Separation of Proteases from Viscera Extract of Yellowfin Tuna (*Thunnus albacares*) by Ultrafiltration

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Master of Science Thesis in Biotechnology

Prince of Songkla University

2004

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เลขหมู่ TP248.65F66 L5 2004 C.1
Bib Key 242756
14 ก.ค. 2547

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Major Program	Biotechnology
Academic Year	2003

Abstract

Yellowfin tuna viscera are major waste products of tuna canning industry. Recovery of enzymes from these waste products can not only improve economic value but also reduce environmental pollution. Separation of proteases by ultrafiltration was studied. The membrane with molecular weight cut off (MWCO) 100 kDa had a higher transmission for enzymes than membrane with MWCO 30 kDa. The transmission ranges of enzymes were 0.6 to 0.8 which depended on the type of enzymes and operation conditions by using regenerated cellulose membrane with MWCO 100 kDa and 0.01 to 0.18 by using MWCO 30 kDa membrane. Temperature at 4°C did not show any difference for enzyme separation compare to the room temperature. It was found that pre-incubation of crude extract at 50°C before ultrafiltration enhanced the average permeate flux. Pre-incubation for 1 hour at 50°C provided the highest enzyme activities which were 58.43 U/ml, 14.35 U/ml and 15.73 U/ml and specific activity which were 5.35 U/mg, 1.46 U/mg and 1.44 U/mg in the retentate for general protease, trypsin and chymotrypsin, respectively by ultrafiltration using regenerated cellulose membrane with MWCO 30 kDa. The effects of transmembrane pressure and cross-flow rate during ultrafiltration using regenerated membrane with MWCO 30 kDa and 100 kDa were also studied. Transmembrane pressure (TMP) and cross-flow rate had little effect on protein and enzyme transmission. Increasing cross

flow rate and TMP increased permeate flux. The highest permeate flux of 54.72 L/m^2.h for MWCO 30 kDa membrane by using TMP 3.5 bar and cross flow rate 360 L/h and 68.4 L/m^2.h for MWCO 100 kDa membrane by using TMP 2.5 bar and cross flow rate 360 L/h were achieved. Higher TMP could not further increase the permeate flux. Continuous diafiltration increased purity factor of these enzymes more than ten times by using TMP at 1.5 bar and cross flow rate of 360 L/h for extract of spleen. It also increased purity factor of these enzymes more than five times for extract of mixed viscera. Concentration of purified extract achieved by using dead-end model ultrafiltraion with MWCO 10 kDa membrane. The gel electrophoresis with both silver staining and activity staining proved that the trypsin and chymotrypsin were kept in the retentate after ultrafiltration. All of the results proved that the separation, purification and concentration of these enzymes were achieved by using ultrafiltration.