

ABSTRAK

Kacang merah merupakan sumber protein nabati yang cukup tinggi sebesar 16-33%, sehingga dapat menghasilkan peptida bioaktif yang memiliki aktivitas antioksidan. Protein hidrolisat kacang merah diperoleh dengan hidrolisis menggunakan protease dari bakteri *Bacillus subtilis* B298. Tujuan penelitian ini yaitu untuk mengetahui aktivitas spesifik fraksi-fraksi protease; kondisi optimum fraksi protease F30; pengaruh waktu hidrolisis terhadap derajat hidrolisis dan aktivitas antioksidan protein hidrolisat kacang merah; IC_{50} , AAI (*Antioxidant Activity Index*); dan persentase hemolisis dari fraksi protein hidrolisat dengan aktivitas antioksidan tertinggi. Tahap penelitian yang dilakukan yaitu produksi isolat protein kacang merah; produksi ekstrak kasar enzim protease; fraksinasi bertingkat ekstrak kasar enzim protease dengan ammonium sulfat; karakterisasi suhu dan pH terhadap fraksi protease dengan aktivitas spesifik tertinggi; hidrolisis protein menggunakan enzim protease dengan variasi waktu 10, 20, 30, 40, 50, dan 60 menit. Protein hidrolisat ditentukan derajat hidrolisis; uji aktivitas antioksidan menggunakan metode DPPH dan uji hemolisis. Hasil penelitian menunjukkan bahwa F30 merupakan fraksi protease dengan aktivitas spesifik tertinggi sebesar 0,700 U/mg yang optimum pada suhu 45 °C dan pH 8. Nilai derajat hidrolisis protein hidrolisat tertinggi didapatkan pada waktu 60 menit sebesar 22%. Protein hidrolisat pada waktu hidrolisis 10 menit memiliki aktivitas antioksidan tertinggi dengan persentase inhibisi sebesar 66%. Nilai IC_{50} diperoleh sebesar 29,21 ppm, sehingga termasuk kategori aktivitas antioksidan kuat. Nilai AAI diperoleh sebesar 0,674 yang termasuk kategori antioksidan sedang. Persentase hemolisis protein hidrolisat yang didapatkan yaitu 1,8% dengan kategori tidak terjadi hemolisis.

Kata Kunci: antioksidan, *Bacillus subtilis*, kacang merah, fraksinasi protease, hidrolisis protein.

ABSTRACT

*Kidney beans are a fairly high source of vegetable protein at 16-33%, so they can produce bioactive peptides that have antioxidant activity. Kidney bean hydrolysate protein is obtained by hydrolysis using proteases from the bacterium *Bacillus subtilis* B298. The purpose of this study is to determine the specific activity of protease fractions; optimal condition of F30 protease fraction; effect of hydrolysis time on the degree of hydrolysis and antioxidant activity of kidney bean hydrolysate protein; IC₅₀, AAI (Antioxidant Activity Index); and the percentage of hemolysis from the hydrolysate protein fraction with the highest antioxidant activity. The research stage carried out is the production of red bean protein isolate; production of crude extracts of protease enzymes; graded fractionation of crude extract of protease enzymes with ammonium sulfate; temperature and pH characterization of the protease fraction with the highest specific activity; Protein hydrolysis uses protease enzymes with time variations of 10, 20, 30, 40, 50, and 60 minutes. Hydrolyzed proteins are determined by the degree of hydrolysis; antioxidant activity test using the DPPH method and hemolysis test. The results showed that F30 was the protease fraction with the highest specific activity of 0.700 U/mg which was optimal at 45 °C and pH 8. The highest value of hydrolysate protein hydrolysis was obtained at 60 minutes of 22%. Protein hydrolysate at 10 minutes of hydrolysis had the highest antioxidant activity with an inhibition percentage of 66%. The IC₅₀ value was obtained at 29.21 ppm, so it is included in the category of strong antioxidant activity. The AAI value was obtained at 0.674 which is in the category of moderate antioxidants. The percentage of hemolysis of hydrolysate protein was 1.8% with the category of no hemolysis.*

Keywords: *antioxidants, *Bacillus subtilis*, kidney beans, protease fractionation, protein hydrolysis.*