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Processing Effect on Phytase in Selected Indian Wheat Varieties (*Triticum aestivum* L.)

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A B S T R A C T

The effect of different process on phytase activity in four wheat varieties DBW-17, HD-2894, HUW-234 and LOK-1were evaluated. Interestingly, maximum phytase activity was observed in LOK-1 wheat variety in all the five different process *i.e.* 2675.44±0.56 nM/min/g 10th day of germination, 771.07±0.58 nM/min/g 3rd day of soaking, 373.77±0.62 nM/min/g 6th day of kilning, 342.38±0.33 nM/min/g in grinding as coarse flour and 9.37±0.37 nM/min/g at 90^{o}C in roasting, respectively.

Introduction

The main source of world's food energy contains significant amounts of proteins, minerals and vitamins, which are essential nutrients for human health (Piironen *et al.*, 2009). However, their absorption in humans is significantly inhibited by the presence of substances such as phytate. This is the primary storage form of phosphorus in seeds upto 85% of total seed phosphorus. The negatively charged phosphates in phytate bind strongly to metallic cations such as potassium, magnesium and iron at physiological pH to form a mixed salt called phytin or phytate, which reduces bioavailability to humans (Greiner and Konietzny, 2006). Soaking is a pre-treatment to enhance processing of seeds. In household activities, cereals and pulses are typically soaked in water at room temperatures overnight for consumption (Sandberg, 1991). Germination is used to breakdown antinutrients in seed such as phytate and protease inhibitors, which helps in significant increase in mineral uptake after 6 to 10 days of germination. Phytate is hydrolyzed during germination. Whereas, in non-germinated grains and cereals, only little intrinsic phytate-degrading activity is found (Egli *et al.*, 2002). Malting is a process during which the whole

grain is soaked and then germinated. Otherwise, if malted grains of wheat, rye and oats were used as such there was only slightly or not at all reduction of phytate content. Oat germination requires 5 days at 11^0C followed by incubation at 37-40^{o}C, then phytate content of oats was reduced by 98% (Larsson and Sandberg, 1992). Roasting can improve protein digestibility but has little or no effect during preparation (Nout, 1993) as well as this is an important unit operation in processing of grain for making *sattu* due to its significant effect on the odour in the final product which is the most desired quality of *sattu* (Mridula *et al.,* 2008). Milling process defines the chemical composition of any flour. Flours with higher extract ion contain increasing amount of bran. Nowadays, as consumption of whole grain breads are increased so it would be beneficial and attractive to improve the mineral status and nutrition (Turk *et al.,* 1996).

Materials and Methods

Four locally available wheat varieties were purchased from Alopibagh market, Prayagraj, India that is namely DBW-17, HD-2894, HUW-234 and LOK-1. For experiment purpose, native of all the four wheat varieties as well as processed seeds *i.e.* soaked (overnight), germinated (after overnight soaking kept for 10 days), kilned (after overnight soaking, kept for 7 days and 2 hrs air dry at 40^{o}C), heated (30^{o}C heating) and ground seeds were used for sample preparations.

Phytase assay

0.5g fresh samples of all wheat varieties were separately homogenized in 10ml of sodium acetate buffer (0.1M, pH 5.0). The homogenized samples were centrifuged at 12000g for 5min and supernatants were used for enzyme assay (Senna *et al.,* 2006). The assay mixture consisted of 350µl of sodium acetate buffer (0.1M, pH 5.0)and 100µl of sodium phytate (2mM). This mixture was preincubated for 10min at 40^{o}C and the enzymatic reactions were started by adding 100µl of the crude enzyme to preincubated assay mixture. After incubation at 40^{o}C for 30min, the liberated phosphate was measured by using the ammonium molybdate. For this, to the assay mixture, 1.5ml of a freshly prepared solution of acetone/5N H_2SO_4/10mM ammonium molybdate (2:1:1 v/v/w) and 100µl of 1.0M citric acid were added. Any cloudiness was removed by centrifugation to measurement of the absorbance at 355nm against blank as ammonium molybdate solution (Heinonen and Lahti, 1981).The data were analyzed by SPSS ver. 20.0, two-way analysis of variance (ANOVA) at 5% level of significance

Results and Discussion

The data pertaining to phytase assay four wheat varieties through different processing germination, soaking, heating, kilning and grinding are presented under present study. Effect of germination on phytase activity was studied at 5th, 10th and 15th day of germination of four wheat varieties from Fig. 2. On 5th day of germination, maximum phytase activity (nM/min/g) was observed in LOK-1 (2342.13±0.52) followed by DBW-17 (2065.78±0.390), HD-2894 (2133.51±0.46) and HUW-234 (2041.17±0.66). On 10th day of germination, maximum phytase activity (nM/min/g) was in LOK-1 (2675.44±0.56) followed by DBW-17 (2418.59±0.48), HD-2894 (2471.44±0.39) and HUW-234 (2372.81±0.37). On 15th day of germination, maximum phytase activity (nM/min/g) was in LOK-1 (2382.07±0.55) followed by DBW-17 (2205.17±0.52), HD-2894 (2233.17±0.46) and HUW-234 (2173.91±0.31). Germination mobilizes reserve nutrients required for growth and development therefore may help in

the removal of some of the unwanted compounds (Sathe and Salunkhe, 1989). Germination of seeds leads to a rapid disappearance of phytin inclusions accompanied by a large increase in activity of the phytase enzyme responsible for phytin degradation (Loewus *et al.*, 1990). Germination triggers the enzymatic activity of sprouting grains, leading to the breakdown of proteins, carbohydrates and lipids into simpler forms. This processing method activates proteases, which are active in degrading proteins, thereby increasing nutrient bioavailability (Elkhalifa and Bernhardt, 2010).

Effect of soaking on phytase activity was studied at 1st, 2nd and 3rd day of soaking of four wheat varieties from 3. In 1st day of soaking, maximum phytase activity (nM/min/g) was observed in LOK-1 (734.14±0.07) followed by DBW-17 (725.45±0.34), HD-2894 (730.51±0.25) and HUW-234 (691.73±0.32). On 2nd day of soaking, maximum phytase activity (nM/min/g) was in LOK-1 (765.24±1.45) followed by DBW-17 (741.06±0.24), HD-2894 (745.51±0.33) and HUW-234 (705.44±0.28). On 3rd day of soaking, maximum phytase activity (nM/min/g) was in LOK-1 (771.07±0.58) followed by DBW-17 (747.88±0.35), HD-2894 (751.41±0.70) and HUW-234 (711.37±0.32). Soaking usually forms an integral part of processing methods such as germination, fermentation, cooking and toasting (Muliman and Vadiraj, 1994). Temperature and pH value have been shown to have a significant effect on enzymatic phytate hydrolysis during soaking. If the soaking step is carried out at temperatures between 45 to 65^{o}C and pH values between 5.0 to 6.0, which are close to the optimal conditions for phytate dephosphorylation by the intrinsic plant phytases, a significant percentage of phytate (26–100%) was enzymatically hydrolyzed (Greiner and Konietzny, 1999). Effect of kilning on phytase activity was studied at 2nd, 4th and 6th day of germination of four wheat varieties from Fig. 4. On 1st day of kilning, maximum phytase activity (nM/min/g) was observed in LOK-1 (342.81±0.34) followed by DBW-17 (323.10±0.52), HD-2894 (324.70±0.35) and HUW-234 (321.75±0.37). On 2nd day of kilning, maximum phytase activity (nM/min/g) was in LOK-1 (352.77±0.38) followed by DBW-17 (341.67±0.88), HD-2894 (346.04±0.58) and HUW-234 (345.71±0.29). On 3rd day of kilning, maximum phytase activity (nM/min/g) was in LOK-1 (373.77±0.62) followed by DBW-17 (362.34±1.21), HD-2894 (374.70±0.70) and HUW-234 (364.71±1.29).

Effect of roasting on phytase activity was studied at 30^{o}C, 60^{o}C and 90^{o}C for four wheat varieties from 5. On 30^{o}C roasting, maximum phytase activity (nM/min/g) was observed in LOK-1 (287.08±0.58) followed by DBW-17 (281.33±0.88), HD-2894 (255.13±0.47) and HUW-234 (273.14±0.09). On 60^{o}C roasting, maximum phytase activity (nM/min/g) was found in LOK-1 (90.41±0.71) followed by DBW-17 (82.67±0.33), HD-2894 (85.67±0.33) and HUW-234 (62.02±0.51). On 90^{o}C roasting, maximum phytase activity (nM/min/g) was in LOK-1 (9.37±0.37) followed by DBW-17 (8.60±0.35), HD-2894 (8.90±0.35) and HUW-234 (6.62±0.25).

Breakfast cereal is defined as food obtained by soaking, swelling, roasting, toasting, grinding, rolling/flaking and shredding of puffing of any cereal and which is usually eaten at breakfast. Flaking is one of the methods of processing breakfast cereal, which involves cleaning, and conditioning to suitable moisture content of the whole grain and lightly rolled between smooth rolls to fracture the outer layer (Kent, 1975).

Fig.1 Wheat native phytase

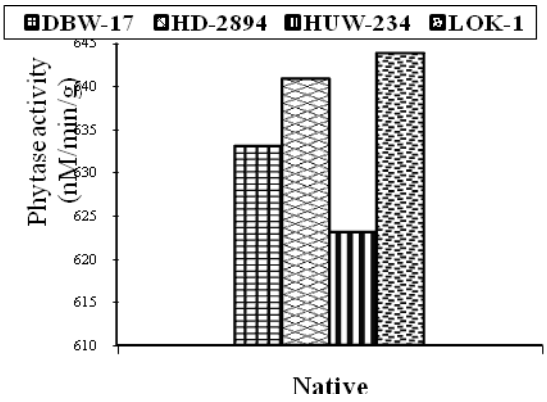


Fig.2 Effect of germination on phytase activity

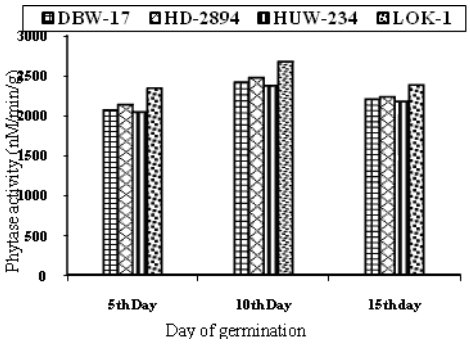


Fig.3 Effect of soaking on phytase activity

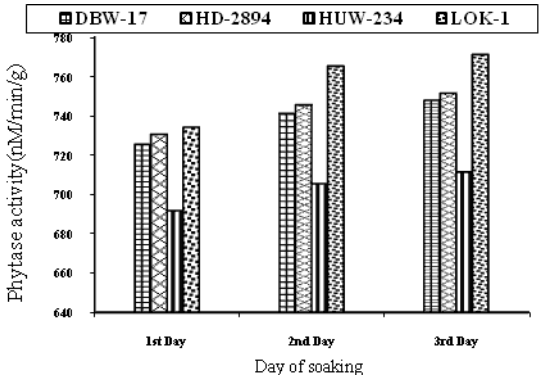


Fig.4 Effect of kilning on phytase activity

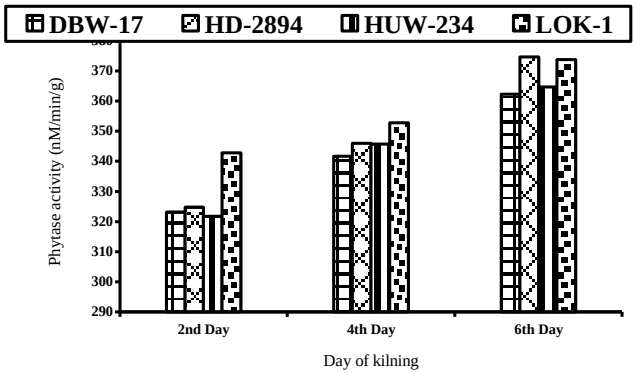


Fig.5 Effect of roasting on phytase activity

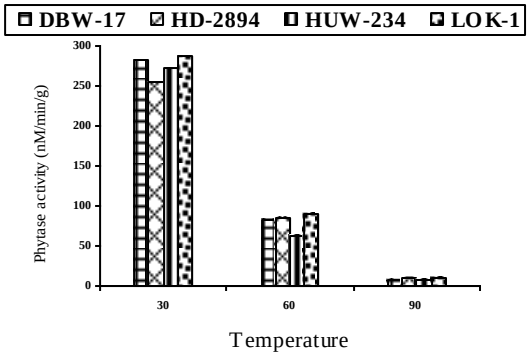
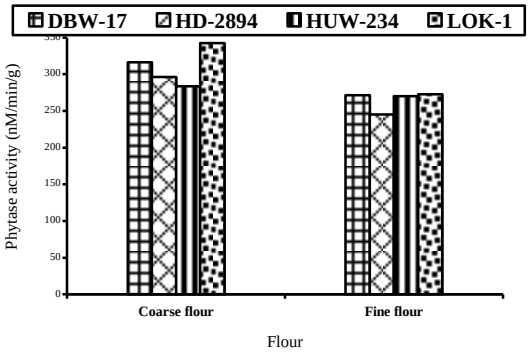


Fig.6 Effect of grinding on phytase activity



Effect of grinding on phytase activity was studied in coarse and fine flour of four wheat varieties from 6. In coarse flour, maximum phytase activity (nM/min/g) was observed in LOK-1 (342.38±0.33) followed by DBW-17 (316.15±0.18), HD-2894 (295.97±0.09) and HUW-234 (283.40±0.30). In Fine flour, maximum phytase activity (nM/min/g) was seen in LOK-1 (272.76±0.79) followed by DBW-17 (271.33±0.88), HD-2894 (245.18±0.43) and HUW-234 (269.81±0.19). Grinding increased the concentration of dialyzable phosphorus, but samples that were not autoclaved. Autoclaving decreased amounts of phosphorus liberated from feed. This leads to significant part of phosphorus liberated from wheat-based feed under simulated conditions of poultry intestine may be attributed to the action of endogenous wheat enzymes (phytase and acid phosphatase). Mechanical breakdown of plant tissues improves access of those enzymes to wheat phytate (Fuller, 1991).

Similarly, 1759±73 nM/min/g phytase activity in Aglika wheat variety of Bulgaria was observed by Chalova *et al.,* (2012) but 2670±0.11 in Roshan, 2860±0.15 in Ghods and 3100±0.20 nM/min/g phytase in Mahdavi Iranian wheat variety were reported (Sedaghati *et al.,* 2011). A range 400 to 6000 nM/min/g phytase for oats, barley, triticale, rye and wheat was reported (Steiner *et al.,* 2007) and 520 to 1400 nM/min/g phytase in wheat was found (Cossa *et al.,* 2000) and 710 nM/min/g phytase activity in one day seedlings of Nigerian wheat was reported (Azeke *et al.,* 2011). Phytase exists in most cereals, but their activity varies widely among cereals (Bartnik and Szafranska, 1987). It appears that phytase activity usually increases on germination (Sung *et al.,* 2005) and germination has been used to induce phytase activity in cereals (Senna *et al.,* 2006). In Iranian whole wheat, 340 nM/min/g phytase in Hot weather aera, 442 in Pishtaz, 490 in S-78-11, 410 in Niknejad, 410 in Shiraz, 460 in S-79-10, 370 in Keras-Adl, 450 in Chamran, 320 in Estar, 520 in Shahriar, 440 in Falat, 430 in Kavir, 430 in Marvdasht, 320 in Pavarus, 360 in Azady, 440 in Darab2, 450 in Zarin and 500 nM/min/g phytase were reported by Tavajjoh *et al.,* (2011). In the anaphase of germination, the biosynthesis of phytase in malt of wheat decreases. In the course of germinating, variation of phytase activity presented low-high-low trend. In the germinating period's seeds grow only by consuming its storing matter with a low conversation rate of nutrient. Therefore, the dry matter loss is very great. The germinating condition should be optimized to gain the highest activity of phytase and the most of dry matter at the same time (Ma and Shan, 2002). The first intermediate myo-inositol was also identified as the main reaction product of F_1 phytase from wheat bran (Lim and Tate, 1973) and soybean phytase (Gibson and Ullah, 1990), which were described as 6-phytases. They are completely different from the 3-phytase or 5-phytase from *Aspergillus ficuum* (Ullah and Phillippy, 1988) or lily pollen (Barrientos *et al.,* 1994), respectively. Other phytases with broad substrate specificity have low specific activity for phytic acid (23 to 43 nM/min/g), whereas phytases with narrow substrate specificity generally have high specific activities of 103 - 811 nM/min/g (Greiner *et al.,* 1993). In soybean seed germination, a pronounced increase in phytase activity accompanies a concomitant decrease in phytic acid, with maximal phytase activity attained at approximately 10 d after germination (Gibson and Ullah, 1988).

The data in this study indicate that the significant differences ($P<0.05$) were obtained in the phytase activities of wheat varieties screened. The phytase activity was increased during soaking and germination but significantly decreased was observed in kilned, roasted and ground seeds. Among four

wheat varieties, LOK-1 wheat varieties have higher phytase activity in all different process soaking, germination, kilning, roasting and grinding.

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