“Characterization of potential antidiabetic-related proteins from Malaysia soursop *Annona muricata* leaf”

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Soursop (*Annona muricata*) is a small deciduous tropical evergreen fruit tree, which has shown promising medicinal value. Due to their curative and preventative properties, all parts have been well-studied in literature, especially the leaves. However, knowledge of the antidiabetic-related proteins from soursop leaves is limited. This study aimed to characterize and evaluate the antidiabetic activities of soursop leaves protein hydrolysate (SLH) obtained by enzymatic hydrolysis. Therefore, an amounting of 17.50 ± 0.37 mg BSA/g of soursop leaves protein was extracted via ultrasound mechanical stirrer-assisted extraction (UMSAE) technique. This protein was further hydrolysed by three different enzymes (alcalase, flavourzyme and trypsin). Flavourzyme-generated SLH showed higher peptide content (28.89 ± 5.52%) compared to alcalase and trypsin-generated hydrolysate. In order to get the highest yield of peptides, the enzymatic hydrolysis of soursop leaves protein using flavourzyme was optimized with the response surface methodology (RSM). The optimal hydrolysis conditions were as follows: enzyme to substrate ratio of 4%, hydrolysis time of 4 h, the temperature of 50°C and pH of 7. Under these conditions, the peptide content of SLH was 40.55 ± 4.85%. The anti-diabetic effects were investigated by evaluating inhibitory properties of flavourzyme-generated SLH towards carbohydrate hydrolysing enzymes (α-glucosidase and α-amylase). Results revealed that upon hydrolysis, SLH exhibited superior antidiabetic activities compared with the soursop leaves protein. Besides, to verify the effectiveness of enzymatic hydrolysis, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) also indicated that a large proportion of protein bands in the hydrolysate was diminished, which was different from the original soursop leaves protein confirming that soursop leaves protein was effectively hydrolysed by flavourzyme. Peptide sequencing revealed a total of 14 bioactive peptides with a score of 0.60 or more on the peptide ranker and was categorized as potential inhibitors for diabetes treatment. Taken together, these findings suggest that SLH released by the flavourzyme might be a potential resource for the natural anti-diabetic component.

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