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A novel antioxidative peptide derived from conger eel (*Conger myriaster*) muscle protein and its antioxidative properties

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Introduction

Free radicals are mediated in lipid oxidation of foods and deleterious diseases and antioxidants are used to protect against them. New interest has been developed to search for natural and safe antioxidative since synthetic antioxidants may cause deleterious effects on human health though their effectiveness are high. In this research Conger eel, *Conger myriaster*, one of the most valuable fishery resources in East Asia was used to purify antioxidative peptide and to assess its in-vitro antioxidative properties against lipid peroxidation.

Methods and Materials

Purification of antioxidative peptide

Conger eel (*Conger myriaster*) muscle protein was purified using different techniques and during each step fractions were selected based on ferric thiocyanate method (Mitsuda et al., 1966) associated with 7th day in-vitro linoleic acid peroxidation (Osawa et al., 1985). One enzymatic hydrolysate of conger eel (*Conger myriaster*) muscle protein, out of four enzymatic hydrolysates (Alcalase, alpha-chymotrypsin, trypsin and pepsin), was fractionated according to the molecular weights using ultrafiltration (UF) membrane system. Among them, the selected molecular weight fraction was subjected to different, consecutive chromatographic techniques, ion exchange chromatography (SP-Sephadex C-25 column), gel filtration chromatography (Sephadex G-25 column), and high performance liquid chromatography (Capcell Pak C18 UG 120 and Nucleosil 100-5 C18column) and finally one representative peptide was purified.

Synergistic antioxidative effect

Selected molecular weight fraction and alpha-tocopherol were mixed at different weight ratios (1:1, 2:1, 4:1, 1:2 and 1:4) and applied to the linoleic acid emulsion system as described above. Degree of lipid peroxidation was measured according to the ferric thiocyanate method.

Characterization of purified peptide

Purified peptide was subjected to determine its molecular mass and sequence. Also it was characterized according to antioxidant activity and radical scavenging potencies against hydroxyl and carbon-centered radicals using ESR spectroscopy.

Results and Discussion

Potent molecular weight fraction obtained following ultrafiltration separation, expressed synergistic antioxidative effects with alpha-tocopherol. Among the tested combinations, 4:1 weight ratio of potent fraction:alpha-tocopherol expressed the highest significant antioxidative activity ($P < 0.05$) compared to other tested weight ratios. Therefore, it can be suggested that antioxidative activity of low molecular weight protein hydrolysate of conger eel can be improved by introducing less amount of alpha-tocopherol.

The purified peptide was designated conger eel antioxidative peptide (CEAP). Sequence determination revealed that it contained nine amino acids in its sequence (LGLNGDDVN) and the molecular mass was identified to be 928 Da. CEAP performed better than the natural antioxidant, alpha-tocopherol for the prevention of lipid peroxidation in vitro. Additionally, it scavenged hydroxyl radicals and carbon-centered radicals at IC_{50} values of 74.1 μ M and 78.5 μ M respectively. Therefore these results suggested that, the peptides derived from conger eel protein hydrolysates are responsible for higher antioxidative properties and molecular weight as well as presence of hydrophobic amino acids highly contributed for their antioxidation.

References

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