ABSTRACT
STUDY THE EFFECT OF ADDITION SORBITOL TOWARD STABILITY OF CELLULASE ENZYME FROM *Rhizopus oryzae*

By

Diani Iska Miranti

This research aims to study the effect of addition sorbitol toward stability cellulose enzyme from *Rhizopus oryzae*. To approach this aims, the enzyme has been done to produce, to isolate and to purify. The purification of cellulose enzyme was done by fractination using the ammonium sulfat and dialysis. The purified enzyme was mixed by sorbitol. Determination of the cellulose enzyme activity performed by the method of Mandels, while the measurement of protein concentration performed by the method of Lowry.

The results showed that the purified enzyme has specific activity 9.18 U/mg, increased 4.03 times than the native enzyme. The test of thermal stability of the enzyme at temperature 50°C for 60 minutes has k_i = 0.02 min^{-1}; $t_{1/2}$ = 34.65 min and ΔG_i = 99.7 kJ mol^{-1}. The test of thermal stability of the enzyme mixed by sorbitol 0.5; 1.0; and 1.5 M at temperature 50°C for 60 minutes each has $t_{1/2}$ = 49.5 min, k_i = 0.014 min^{-1} and ΔG_i = 100. kJ mol^{-1};
$t_{1/2}$= 24.75 min, k_i = 0.028 min^{-1} and ΔG_i = 101.8 kJ mol^{-1}; $t_{1/2}$ = 22.35 min,
ki = 0.031 min^{-1} and ΔGi = 100.8 kJ mol^{-1}.

The results showed that the purified enzyme has K_m and V_{max} values
74.02 mg/mL substrate and 6.57 μmol/mL.min, and the enzyme mixed by sorbitol 0.5; 1.0; and 1.5 M each has 24.89 mg/mL substrate and 0.92 μmol/mL.min; 18.39 mg/mL substrate and 0.82 μmol/mL.min; 14.83 mg/mL substrate and 0.42 μmol/mL.min.

Although the optimum of pH and temperature of the mixed enzyme by sorbitol did not changed, at pH 7 and at temperature 60^oC but the thermal stability of modified enzyme was increased and showed by the decrease in the value of k_i in accordance with the increase half life and ΔG_i.

Key word: Cellulase enzyme, sorbitol, *Rhizopus oryzae*

ABSTRAK

STUDI PENGARUH PENAMBAHAN SORBITOL TERHADAP STABILITAS ENZIM SELULASE DARI *Rhizopus oryzae*

Oleh

Diani Iska Miranti

Penelitian ini bertujuan untuk mempelajari pengaruh penambahan sorbitol terhadap stabilitas enzim selulase dari *Rhizopus oryzae*. Untuk mencapai tujuan tersebut dilakukan produksi, isolasi, dan pemurnian enzim. Pemurnian enzim selulase dilakukan dengan menggunakan fraksinasi dengan ammonium sulfat dan dialisis. Enzim hasil pemurnian kemudian ditambahkan sorbitol dengan konsentrasi 0,5 M; 1,0 M; dan 1,5 M. Penentuan aktivitas enzim selulase dilakukan dengan metode Mandels sedangkan pengukuran kadar protein dilakukan dengan metode Lowry.

Hasil penelitian menunjukkan bahwa enzim hasil pemurnian memiliki aktivitas spesifik sebesar 9,18 U/mg, meningkat 4,03 kali dibandingkan ekstrak kasar enzim. Uji stabilitas termal enzim hasil pemurnian pada suhu 50^{o}C selama 60 menit memiliki k_i = 0,02 menit^{-1} ;$t_{1/2}$ = 34,65 menit; dan ΔG_i = 99,7 kJmol^{-1}. Uji stabilitas termal enzim setelah penambahan sorbitol 0,5; 1,0; dan 1,5 M pada suhu 50^{o}C selama 60 menit memiliki berturut-turut $t_{1/2}$ = 49,5 menit, k_i = 0,014 menit^{-1} dan ΔG_i = 100 kJmol^{-1}; $t_{1/2}$ = 24,75 menit, k_i = 0,028 menit^{-1} dan ΔG_i = 101,8 kJmol^{-1}; $t_{1/2}$ = 22,35 menit, k_i = 0,031 menit^{-1} dan ΔG_i = 100,8 kJmol^{-1}.

Enzim hasil pemurnian memiliki nilai Km dan Vmaks berturut-turut sebesar 74,02 mg/mL substrat dan 6,57 µmol/mL.menit, sedangkan enzim setelah peambahan sorbitol 0,5; 1,0; dan 1,5 M memiliki nilai Km dan Vmaks berturut-turut sebesar 24,89 mg/mL substrat dan 0,92 µmol/mL.menit; 18,39 mg/mL substrat dan 0,82 µmol/mL.menit; 14,83 mg/mL substrat dan 0,42 µmol/mL.menit.

Walaupun suhu optimum dan pH optimum enzim setelah penambahan sorbitol tidak mengalami perubahan, yakni tetap pada pH 7 dan suhu 60^{o}C tetapi terjadi peningkatan stabilitas termal karena adanya penurunan nilai k_i serta peningkatan waktu paruh dan ΔG_i.

Kata Kunci: Enzim selulase, sorbitol, *Rhizopus oryzae.*