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Pumpkin leaf-isolated RuBisCO as a protein source for bioactive peptides

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RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) is a non-allergenic, easily digestible protein found in the leaves of C3 plants, accounting for up to 50% of the total. It is similar to the FAO's ideal protein and is offered as a valuable alternative for meeting nutritional requirements. Plant-based proteins provide amino acids for human cell development and act as precursors of bioactive peptides. Protein sources and proteolytic enzymes are crucial for producing bioactive peptides effectively.

This research aimed to optimize the hydrolysis of pumpkin leaf-isolated protein in terms of time and type of process, one or two-step process, by using endo- and exo-peptidases. The efficiency was assessed using SDS-PAGE electrophoresis, quantitative hydrolysis analysis, and peptides' capacity to chelate Fe^{2+} ions and scavenge $ABTS^{\bullet+}$ radical cations. The peptide molecular weight was determined by implementing the size-exclusion UFLC method.

The highest degree of hydrolysis, which is correlated with higher antioxidant activity was shown by Alcalase and Everlase (19.5%), and Neutrase (21.5%) with a tendency to favor Neutrase due to more favorable process conditions and a more sensory-acceptable product. Hydrolysis with Neutrase-Flavourzyme during 225 min yielded hydrolyzates with a 43.5% degree of hydrolysis, and antioxidant activities of 0.74 μmol TEAA/mg (*i.e.* 48%) and 0.30 μmol EEAA/mg (*i.e.* 44%). Five peptide fractions were identified as follows: F1 (> 27 kDa), F2 (20-27 kDa), F3 (10-20 kDa), F4 (3-10 kDa), and F5 (< 3 kDa). By establishing a correlation with antioxidant activity, it has been confirmed that a large proportion of peptide fractions F3, F4, and F5 were accountable for Neutrase-Flavourzyme hydrolyzate's good antioxidant activity. Examined enzymatic approaches contributed to generating the antioxidant peptides from pumpkin-leaf proteins with diverse peptide profiles.

Keywords: Plant-based proteins; RuBisCO; Protease; Bioactive peptides; Size-exclusion UFLC

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