EVALUATION OF ANTIOXIDATIVE ACTIVITY OF MEAT DERIVED COMPOUNDS AND THEIR SYNERGISTIC EFFECT WITH PLANT ANTIOXIDANTS

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Background

Reactive oxygen and free radicals, produced in human body, attack biological components such as lipid, protein, sugar, DNA, and cause oxidative damage. Despite of the protective systems in human body from the oxidative radicals, the accumulated oxidative damage may cause many kinds of diseases and aging. Intake of foods which contain antioxidative compounds may be helpful for inhibition of the oxidative damage. Many antioxidative compounds have been found in plant foods, but few works were done on meat [1)] It is very important to screen antioxidative compounds in meat and to evaluate the synergistic effect with plant antioxidants.

Objectives

To screen free radical scavenging activity in meat using DPPH radical-scavenging assay, [2)] and
To estimate the synergistic effect with thirteen known antioxidative compounds derived from plant foods.

Methods

Beef, pork and chicken purchased from a market were used in this study . After fat and connective tissues were removed, each of the meat was cut into small pieces. They were homogenized with an equal weight of Tris-HCl buffer (100 mM, pH 7.4) and each homogenate was centrifuged. The supernatant was used as a testing material. Characteristic components in meat (myoglobin, creatinine, creatine, taurine, carnosine, carnitine, inosine, hypoxanthine) and thirteen antioxidants derived from plant were purchased commercially.

Purchased carnosine contaminated by hydrazine, which had strong antioxidative activity. We removed contaminated hydrazine using Sep-Pak® Silica cartridges. Ten ml of carnosine (10 mM in water) was passed through the cartridge, which was pre-equilibrated with water. Hydrazine was adsorbed with the column. Then 10 ml of water was submitted to the column. The first 2.5ml of eluted solution was thrown away and the next 7.5 ml was used as the purified carnosine solution.

Meat extracts or test compounds were dissolved in Tris-HCl buffer. Each solution was vigorously mixed with the same volume of DPPH (water-ethanol, 1:1). After 20 min, the decrease of absorbance at 517 nm of DPPH was measured and the radical scavenging activity was calculated using trolox as a positive standard compound. The radical trapping activity of the mixture of either of carnosine or creatinine with each of 13 antioxidants was evaluated using the same method as above mentioned. Thirteen antioxidants used here were as follows: caffeic acid, ferulic acid, gallic acid, sinapic acid, protocatechuic acid, cyanidin, epicatechin, pelargonidin, resveratrol, ascorbyl palmitate, ascorbic acid, δ-tocopherol, (±)-α-tocopherol.

Results and discussion

Purification of carnosine: Commercial carnosine contains 0.78 mol% hydrazine. The DPPH radical scavenging activity of this amount of hydrazine was 13 mmol trolox eq / mol. After purification of commercial carnosine using Sep-Pack Silica cartridge, the concentration of hydrazine decreased to 0.0074 mol%. This concentration of hydrazine had little or no effect to evaluate the radical scavenging effect of carnosine.

DPPH radical trapping activity of meat and the meat-derived compounds: The activities of three kinds of meats were between 2.0-2.5 mmol trorox eq/kg (Table 1). Khanum et al [3)]. reported DPPH radical scavenging activities of fish and fishery products. The activities of octopus, flatfishes, cod, stromateoides were between 2.0 to 2.6 mmol trolox eq/kg. Yamaguchi et al.[3)] reported DPPH radical-scavenging activity of cabbage and chinese cabbage were 3.0 and 1.2 mmol trolox eq/kg, respectively. We found that meats had the similar activity as some fishes and vegetables. Because the order of the activity was beef > pork > chicken, we thought myoglobin had no positive role in the activity. Table 1. also shows the radical scavenging activities of meat derived compounds. Among nine tested compounds, anserine, carnosine and creatinine had rather strong activity. Myoglobin, creatine, inosine, inosinic acid, and hypoxanthine had no activity in this experiment.

Combination effect of carnosine or creatinine with antioxidant derived from plant foods: Next, we tried to determine the combination effect of carnosine or creatinine with each of the 13 antioxidants. As shown in Table 2, antioxidative activity of a combinations of carnosine / caffeic acid showed synergistic activity. The combination of carnosine / ferulic acid, and creatinine / caffeic acid showed weak combination effect.

Pertinent literature

1) MIWA M. and HONGO Y., Application of a Single Gel Electrophoresis (Comet) Assay to Screen the Antimutagenic Activity in Foods, *Biosci.Biotechnol.Biochem.*, **64**, 1292-1294 (2000)
2) YAMAGUCHI T. TAKAMURA H. MATOBA T. TERAO J., HPLC Method for Evaluation of the Free Radical–scavenging Activity of Foods by using 1,1-Diphenyl-2-picrylhydrazyl, *Biosci.Biotechnol.Biochem.*, **62**, 1201-1204 (1998)
3) KHNUM MN, YAMAGUCHI T, HIROISHI S, MURAOKA F, TAKAMURA H. MATOBA T., Radical-scavenging Activities of Fish and Fishery Products, *Food Sci.Technol.Res*, **5**, 193-199 (1999)

Table 1. DPPH radical scavenging activity of meats and their components

	Activity (mmol trolox eq /kg)
Beef	2.5 ± 0.25
Pork	2.3 ± 0.15
Chicken	2.0 ± 0.16
	(mmol trolox eq /mol)
Anserine	7.7 ± 0.03
Creatine	3.6 ± 0.17
Carnosine	3.5 ± 2.9
Carnitine	0.067 ± 0.006
Taurine	0.028 ± 0.01
Myoglobin	0

Table 2. Combination effect of carnosine or creatinine with plant derived antioxidants

	Sum of the activity of two compounds (calculated)	Combination Activity (actually measured)
	(mmol trolox eq /ml)	
Carnosine (6.5)	-	-
+ Caffeic acid (12.3)	18.8	28.7
+ Ferulic acid (12.9)	19.4	22.2
+ Epicatechin (10.4)	16.9	18.4
Creatinine (7.0)	-	-
+ Caffeic acid (12.3)	19.3	22.1

Numbers in parenthesis show the radical scavenging activity of each compound