Enzymatic degradation of phytic acid in low-calore bread with different sources of phytases

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Abstract

The present investigation was carried out to study the possibility of utilization of high phytase activity extracted from different plant sources to degradation of phytic acid in low calore (diabetic bread) bread contained high levels of phytates. The reduction of phytate by using the ordinary water without adding phytase extract (control) in dough mixing then, fermented to 45 min., was 17.2 and 22.1% of its initial value after fermentation and baking, respectively. The addition of phytase extract from germinated wheat, wheat germ and rice bran during dough mixing led to increase the hydrolysis of phytate and reduction its content in final diabetic bread. The obtained results by addition phytase extract from wheat germ showed highly increase in phytic acid degradation compared to germinated wheat extract. When half amount of water required for dough mixing was replaced by equal amount of phytase extract from defatted wheat germ, the phytate phosphorus levels in diabetic bread decreased by 90.6 % of its initial values. Addition of phytase extract prepared from rice bran to the doughs minimized the phytate content during fermentation by 62.7, 65.7 and 77.2% in the presence 10, 20 and 30 ml of rice bran phytase extract, respectively. It can be concluded that, the use of wheat germ phytase extract is better to reduce the phytate content in diabetic bread compared with germinated wheat and rice bran phytase extracts.

Keywords:

Phytase activity, Phytic acid, Phosphorous compounds, Diabetic bread.

INTRODUCTION

Phytase are widely distributed in plants (Viveros *et al.*, 2000; Konietzny and Greiner, 2002), animal (Zhang *et al.*, 2005), human tissues (Igbal *et al.*, 1994) and microorganisms (Mullaney and Ullah, 2003). Many species of bacteria (Simon and Igbasan, 2002), yeasts (Lambrechts *et al.*, 1993) and molds are considered to be other sources of the same enzyme.phytate-degrating enzymes [phytases] catalyze the hydrolysis of phytate (*myo*-inositol hexa-phosphate, IP6), the major storage form of phosphorus in plant kingdom. Phytases belong to a special group of phosphatases, that are chemically known as *myo*-inositol (1.2.3.4.5.6) hexakisphosphate phosphohydrolase, and catalyze the sequential release of phosphate from phytate (Frias *et al.*, 2003; Trann *et al.*, 2011). The degradation of phytate by phytase is of nutritional importance because the mineral binding strength of phytate decreases

and the solubility increases when phosphate groups are removed from the inositol ring resulting in an increased bioavailability of essential dietary minerals (Sandberg and Andlid, 2002; Debnath *et al.*, 2005 and Ma *et al.*, 2009). In an in vitro study, Leenhardt *et al.* (2005) found that by acidifying phytic-acid-rich whole wheat dough via sourdough fermentation or by adding lactic acid to the dough, it showed a large phytate breakdown (around 70% compared to 40% in control). A slight drop in pH (meaning higher acid) is sufficient to reduce phytate content of whole meal flour and magnesium bioavailability was improved. Phytate must be reduced to very low levels to increase mineral bioavailability, especially of iron (Hurrell, 2003). For this purpose addition of exogenous phytase is desired. So far, commercial phytase products have been mainly used as animal feed additives in diets, largely for swine and poultry, and to some extent for fish. But in spite of its immense potential in processing and manufacturing of food for human consumption, no phytase product for human food application has found its way to the market. Conclusively, many researchers have reported a convincing improvement of food products by adding microbial-based phytase during food processing for maize wet milling (Antrim *et al.*,1997), plant protein isolates (Fredrikson *et al.*, 2001), bread making (Haros *et al.*,2001), and the fractionation of cereal bran (Kvist *et al.*, 2005). A major part of the phytate in wheat grains is found in the aleurone layer. During the milling process, most of these aleuronic cells remains with particles of pericarp, hence phytate becomes concentrated in the bran fraction. Whole wheat contain about 0.3 % phytate and the bran contain about 5 % (O'Dell *et al.*, 1972; Wada and Maeda, 1980).Addition of phytase can improve the nutritional value of plant-based foods by enhancing protein digestibility and mineral availability through phytate hydrolysis during digestion in the stomach or during food processing (Sandberg and Andlid, 2002). Phytase offers excellent possibilities as a bread making improver, with two main advantages: first, the nutritional improvement produced by decreasing phytate content, and second, all the benefits produced by alpha-amylase addition can be obtained by adding phytase, which promotes the activation of endogenous alpha-amylase (Haros *et al.*, 2001). Thus, phytases have an important application in human nutrition both for degradation of phytate during food processing and in the gastrointestinal tract. However, the capability to dephosphorylate phytate differs greatly among different plant and microbial species due to differences in their intrinsic phytate-degrading activities.The aim of this study was an attempt to use partially purified phytase enzyme extracted from germinated legumes, germinated wheat, wheat germ, and rice bran to reduce the phytate content during diabetic bread making.

Materials and Methods

Materials: Grain samples of wheat *(Triticum aestivum),* Seds1 variety was obtained from (Field Crops Research Institute, Shandaweel Agriculture Research Center, Sohag, Egypt). All samples were collected during the season of 2014-2015. Wheat gray shorts flour and wheat germ were obtained from Roller Mills at Sohag; Middle Egypt Flour Mills, Assiut, Egypt and rice bran of variety Giza 78 *(Oryza sativa)* was provided by Rice Milling of Middle Delta during the season 2015.

Milling of wheat grains and maize: Wheat grains were conditioned by raising its moisture content up to 14 percent then left for 24 hrs as tempering time. Milling of wheats was run in a Buhler experimental mill (type 212) by progressively receiving the pollard and then regrinding and receiving the pollard and bran, one flour representing extraction 82% was obtained from each wheat variety and maize. Extraction rates were calculated as percentage of the total products according to the following formula:

$$\text{Extraction rate} = \frac{\text{Flour}}{(\text{Flour} + \text{Offals})} \times 100$$

Chemicals: Dodeca sodium phytate, phenyl phosphate disodium salt, acetic acid, acetone, trichloroacetic acid, sulfuric acid, nitric acid, sodium hypochlorite, sodium chlorite and sodium acetate were obtained from Sigma (Germany).

Methods:

Defatting of wheat germ, maize germ, and rice bran: The phytase activity was assayed only in the germ and rice bran after separation from the whole ungerminated, soaked and germinated maize grains. Therefore the samples were defatted by shaking the dried maize germ, wheat germ and rice bran with hexane (1:6 W/V) for 6 hrs. at 25^0C. The suspension was filtered under vacuum; the residue was re-extracted for 2 hrs. as previous described (Abdel-Gawad and Hamada, 2002) then residue was air dried and finally stored under refrigeration until used for enzyme assay.

Low calore bread making: Bread for diabetics was prepared according to the method described by Asad (1992). 1000 g wheat gray short flour mixed with the required amount of water for optimum absorption. Sodium chloride of 10 g as well as compressed yeast of 5 g was used. The previous ingredients were mixed in a mixer for 25 min, and then fermented for one hour at 30^0C and 85% relative humidity. For the treated dough samples, the phytase extracts were added by 0, 10, 20, 30 and 50% of the added water to the doughs. The dough was divided into 100 g pieces. Each piece was arranged on a wooden board sprinkled with a thin layer of bran and left to ferment for 45 min at the same temperature and relative humidity. The fermented dough pieces were flattened to about 30 vm diameter. The flattened loaves were proofed for 15 min under the same

conditions, then baked at 400-450^{0}C for 1-2 min.

Moisture content: Moisture content of soaked and germinated legume seed samples which previously dried (at 60^{0}C, for 48 hrs) were finally performed at 105^{0}C for 3 hrs according to A.OA.C. (1990) methods.

Extraction of phytase: The crude enzyme was extracted as described by Abdel-Gawad and Hamada (20002) by stirring samle in 0.1 M acetate buffer, pH 5.2, (using 1 flour : 10 buffer, W/V) at 5-10^{0}C for 30 min, then centrifuged for 20 min at 4200 *xg* and finally filtering the supernatant through four layers of filter cloth. The obtained filtrate was mixed with cold acetone to precipitate the enzyme. The produced precipitate was re-dissolved in acetate buffer (pH 5.2), dialyzed over night against the same buffer and centrifuged as mentioned above. The obtained supernatant was the partial purified phytase then using in food processing.

Partial purification of phytase by acetone precipitation: The partial purification of phytase was followed by precipitation enzyme from crude extract by acetone as the method described by Abdel-Gawad and Yokoyama (2004).

Determination of phytase activity: The activity of partial purified phytase was measured as described by Lolas and Markakis (1977).

Determination of phosphorus compounds: Total phosphorus (TP), inorganic phosphorus (Pi) and phytate phosphorus (PP) were extracted according to the method described by Tangkonchitr *et al.,* (1981). The ammonium molybdate colorimetric method for phosphorus determination was used in this study (Jackson, 1973). The native inorganic phosphorus present in the samples was extracted according to the procedure of Tangkonchitr *et al.* (1981). Phytate phosphorus was extracted as described below in determination of phytic acid and digested using mixture of 1 ml sulfuric acid and 6 ml nitric acid to release the phosphorus as described in A.O.A.C. (2000). The phytate phosphorus was measured colorimetrically as described above. Phytic acid (*myo*-inositol hexaphosphate) was calculated by multiplying the value of phytate phosphorus × 3.546 factor.

Results and Discussion

Phytase activity: Phytase activity in raw wheat grains, soaked wheat, germinated wheat, wheat germ and rice bran are illustrated in Fig. (1). The values of phytase activity in raw and soaked wheat were 2.33 and 4.63 µM/g/min in crude extract, while after purification by acetone 80% the activity values were increased. The highest phytase activity was recorded in germinated wheat(21.36 µM/g/min) for 120 hr compared with raw and soaked wheat samples. Centeno *et al.* (2003) reported that the germination process caused a significant increase of phytase activities in spring and winter wheat up to 275% and 250%, respectively

and a reduction in the phytate phosphorus content up to 35%. Oraby (2005) reported that the activity of phytases and acid phosphatase from germinated-120 hrs. of wheat was 20.75 and 37.40 µM/g/min, respectively. The results ilustrated in Fig. (1) showed that the activity values of phytase in crude extract of defatted wheat germ and rice bran were 18.60 and 16.60 µM/g/min, respectively. Purification process of crude extract by acetone 80% led to increases of phytase activity in same samples under investigation. The activity values of purified phytase in defatted wheat germ, maize germ and rice bran were 29.60, 22.20 and 26.30 µM/g/min, respectively. Abdel-Gawad *et al.* (2013) reported that, the activity of phytase from germinated-96 hrs of maize germ was 20.50 µM/g/min.

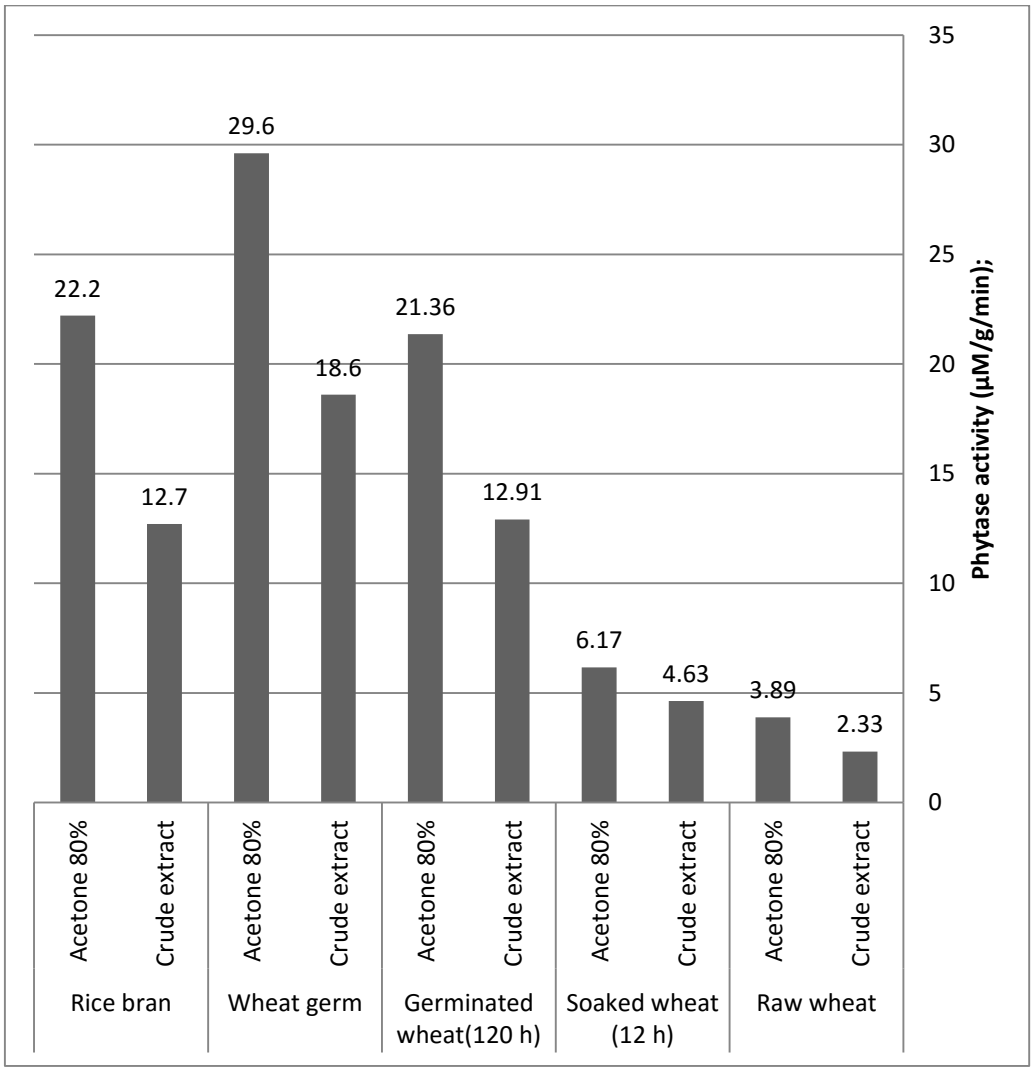

Fig. 1: Phytase activity in wheat and some cereal by-products.

Phytate and phosphorus compounds in milling fractions of wheat: In view the voluminous literature data, the total quantity of phytic acid and salts of phytic acid present in cereal grains varies with the variety of grain type and other factors. The rates of flour extraction influence greatly the contents of phytic acid and total phosphorus in flours and the resultant bread (Sorour 1997). Phytic acid, phytate phosphorus and total phosphorus contents in milling fraction of Seds1wheat are shown in Table (1). Phytic acid contents in whole flour of wheat Seds1 variety were 0.77%. Phytate phosphorus contents in milling fractions of wheat Seds1 variety were; 217.0, 134.4, 275.7, 439.2, 1176.2 and 1151.3 mg/100g in whole flour, 82% extraction flour, fine shorts, coarse shorts, fine bran and coarse bran, respectively. The same observations were reported by Fretzdorff and Brummer (1992). The values of inorganic phosphorus and total phosphorus in wheat Seds1 were higher in shorts and bran than that in the whole flour. These results are in the line with those reported by Knorr et al. (1981) and Sorour (1997), they reported that more than 90 % of the total phytic acid of wheat is localized in the aleuronic layer which consists entirely of phytin. Okazaki and Katayama (2005) reported that, the accumulation site of phytic acid in monocotyledonous seeds (wheat, millet, barley and rice) is the aleurone layer, particularly the aleurone grain. Maize differs from other cereals as more than 80% of phytic acid is concentrated in germ. Phytic acid content of cereals varies from 0.5 to 2.0%.

Table (1): Phytic acid, phytate phosphorus and total phosphorus contents in milling of wheat*.

Fractions	Phytic acid (%)	Phytate-p* (mg/100 g)	Total-p* (mg/100 g)	Phytate-p as % of Total-p
Whole wheat flour	0.77	217.0	376.2	57.7
82 % extr. flour	0.48	134.4	334.6	40.2
Fine shorts	0.98	275.7	417.4	66.1
Coarse shorts	1.56	439.2	623.2	70.5
Fine bran	4.20	1176.2	1518.6	77.5
Coarse bran	4.10	1151.3	1496.7	76.9

*: All values expressed on dry weight basis and the samples analyzed in duplicate.

* Phytate phosphorus, Total phosphorus.

Reduction of phytate content in low calore bread*:* In most diabetic breads, the ingredients may contain wheat bran and gray shorts as a traditional source of dietary fiber to substitute wheat flour for processing diabetic bread. A major part of the phytate in wheat grains is found in the aleurone layer. During the milling process, most of these aleuronic cells remains with particles of pericarp, hence phytate becomes concentrated in the bran fraction. Whole wheat contain about 0.3 % phytate and the bran contain about 5 % (O'Dell *et al.*, 1972; Wada and Maeda, 1980). The diabetic bread has normal low starchy carbohydrates and high non-starchy polysaccharides (dietary fiber) contents and therefore may contain relatively considerable amounts of phytate. The present study compares phytate degradation in diabetic bread doughs by adding phytase from various sources.

Addition of phytase extracted from germinated wheat*:* The utilization of phytase extract from the germinated wheat during the diabetic bread making are illustrated in Fig.(2). The using of ordinary water without adding phytase extract (control) in dough mixing then, fermented to 45 min., the reduced the phytate by 17.2 and 22.1% of its initial value after fermentation and baking, respectively. The addition of phytase extract from the germinated wheat during dough mixing led to increase the hydrolysis of phytate and reduction its content in final diabetic bread. The hydrolysis of phytate was enhanced with increasing the amount of added wheat phytase extract (Figure 2). When half amount the required water for dough mixing was replaced by equal amount of wheat phytase extract, the phytate phosphorus levels in diabetic bread decreased to 83.1 % of its initial values. The obtained results are in the line with those reported by Sorour (1997) who found that, the hydrolysis of phytate was enhanced with increasing the amount of added wheat bran phytase extract to flour during mixing of dough. Up to 90.2% of phytate was reduced in Balady bread when added 30 ml wheat bran phytase extract during dough mixing.

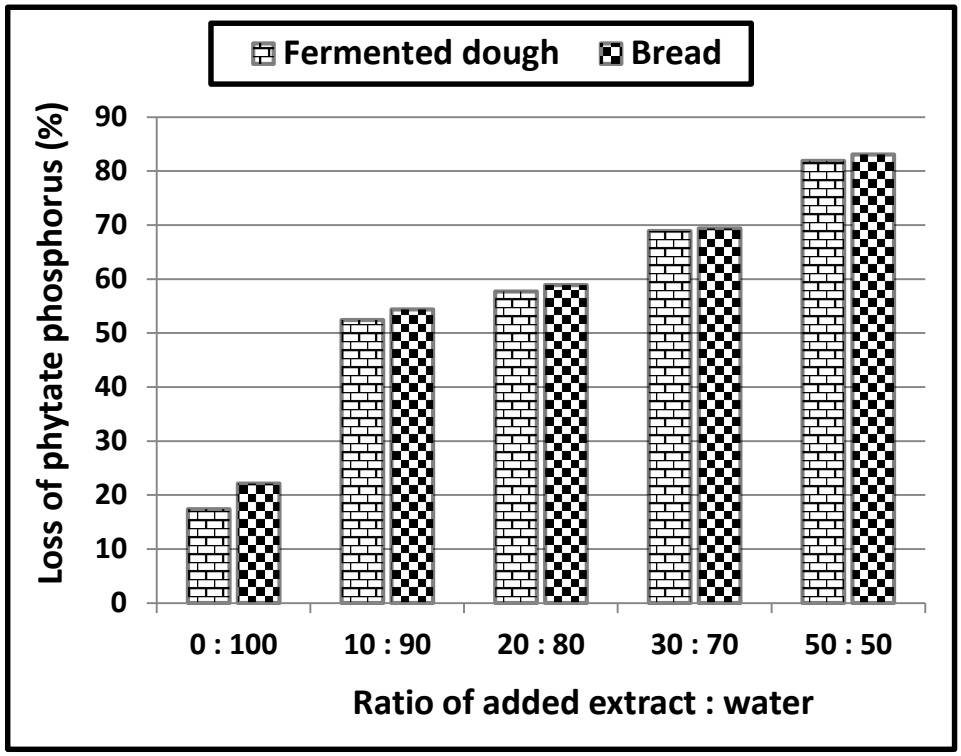

Figure 2: Effect of addition the extracted phytase from germinated wheat on hydrolysis of phytate during diabetic bread making.

Addition of phytase extracted from the defatted wheat germ: The addition of phytase extract from defatted wheat germ during dough mixing led to increase the hydrolysis of phytate and reduction its content in produced diabetic bread. The obtained results of phytase extract from wheat germ addition showed highly increase in phytic acid degradation compared to previous data when adding phytase extract from germinated wheat extracts (Fig. 3). When half amount the required water for dough mixing was replaced by equal amount of phytase extract from the defatted wheat germ, the phytate phosphorus levels in diabetic bread decreased to 90.6 % of its initial values. The results are in the line with those reported by Sorour (1997) who found that, the reduction of phytate in dough after fermentation by addition of wheat germ phytase extract was greater than that fount in corresponding doughs prepared by addition of phytase extract from other sources.

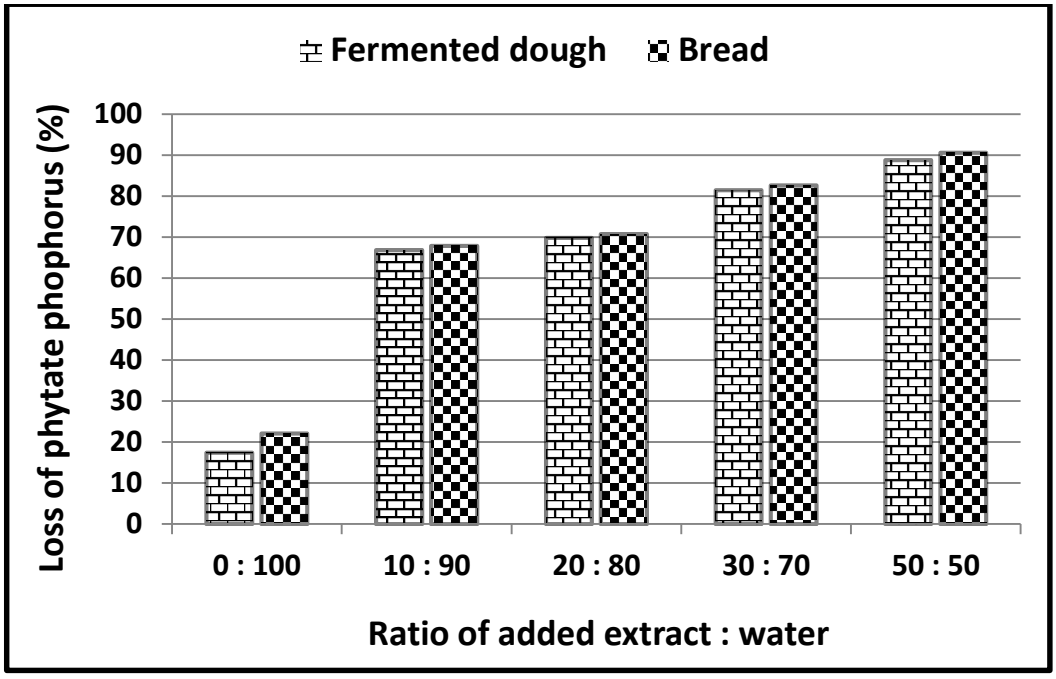

Figure 3: Effect of addition of the extracted phytase from defatted wheat germ on hydrolysis of phytate during diabetic bread making.

Addition of phytase extracted from defatted rice bran: Addition of phytase extract prepared from rice bran to the doughs (Fig. 4) minimized the phytate content during fermentation by 62.7, 65.7 and 77.2% in the presence 10, 20 and 30 ml of rice bran phytase extract, respectively. By increasing the volumes of rice bran phytase extract to 50% of the added water, the loss of phytate accounted to 85.9% after fermentation. These results are in the line with those reported by Sorour (1997) who found that, addition of

rice bran phytase extract with different volumes (5-30 ml) to the Balady bread doughs made from flour 82% extraction reduced the phytate content during fermentation to 35.6 - 83.3% of its initial values, respectively.The high hydrolysis of phytate during fermentation may be due to the added phytase extract from

rice bran to wheat flour and fine branmixtures during dough mixing. The decreasing rate of phytate was more noticeable than that observed for phytase extract from wheat germ. Addition of phytase extract from rice bran with different volumes (10-50 ml) to the diabetic bread doughs made from flour 82% extraction and fine bran mixtures reduced the phytate content in the final bread to (63.6-86.4%) of its initial value, respectively. Konietzny and Greiner (2002) reported that, the reduction of phytates in foods can be achieved through both enzymatic and non-enzymatic removal. Enzymatic degradation includes addition of either isolated form of wild-type or

recombinant exogenous phytate-degrading enzymes microorganisms in the food matrix. Non-enzymatic hydrolysis of phytate occurred in the final food during food processing.

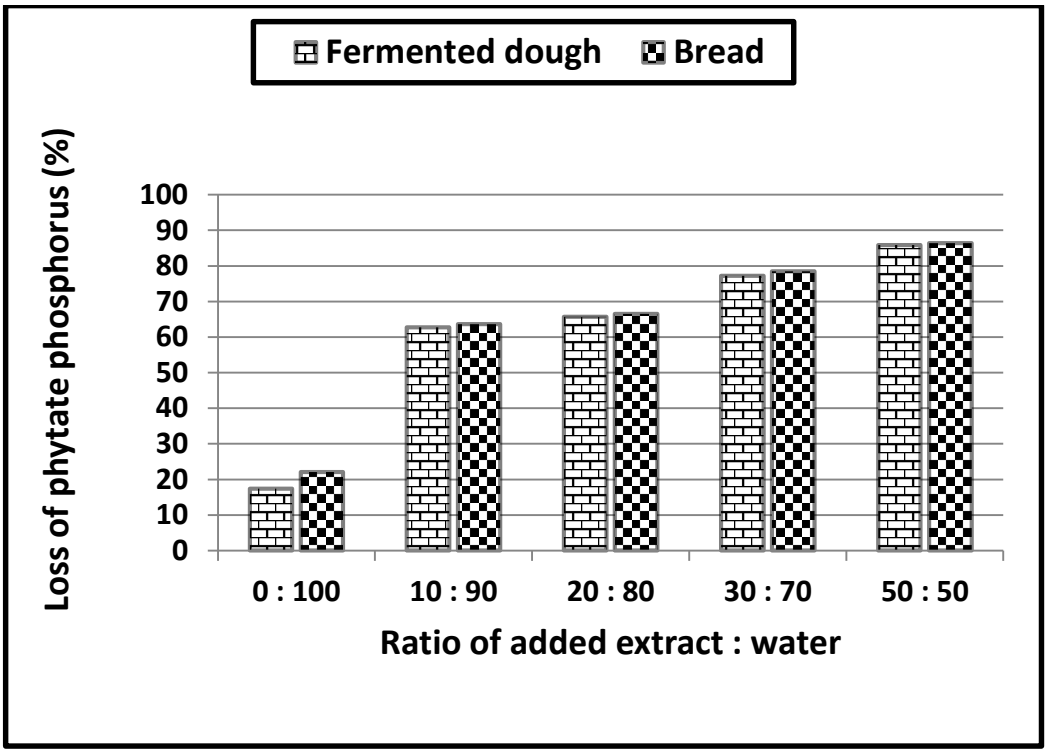

Figure 4: Effect of addition the extracted phytase from defatted rice bran on hydrolysis of phytate during diabetic bread making.

REFERENCES

Abdel-Gawad, A.S. and Hamada, J.S. (2002). Inositol phosphates hydrolyzing enzymes in rice bran.1. Purification and characterization of phytase, The third Scientific Conference of Agricultural Sciences, Assiut University, Assiut, Egypt, 20-22 October, 2002, p.p. 163-178.

AAbdel-Gawad, A.S. and Yokoyama, W. (2004). Commercial-scale production of functional inositol polyphosphates from rice bran. Final report No. 101 ID code MAN4-008-020.

Abdel-Gawad, A.S.; Ramadan, B.R. and Oraby, R.E.A. (2013). Legume phytases: Characteristics and changes in activity during germination. *International Journal of Agricultural Policy and Research,* 1 (4): 093-102.

Afinah, S.; Yazid, A.M.; Anis Shobirin, M.H. and Shuhaimi, M. (2010). Review Article, Phytase: application in food

industry. *International Food Research Journal*, 17: 13-21.

Antrim, R.L.; Mitchinson, C. and Solheim, L.P. (1997). Method for liquefying starch. *US patent application*, -5652127.

A.O.A.C. (1990). Official Methods of Analysis (15th Ed.). Association of official Analytical Chemists. Washington, D.C.

A.O.A.C. (2000). Official methods of analysis (17th Ed.). Association of Official Analytical Chemists. (by Dr. William Horwitz). Gaithersburg, MD, USA.

Asad, I.A. (1992). Production of Improved Egyption bread types and diabetic bread. Ph.D.Thesis, Fac. of Agric. Cairo University, Cairo, Egypt.

Centeno, C.; Viveros, A.; Brenes, A.; Lozano, A. and Cuadra, C.D. (2003). Effect of several germination conditions on total P, phytate P, phytase, acid phosphatase activities and inositol phosphate esters in spring and winter wheat. *J. Agric. Sci.*, 141: 313-321.

Dahiya, S. (2016). Role of phytate and phytases in human nutrition. *International Journal of Food Science and Nutrition*, 1 (1): 39-42.

Debnath, D.; Sahu, N.P.; Pal, A.K.; Baruah, K.; Yengkokpam, S. and Mukherjee, S.C. (2005). Present scenario and future prospects of phytase in aqua feed -Review, Asian-Australian *J. Anim. Sci.*, 18 (12): 1800-1812.

Fredrikson, M.; Biot, P.; Alminger, M.; Larsson Carlsson, N.G. and Sandberg, A.S. (2001). Production process for high-quality pea-protein isolate with low content of oligosaccharides and phytate. *Journal of Agriculture and Food Chemistry*, 49: 1208-1212.

Fretzdorff, B. and Brummer, J.M. (1992). Reduction of phytic acid during bread making of whole meal breads. Cereal Chem., 69: 256-275.

Frias, J.; Doblado, R.; Antezana, J.R. and Vidal-Valverde, C. (2003). Inositol phosphate degradation by the action of phytase enzyme in legume seeds. *Food Chem.*, 81 (2): 233-239.

Greiner, R.; Muzquiz, M.; Burbano, C.; Cuadrado, C.; Pedrosa, M.M. and Goyoaga, C. (2001). Purification and characterization of a phytate-degrading enzyme from germinated faba beans (*Vicia faba* Var. Alameda). *J. Agric. Food Chem.*, 49 (5): 2234-2240.

Haefner, S.; Knietsch, A.; Scholten, E.; Braun, J.; Lohscheidt, M. and Zelder, O. (2005). Biotechnological production and applications of phytases. *Appl. Microbiol Biotechnol.*, 68 (5): 588-597.

Haros, M.; Rosell, C.M. and Benedito, C. (2001). Use of fungal phytase to improve bread making performance of whole wheat bread. *J. Agric. Food Chem.,* 49 (11): 5450-5454

Iqbal, T.H.; Lewis, K.O. and Cooper, B.T. (1994). Phytase activity in the human and rat small intestine. *Gut.*, 35: 1233-1236.

Jackson, M.L. (1973). "Soil Chemical Analysis" Prentice Hall India Private Limited, *New Delhi*. pp. 141-149.

Knorr, D.; Watkins, T. R. Carlson, B. L. (1981). Enzymatic reduction of phytate in whole wheat breads. *J. of Food Sci.* 46: 1866-1869.

Konietzny, U. and Greiner, R. (2002). Molecular and catalytic properties of phytase-degrading enzyme (phytase). *Int. j. Food Sci. Technol.,* 37: 791-812.

Kvist, S.; Carlsson, T.; Lawther, J.M. and De Castro, F.B. (2005). Process for the fractionation of cereal brans. *U.S.patent application,* -0089602.

Lambrechts, C.; Boze, H.; Segueilha, L. and Gaizy, P. (1993). Influence of culture conditions on the biosynthesis of Schwanniomyces castelii phytase. *Biotech. Lett.*, 15: 399-404.

Leenhardt, F.; Levrat-Verny, M.A.; Chanliaud, E. and Rémésy, C. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. J. *Agric Food Chem.*, 53 (1): 98-102.

Lolas, G.M. and Markakis, P. (1977).The phytase of Navy bean (*Phaseolus*). *J. Food Sci.*, 42: 1094-1099.

Luo, Y. and Xie, W. (2012). Effect of phytase treatment on iron bioavailability in Faba bean (*Vicia faba* L.) flour. *Food Chem.,* 134: 1251-1255.

Ma, X.F.; Wright, E.; Ge, Y.; Bell, J.; Xi, Y.; Bouton, J.H. and Wang, Z. (2009). Improving phosphorus acquisition of white clover (*Trifolium repens* L.) by transgenic expression of plant-derived phytase and acid phosphatase genes. *Plant Sci.*, 176: 479-488.

Marklinder, I.M.; Larsson, M.; Fredlund, K. and Sandberg, A.S. (1995). Degradation of phytate by using varied sources of phytases in an oat-based nutrient solution fermented by Lactobacillus plantarum. *Food Microbiology*, 12: 487-495.

Mullaney, E.J. and Ullah, A.H. (2003). The term phytase comprises several different classes of enzymes. *Biochem.*

Biophys. Res. Coummun., 312: 179-184.

O'Dell, B.L.; Deboland, A.R. and Koirtyohann, S.R. (1972). Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. *J. Agric. Food Chem.,* 20: 718-723.

Okazaki, Y. and Katayama, T. (2005). Reassessment of the nutritional function of phytic acid, with special reference to *myo*-inositol function. *J. Jpn. Soc. Nutr. Food Sci.,* 58: 151-156.

Oraby, R.E.A. (2005). Characteristics of phytases and acid phosphatases extracted from differend plant sources. M.Sc. Thesis, Food Science and Technology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Sandberg, A.S. and Andlid, T. (2002). Phytogenic and microbial phytases in human nutrition. *Inter. J. Food Sci. and Tech.,* 37: 823-833.

Sayed, E.M. (1992). Studies on distribution and location of phytase in different seeds and cereal grains. M.Sc. Thesis, Food Science and Technology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Simon, O. and Igbasan, F. (2002). In vitro properties of phytases from various microbial origins. *Inter. J. Food Sci. and Tech.*, 37: 813-822.

Sorour, M.A.H. (1997). Studies on the reduction of phytic acid contents in egyptian bread by different fermentation methods. Doct. Sc. Thesis, Food Science and Technol. Depart. Faculty of Agric., Assiut Uuiv., Assiut, Egypt.

Tangkongchitr, U.; Seib, P.A. and Hoseney, R.C. (1981). Phytic acid. I. Determination of three froms of phophours in flour, dough and bread. *Cereal Chem.*, 58: 226-230.

Trann, T.T.; Hatti-Kaul, R.; Dalsgaard, S. and Yu, S. (2011). A simple and fast kinetic assay for phytases using phytic acid-protein complex as substrate. *Anal. Biochem.*, 410: 177-184.

Qasim, S.S.; Shakir, K.A. and Al-Shaibani, A.B. (2015). Isolation, screening and production of phytate degrading enzyme (phytase) from local fungi isolate.*Iraqi Journal of Agricultural Sciences,* 74: 121-128.

Viveros, A.; Centeno, C.; Brenes, A.; Canales, R. and Lozano, A. (2000). Phytase and acid phosphatase activities in plant

feeds tuffs. *J. Agric. Food Chem.,* 48: 4009-4013.

Wada, T. and Maeda, E. (1980). Acytological study on the phosphorus accumulation tissues in graminaceous seeds. JPN. J Crop. Sci., 49: 457-465.

Zhang, W.; Aggrey, S.E.; Pesti, G.M.; Bakalli, R.I. and Edwards, H.M. (2005). Correlated responses to divergent selection for phytate phosphorus bioavailability in a random bred chicken population. *Poultry Science,* 84: 536-542.