8-P17

ANTIOXIDANT ACTIVITY OF PORCINE MYOFIBRILLAR PROTEIN HYDOLYSATES OBTAINED BY PROTEASE TREATMENT

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Background:

The peroxidation of lipids and fatty acids affects food qualities. In meat and meat products, their peroxidation causes deterioration of their qualities and limit of their shelf-lifes. The peroxidation also makes off-flavor or warmed-over flavor during storage of cooked meat. Therefore, it is very important to protect lipids and fatty acids in meat and meat products from peroxidation.

Various antioxidants to control lipid peroxidation have been synthesized and found out in natural substances. α-tocopherol (Vitamin E), β-carotene, polyphenols and so on are well known as natural antioxidants. Recently, carnosine and protein hydrolysates have been also reported to possess antioxidant activities. Carnosine is a dipeptide, β-alanyl-L-histidine, and occurs at the concentration of 1-20 mM in skeletal muscle. It is suggested to exhibit antioxidant activity by the combination of metal-ion chelation, free radical scavenging and singlet oxygen quenching[1, 2]. In addition, the hydrophobic modification of carnosine is shown to enhance its activity[3]. On the other hand, peptides in proteolytic hydrolysate of ovalbumin[4], soybeans protein[5] or elastin[6] have been also shown to possess antioxidant activities. However, there are very few studies on antioxidant activities of peptides derived from proteins.

Objectives:

This study is accomplished to examine antioxidant activities of peptides prepared from porcine myofibrillar proteins on the treatment with two kinds of proteases, papain and actinase E. And their antioxidant activities were compared with that of carnosine.

Materials and methods:

Preparation of Myofibrillar Protein Hydrolysate-Myofibrillar proteins were prepared according to the method reported by Yang *et al.* [7]. A hundred parts by weight of these proteins were incubated with 1 part by weight of actinase E or papain in distilled water (pH 7) at 37 ℃ for 24 h. After incubation, ethanol was added into the reaction mixture in order to remove unhydrolyzed proteins. This solution was centrifuged at 2,000×g for 10min. The supernatant was concentrated by evaporation at 45℃.

Amino Acid Analysis-Proteolytic hydrolysates of myofiblillar proteins were hydrolyzed in 6 N HCl at 110℃ for 24h. Amino acid composition of peptides in the hydrolysate was analyzed with an amino acid analyzer (SHIMAZU Co., Japan).

Peroxidation System-We used the peroxidation system reported by Chen *et al.* [5] with slight modification. Ten milligrams of linolenic acid in 4 ml of 0.1 M K-phosphate buffer (pH 7.0) containing 0.5 % Triton X-100 (w/v) and 0.05 mM $FeCl_2$ (accelerator of oxidation) were sonicated and heated in the water bath at 80 ℃ for 60 min.

Measurement of Hydroperoxides and 2-hiobarbituric Acid Reactive Substances (TBARS) -The hydroperoxides in peroxidation system before and after heating for 60 min were measured according to the method described by Osawa and Namiki[8]. That is, 100 μl of reaction mixture was mixed with 4.5 ml of 75 % ethanol, 100 μl of 30 % ammonium thiocyanate, 100 μl of 0.1 N HCl, and 100 μl of 20 mM ferrous chloride in 3.5 % HCl solution. After 3min, the absorbance of the colored solution at 500nm was measured. The hydroperoxide content before heating was subtracted from that after heating.

The 2-thiobarbituric acid reactive substances (TBARS) in peroxidation system before and after heating for 60 min were also measured according to the method described by Tarladgis *et al.* [9]. That is, 0.5 ml of the solution in peroxidation system was taken into microtube, and then mixed with 5 μl of 3 % BHT in ethanol, 0.25 ml of 0.25 % trichloroacetic acid and 0.5 ml of 0.67 % 2-thiobarbituric acid. This mixture was boiled for 10min and centrifuged at 15,000rpm for 15 min. The absorbance of the supernatant at 535nm was measured. The value of TBARS before heating was subtracted from that after heating.

Results and discussions:

Amino Acid Composition of Proteolytic Hydrolysates of Myofibrillar Proteins-Amino acid composition of proteolytic hydrolysate of myofibrillar proteins on the treatment with actinase E or papain was very similar. Major constitute amino acids in both hydrolysate were Glu, Asp and Lys. Some peptides containing Tyr, Met, His, Lys and Trp were shown to possess antioxidant activities[10]. As both hydrolysates contained a lot of Lys, they seemed to be hopeful antioxidants. However, the contents of hydrophobic amino acids in papain hydrolysate were a little larger than those in actinase E one.

Effects of Hydrolysate Concentration on Antioxidant Activities-Effects of hydrolysate concentration on antioxidant activities toward lipid peroxidation were examined. Addition of 0.02, 0.2 and 2 % of actinase E or papain hydrolysates exhibited antioxidant activities, addition of 0.2 % each hydrolysate depressing the production of TBARS most strongly. The production of hydroperoxides was more strongly inhibited as the concentration of actinase E hydrolysate was higher.

Effect of pH on Antioxidant Activities of Protein Hydrolysates-Antioxidant activities of both actinase E and papain hydrolysates at pH 5.4-7.8 were examined. Both hydrolysates depressed the production of hydroperoxides more strongly as their pH was higher. As shown in Fig. 1, the actinase E hydrolysate also suppressed the production of TBARS more strongly as its pH was higher. On the other hand, the papain hydrolysate depressed the production of TBARS to the almost same level at pH 5.4-7.8, suggesting that the mechanism of antioxidant activity of papain hydrolysate was different from that of actinase E one.

Comparison of Antioxidant Activities of Protein Hydrolysates with That of Carnosine-Antioxidant activities of actinase E and papain hydrolysates were compared with that of carnosine at the same concentration (0.2 %) (Fig. 2). The strength of antioxidant activity of actinase E was almost same as that of carnosine, while the strength of antioxidant activity of papain hydrolysate was a little larger than that of carnosine. As both hydrolysates contained various peptides, peptides possessing strong antioxidant activities were expected to occur in the hydrolysates.

Conclusion:

Hydrolysate of porcine myofibrillar proteins on treatment of actinase E or papain exhibited antioxidant activity in linolenic peroxidant system. The strength of activity of actinase E hydrolysate was almost same as that of carnosine, while the strength of papain one was a little stronger than that of carnosine.

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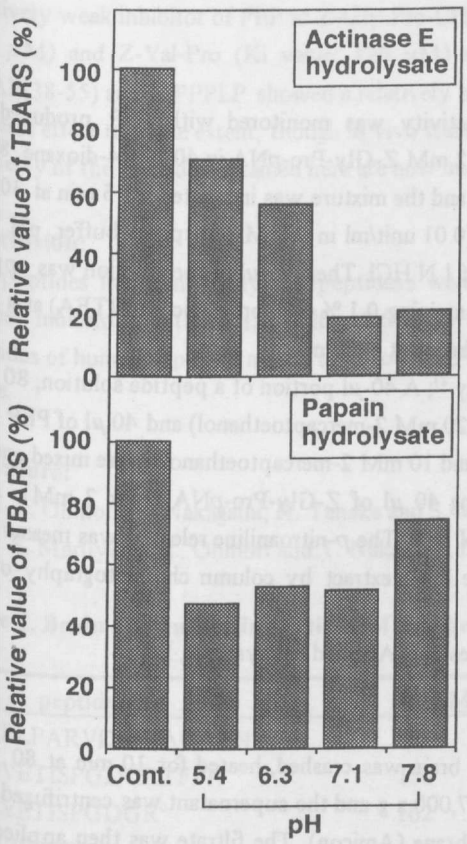

Fig. 1 Effect of pH on Antioxidant Activities of Protein Hydrolysates

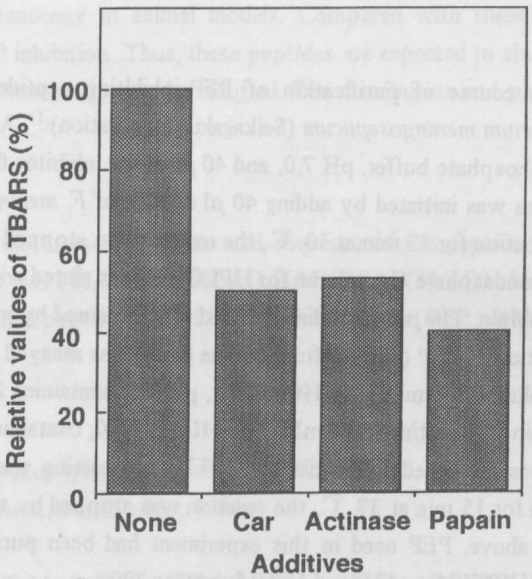

Fig. 2 Comparison of Antioxidant Activities of Protein Hydrolysates with That of Carnosine

Antioxidant activities of carnosine (Car), actinase E hydrolysate (Actinase), and papain hydrolysate (Papain) were measured by the production of TBARS in linolenic acid peroxidation system (pH 7).