determination. *Journal of Agricultural and Food Chemistry*, 28(6), 1313-1315. https://doi.org/10.1021/jf60232a049.

O'Callaghan, Y. C., Shevade, A. V., Guinee, T. P., O'Connor, T. P., & O'Brien, N. M. (2019). Comparison of the nutritional composition of experimental fermented milk:wheat bulgur blends and commercially available kishk and tarhana products. *Food Chemistry*, 278, 110–118. https://doi.org/10.1016/j.foodchem.2018.11.026.

Palacios, M. C., Haros, M., Sanz, Y., & Rosell, C. M. (2008). Selection of lactic acid bacteria with high phytate degrading activity for application in whole wheat breadmaking. *LWT - Food Science and Technology*, 41(1), 82–92. https://doi.org/10.1016/j.lwt.2007.02.005.

Rezaei, S., Najafi, M. A., & Haddadi, T. (2019). Effect of fermentation process, wheat bran size and replacement level on some characteristics of wheat bran, dough, and high-fiber Tafton bread. *Journal of Cereal Science*, 85(October 2017), 56–61. https://doi.org/10.1016/j.jcs.2018.11.019.

Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral elements for humans , domestic animals and plants. *African Journal of Food Science*, 4(5), 200–222. https://doi.org/10.1186/s12302-017-0116-y.

Stein, A. J. (2010). Global impacts of human mineral malnutrition. *Plant and Soil*, 335(1), 133–154. https://doi.org/10.1007/s11104-009-0228-2.

Tamang, J. P., Tamang, B., Schillinger, U., Guigas, C., & Holzapfel, W. H. (2009). Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas. *International Journal of Food Microbiology*, 135(1), 28–33. https://doi.org/10.1016/j.ijfoodmicro.2009.07.016.