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Antioxidative activity of enzymatic hydrolysates from *Russula cutefracta*

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청버섯으로부터 효소적 가수분해물의 항산화 활성

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Objectives

Russula cutefracta a foliose type macrolichen, has been regarded as an edible mushroom and used for a traditional food or a medicine in the Far East such as Korea, Japan and China. Extracts or prescriptions of *Russula cutefracta* were known to have effects on treating many kinds of inflammation, bleeding and poisoning. It was also reported that *Russula cutefracta* had activities on free radical scavenging. These results, indicate that enzymatic extracts of *Russula cutefracta* possess potent antioxidative activity.

Materials and Methods

○ Materials

Russula cutefracta was sourced from a local market (Chungju, Korea).

○ Methods

Free radical scavenging activity DPPH radical - A sample solution of 30 μL of each enzymatic extracts, was added to 30 μL of DPPH (30 μM) in methanol solution. After mixing vigorously for 10 sec, the solution was then transferred into a 100 μL Teflon capillary tube, and the scavenging activity of each enzymatic extract on DPPH radical was measured using a JES-FA ESR spectrometer (Jeol Ltd., Tokyo, Japan).

Alkyl radical - Alkyl radicals were generated by AAPH. The phosphate-buffered saline (PBS, pH 7.4) reaction mixtures containing 10 mM AAPH, 10 mM 4-POBN, and indicated concentrations of tested samples were incubated at 37℃ in a water bath for 30 min and then transferred to a 100 μL teflon capillary tube. The spin adduct was recorded on an ESR spectrometer.

Hydroxyl radical - Hydroxyl radicals were generated by iron-catalysed Haber - Weiss reaction (Fenton-driven Haber - Weiss reaction), and the generated hydroxyl radicals rapidly reacted with nitron spin-trap DMPO. The resultant DMPO-OH adduct was detectable with an ESR spectrometer. Briefly, 0.2 ml of each enzymatic extract at various concentrations was mixed with 0.2 ml of DMPO (0.3 M), 0.2 ml of FeSO4 (10 mM) and 0.2 ml of H2O2 (10 mM) in a phosphate buffer solution (pH 7.2), and then transferred into a 100 ll Teflon capillary tube. After 2.5 min, an ESR spectrum was recorded using a JES-FA ESR spectrometer (JEOL Ltd., Tokyo, Japan). Experimental conditions were as follows: central field, 3475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 1 mW; gain, 6.3 105 and temperature, 298 K.

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Results

The *Russula cutefracta* were enzymatically hydrolyzed by 8 proteases (α-chymotrypsin, Alcalase, Flavourzyme, Neutrase, Papain, Pepsin, Protamax and Trypsin) and 7 carbohydrases (Dextrozyme, AMG, Promozyme, Maltogenase, Termamyl, Viscozyme, and Celluclast). The DPPH radical scavenging activity of Alcalase and AMG extracts was the highest, and the IC_{50} value was 155 and 320 μg/mL. The Papain and Maltogenase extracts showed the highest alkyl radical scavenging activity, and the IC_{50} value was 413 and 392 μg/mL.

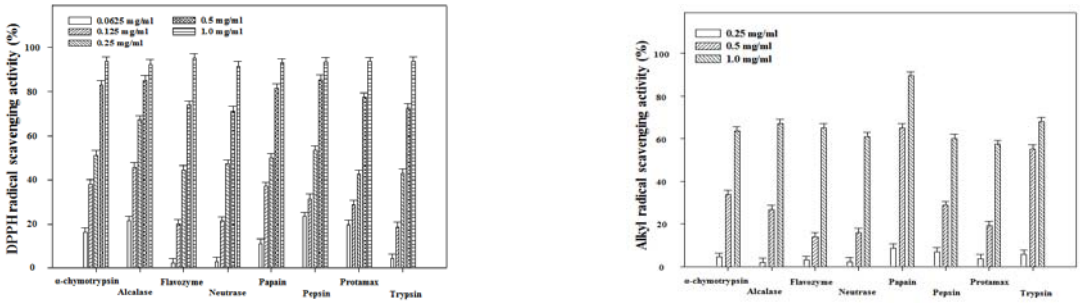

Fig 1. DPPH (left) and alkyl (right) radical scavenging activity of various enzymatic extracts by protease hydrolysis from *Russula cutefracta*.

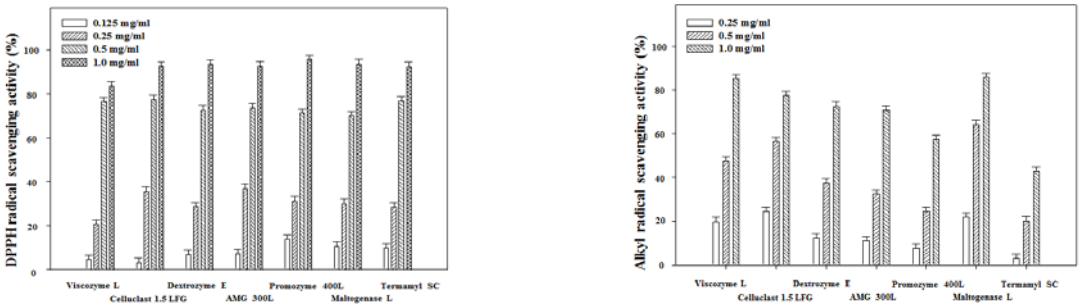

Fig 2. DPPH (left) and alkyl (right) radical scavenging activity of various enzymatic extracts by carbohydratic hydrolysis from *Russula cutefracta*.