Purification of β-1,3-Endoglucanase from Cabbage (*Brassica oleracea* cv. c*apitata* L.) by Ion Exchange Chromatography

Y. Sri Wulan Manuhara[1], Ni Nyoman Tri Puspaningsih[2], Sri Pudji Astuti W.[1]
[1]Departement of Biology, Faculty of Sains dan Teknologi, Universitas Airlangga, Kampus C Jl. Mulyorejo, Surabaya, Indonesia
[2]Departement of Chemistry, Faculty of Sains dan Teknologi, Universitas Airlangga, Kampus C Jl. Mulyorejo, Surabaya, Indonesia
Email: wulanmanuhara@unair.ac.id

Abstract
In this research, there was purified of β-1,3-endoglucanase from cabbage, having the aim to calculate purification level of enzyme by ion exchange chromatography methods. That process was started by producing enzyme isolated from Gloria osena hybrid of cabbage, then precipitate it using ammonium sulphate with concentration of saturated 40%. The sediment of enzyme was diluted by phosphate citrate then purified by dialysis in order to separate enzyme from other proteins and ammonium sulphate. The next, hydrophobic interaction chromatography eluted by ammonium suphate concentration of saturated 40% in Tris HCl pH 7 (high concentration to low concentration gradient) with Butyl-topearl 650M in ethanol as matrix was done to purify enzyme based on hydrophobic group interaction of protein and absorbent. After that, there was purifying enzyme by ion exchange chromatography in order to separate enzyme based on it's ion. Enzyme was eluted by NaCl (0-0.5) M in Tris HCl (low concentration to high concentration gradient) with DEAE-toyopearl 650 M in ethanol as the matrix. The best result of this ion exchange chromatography on first fraction pH 7 had purification level 3.534,1 of initial extract.

Keywords: *endoglucanase, Brassica oleracea cv. capitata L., ion exchange chromatography*

INTRODUCTION
β-1,3-glucanase could degrades the cell wall of pathogenic fungi, so it can be included as one of pathogenesis-related protein (PR-protein) and it's a group of PR-2 protein which has activity of (1,3)β-endoglucanase in vitro. Antifungal activity of β-1,3-glucanase on plants can happen on its ability to hydrolyze β-glucane on the cell wall of fungi, especially on the tip of hife [5]. Beside that, the activity of antifungal β-1,3-glucanase on the cell wall of fungi will caused the released of elicitor, which is like oligosaccharide that will induce antifungal phytoalexin. The induction of phytoalexins in infected plants is presumed to be mediated by an initial recognition process between plants and pathogens which involves detection of certain unique molecules in plants [7,8]. We have already done to extraction of protein from six hybrid of cabbage (3 local hybrid, and 3 import hybrid). From the extraction we have same protein profile, which have molecular weight are 200 kD, 150 kD, 100 kD, 50 kD, 40 kD, 30 kD [4]. In this research we will do the purification of β-1,3-endoglucanase from cabbage cv. Gloria osena by hydrophobic interaction chromatography and ion exchange chromatography.

Hydrophobic interaction chromatography is best used as an intermediate purification technique. For example, after precipitation with ammonium sulphate, a mixture of protein may be brought back in solution by decreasing the concentration of ammonium sulphate to about 1.5M, and applied to the column. Washing with this solution will remove unbound material. Gradually decreasing the concentration of the eluting solution will then elute the different components. Ion exchange chromatography involves two primary step, first the binding of a protein to a charged resin and second the elution or displacement of protein from the charges of the resin. Critical to the former are the pH of the buffer used to equilibrate and load the proteins onto the

ISBN: 978-983-9805-76-5