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Research Article

Effect of Phytase Treatment on Phosphate Availability in the Potential Food Supplement Corn Distillers' Grains with Solubles

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The most effective treatment of the potential food supplement corn distillers' grains with solubles using a fungal phytase to degrade phytic acid and release phosphorus was explored. Compared to the untreated grains with solubles, the phytic acid level in the grains with solubles was reduced by phytase treatment but treatment with 4 units of enzyme/g was more effective than 2 units of enzyme/g after 2 h. At 30°C or 45°C, 4 units of phytase/g reduced the phytic acid content of the grains with solubles by at least 94% after 2 h. The available phosphate in the grains treated for 2 h with 4 units of enzyme/g was increased by at least 1.5-fold compared to the untreated grains. Although phytic acid levels in the grains with solubles was reduced at 28°C, 30°C, 37°C, 40°C, or 45°C using 4 units of phytase/g for 2 h, the greatest reduction (96%) in phytic acid concentration occurred at 40°C. The available phosphate in the phytase-treated grains at 40°C was increased after 2 h by 1.9-fold compared to the control grains. Overall, phytase treatment of this potential food supplement improved it by increasing its phosphate content while reducing its phytic acid content.

1. Introduction

Corn distillers' grains with solubles is a major coproduct from the dry grinding of corn for ethanol fermentation [1]. Approximately 16 pounds of dried corn distillers' grains with solubles is produced from each bushel of corn processed at ethanol plants [1]. The ability of the grains to serve as an additive in food has been examined and has been shown to provide fiber and other nutrients to the foods being studied [2, 3]. The use of grains with solubles has been noted to be effective in extrusion doughs [4, 5]. When grains with solubles are supplemented into breads, cookies, muffins, doughnuts, rolls, hush puppies, snack food, spaghetti, or blended foods, the quality of the foods is not adversely affected [6–15]. It has also been shown that grains with solubles can be added to beef stew, chili, and hotdog sauce without affecting the taste quality of the foods [16].

One obstacle of using corn distillers' grains with solubles as a supplement in foods is that it contains a high concentration of phytic acid [17]. In corn, phytic acid (inositol hexaphosphate) is the primary compound used to store phosphate and ranges from 60 to 82% of the total phosphorus [18]. The phytic acid content in coproducts resulting from the dry milling of corn to produce ethanol ranges from 50 to 80% of the total phosphorus available [19]. The phytic acid content of corn distillers' grains with solubles has been shown to represent 27–57% of the total phosphorus present [20]. Phytic acid has low digestibility and acts as a chelator since it binds calcium, ferric, magnesium, and zinc ions as well as starch and proteins [21]. With phytic acid acting as a chelating agent, it hinders the nutritional value of corn distillers' grains with solubles [21]. A solution to the high phytic acid content in corn distillers' grains with solubles is treating it with the enzyme phytase [20, 22]. Phytase degrades phytic acid to inositol and phosphate [20, 22]. Therefore, the enzyme not only decreases the level of phytic acid in the grains but also increases its phosphate content. The phytase from the fungus *Aspergillus niger* has been shown previously to effectively degrade phytic acid in coproducts from corn wet milling as well as in corn-soybean meal used in animal diets [22, 23]. The phytase from *Aspergillus ficuum* has been used to treat corn distillers' grains with solubles or thin stillage which showed that each coproduct contained at least 50% phytase-hydrolyzable phosphorus [24, 25].

In this investigation, the phytase from *A. ficuum* was utilized to treat corn distillers' grains with solubles to reduce the phytic acid content and increase the phosphate content of the grains with solubles to improve the grains with solubles as a food supplement. The effect of enzyme concentration and incubation temperature on the ability of the fungal phytase to degrade the phytic acid in the grains was examined. Two concentrations of phytase were tested for their effectiveness in degrading phytic acid and releasing phosphate in the grains with solubles relative to incubation temperature. The most effective phytase concentration was further studied relative to incubation temperature.

2. Materials and Methods

2.1. Treatment of Corn Distillers' Solubles with Grains. The corn distillers' grains with solubles were obtained from the Dakota Ethanol Limited Liability Company Plant, Wentworth, SD, USA. To enzymatically treat the corn dried distillers' grains with solubles, 15 mL of sodium acetate buffer, pH 5.4, and 2 or 4 units of *Aspergillus ficuum* phytase (Sigma Chemical Company, St. Louis, MO, USA) were added to grains with solubles (1 g) at 30°C or 45°C. Once it was determined that treatment of the corn distillers' grains with soluble (1 g) with 4 units of phytase was more effective than 2 units of phytase in reducing the phytic acid content of the grains with solubles, the subsequent experiments were undertaken using 4 units/g of phytase. When enzymatically treating the corn dried distillers' grains with solubles with 4 units of *Aspergillus ficuum* phytase, 15 mL of sodium acetate buffer, pH 5.4, and 4 units of phytase were added to grains with solubles (1 g) at 28°C, 30°C, 37°C, 40°C, or 45°C. No enzyme was added to the control samples during all experiments performed. A unit of *A. ficuum* phytase activity will degrade 1 μmole of inorganic phosphorus from 42 mM magnesium-potassium phytate per min at 37°C (Sigma Chemical Company, St. Louis, MO, USA). The mixtures were shaken (200 rpm) for 2 h at the selected incubation temperature. Following 2 h of shaking, the grains with solubles were collected by centrifugation at 20,000 ×g for 30 min at 25°C. The grains with solubles were processed to analyze phytic acid and phosphate concentrations.

2.2. Processing of Phytase-Treated Grains with Solubles. The phytase-treated grains with solubles were suspended in 0.65 M HCl (20 mL) and shaken (200 rpm) for 2 h at 25°C. The suspension was centrifuged at 20,000 ×g for 30 min at 25°C. The supernatant was filtered through a Whatman No. 1 filter. Each filtrate was stored in sealed tubes until being subjected to ion-exchange chromatographic separation [26, 27]. The filtrates were diluted 1 : 5 in 0.65 M HCl and 10 mL of the diluted sample was applied to 1 cm × 10 cm columns (mL) of the ion-exchange resin Dowex 1 × 8 chloride form (200–400 mesh) anion exchange resin (Bio-Rad Laboratories, Hercules, CA). After washing the column with water (25 mL), bound phosphate was eluted from the column using 0.10 M NaCl. After collecting the phosphate, bound phytic acid was eluted from the column using 0.70 M NaCl and collected.

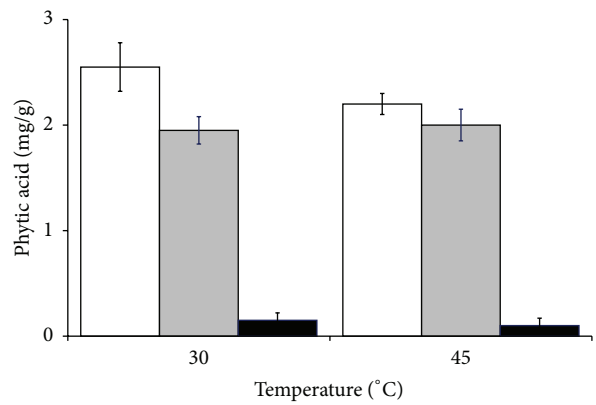

Figure 1: Phytic acid levels (mg/g of grains) determined in untreated corn distillers grains with solubles (white square), grains treated with 2 units of phytase/g (gray square), or grains treated with 4 units of phytase/g (black square) at either 30°C or 45°C for 2 h. Error bars indicate the standard deviations of mean data values.

2.3. Assays and Statistical Analysis. The phytic acid concentration present in each sample was determined colorimetrically at 500 nm using Wade reagent (1.11 mM ferric chloride hexahydrate and 13.75 mM sulfosalicylic acid) [27]. The phytic acid concentration in each sample was derived from a standard curve using authentic phytic acid (Sigma Chemical Company, St. Louis, MO, USA). The phosphate concentration in the samples was also measured colorimetrically at 350 nm using a zinc acetate modified ammonium molybdate-based assay [28]. A phosphate standard curve was used to calculate the phosphate level present in the samples. The results represent the mean of three independent trials. The control samples were untreated with enzyme. Student's t-test was used during statistical analysis of the data.

3. Results and Discussion

The phytic acid concentration in corn distillers' grains with solubles has been reported to be 0.257% in a prior investigation [29]. In this study, the phytic acid content of the corn distillers' grains with solubles was found to range from 0.220% to 0.255% which is very similar to the value determined in the previous study [29]. Initially, the effect of treating the grains with solubles with 2 or 4 units of fungal phytase activity per g of grains with solubles was explored relative to temperature and compared to untreated samples of corn distillers' grains with solubles. The grains with solubles were incubated with the phytase activity at 30°C or 45°C for a period of 2 h. Treatment with 2 units of fungal phytase/g grains with solubles at 30°C only reduced phytic acid content by 23% while treatment with 4 units of fungal phytase/g grains with solubles at 30°C reduced the concentration of phytic acid by 94%. This can clearly be seen in Figure 1 where treatment using 4 units of fungal phytase activity/g grains with solubles was far more effective in degrading phytic acid levels than using 2 units of fungal phytase activity/g of grains with solubles at either selected temperature after 2 h of incubation. The reduction in phytic acid concentration was significantly

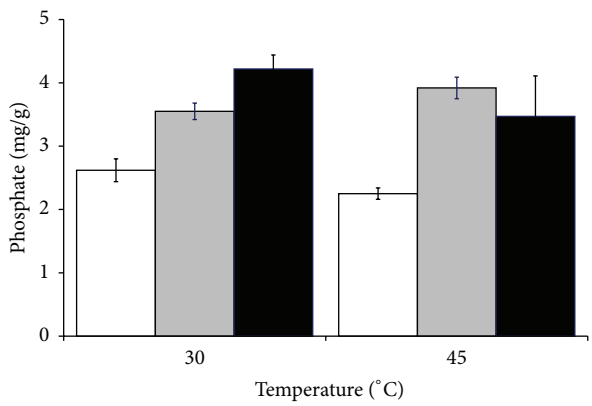

Figure 2: Inorganic phosphate levels (mg/g of grains) measured in untreated corn distillers grains with solubles (white square), grains treated with 2 units of phytase/g (gray square), or grains treated with 4 units of phytase/g (black square) at either 30°C or 45°C for 2 h. Error bars indicate the standard deviations of mean data values.

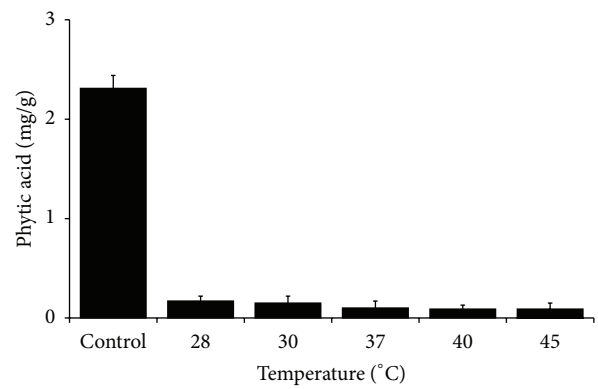

Figure 3: Phytic acid levels (mg/g of grains) determined in untreated corn distillers' grains with solubles (control) or grains treated with 4 units of phytase/g at selected temperatures for 2 h. Error bars indicate the standard deviations of mean data values.

($P < 0.01$) higher statistically when 2 units or 4 units of fungal phytase/g of grains with solubles were used during the treatment compared to the level detected in the untreated grains with solubles at 30°C. A statistically significant ($P < 0.01$) difference in phytic acid levels between the grains with solubles treated at 30°C for 2 h with 2 units of phytase/g and the grains with solubles treated with 4 units of phytase/g for 2 h at 30°C was also noted. In contrast, treatment of the grains with solubles at 45°C for 2 h with 2 units of phytase/g did not significantly reduce the concentration of phytic acid compared to the level measured in the untreated grains with solubles (Figure 1). If the concentration of fungal phytase used to treat the grains with solubles at 45°C for 2 h was increased to 4 units of phytase/g, a significant decrease ($P < 0.01$) in the phytic acid content of the grains with solubles was found to occur relative to the phytic acid content in the untreated grains with solubles. There was also a statistically significant ($P < 0.01$) difference between the phytic acid concentration present in the grains with solubles treated with 2 units of phytase/g at 45°C for 2 h and the phytic acid concentration in the grains with solubles treated with 4 units of phytase/g at 45°C for 2 h.

It was also of interest to learn how treatment with phytase of the grains with solubles affected the phosphate concentration present in the grains with solubles. The treatments of the grains with solubles for 2 h at 30°C with either 2 units or 4 units of fungal phytase/g were both effective in increasing the phosphate content in the grains with solubles compared to the phosphate concentration measured in the untreated grains with solubles with the difference in phosphate concentrations being statistically significant ($P < 0.01$). When the grains with solubles were treated with 4 units of phytase/g for 2 h at 30°C, a 1.2-fold higher level of available phosphate was detected in the grains with solubles than when 2 units of phytase/g were used to treat the grains with solubles for 2 h (Figure 2). A statistically significant ($P < 0.01$) difference in the phosphate concentration present in the grains with solubles treated for 2 h at 30°C with 4 units of phytase/g relative to the grains with solubles treated with 2 units of phytase/g of grains with solubles was noted. When the incubation temperature was elevated to 45°C, treatment of the grains with solubles using either 2 units or 4 units of the fungal phytase/g for 2 h resulted in at least a 1.5-fold higher phosphate concentration compared to the phosphate concentration present in the untreated grains with solubles (Figure 2). The difference in phosphate levels between the phytase-treated grains and the untreated grains was statistically significant ($P < 0.01$). No statistical difference in the phosphate level of the grains with solubles treated at 45°C for 2 h with 2 units or 4 units of fungal phytase/g was found.

With the corn distillers' grains with solubles having a lower phytic acid concentration following treatment with 4 units of the fungal phytase/g of grains with solubles compared to treatment with 2 units of phytase/g of grains with solubles, it was chosen to use 4 units of phytase activity/g of the grains with solubles for the 2 h treatment process. The effect of selected incubation temperatures upon phytase degradation of the phytic acid in the grains with solubles was next studied (Figure 3). The treatment for 2 h of the grains with solubles with the fungal phytase was very effective at all the incubation temperatures selected (Figure 3). The difference in phytic acid concentrations between the untreated grains with solubles and the phytase-treated grains incubated at 28°C, 30°C, 37°C, 40°C, or 45°C was statistically significant ($P < 0.01$). The greatest decrease (96%) in phytic acid concentration of the grains with solubles was observed when treated with the fungal phytase/g at 40°C or 45°C for 2 h compared to the untreated grains. The difference in the phytic acid concentration present in the grains with solubles treated with 4 units of phytase/g at 28°C relative to the grains with solubles treated with 4 units of phytase/g at 40°C for 2 h was statistically significant ($P < 0.05$).

As can be seen in Figure 4, the phosphate content of the corn distillers' grains with solubles following fungal phytase treatment relative to no treatment was next studied. With the phytic acid level being reduced in the grains with solubles treated with 4 units of phytase/g for 2 h at the incubation

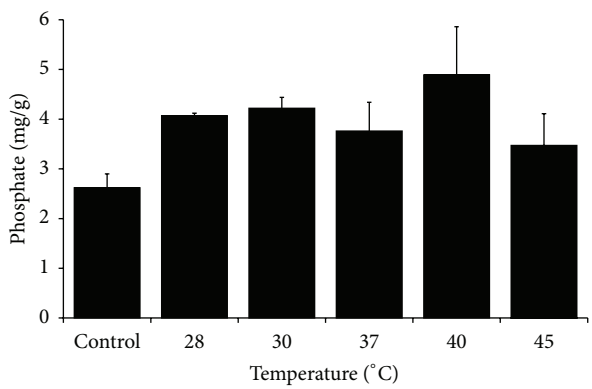

Figure 4: Inorganic phosphate levels (mg/g of grains) measured in untreated corn distillers' grains with solubles (control) or grains treated with 4 units of phytase/g at selected temperatures for 2 h. Error bars indicate the standard deviations of mean data values.

temperatures selected, it was expected that the phytase treatment of the grains with solubles would also have an effect on its phosphate content. A significant increase in the phosphate concentration of the corn distillers' grains with solubles was seen following treatment with 4 units of *A. ficuum* phytase/g for 2 h compared to the concentration in the untreated corn distillers' grains with solubles (Figure 4). Relative to the untreated corn distillers' grains with solubles, a statistically significantly ($P < 0.01$) higher concentration of inorganic phosphate was present following phytase treatment of the grains with solubles at 28°C, 30°C, 37°C, 40°C, or 45°C for 2 h. A 1.9-fold higher concentration of phosphate in the grains was released following phytase treatment at an incubation temperature of 40°C relative to the control grains. The difference in the phosphate concentration present in the grains with solubles treated with 4 units of fungal phytase/g at 40°C relative to the grains with solubles treated with 4 units of phytase/g at 45°C for 2 h was statistically significant ($P < 0.05$). A 1.4-fold higher phosphate concentration was present in the grains with solubles treated with 4 units of fungal phytase/g at 40°C relative to the grains with solubles treated with 4 units of phytase/g at 45°C for 2 h. The results indicated that a treatment using 4 units of the phytase from *A. ficuum* to treat the corn distillers' grains with solubles (1 g) was very effective in reducing the phytic acid level in the grains with solubles as well as increasing the available phosphorus present in the grains with solubles.

Prior studies have investigated the treatment of coproducts from corn-based ethanol production and corn wet milling with *A. niger* phytase to reduce their phytic acid content and improve their available phosphate [20, 22]. It was found that treatment with 2 units of an *A. niger* phytase/g of light steepwater (an intermediate in corn gluten feed production) substantially increased the phosphate content by 3.8-fold to 90% [20]. Optimization of *A. niger* phytase treatment of light steepwater involved the use of 1 unit of phytase/g steepwater at 35°C for 2 h [22]. Similar to the present study where a 1.9-fold increase in the phosphate concentration of the grains with solubles was observed after *A. ficuum* treatment for 2 h at 40°C, the use of 4 units of *A. niger* phytase/g at 35°C to treat whole stillage (an intermediate in corn distillers' grains production) was very effective in increasing the phosphate content by 1.2-fold [20]. The phosphate level rose from 54% in the control stillage to 66% in the treated whole stillage [20]. Relative to incubation temperature, little difference in the phosphate content of the whole stillage treated with 4 units of phytase activity/g stillage at 45°C relative to 35°C was observed [20]. Another study explored the use of low concentrations of phytase from *A. niger* to release inorganic phosphorus from corn-soybean meal used in animal diets [23]. The fungal phytase concentration used to treat the meal ranged from 0.15 to 0.45 units/g of meal [23]. The most effective concentration of *A. niger* phytase used to treat the meal was 0.45 units/g of meal since the phosphate level of the meal was improved [23]. A higher concentration of the *A. ficuum* phytase was required to improve the phosphate level of the grains with solubles in this study. Other studies have used the *A. ficuum* phytase to treat corn distillers' grains with solubles or thin stillage in order to learn the concentration of phosphorus existing within the phytic acid present in each coproduct [24, 25]. The coproduct thin stillage was found to contain 49.8% phytase-hydrolyzable phosphorus following treatment with the *A. ficuum* phytase [24, 25]. With corn distillers' grains with solubles containing 54.2% phytase-hydrolysable phosphorus, it was clear that much of the phosphorus content of the grains with solubles was trapped within its phytic acid component [25]. During this investigation, treatment of the grains with solubles with 4 units/g of *A. ficuum* phytase for 2 h indicated that the grains with solubles contained 49% phytase-hydrolyzable phosphorus similar to the prior study treating the grains with solubles with the *A. niger* phytase [25]. The findings from the current study also found that phosphorus can be released from the phytic acid present in the corn distillers' grains with solubles following the *A. ficuum* phytase treatment but the effectiveness of the phytase treatment was dependent upon incubation temperature and enzyme concentration.

4. Conclusions

It was concluded that the most effective treatment of the potential food supplement corn distillers' grains with solubles was using the highest concentration of *A. ficuum* phytase tested. Compared to the untreated grains with solubles, treatment of the grains with solubles with 4 units phytase/g reduced the phytic acid concentration by about 26-fold when incubated at 40°C for 2 h. The phosphate concentration present in the phytase-treated grains with solubles was increased by about 1.9-fold at 40°C relative to its concentration in the untreated grains with solubles. It was clear that both the concentration of fungal phytase applied to the grains with solubles and the incubation temperature utilized were critical in converting the phytic acid present to available phosphate to improve the nutritional value of this possible food supplement.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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