Effect of Germination and Heating on Phytase Activity in Cereal Seeds

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ABSTRACT : The effect of germination on phytase activity in wheat NEAU123, triticale5305 and rye2 was studied in the present study. Germination significantly increased phytase activity by 2.04 times for wheat NEAU123 (3 d), 1.82 times for triticale 5305 (1 d) and 2.45 times for rye2 (1 d), respectively. It was safe for phytase in fresh malts kilned for 2 h at 40°C. Phytase in cereal seeds had strong heat stability. There was no loss of phytase activity in cereal seeds heated at 70°C for 1 h, a little loss (≤5.46%) at 80°C or 90°C. Even heated at 100°C, the phytase activity in wheat NEAU123, triticale5305 and rye2 remained 89.47%, 86.44% and 104.64%, respectively. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 1036-1039)***

Key Words : Cereal Seeds, Germination, Phytase Activity, Heating Stability

INTRODUCTION

The availability of phosphorus in plant feedstuffs to pig and poultry varies widely. It has been determined as ranging from 0 (Nelson, 1976) to more than 50% (Edwards, 1983; Lenis et al., 1999). Ballam et al. (1985) reported that the hydrolysis values of phytate ranged from 3 to 42%. The biological availability of plant phosphorus for pigs and poultry was related to its endogenous phytase activity (Cromwell, 1980; Nelson, 1980). High phytase activity result in high biological availability of phosphorus in plant feeds. Furthermore, the endogenous phytase acts on phytate from other ingredients (Scheuermann et al., 1988). It is possible to reduce inorganic phosphorus supplements and the costs of production by use of cereals rich in phytase.

Phytase exists in most cereals, but their activity varies widely among cereals (Bartnik and Szafranska, 1987). Wheat, triticale and rye (and their by-products such as bran and pollard) are high in phytase, barley is somewhat lower while maize has very little phytase (Pointillart et al., 1993; Eeckhout et al., 1994). In nature phytase is activated during germination of the seeds. Chen (1977) reported that phytase activity in the seeds of soybean, Dwarf bean and Alaska bean attained 2.27, 8.07 and 37.56 times high after 5 day's germination. Endogenous phytase is also heat labile and is readily inactivated at 70-80°C, the temperatures commonly used in feed processing (Jongbloed and Kemme 1990). But there were also contrary reports that plant phytase had high heat resistance (Ranhotra et al., 1975; Han Yanming et al., 1995).

Therefore, to improve the utilization of phytase from plant feedstuffs, extensive research should be conducted. Our objective of the present study was to increase the phytase activity in the cereal seeds by germination and investigate their kilning and heating stability.

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MATERIALS AND METHODS

Cereal seed samples

Wheat (NEAU123), Triticale 5305 and Rye 2 were analyzed in the present study.

Germinating procedure

Germination was performed at 25°C, with 100% humidity for 6 days. The malts were watered 4 times per day with deionized water. Two samples of every kind of cereal were picked per day. One sample of the two was for determination of phytase activity in fresh samples, the other was for determination of the dry matter.

Kilning procedure

The first day's malts of triticale 5305 and rye 2 and the third day's malts of wheat NEAU123 were air dried at room temperature after 2 hour's kilning at 40°C.

Heating procedure

The ground cereal seeds were heated at 60, 70, 80, 90, 100°C for 1h respectively. Then these samples were stored at room temperature for 2 d. Phytase activity of treated samples was analyzed.

Extraction procedure

Cereal seeds were ground to less than 0.25 mm, and were suspended in 0.25 mol/L sodium acetate buffer (pH 5.50), stirring gently for 1 h at 4°C. Then the suspension was filtrated. The filter liquor that was extract of phytase was to be assayed.

The fresh malts were homogenated firstly. The subsequent procedure was the same as that of dry samples.

Phytase activity assay

The phytase activity was measured following the method described by Zhang ruohan (1997), Lu wenqing et al. (2000). Phytase acts on sodium phytate and releases inorganic P. Phytase activity is determined by measuring the quantity of released inorganic P. 1 unit of phytase activity is defined as that the quantity of enzyme required to produce 1 μmol of inorganic P 1 min from 0.0051 mol/L of sodium phytate at a pH of 5.50 and at a water bath temperature of 37°C. 4 parallel controls were set for every sample.

Statistical analysis

One way analysis of variance was performed by the GLM procedure of SAS6.12. Data are presented as means± SEM (n=4).

RESULTS

Effect of germination on phytase activity in cereal seeds

Germination greatly enhanced ($p<0.01$) phytase activity (table 1), but the variation of activity were different among cereals. At 1 d phytase activity in malts of triticale 5305 and rye2 were highest in the course of germination. However, it was at 3 d that the activity attained the highest value in the malts of wheat NEAU123. Phytase activity in malts (1 d) of triticale 5305 and rye 2 and in malts (3 d) of wheat NEAU123 were 1.82, 2.45 and 2.04 times as high as that in ungerminated seeds, respectively. There were no statistical difference ($p>0.05$) among different day's malts of wheat NEAU123, but the activity in different day's malts of triticale 5305 and rye 2 presented significant or great significant difference ($p<0.05$ or $p<0.01$).

Kilning stability of phytase in fresh malts

The variation of phytase activity in fresh malts during kilning is presented in figure 1. Through 2 hour's kilning at 40°C and air drying at room temperature, phytase activity had slight decrease. In comparison with the fresh malts, the phytase activity in dry malts of wheat NEAU123, triticale 5305 and rye 2 lost 7.25%, 9.26% and 5.28% respectively.

Heat stability of phytase in dry malts

The effect of heating on stability of phytase in dry cereal malts is presented in table 2. The results indicated that phytase in cereal seeds had stong heat resistance. There was no loss of phytase activity in dry malts heated at 70°C for 1h, a little loss (≤5.46%) at 80°C or 90°C. Even heated at 100°C, the phytase activity in wheat NEAU123, triticale 5305 and rye2 remained 89.47%, 86.44% and 104.64%, respectively. So at 70-80°C that the temperatures commonly used in feed processing, such as pelleting, most phytase activity in cereal seeds is remained.

DISSCUSSION

Effect of germination on the phytase activity in cereal seeds

Phytase in dry seeds is inactive. When absorbing water and germinating, phytase in seeds is activated and hydrolyses phytate and releases phosphorus to meet the need for growing plants. Furthermore, lots of new phytase are biosynthesized in germination. So, the activity of phytase in cereal seeds increases in the course of germinating. This regularity was also found in our study. However, the improved degree was different among different cereals. The increase of phytase activity was highest in rye 2, lowest in wheat NEAU123. But all these were far lower than that Chen (1977) reported. The reason

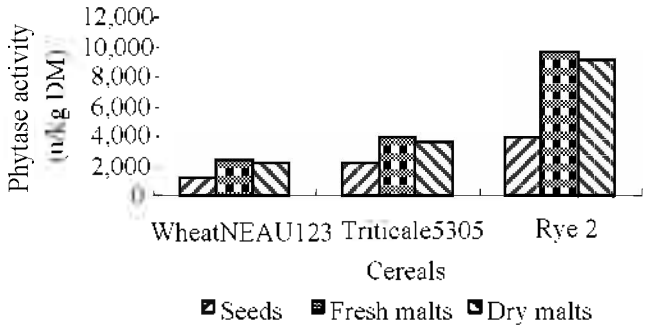

Figure 1. The phytase activitiese in seeds, fresh malts and dry mals (n=4)

Table 1. The effect of germination on phytase activity in cereal seeds (u/kg DM)

Germinating days (d)	0	1	2	3	4	5	6
Wheat NEAU123	1,168.03 ±64.88^{B}	2,082.26 ±162.41^{Aa}	2,319.83 ±248.93^{Aa}	2,388.61 ±88.23^{Aa}	2,182.55 ±244.04^{Aa}	2,035.74 ±47.99^{Aa}	2,017.01 ±152.07^{Aa}
Triticale 5305	2,147.24 ±52.63^{BCd}	3,918.75 ±152.62^{Aa}	3,916.89 ±192.19^{Aa}	3,399.59 ±154.59^{Ab}	3,450.82 ±123.02^{Aab}	2,652.77 ±27.62^{Bc}	1,987.56 ±150.73^{Cd}
Rye 2	3,913.79 ±60.37^{BCc}	9,575.97 ±203.99^{Aa}	8,237.57 ±151.71^{Aa}	5,686.09 ±208.81^{Bb}	3,471.23 ±131.54^{BCc}	3,590.45 ±218.52^{BC}c	2,723.28 ±172.87^{Cc}

Values are presented as means±SEM (n=4).

^{a,b,c} means in the same row with no common superscripts differ significantly ($p<0.05$).

^{A,B,C} means in the same row with no common superscripts differ great significantly ($p<0.01$).

Table 2. The relative activities of phytase in dry malts heated at different temperature (n=4)

	Relative activity (%)					
	Unheated	60°C	70°C	80°C	90°C	100°C
Wheat NEAU123	100	91.63	106.49	99.52	95.80	89.47
Triticale 5305	100	99.74	109.51	94.88	94.54	86.44
Rye 2	100	99.17	101.00	105.22	101.52	104.64

may be that the increased degree of phytase activity varied depending on the cultivar and/or drying and storage condition of cereal seeds. In addition, the condition of germinating also is a very important factor. The day that phytase gained highest activity was different among cereals. It was very short in present study. In this study we employed 25°C as germinating temperature which was higher for common condition for germinating. Therefore the seeds germinated faster and the days when phytase activity attained highest value was short.

In the anaphase of germination, the biosynthesis of phytase in malts decreases. In the course of germinating, the activity of protease also increases to hydrolyze storage protein to meet the needs of crop's growth. Being a kind of protein, phytase also could be hydrolyzed by protease. Variation of phytase activity presented low-high-low trend during germinating. In the germinating periods 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.

According to the results of this study, the elevated degree of phytase activity in cereal seeds by germinating was very low. Extraction of phytase from cereal malts maybe not economical. So, choosing special kind of seeds with high phytase activity or its products such as brand and direct using maybe a practicable way.

Kilning stability

When the phytase activity in malts attains maximum, the germination should be terminated rapidly. So setting a fast and safe kilning procedure is needed. In this study, we employed two periods of procedure to dry fresh malts. In the first period, the fresh malts were kilned at 40°C for 2 h in order to decrease the quantity of water and stop the germination quickly. In the second period, the treated malts were dried at room temperature to minimize the loss of phytase. This study's result showed that the two-periods kilning way was effective and safe.

Heat stability

Peers (1953) reported that the phytase in wheat inactivated partially when heated at 80°C for 10 min. Pointillart (1988) reported that plant phytase was stable at temperature between 40-60°C, but higher temperature (70-80°C) caused partial or total inactivation. Jonbloed et al. (1990) found that pelleting led to inactivation of the phytase in wheat when temperature was above 80°C. The result of our study didn't support the above-described reports. In this study, compared with that of unheated control, the loss of phytase activity was no more than 13.56% even heated at 100°C for 1 h. Ranhotra et al. (1975) obtained the similar results. When they heated wheat at 100°C for 1.5 h, the phytase activity lost 25%, but when heated for 3 h, it lost nearly 90%. The strong heat stability of plant phytase may result from the condition of phytase in plant seeds. Plant phytase exists together with other materials in seeds. These combined materials have protective effect on phytase when it is under heating or in other disadvantageous condition. Enzyme is more stable in dry condition than in wet condition. In this study, plant phytase was heated at dry condition, but in the above researches samples were heated with water vapor. Perhaps this was another important reason that plant phytase had higher stability in this study. In addition, Enzyme in low purity has higher heat stability than in high purity. The purity of phytase in seeds in natural state is low compared with microphytase, so it has high heat resistance. As yet, there is no report about heat stability of pure plant phytase.

One of important factors that restrict the use of phytase is that heat stability of phyase is too low. The common pelleting temperature is at 70-90°C. At this temperature most microphytase presents great inactivation. Plant phytase may be a good choice at higher temperature.

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