World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:8, No:08, 2014

Characterisation of Chitooligomers Prepared with the Aid of Cellulase, Xylanase and Chitosanase

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Abstract : The aim of this study was to obtain chitooligosaccharides from chitosan with better functional properties using three different enzyme preparations and compare the products of enzymatic hydrolysis. Commercially available cellulase (CL), xylanase (X) and chitosanase (CS) preparations were used to investigate hydrolytic activity on chitosan (CH) with low molecular weight and DD of 75-85%. It has been reported that CL and X have side activities of other enzymes, such as β-glucanase or β-glucosidase. CS enzyme has a foreign activity of chitinase. Each preparation was used in 1000 U of activity and in the same reaction conditions. The degree of deacetylation and molecular weight of chitosan were specified using titration and viscometric methods, respectively. The hydrolytic activity of enzymes preparations on chitosan was monitored by dynamic viscosity measurement. After 4 h reaction with stirring, solutions were filtered and chitosan oligomers were isolated by methanol solution into two fractions: precipitate (A) and supernatant (B). A Fourier-transform infrared spectroscopy was used to characterize the structural changes of chitosan oligomers fractions and initial chitosan. Furthermore, the solubility of lyophilized hydrolytic mixture (C) and two chitooligomers fractions (A, B) of each enzyme hydrolysis was assayed. The antioxidant activity of chitosan oligomers was evaluated as DPPH free radical scavenging activity. The dynamic viscosity measured after addition of enzymes preparation to the chitosan solution decreased dramatically over time in the sample with X in comparison to solution without the enzyme. For mixtures with CL and CS, lower viscosities were also recorded but not as low as the ones with X. A and B fractions were characterized by the most similar viscosity obtained by the xylanase hydrolysis and were 15 mPas and 9 mPas, respectively. Structural changes of chitosan oligomers A, B, C and their differences related with various enzyme preparations used were confirmed. Water solubility of A fractions was not possible to filter and the result was not recorded. Solubility of supernatants was approximately 95% and was higher than hydrolytic mixture. It was observed that the DPPH radical scavenging effect of A, B, C samples is the highest for X products and was approximately 13, 17, 19% respectively. In summary, a mixture of chitooligomers may be useful for the design of edible protective coatings due to the improved biophysical properties.

Keywords : cellulase, xylanase, chitosanase, chitosan, chitooligosaccharides
Conference Title : ICFNT 2014 : International Conference on Food and Nutrition Technology
Conference Location : Paris, France
Conference Dates : August 28-29, 2014