# CHAPTER IV

# CONCLUSIONS

Serum from skim latex of para rubber tree (*Hevea brasiliensis*) was investigated for its potential use in the preparations of *N*-acetyl-D-glucosamine and *N,N'*-diacetylchitobiose by enzymatic hydrolysis of fibrous β-chitin from squid pen. The serum obtained from simple filtration of acidified skim latex from Pan Asia Biotechnology Co., Ltd., (Rayong, Thailand) contained protein 6.0 mg/mL and chitinolytic activity 108 mU/mL (specific activity of 18 mU/mg protein). The optimum enzyme/chitin mole ratio of 0.22 mU/mg at chitin concentration of 60 mg/mL. The optimum pH range for the enzyme was 2.0-4.0. Sodium acetate buffer pH 4.0 (0.1 M) was the most cost effective to be used. The optimum temperature was 45 °C. The chitinolytic susceptibility of β-chitin did not seem to depend on the size of substrate particles as long as it had over all a dimension of less than 100 μm or it was swollen before use. The chitinolytic susceptibility of chitin was however depended greatly on the degree of acetylation (%DA). High degree of hydrolysis could only be achieved on the chitin with extremely high %DA (>90%). β-chitin (60 mg/mL) with 91 %DA was hydrolyzed by the serum (13 mU/mL) at optimum condition to give a mixture of GlcNAc (36 mM) and $(GlcNAc)_2$ (51 mM), corresponding to HPLC yield of 11.6% GlcNAc, 35.8% $(GlcNAc)_2$ and 47.4% total yield.

In a preparative scale using β-chitin (3.6 g) and the serum (1.58 U.) in dialysis bags, the hydrolysis gave a mixture of GlcNAc (0.18 mg/mL) and $(GlcNAc)_2$ (0.83 mg/mL) with a total hydrolysate volume of 1,250 mL, corresponding to HPLC yields of 6.3% GlcNAc, 29.0% $(GlcNAc)_2$ and 35.3% total yields. Partial purification of $(GlcNAc)_2$ was achieved by gel filtration chromatography using Totopearl packing material as a stationary phase and water as an eluent. $(GlcNAc)_2$ was recovered in 92% with 36% purity. The major impurity was the sodium acetate and less than 0.5% of GlcNAc remained.

A mixing enzyme of pectinase from *Aspergillus nigur* with the serum *Hb* at the chitobiose unit/chitinase unit ratio of 1.65 converted all of $(GlcNAc)_2$ to GlcNAc, suggesting a technique of enzyme combination to use the serum *Hb* for production of GlcNAc from chitin. This hydrolysis showed a maximum HPLC yield of 52% GlcNAc in 4 days.

The hydrolysis of chitosan (13-21% DA, $M_w = 5.1\times10^5$ - $6.1\times10^6$) with the serum *Hb* gave low molecular weight chitosan with $M_w$ $5.4\times10^4$ - $1.5\times10^5$ rather than the expected chitooligosaccharide $(GlcNAc)_2$ – $(GlcNAc)_7$). This result and previous results strongly indicated that hevamine, a major chitinase in the serum *Hb*, hydrolyzed chitin or chitosan chain where the acetylated units presented in at least 4 consecutive units, $(GlcNAc)_4$.

In conclusion, the serum *Hb* from skim latex of rubber tree may be used for hydrolysis of chitin to produce GlcNAc and $(GlcNAc)_2$ with limited yields, no better that 50%. Purification of $(GlcNAc)_2$ need further investigation as the gel filtration chromatography can not completely eliminate the acetate salt used as a buffer in the reaction. The serum *Hb* may also be used to produce low molecular weight chitosan ($M_w$ ~$5.4\times10^4$ - $1.5\times10^5$) from high molecular weight chitosan ($5.1\times10^5$ - $6.1\times10^6$) that may find application in agriculture. Owing to its availability and low cost, the application of the serum *Hb* for production of GlcNAc and $(GlcNAc)_2$ remains merit for further investigation, especially for improving its production yield.