PD-7

Isolation and Characterization of an Antioxidant from Enzymatic Hydrolysates of Yellowfin Sole (*Limanda aspera*) Frame Protein

Se-Kwon Kim[1], Pyo-Jam Park[1], Won-Kyo Jung[1], Jae-Young Je[1], Hee-Guk Byun[1], Jong-Bae Kim[2], and Soo-Hyun Chang[2]

[1] Department of chemistry, Pukyong National University, Busan, 608-737
[2] Department of Food Science & Technology, Kunsan National University, Kunsan, 573-701

Introduction

The term antioxidant is defined as any substance that, when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tertiary*-butylhydroquinone (TBHQ) and propyl gallate (PG) may be added to food products to retard lipid oxidation . However, use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds. Therefore, search for natural antioxidants as alternatives to synthetic ones is of great interest among researchers. Recently, six antioxidative peptides were isolated from the hydrolysate of a soybean protein, β-conglycinin. These peptides were composed of 5 to 16 amino acid residues and included hydrophobic amino acids, Val and Leu, at N-terminus and Pro, His, or Tyr in their sequences. However, little is known about the structural information on antioxidative peptides from marine organism. In this study, we examined the antioxidative effect of enzymatic hydrolysate of Yellow Fin sole frame protein, a by-product of fish processing plant. An antioxidative peptide was isolated from the hydrolysate so obtained, and their amino acid sequences were determined.

Materials and Methods

In order to utilize protein hydrolysate produced from yellowfin sole (*Limanda aspera*) frame as industrial waste in the process of fish manufacture, yellowfin sole frame hydrolysates including peptides and low molecular proteins (YFPs) were recovered after hydrolysation with pepsin and MICE (Mackerel Intestines Crude Enzyme). According to their relative molecular weights, 30-10kDa, 10-5kDa, 5-3kDa, 3-1kDa, and 1kDa, YFPs were separated into five major types, YFP I, YFP II, YFP III, YFP IV, and YFP V by molecular weight cut-offs of ultrafiltration membrane, respectively. The antioxidative activity of YFPs was investigated and compared with anti-peroxidation activity in linoleic acid water-alcohol emulsion and radical scavenging activity against 1,1-Diphenyl-2-picrylhydrazyl (DPPH) used α-tocopherol as a control. Furthermore, the fraction showing strong antioxidative activity was isolated from the YFPs using consecutive chromatographic methods on a SP-Sephadex C-25 column, a Sephadex G-75 column, and an octadecylsilane column. The purity and molecular mass of the antioxidant was identified using 12.5% SDS-PAGE, and then N-terminal amino acid sequence analysis was performed by automated protein sequencer after electrophoretical transfer onto a polyvinylidene difluoride (PVDF) membrane.

Results and Summary

The antioxidative activity of YFP I (M.W. 30-10kDa) was stronger than those of YFP II, YFP III, YFP IV, and YFP V, and the optimum substrate concentration for antioxidative activities of the hydrolysate was 1% (w/w) in linoleic acid water-alcohol emulsion. The synergistic effect was significantly increase in using hydrolysates with α-tocopherol. The estimated molecular weight of hydrolysates on SDS-PAGE was 13 kDa.

References

Park, P.Y., W.K. Jung, K.S. Nam, F. Shahidi and S.K. Kim. 2001. Purification and characterization of antioxidative peptide from protein hydrolysate of licithin-free egg yolk, *J. Am. Oil Chem. Soc.* 10:34-41.

Wanita, A. and K. Lorenz. 1996. Antioxidant potential of 5-N-pentadecylresorcinol, *J. Food Process. Preserv.* 20:417-429.

Yukami, S. 1972. Autoxidation of sodium linoleate in a protein solution. *Agric. Biol. Chem.* 36:871-874.