

Usa Yonkoksung 2008: Characterization and Purification of Linamarase from Cassava Latex.  
Master of Science (Biotechnology), Major Field: Biotechnology, Department of Biotechnology.  
Thesis Advisor: Associate Professor Klanarong Sriroth, Dr. Ing. 125 pages.

Linamarase (E.C.3.2.1.21) is a  $\beta$ -glucosidase enzyme, capable to hydrolyze linamarin, a toxic cyanogenic glucoside compound naturally occurred in cassava. In this work, purification and characterization of linamarase from cassava latex, being collected from petiole were studied. The specific activity of crude linamarase from the latex was 21.28 unit/mg protein and used for further purification by 2 different methods, i.e. salt precipitation (60 %  $(\text{NH}_4)_2\text{SO}_4$ ) or anion exchange chromatography using DEAE-cellulose (pH 6.0), yielding the enzyme with increased specific activities by 1.21 and 2.98 folds, respectively. When compared with the commercially available enzyme (Linamarase™), only the enzyme obtained from anion exchange chromatography with the molecular weight size of 70 kDa was pure, as determined by SDS-PAGE electrophoresis. The optimum temperature, as determined by using *p*-nitrophenyl- $\beta$ -D-glucoside (pNPG), of all tested enzymes including crude, purified by salt precipitation, purified by anion exchange chromatography and the commercial ones was similar, i.e. at 60°C. However, the enzymes were not stable at that high temperature. All tested enzymes showed the highest activity at pH 7.0 when evaluated by *p*-nitrophenyl- $\beta$ -D-glucopyranoside and the enzymes were quite stable in acidic (pH > 3) and alkali (pH < 9) conditions.

Three enzymes, i.e. crude, purified by salt precipitation and purified by anion exchange chromatography were used to determine the total cyanide contents of cassava samples including peels and parenchyma of fresh roots, boiled roots, flour and starch (5 samples each) and compared with the commercial one for analytical purpose. The results suggested that purified enzymes by anion exchange chromatography provided the most equivalent comparable results as the commercial enzymes ( $p \leq 0.05$ ). Yet, in some samples both crude and purified enzymes by salt precipitation were still applicable for analytical works as the analytical results were not significantly different ( $p \leq 0.05$ ).

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