

## ABSTRACT

The Utilization of nyamplung seeds for oil resources, produce seedcake as a waste. The seed cake still contain high protein. Recent studies shows that protein hydrolysate which obtained by hydrolysis has many useful effect, such as antioxidant. The hydrolysis was controlled by kinetic reaction approach.

Fat reduction was performed on the seedcake before protein isolation. The protein further on hydrolyzed into short chain peptides. The chemical composition of seedcake and the protein content of isolated nyamplung was analyzed. The functional group of the isolated nyamplung protein and molecular weight were determined by FTIR and SDS PAGE respectively. The hydrolysis of isolated nyamplung protein was carried out in various condition such as substrate concentration, pH, and temperature for different time of hydrolysis, and the degree of hydrolysis was determine by TNBS method.

The protein isolation process performed on the defatted seedcake, produced the brown colour of isolated nyamplung protein, with the L, a, b value were  $44.37 \pm 1.29$ ,  $9.68 \pm 0.3$ , and  $12.4 \pm 0.78$ , respectively. Chromatogram of FTIR showed that the isolated nyamplung protein have a group as an amide are characterize of C = O stretching at  $1658.78 \text{ cm}^{-1}$ , N-H stretching at  $3371.57 \text{ cm}^{-1}$  and NH bending at  $1543.05 \text{ cm}^{-1}$ . Electrophoresis SDS PAGE analysis for molecular weight showed the isolated nyamplung protein separated to seven band, molecular weight were 9.73, 12.04, 15.21, 18.03, 23.27, 27.36 and 37.96 kDa, where the molecule size of 12.5 kDa was dominant.

Papain was selected to the rest of hydrolysis since it showed the higher DH in long term hydrolysis. The degree of hydrolysis (DH) were increased by increasing of time of hydrolysis and showed the optimum DH with 6.15mg/ml substrate concentration. The increase of temperature also increase the DH, however at  $70^\circ\text{C}$  with 240 min time reaction, the DH tend to decrease. The pH of reaction showed the similar DH among various pH of reaction, with pH 7 showed the stable increasing DH till 240 min of reaction. The antioxidant activity showed an increase to 90% with the addition of the hydrolysate into the system until 1300 ppm. However the addition of more than 1300 ppm showed a decrease in activity. The addition of 250 ppm of hydrolysate from various duration of hydrolysis, showed the DPPH scavenging activity more than 50% in all treatment. However, it displayed a maximum DPPH free radical scavenging activity at first hours of hydrolysis.

The reaction rate constant value was 0.0751 (mg/unit.min) enzymic deactivation constant was 0.0466 mg protein/unit.min and energy activation ( $E_a$ ) were 77.35 kJ/mol for  $E_{a1}$ , 5.8 kJ/mol for  $E_{a-1}$ , and 11.47 kJ/mol for  $E_{a2}$  while the value of Arrhenius factor were  $1.2e11/\text{sec}$  for  $A_1$ ,  $2.2/\text{sec}$  for  $A_{-1}$  and  $5.7/\text{sec}$  for  $A_2$

The kinetic equation can be used to fit the enzymic hydrolysis process of nyamplung protein and to optimise the operating parameters of bioactive peptides preparation for the bioreactor design.

Keyword: isolated nyamplung protein, hidrolisis, degree of hidrolisis, antioxidant, kinetics

## INTISARI

Pemanfaatan biji nyamplung untuk sumber minyak, menghasilkan bungkil/*seedcake* dengan kandungan protein yang masih cukup tinggi. Protein ini bisa dimanfaatkan sebagai hidrolisat protein untuk mendapatkan manfaat yang lebih besar, seperti antioksidan, dengan cara hidrolisis. Proses hidrolisis dikontrol untuk mendapatkan hidrolisat dengan derajat hidrolisis (DH) tertentu. Untuk menentukan kondisi yang sesuai didekati dengan kinetika reaksi.

Bungkil/*seedcake* yang digunakan dianalisa kadar air, protein, lemak, karbohidrat, dan kadar abunya. Protein hasil isolasi diuji gugus fungsionalnya dengan FTIR dan berat molekul diuji dengan elektroforesis SDS-PAGE. Hidrolisis protein nyamplung hasil isolasi dilakukan dalam berbagai kondisi sementara derajat hidrolisis ditentukan dengan metode TNBS.

Proses isolasi protein dilakukan pada *defatted seedcake*, menghasilkan protein nyamplung berwarna coklat dengan nilai L,  $44,37 \pm 1,29$ , nilai a  $9,68 \pm 0,3$ , dan nilai b  $12,4 \pm 0,78$ . Kromatogram dari FTIR menunjukkan bahwa ekstrak protein dari nyamplung memiliki kelompok amida dengan C=O peregangan di  $1.658,78 \text{ cm}^{-1}$ , N-H peregangan di  $3.371,57 \text{ cm}^{-1}$  dan NH *bending* di  $1.543,05 \text{ cm}^{-1}$ . Pemisahan berat molekul dari hasil isolasi protein nyamplung dengan menggunakan elektroforesis SDS menghasilkan tujuh pita, masing-masing pita menunjukkan berat molekul 9,73, 12,04, 15,21, 18,03, 23,27, 27,36 dan 37,96 kDa, di mana ukuran molekul 12,5 kDa dominan.

Papain dipilih untuk hidrolisis selanjutnya karena menunjukkan DH tinggi. Derajat hidrolisis (DH) meningkat dengan meningkatkan waktu hidrolisis dan menunjukkan DH optimum dengan 6.15mg/ml konsentrasi substrat. Peningkatan suhu juga meningkatkan DH, namun pada  $70^{\circ}\text{C}$  dengan waktu reaksi 240 menit, DH cenderung menurun. Derajat hidrolisis pada pH reaksi yang berbeda menunjukkan nilai DH yang hampir sama, dimana pH 7 menunjukkan peningkatan DH stabil sampai 240 menit dari waktu reaksi. Aktivitas antioksidan yang dievaluasi dengan menggunakan metoda DPPH, menunjukkan peningkatan sampai 90% dengan penambahan hidrolisat ke dalam sistem sebanyak 1300 ppm. Namun penambahan lebih dari 1300 ppm menunjukkan penurunan aktivitas. Penambahan 250 ppm dari hidrolisat dari berbagai durasi hidrolisis, menunjukkan penghambatan oksidasi yang lebih dari 50% di semua perlakuan. Namun, aktivitas antioksidan maksimum pada jam pertama *hyrolysis*.

Dari persamaan matematika dan asumsi-asumsi yang digunakan, mekanisme hidrolisis protein nyamplung melibatkan peristiwa inhibisi dan deaktivasi. Pada peristiwa deaktivasi, konstanta laju reaksi adalah sebesar 0.0751 (mg/unit.min), konstanta deaktivasi enzim, enzymic deactivation constant of 0.0466 mg protein/unit.min. Energi aktivasi ( $E_a$ ) adalah  $E_{a1}$  sebesar 77.35 kJ/mol untuk,  $E_{a-1}$  sebesar 5.8 kJ/mol untuk, and  $E_{a2}$  sebesar 11.47 kJ/mol, dan nilai konstanta Arrhenius adalah  $A_1$  ( $1,2 \cdot 10^{11}$ /detik),  $A_{-1}$  (2.2/detik) dan  $A_2$  (5.7/detik)

Kata kunci: protein nyamplung, hidrolisis, derajat hidrolisis, antioksidan, kinetika