

3736282 SCBC/M ; MAJOR : BIOCHEMISTRY : M.Sc. (Biochemistry)

KEY WORD : α -MANNOSIDASE / REVERSE HYDROLYSIS
/ MANNOSYL-RAFFINOSE

DUMRONGKIET ARTHAN : ISOLATION AND REVERSE HYDROLYSIS OF α -MANNOSIDASE FROM *Hibiscus sabdariffa* L . var. *sabdariffa*. THESIS ADVISOR : M.R. JISNUSON SVASTI, Ph.D., PRAYAD KOMARATAT, Ph.D., 135 p. ISBN 974-588-991-1.

α -Mannosidase was partially purified from seeds of germinated *Hibiscus sabdariffa* L. var. *sabdariffa* (roselle) by ammonium sulfate fractionation followed by DEAE-cellulose, Sephadex G-150, and Phenyl-Sepharose chromatography. The partially purified α -mannosidase was free from other glycosidases. α -Mannosidase had subunits of three molecular weight 121, 67 and 56 kDa on SDS-PAGE. Thus, the purified α -mannosidase had two forms (pI = 4.8 and 4.9) when analysed by using isoelectric focusing pH 3/10. Manno-oligosaccharide synthesis by roselle α -mannosidase was studied at high mannose concentration and elevated temperature. Roselle α -mannosidase showed regiospecificity in synthesis towards man(α 1-6)man. Variation of synthesis conditions such as pH, temperature and mannose concentration did not effect the regioselectivity of α -mannosidase. The optimal conditions for manno-oligosaccharide synthesis were 50%-60% w/w D-mannose incubated at 50 °C and pH 4.0-4.5. Oligosaccharides larger than raffinose have been synthesised by the condensation of mannose and rafffinose. The oligosaccharides of mannosyl-raffinose synthesis were fractionated by activated carbon column chromatography eluted with increasing concentrations of ethanol. Two products were shown to be two tetrasaccharides well separated from substrate and other products. The products were analysed by digestion with three glycosidases, the results suggested that the first product was composed of two components of mannosyl-raffinose where all of fructose terminals of raffinose were completely mannosylated. The second product peak was also mannosyl-raffinose and composed of one component of a tetrasaccharide which most of the galactose terminals of raffinose were mannosylated.