POTENSI PROTEASE NETRAL TERMOSTABIL DARI *Geobacillus* sp. DS3 DALAM MENGHIDROLISIS *DEAMINATED* GLIADIN DAN SUBSTITUSI RESIDU SISI AKTIFNYA SECARA *IN SILICO*

INTISARI

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Gluten atau protein gandum merupakan produk sampingan dari industri tepung-tepungan yang sangat potensial untuk pengolahan pangan. Peningkatan fungsionalitas gluten dapat dilakukan melalui deaminasi dan hidrolisis enzimatik, salah satunya menggunakan enzim protease. Protease netral termostabil (NPr) yang diisolasi dari *Geobacillus* sp. DS3 di Kawah Sikidang, Dieng, Jawa Tengah diketahui memiliki aktivitas proteolitik. Penelitian sebelumnya telah dilakukan pemodelan struktur tiga dimensi dari sekuens NPr dan penambatan inhibitor phosporamidon untuk mengetahui sisi aktif enzim namun, belum didapatkan informasi mengenai mekanisme katalisis dengan substrat. Penelitian *in silico* penting dilakukan untuk meningkatkan efisiensi dalam memprediksi penemuan baru. Oleh karena itu, tujuan penelitian ini adalah melakukan penambatan substrat *deaminated* gliadin dan substitusi residu aktif untuk mengetahui kemampuan enzim NPr dalam melakukan aktivitas katalisis dengan substrat secara *in silico*. Analisis penambatan substrat dilakukan dengan metode *Molecular docking* menggunakan web server HADDOCK dan Prodigy. Hasil penambatan menunjukkan bahwa residu Glu139 enzim NPr *Geobacillus* sp. DS3 mampu menggeser residu Tyr153 ke arah substrat sehingga membentuk ikatan hidrogen pada N-terminal residu Ser9 substrat *deaminated* gliadin yang memungkinkan terjadinya hidrolisis dan mutasi terarah residu aktif Tyr153 menjadi Phe153 menyebabkan penurunan nilai ΔG dan hilangnya ikatan hidrogen yang mengakibatkan hilangnya aktivitas katalisis NPr. Dengan demikian, enzim NPr diduga dapat meningkatkan fungsionalitas dan kelarutan protein *deaminated* gluten untuk digunakan dalam pembuatan produk pangan.

Kata kunci: protease netral termostabil (NPr), *Geobacillus* sp. DS3, gluten, *deaminated* gliadin, mutasi terarah

THERMOSTABLE NEUTRAL PROTEASE FROM *Geobacillus* sp. DS3 CAPABILITY IN HYDROLYZING *DEAMINATED* GLIADIN AND ACTIVE SITE RESIDUE SUBSTITUTION BY *IN SILICO*

ABSTRACT

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Gluten or wheat protein is a by-product of the flour industry which has great potential for food processing. Increasing the functionality of gluten can be done through deamination and enzymatic hydrolysis by, the protease enzyme. Thermostable neutral protease (NPr) isolated from *Geobacillus* sp. DS3 in Sikidang Crater, Dieng Plateau, Central Java showed have proteolytic activity. Previous studies have carried out modelling of the NPr three-dimensional structure and inhibition by phosphoramidon to determine the active site of the enzyme. However, no information was studied regarding the mechanism of catalysis with the substrate. In silico research is important to do to increase efficiency in predicting new discoveries. Therefore, the aim of this study was to dock NPr with deaminated gliadin substrate and substitution analysis of the active residue by *in silico*. Active site enzyme and possible catalytic mechanism were determined. Substrate docking analysis was carried out using the Molecular Docking method using the HADDOCK and Prodigy web servers. The results showed that the Glu139 residue of the NPr of *Geobacillus* sp. DS3 bound to the side chain of the deaminated gliadin substrate was able to shift the Tyr153 residue towards the substrate so as to form a hydrogen bond at the N-terminal of the Ser9 residue on the deaminated gliadin substrate which allows hydrolysis to occur. The directional mutation of the active residue Tyr153 to Phe153 caused a decrease in ΔG value and loss of hydrogen bonds which resulted in loss of NPr catalytic activity. Thus, the NPr enzyme is predicted to be able to increase the functionality and solubility of gluten deaminated proteins for use in the manufacture of food products.

Keywords: thermostable neutral protease (NPr), *Geobacillus* sp. DS3, gluten, deaminated gliadin, site-directed mutagenesis