World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:9, No:06, 2015

Change of Substrate in Solid State Fermentation Can Produce Proteases and Phytases with Extremely Distinct Biochemical Characteristics and Promising Applications for Animal Nutrition

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Abstract : Utilization of agricultural by-products, wheat ban and soybean bran, as substrate for solid state fermentation (SSF) was studied, aiming the achievement of different enzymes from Aspergillus sp. with distinct biological characteristics and its application and improvement on animal nutrition. Aspergillus niger and Aspergillus oryzea were studied as they showed very high yield of phytase and protease production, respectively. Phytase activity was measure using p-nitrophenilphosphate as substrate and a standard curve of p-nitrophenol, as the enzymatic activity unit was the quantity of enzyme necessary to release one µmol of p-nitrophenol. Protease activity was measure using azocasein as substrate. Activity for phytase and protease substantially increased when the different biochemical characteristics were considered in the study. Optimum pH and stability of the phytase produced by A. niger with wheat bran as substrate was between 4.0 - 5.0 and optimum temperature of activity was 37oC. Phytase fermented in soybean bran showed constant values at all pHs studied, for optimal and stability, but low production. Phytase with both substrates showed stable activity for temperatures higher than 80oC. Protease from A. niger showed very distinct behavior of optimum pH, acid for wheat bran and basic for soybean bran, respectively and optimal values of temperature and stability at 50oC. Phytase produced by A. oryzae in wheat bran had optimum pH and temperature of 9 and 37oC, respectively, but it was very unstable. On the other hand, proteases were stable at high temperatures, all pH's studied and showed very high yield when fermented in wheat bran, however when it was fermented in soybean bran the production was very low. Subsequently the upscale production of phytase from A. niger and proteases from A. oryzae were applied as an enzyme additive in fish fed for digestibility studies. Phytases and proteases were produced with stable enzyme activity of 7,000 U.g-1 and 2,500 U.g-1, respectively. When those enzymes were applied in a plant protein based fish diet for digestibility studies, they increased protein, mineral, energy and lipids availability, showing that these new enzymes can improve animal production and performance. In conclusion, the substrate, as well as, the microorganism species can affect the biochemical character of the enzyme produced. Moreover, the production of these enzymes by SSF can be up to 90% cheaper than commercial ones produced with the same fungi species but submerged fermentation. Add to that these cheap enzymes can be easily applied as animal diet additives to improve production and performance.

Keywords : agricultural by-products, animal nutrition, enzymes production, solid state fermentation
Conference Title : ICAFE 2015 : International Conference on Agricultural and Food Engineering
Conference Location : New York, United States
Conference Dates : June 04-05, 2015