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RICE BRAN BASED NANOFIBERS FOR STABILIZATION OF PHYTASE ENZYME

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Degree of Master of Science

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Thesis/ Dissertation submitted in partial fulfillment of the requirements for the degree Master of Science

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DECLARATION OF THE CANDIDATE & SUPERVISOR

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U.A.Rathnayake

Abstract

Phytases is an enzyme belonging to the histidine acid phosphatase family which has the capability to initiate the stepwise dephosphorylation of phytate, the primary storage form of phosphorus in most seeds and cereal grains. Phytase enzyme has become the most widely used enzyme in the world, particularly in poultry and swine industries as a source for the dephosphorylation of phytic acid, the primary storage form of phosphorus in most seeds and cereal grains. The problem involved with the phytase applications in animal feed is its thermal instability. Hence, a substantial thermal stabilization of the enzyme is required to increase its practical applications.

In this research, phytase enzyme was stabilized in to agro waste based nanaofibers. Nanofibers with a fiber diameter ranging from 30 – 50 nm were produced successfully using electrospinning techniques. For the best of our knowledge only few researches have reported on the nanofiber synthesis from agro waste and probably this could be the first study that reports the use of rice bran for this purpose.

First, rice bran received from a local mill was characterized and the soluble fiber fraction without any fat was isolated. This fiber solution was used for electrospinning. The apparatus containing the high voltage supply, electrodes, solution input compartment and the collector plates were fabricated in-house and the instrument was automated prior to electrospinning of fibers. The initial polymer solution parameters such as pH, viscosity, and conductivity were adjusted in order to facilitate the spinning process. The solution viscocity was modified using a food grade bio-polymer, polyvinyl alcohol (PVA). Then, the spinning parameters such as accelerating voltage, distance between the two electrodes, PVA content were optimized to result in nanofibers with uniform diameter.

After achieving the best conditions for electrospinning, two different techniques were investigated to encapsulate phytase enzyme in to nanofibers synthesized from rice bran. First, attempts were made to in-situ encapsulatie the phytase enzyme into nanofibers. Here, electrospinnig process was carried out in the presence of phytase enzyme in the initial polymer solution followed by cross-linking of the fibers using

boric acid. Secondly, phytase enzyme was immobilized into the nanofibers as a post modification after spinning followed by cross linking with sodium tripolyphosphate. The activity of the phytase enzyme in the encapsulated product was established at the gutter pH. 2.5 and at different temperatures up to 170 °C .

The presence of nanofibers was essential to offer a large surface area for large amount of enzyme encapsulation. The morphology and the size of the fibers were studied using scanning electron microscopic technique and the elemental composition was determined using energy dispersive X-ray analysis. It was possible to produce nanofibers with uniform diameter between 30 – 50 nm by this technique. However, when the phytase enzyme was encapsulated a beaded-string like morphology was observed. The bonding and extent of encapsulation was studied using Fourier transform infra-red spectroscopy FT-IR). Any changes in the peak positions or shape allowed predicting about the nature of bonding. Based on the FT-IR data it was confirmed that the enzyme was H-bonded to the nanofiber surface. Differential scanning calorimetric studies further confirmed the successful encapsulation of the phytase enzyme. Melting point of the enzyme has increased by 40 °C due to the encapsulation. Thermal stability of the final product was investigated using thermogravimetric analysis (TGA) technique. It was observed that the decomposition temperature of the enzyme has increased from 238 °C to 348 °C due to the encapsulation.

Interestingly, as expected it was found that the thermal stability of the enzyme has increased almost by 100% after encapsulated into the nanofibers followed by crosslinking. It was found that the pure enzyme loses its activity at 70 °C while after encapsulation into nanofibers based on rice bran, it is active up to 170 °C.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree Celsius
A	Angstron
cm	Centimeter
DI	Deionized
DSC	Differential Scanning Calorimetry
DTG	Derivative Thermogravimetric Analysis
EDX	Energy Dispersive X-ray analysis
FT-IR	Fourier Transform Infrared Spectroscopy
g	Gram
Hz	Hertz
kV	Kilo Volts
M	Molarity
mg	Milligram
ml	Milliliter
mm	Millimeter
Mt	Metric Tons
nm	Nanometer
PDF	Portable Document Format
PXRD	Powder X-ray Diffraction
rpm	Revolutions per minute
SEM	Scanning Electron Microscope
SLINTEC	Sri Lanka Institute of Nanotechnology
STPP	Sodium tripolyphosphate
TGA	Thermo Gravimetric Analysis/ Analyzer
USA	United State of America
UV-VIS	UltraViolet-Visible
wt%	Weight percentage
λ	Wavelength

μm	Micrometer