Optimization of a protease extraction using a statistical approach for the production of an alternative meat tenderizer from *Spondias cytherea* roots

Mohammad Norazmi Ahmad[a], *Nor Aini Mat Noh*[a], *Erna Normaya Abdullah*[a], *Mohd Ambar Yarmo*[b], *Mohd Bijarimi Mat Piah*[c], *Ku Halim Ku Bulat*[d]

[a] Experimental and Theoretical Research Laboratory, Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, Kuantan, Malaysia
[b] School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia
[c] Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, Kuantan, Malaysia
[d] Department of Chemistry, Faculty of Science, University Malaysia Terengganu, Kuala Terengganu, Malaysia

ABSTRACT

In this research, a novel protease from Ambarella (*Spondias cytherea*) was identified as a new potential *halal* meat tenderizer. Optimization of protease extraction by response surface methodology has found the optimized variables at pH 8.22, 4.95% of TX-100, 6.80 mM of 2-mercaptoethanol, and 1.71 min of mixing time at 12.37 U/g of protease activity. The overall model was significant ($p < .05$) with an R^2 value of 0.9885. Verification test results (Tukey's test) showed that no significant difference between the expected and experimental protease activity value (98%). The enzyme was stable at pH 8.0–10.0 and temperature 50–60°C. Incubation of enzyme with organic solvents showed higher activity in hydrophobic than hydrophilic phases. In addition, prolonged storage time of enzyme decreased the activity by 32%. Protein bands of muscle proteins and firmness of muscle samples were reduced upon protease treatment due to breaking of tissue fibers and loosening of myofibrils. Practical applications: Treatment by exogenous proteases is one of the various tenderization techniques used in the meat industry to improve meat tenderness. Myofibrillar and connective tissue proteins which cause toughness in meat were effectively hydrolyzed by the protease enzymes. The results of the present study should be useful to the meat industry for discovering new source of plant protease which is able to overcome the shortcoming of animal and microbial proteases as meat tenderizer.

KEYWORDS

2-mercaptoethanol; Connective tissues; Microbial protease; Protease activities; Protease treatment; Response surface methodology; Statistical approach; Verification tests

ACKNOWLEDGEMENT

The project was funded by the Ministry of Higher Education (MoHE) (Grant No. FRGS16-058-0557) and International Islamic University Malaysia (Grant No. RIGS 15-133-0133).