

OPTIMIZATION OF PROTEASE EXTRACTION
AND CHARACTERIZATION OF PROTEASE
FROM *Syzygium polyanthum* AS POTENTIAL
MEAT TENDERIZER

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ABSTRAK

Kelembutan daging merupakan satu sifat penting yang menyumbang kepada kepuasan makan dan penerimaan pengguna dalam pasaran produk daging. Oleh itu, satu teknik pelembutan daging diperlukan dengan meneroka protease berasaskan tumbuhan sebagai pelembut daging. Walaupun pelembut daging seperti bromelain dan papain mudah didapati, daun *Syzygium polyanthum* masih belum diterokai oleh industri daging disebabkan oleh kekurangan kajian saintifik. Selain itu, kepekatan bahan-bahan yang digunakan dalam penampunan pengekstrakan semasa proses pengekstrakan protease daripada daun *S. polyanthum* juga tidak dioptimumkan. Oleh itu, satu 2^4 rekabentuk pemfaktoran penuh (FFD) telah dijalankan untuk menyaring kepekatan kalium fosfat (KPO_4), Triton X-100, gliserol dan dithiotreitol (DTT) dalam penampunan pengestrakan yang memberi kesan kepada aktiviti protease. Keputusan menunjukkan bahawa kepekatan KPO_4 dan DTT menyumbang secara signifikan kepada aktiviti protease. Kemudian, pengekstrakan protease telah dioptimumkan menggunakan kaedah permukaan tindak balas (RSM) dengan mempelbagaikan kepekatan KPO_4 dan DTT dalam penampunan pengekstrakan. Keputusan mendapati bahawa penampunan pengekstrakan yang mengandungi 3.18 M KPO_4 dan 1.81 M DTT menunjukkan aktiviti protease optimum dengan nilai R^2 iaitu 0.8357. Didapati bahawa tiada perbezaan yang signifikan (0.082%) antara keputusan yang diramalkan (0.5004 U/ mL) dan keputusan eksperimen (0.5008 ± 0.0500 U/ mL). Suhu dan pH optimum bagi protease yang diekstrak daripada daun *S. polyanthum* adalah pada suhu 60°C dan pH 7. Protease *S. polyanthum* telah dikenalpasti sebagai protease sisteina kerana ia telah direncatkan oleh iodoasetamida, sejenis perencat protease sisteina. Dalam kajian ini, protease *S. polyanthum* menunjukkan kebolehan yang setara dengan papain sebagai pelembut daging pada unit aktiviti yang berbeza (U/ mL) dengan menunjukkan pengurangan dalam bilangan pita protein dalam analisis elektroforesis. Didapati bahawa terdapat kenaikan dalam kapasiti menahan air (WHC) dan penurunan dalam pH telah dikesan dengan pertambahan kepekatan bagi kedua-dua protease (protease *S. polyanthum* dan papain). Hasil kajian ini membuktikan bahawa *S. polyanthum* boleh digunakan sebagai alternatif pelembut daging yang berpotensi.

ABSTRACT

Meat tenderness is a crucial trait that contributes to the consumer eating satisfaction and acceptance in meat product marketability. Therefore, an effective meat tenderization technique is required by exploring plant-based protease as meat tenderizer. In spite of easy availability of commercial meat tenderizer including bromelain and papain, *Syzygium polyanthum* leaves have not been explored by the meat industry due to lack of scientific literature. Besides, the concentration of components used in the extraction buffer during the extraction of protease from the leaves of *S. polyanthum* have not been optimized. Hence, a 2^4 full factorial design (FFD) was performed to screen the concentration of potassium phosphate (KPO₄), Triton X-100, glycerol and dithiotreitol (DTT) in the extraction buffer that affect the protease activity. The results demonstrated that the concentration of KPO₄ and DTT contributed significantly to the effect of protease activity. Later, the extraction of protease was optimized using response surface methodology (RSM) by varying the concentration of KPO₄ and DTT in the extraction buffer. The results showed that the extraction buffer formed by 3.18 M of KPO₄ and 1.81 M of DTT exhibited the optimal protease activity with R^2 of 0.8357. There was no significant difference (0.082 %) between the predicted (0.5004 U/ mL) and experimental results (0.5008 ± 0.0500 U/ mL). The optimum temperature and pH for the protease activity of *S. polyanthum* protease were found to be at 60 °C and pH 7. The *S. polyanthum* protease was identified as cysteine protease because it was inhibited by iodoacetamide, a cysteine protease inhibitor. In the present study, the *S. polyanthum* protease exhibited comparable ability with papain as meat tenderizer at different unit activity (U/ mL) by showing a reduction in a number of protein bands in the electrophoretic analysis. There were an increase of water holding capacity (WHC) and a decline of pH with the increase of protease activity for both proteases (*S. polyanthum* protease and papain). These results conclude that *S. polyanthum* protease can be employed as a potential alternative meat tenderizer.

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