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An integrated approach to the degradation of phytates in the corn wet milling process

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ABSTRACT

An integrated process was developed to hydrolyze the phytates in light steep water (LSW) and to simultaneously isolate inorganic phosphate (Pi) and myo-inositol products. The proposed integrated process will be helpful in resolving the environmental and nutritional concerns in the use of corn gluten feed (CGF) in the animal diets. This process comprised of partial and total hydrolysis of LSW and intermediate anion exchange separation technique. The phytates in LSW were initially degraded to negatively charged myo-inositol phosphates ($InsP_2$–$InsP_5$). The optimized experimental parameters for the partial hydrolysis of LSW were determined to be 2 h hydrolysis with 1 FTU *Aspergillus niger*/g substrate at 35 °C. The negatively charged species of the partially hydrolyzed substrate were separated on a strong base anion exchange resin. The negatively charged species, retained by the resin, were eluded with 1 M NaCl solution and were subjected to complete hydrolysis with the *Escherichia coli*, *A. niger* derived phytases and their respective combinations. The maximum amount of myo-inositol released from the anion exchange column was 3.73 ± 0.03 mg/NaCl elution which was detected after 48 h reactions catalyzed by 100 FTU *E. coli*, 150 FTU *E. coli*, and 150 FTU the combination of *A. niger* and *E. coli*. The time course of Pi released showed a similar trend to that of myo-inositol and the released Pi reached a maximum amount of 3.30 ± 0.05 mg/g NaCl elution after 48 h incubation at the enzyme loadings for which the maximum concentration of myo-inositol were reached.

1. Introduction

Corn wet milling is one of the two main ethanol production processes and accounts for about 18–20% of the current ethanol production (RFA, 2008). This process produces several important co-products including CGF, corn gluten meal (CGM), corn germ meal, and corn oil (RFA, 2008). CGF is commonly used as animal feed for dairy and beef cattle, poultry, swine, and pet food (RFA, 2008). CGF contains important nutrients for animals as well as high levels of phosphorous (P). P in CGF is mainly from the P in corn kernel but is at much higher concentrations. Previous studies have shown that, while the P content of the corn was 2.6 ± 0.2 mg/g corn, it was 11.4 ± 0.2 mg/g CGF (Noureddini et al., 2009). P in corn and CGF is mainly in phytate form. P in phytate form cannot be digested by nonruminant animals such as poultry and swine, leaving significant amounts of phytate P in their excreta. This phytate P-rich manure serves as a source of phosphorous and potentially could cause P pollution in soil and underground water resources (EPA, 1998; Parry, 1998; Sharpley et al., 1996). Moreover, to guarantee the skeletal integrity and growth performance of swine and poultry, inorganic P supplements are also added into their diets which result in an added P in the animal excrete and further environmental concerns (Selle and Ravindran, 2007).

Phytates, the salt form of inositol hexakisphosphate, are found in most cereal seeds. Phytates serve several physiological functions, especially in seed germination. This organic complex is the main storage form of plant P, accounting for approximately two-thirds of the total P in plants (Reddy, 2002). Phytates, also known as antinutrients, can strongly bind to divalent minerals (Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{2+}), starch, and proteins, preventing their assimilation through the digestive system of nonruminant animals (Angel et al., 2002). Thus, both nutritional and environmental problems are likely to occur when animals are fed with CGF diets, compromising the quality of CGF as an animal feed.

Most of P and phytate P in CGF are attributed to the addition of the LSW which contains the majority of the P entering the plant. LSW is the liquid stream drained from the corn steeping tank. LSW is concentrated and added to the bran to form CGF. Ninety percent of the phytates in corn is found in the germ portion of the kernel which accounts for approximately 50–80% of the P in corn (Ravindran et al., 1995). Noureddini et al. (2009) and Noureddini and Dang (2009) studied P distribution in corn processing, and found that phytate P accounted for approximately 80% of the total P in the LSW, in which

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inositol hexakisphosphate ($InsP_6$) was the main phytate component, accompanied by a small amount of inositol pentakisphosphate ($InsP_5$). Rausch and coworkers (2005), measured the concentration and flow rate of P in three wet milling plants and concluded that 86% of the P entering the steeping process ended up in the LSW.

Phytases (inositol hexaphosphate hydrolase) have been reported to hydrolyze phytate P and when added to feed has shown to result in an increase in the growth performances of animals (Selle and Ravindran, 2007). Currently, there are two commercially available phytases, *Aspergillus niger* phytase (Natuphos™, BASF, Florham Park, NJ) and *Escherichia coli* phytase (OptiPhos, JBS United Inc., Sheridan, IN). These phytases have been studied extensively, and their catalytic efficacy as animal feed additives have been demonstrated (Nyannor et al., 2007; Selle and Ravindran, 2007). *A. niger* is a 3-phytase and initiates the dephosphorylation of phytate at the three position of the inositol ring. This enzyme has an optimal activation pH of 2.0 and 5.5, and an optimal temperature of 55 °C. Its deactivation occurs at 60 °C (Rao et al., 2009). The effectiveness of *A. niger* phytase on the degradation of phytate P in LSW and the hydrolysis pathway have been demonstrated in a previous study (Noureddini and Dang, 2009). *E. coli* phytase is a 6-phytase and initiates the dephosphorylation of phytate at the six position of the inositol ring. It has an optimal activation pH and temperature of 4.5 and 55 °C, respectively. Its deactivation occurs at 60 °C (Rao et al., 2009). Both *A. niger* and *E. coli* phytases have been reported to degrade phytic acid into myo-inositol 2-monophosphate ($Ins(2)P_1$) as the end product (Wyss et al., 1999). Upon this transformation, the phytate P in CGF would potentially satisfy the P requirement of animal diet with no need for supplemental P in the diet. Moreover, binded minerals, proteins and starch are released for assimilation during the digestion (Selle et al., 2000).

Myo-inositol plays an important role as the structural base for a number of secondary messengers in eukaryotic cells (Thorsell et al., 2008). It has been found to be promising in the treatment of cancer, depression, obsessive compulsive disorder, panic disorder, etc. (Nick, 2004). Its deficiency will cause accumulation of triacylglycerols and abnormal fatty acid metabolism (Eagle et al., 1957; Holub, 1992). It is also considered as an important nutrient for infants with high concentrations of free myo-inositol in human milk compared with infant formulae (Pereira et al., 1990). At about 3 million metric tons production in 2008, CGF or more specifically its LSW ingredient could provide an abundant resource for the production of P and myo-inositol (RFA, 2008).

LSW is the liquid drained after corn steeping and contains most of the P that enters the wet milling process. LSW consists of 5–10% solids which contains 45% protein and many micronutrients (Johnson and May, 2003). The liquid fraction of LSW contains a number of compounds including carbohydrates, amino acids, peptides, organic acids, heavy metals, inorganic ions and myo-inositol phosphates (Hull et al., 1996). The pH of LSW is in the range 3.5–4.3, mainly due to the presence of sulfur dioxide and lactic acid and other fermentation product (Johnson and May, 2003). In this pH range, protein–phytic acid electrostatic interaction occurs, resulting in neutral, insoluble complexes. Moreover, insoluble and/or soluble cation–phytic acid complexes are also likely to form, depending on the cation types existing in the system (Cheryan, 1980; Selle et al., 2000).

Separations of components in the LSW can be done with ion exchange chromatography, based on the differences in electrical charges of the components. Ion exchange chromatography has been widely used as an effective separation method in various fields, e.g. bio-separation, water purification (deionization), waste water treatment, etc. (Ackermann and Waitz, 1976; Flook et al., 2008; Paul, 2000). By using ion exchange columns, Hull and coworkers (1996) separated the components in the LSW into three groups for analysis: anionic fraction (e.g. organic acids), neutral fraction (e.g. carbohydrates), and cationic fraction (e.g. minerals). Anion exchange chromatography was used in the separation of myo-inositol phosphates since they are negatively charged species (Skoglund et al., 1998; Chen and Li, 2003). On a CarboPac PA-100 (Dionex Corp, Sunnyvale, CA) anion exchange analytical column, Chen and Li (2003) separated 27 peaks representing phytic acid, its degraded myo-inositol phosphate (i.e. $InsP_2$–$InsP_6$) and their isomers. Kney and Zhao (2004), successfully removed phosphate from wastewater solutions with polymer ligand ion exchange resins.

The main focus of this work was to develop an integrated process to hydrolyze the phytates in LSW to Pi and myo-inositol as end products. Initially, the phytates in LSW were subjected to a controlled hydrolysis with *A. niger* enzyme. The partially hydrolyzed phytates were separated from LSW in an ion exchange column, and were then subjected to complete hydrolysis with *A. niger* and *E. coli* enzymes. The effectiveness of *A. niger* and *E. coli* as well as the effect of the combined use of the two enzymes in the complete hydrolysis of myo-inositol phosphates were investigated. The developed integrated process will provide for a more digestible feed for the nonruminant animals. There is also potential for the production of myo-inositol as a new value-added product from the wet milling process.

2. Methods

2.1. Materials

LSW samples used in this study were from Cargill, a wet milling corn facility in Blair, Nebraska. All samples were kept in a refrigerator (4 °C) prior to use. As samples contained a mixture of solid and liquid, care was entailed to ensure the homogeneity of the samples taken before experiments.

Natuphos 10,000 liquid enzyme (3-phytase produced from *A. niger*) was purchased from BASF (Florham Park, NJ). OptiPhos 5000 liquid enzyme (*E. coli* AppA2) was generously provided by JBS United Inc. (Sheridan, IN). The nominal activity of this enzyme was 5000 FTU/g.

Myo-inositol standard (99%), sulfuric acid (95–98%), sodium molybdate dehydrate (99.5%), ascorbic acid, hydrochloric acid (37%), potassium dihydrogen phosphate (1 M), zinc oxide (99.9%), potassium hydroxide, phytic acid (50% w/w), perchloric acid (70%), iron (III) nitrate nonahydrate (98%) were purchased from Sigma–Aldrich (St. Louis, MO). DOWEX Marathon MSA anion exchange resin was purchased from Sigma–Aldrich (St. Louis, MO). Deionized (DI) water was further purified by a Simplicity Ultra Pure Water System from Millipore (Billerica, MA). Hydrochloric acid (0.5 M) and a solution of 1 g/L $Fe(NO_3)_3$ in 0.33 M $HClO_4$ were filtered by 0.22 μm membrane filter from Millipore before use.

2.2. Procedures

LSW was first subjected to a controlled hydrolysis step where the phytates were partially hydrolyzed to negatively charged phytic acid and its related myo-inositol phosphates. An anion exchange column was then used to separate the anionic fraction from the neutral and cationic fractions in the system. The anionic fractions were collected and then subjected to complete hydrolysis where myo-inositol phosphates were degraded into P and myo-inositol which could be further separated. The flow diagram of the developed integrated process is presented in Fig. 1.