IDENTIFIKASI PROTEIN PENANDA SPESIFIK METODE PENYEMBELIHAN HEWAN MENGGUNAKAN SPEKTROMETRI MASSA RESOLUSI TINGGI

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INTISARI

Daging merupakan salah satu sumber pangan bagi masyarakat. Khususnya untuk masyarakat Muslim, konsumsi daging harus memenuhi unsur kehalalan tertentu, seperti asal spesies dan proses penyembelihan. Identifikasi asal spesies daging telah banyak dilakukan, namun identifikasi terhadap proses penyembelihan belum banyak dilaporkan. Perbedaan proses penyembelihan dapat mempengaruhi respon fisiologis hewan, yang dapat diidentifikasi dari protein yang tereskpresi. Perbedaan ekspresi protein antara hewan yang disembelih secara halal dan nonhalal dapat dijadikan sebagai kandidat protein penanda spesifik untuk membedakan kedua daging. Pada penelitian ini dilakukan analisis protein secara menyeluruh dan pencarian protein penanda spesifik menggunakan Spektrometri Massa Resolusi Tinggi/*High-Resolution Mass Spectrometry* (HRMS*)*, untuk membedakan daging dari kedua proses penyembelihan. Penelitian menggunakan enam ekor tikus Wistar. Kelompok pertama disembelih dengan metode halal, yaitu dengan pemotongan pembuluh darah leher. Kelompok kedua tidak disembelih secara halal, yaitu dilakukan metode dislokasi servikal. Sampel protein daging diekstraksi dan dilakukan karakterisasi awal menggunakan *Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis* (SDS-PAGE). Perbedaan ekspresi protein daging dari dua metode penyembelihan kemudian dikonfirmasi secara akurat menggunakan HRMS. Dalam menyiapkan sampel untuk HRMS, dilakukan optimasi terhadap hidrolisis enzimatis protein dan gradien pemisahan peptida dengan *Liquid Chromatography* (LC). Analisis dan pengolahan data HRMS dilakukan untuk mengidentifikasi dan mengkuantifikasi protein penanda spesifik.

Berdasarkan hasil analisis, hidrolisis protein secara optimum dapat dicapai dengan menggunakan rasio enzim:substrat 1:50 (b/b), sedangkan pemisahan peptida secara optimum pada LC didapatkan dengan menggunakan sistem pemisahan gradien, dengan fase gerak air dan air:asetonitril 2:8 (v/v) pada komposisi tertentu selama 90 menit. Sebanyak 13 protein terekspresi lebih tinggi di kelompok nonhalal dan 10 protein terekspresi lebih tinggi di kelompok halal. Sebagai tambahan, 3 protein terekspresi spesifik di kelompok nonhalal dan 12 protein di kelompok halal. Protein-protein tersebut dapat disarankan sebagai kandidat protein penanda spesifik metode penyembelihan hewan.

Kata kunci: *biomarker*, halal, nonhalal, proteomik, spektrometri massa

IDENTIFICATION OF SPESIFIC MARKER PROTEIN FOR ANIMAL SLAUGHTER METHODS USING HIGH RESOLUTION MASS SPECTROMETRY

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ABSTRACT

Meat is one of food source among people. Especially for the Muslim community, meat consumption must fulfill certain halal requirements, such as the origin of species and their slaughter process. Identification of meat from its species sources has been widely carried out, but identification of the slaughter process has not been widely reported. Difference in slaughter processes can affect the physiological response of animals, which can be identified from the expressed protein. Differences in the expression of protein between halal and non-halal slaughtered animals can be used as the specific marker protein to distinguish them. In this study, a thorough protein analysis and the search for specific marker protein using High-Resolution Mass Spectrometry (HRMS) were conducted to distinguish meat from the two slaughter processes. The study used six Wistar rats. The first group was slaughtered by halal method, namely by cutting the blood vessels of the neck. The second group was not slaughtered in halal method, it was carried out by cervical dislocation. Meat protein was extracted and initial characterization was performed using Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis (SDS-PAGE). The differences in the expression of meat protein were then confirmed accurately using HRMS. In preparing samples for HRMS, optimization were performed to the enzymatic hydrolysis of protein and the separation gradient of peptides using Liquid Chromatography (LC). Analysis and data processing were done to identify and quantify the specific marker protein.

As result, the optimum hydrolysis of protein was achieved using the enzyme:substrate ratio of 1:50 (w/w), while the optimum peptide separation through LC was obtained by using a gradient separation system of water and water:acetonitrile 2:8 (v/v) mobile phases of certain composition for 90 min. A total of 13 proteins were higher expressed in non-halal group and 10 proteins were higher expressed in halal group. In addition, 3 proteins were specifically expressed in non-halal group and 12 proteins were in halal group. These proteins can be suggested as the candidate of specific marker proteins on animal slaughter methods.

Keywords: biomarker, halal, mass spectrometry, non-halal, proteomic