PENGARUH METODE HIDROLISIS DAN PEMURNIAN PADA ISOLASI ARGININ DARI PROTEIN MEMBRAN CANGKANG TELUR ITIK

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INTISARI

Telah dilakukan isolasi arginin dari protein membran cangkang telur itik melalui variasi metode hidrolisis dengan asam dan kombinasi enzim-asam, serta variasi metode pemurnian dengan kromatografi kolom penukar kation dan ekstraksi fasa padat/*solid phase extraction (SPE)* penukar kation. Tujuan penelitian ini adalah untuk mempelajari pengaruh metode hidrolisis dan metode pemurnian terhadap kadar arginin hasil isolasi dari protein membran cangkang telur itik.

Hidrolisis asam terhadap sampel protein dilakukan menggunakan HCl 6 M pada suhu 110 °C dengan durasi 5 jam, sedangkan hidrolisis kombinasi enzim-asam dilakukan dengan inkubasi sampel protein menggunakan tripsin pada pH 8 dan suhu 37 °C selama 2,5 dan 5 jam kemudian dilanjutkan dengan hidrolisis asam menggunakan HCl 9 M pada suhu 110 °C selama 2,5 dan 5 jam. Untuk memperoleh arginin dari hidrolisat protein, dilakukan pemurnian menggunakan metode kromatografi kolom penukar kation dan SPE penukar kation dengan bufer pencuci pH 9,85 dan bufer pengelusi pH 11. Arginin hasil isolasi kemudian dianalisis secara kuantitatif menggunakan instrumen HPLC.

Hasil penelitian menunjukkan bahwa metode hidrolisis kombinasi enzim-asam 2,5 jam mampu melepaskan residu arginin pada protein dengan lebih banyak dibandingkan hidrolisis asam 5 jam dan kombinasi enzim-asam 5 jam. Metode SPE penukar kation memberikan hasil pemurnian yang lebih efektif dan efisien dibandingkan dengan kromatografi kolom penukar kation. Analisis kuantitatif dengan HPLC menunjukkan bahwa kadar arginin paling tinggi diperoleh pada hidrolisis kombinasi enzim-asam 2,5 jam dan pemurnian menggunakan SPE penukar kation, dengan hasil sebesar 681 mg/g membran kering.

Kata kunci: arginin, hidrolisis protein, kromatografi kolom penukar kation, SPE penukar kation, HPLC.

THE IMPACT OF HYDROLYSIS AND PURIFICATION METHODS IN ARGININE ISOLATION FROM DUCK EGGSHELL MEMBRANE PROTEIN

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ABSTRACT

Isolation of arginine from duck eggshell membrane protein using acid and acid-enzyme combinations as hydrolysis variation methods, with cation exchange column chromatography and cation exchange solid phase extraction (SPE) as purification methods had been done. The purpose of research was to learn about the impact of hydrolysis and purification methods toward arginine isolate degree from duck eggshell membrane protein.

Acid hydrolysis toward protein sample had been performed using 6 M HCl at 110 °C for 5 hours, while acid-enzyme combinations had been performed with protein sample incubation using trypsin at pH 8 and 37 °C for 2.5 and 5 hours followed by acid hydrolysis using 9 M HCl at 110 °C for 2.5 and 5 hours. To obtained arginine from protein hydrolysate, cation exchange column chromatography and cation exchange SPE purification methods had been used with pH 9.85 of washing buffer and pH 11 of elution buffer. Collected arginine isolate then being quantitatively analyzed with HPLC instrument.

Experiment result showed that 2.5 hours acid-enzyme combination method could released more arginine residue of protein compared to 5 hours acid hydrolysis and 5 hours acid-enzyme combination hydrolysis. Cation exchange SPE gave more effective and efficient purification result compared to cation exchange column chromatography. Quantitative analysis using HPLC showed that the highest degree of arginine isolate obtained from 2.5 hours acid-enzyme combination hydrolysis method with SPE purification method, which resulted 681 mg/g dry membrane.

Key words: arginine, protein hydrolysis, cation exchange column chromatography, cation exchange SPE, HPLC.