ABSTRAK

Protease merupakan enzim yang dapat menghidrolisis protein menjadi senyawa sederhana seperti peptida dan asam amino. Enzim protease pada penelitian ini diisolasi dari bakteri *Bacillus subtilis* B298 digunakan untuk menghidrolisis protein kasein dan whey susu sapi. Susu sapi merupakan salah satu sumber protein yang sangat potensial sebagai penghasil peptida bioaktif. Ekstrak kasar enzim protease *B. subtilis* B298 difraksinasi dengan garam amonium sulfat untuk mendapatkan enzim protease yang lebih murni. Hidrolisis susu sapi dilakukan dengan menginkubasi substrat kasein dan whey selama 10, 20, 30, 40, 50, dan 60 menit. Protein hidrolisat yang diperoleh ditentukan aktivitas antioksidannya menggunakan metode DPPH dan diuji pula kemampuan lisisnya terhadap sel darah merah. Hasil penelitian menunjukkan bahwa aktivitas spesifik enzim pada ekstrak kasar sebesar 0,030 U/mg protein sedangkan pada F70 hasil fraksinasi memiliki nilai aktivitas spesifik enzim yaitu 0,008 U/mg dengan faktor pemurnian 0,293 kali. Nilai derajat hidrolisis tertinggi diperoleh pada inkubasi selama 60 menit, yaitu sebesar 74,5% untuk kasein dan 38,6% untuk whey. Protein hidrolisat yang diinkubasi selama 10 menit memiliki persentase inhibisi tertinggi terhadap radikal DPPH. Nilai AAI protein hidrolisat dari kasein dan whey adalah sama yaitu 0,0005 mg/mL yang menunjukkan aktivitas antioksidan lemah. Persentase hemolisis menyatakan protein hidrolisat kasein dan whey masing – masing sebesar 10% dan 3%, kontrol positif tween 80 sebesar 100% dan kontrol negatif sebesar bufer trisalin 0%.

Kata Kunci: antioksidan, *Bacillus subtilis*, fraksinasi, protease, protein hidrolisat

ABSTRACT

Proteases are enzymes that can hydrolyze proteins into simple compounds such as peptides and amino acids. The protease enzyme in this study isolated from the bacterium *Bacillus subtilis* B298 was used to hydrolyze casein and whey proteins in cow's milk. Cow's milk is one of the protein sources with great potential as a producer of bioactive peptides. The crude extract of the protease enzyme *B. subtilis* B298 was fractionated with ammonium sulfate salt to obtain a purer protease enzyme. Hydrolysis of cow's milk was carried out by incubating casein and whey substrates for 10, 20, 30, 40, 50, and 60 minutes. The protein hydrolyzate obtained was determined for its antioxidant activity using the DPPH method and also tested for its lysis ability on red blood cells. The results showed that the specific activity of the enzyme in the crude extract was 0.030 U/mg protein, while the fractionated F70 had a specific enzyme activity value of 0.008 U/mg with a purification factor of 0.293 times. The highest degree of hydrolysis was obtained at 60 minutes of incubation, which was 74.5% for casein and 38.6% for whey. Protein hydrolyzate which was incubated for 10 minutes had the highest percentage of inhibition against DPPH radicals. The AAI value of protein hydrolyzate from casein and whey was the same, namely 0.0005 mg/mL which indicates weak antioxidant activity. The percentage of hemolysis stated that the casein and whey protein hydrolyzate were 10% and 3%, respectively, the positive control of tween 80 was 100% and the negative control was 0% trisaline buffer.

Keywords: antioxidant, *Bacillus subtilis*, fractination, protease,protein hydrolyzate