# Antioxidant Activity of Designed Peptides Based on the Antioxidative Peptide Isolated from Digests of a Soybean Protein

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Antioxidative activities of 28 synthetic peptides, which were designed based on an antioxidative peptide (Leu-Leu-Pro-His-His) derived from proteolytic digests of a soybean protein, against the peroxidation of linoleic acid in an aqueous system were measured by the ferric thiocyanate method. The results for the hydroperoxide levels derived from linoleic acid agreed with those obtained by reversed-phase high-performance liquid chromatography. The deletion of the C-terminal His decreased the activity, whereas the deletion of the N-terminal Leu had no effect. In the peptide sequence, His and Pro played important roles in the antioxidative activity and, among the peptides tested, Pro-His-His was the most antioxidative. The activity decreased on substitution of the second His with D-His. Introduction of Tyr to the positions of Pro or His did not increase the activities of the corresponding peptides. Antioxidative peptides showed synergistic effects with nonpeptidic antioxidants as observed in soybean protein hydrolysates. The magnitude of the effects, however, did not correlate with the antioxidative activities of the peptides.

**Keywords:** *Antioxidative peptides; antioxidant; soybean protein hydrolysate; ferric thiocyanate method; synergistic effect*

## INTRODUCTION

Many proteins have been shown to have antioxidative activities against the peroxidation of lipids or fatty acids upon hydrolysis (Bishov and Henick, 1972, 1975; Yamaguchi et al., 1975b, 1979, 1980; Yee et al., 1980; Tsuge et al., 1991). Thus, the antioxidative activities either of amino acids or peptides have been investigated to gain insight into the antioxidative mechanism of protein hydrolysates. Several amino acids, such as Tyr, Met, His, Lys, and Trp, are generally accepted as antioxidants in spite of their pro-oxidative effects in some cases (Marcuse, 1960, 1962; Karel et al., 1966; Yamaguchi, 1971). The antioxidative activities of some dipeptides were demonstrated in an oil system by Kawashima et al. (1979), and in a metal-catalyzed liposomal suspension system by Yamashoji and Kajimoto (1980). Dipeptides consisting of Ala, Tyr, His, and Met at the N-terminus on linoleic acid were investigated by Yamaguchi et al. (1975 a) and by Kawashima et al. (1979). The dipeptides showed higher antioxidative activities than the constituent amino acid mixtures in an aqueous system. Recently, the antioxidative activity of carnosine, a histidine-containing dipeptide, was reviewed extensively (Chan and Decker, 1994). The antioxidant mechanism of the peptide has been postulated to be metal chelation or free radical scavenging.

More recently, six antioxidative peptide fragments were isolated from digests of a soybean protein, $\beta$-conglycinin (Chen et al., 1995). The peptides were composed of 5−16 amino acid residues, including hydrophobic amino acids, Val or Leu, at the N-terminal positions, and Pro, His, or Tyr in the sequences. In the present study, the smallest peptide, Leu-Leu-Pro-His-His (LLPHH), of the aforementioned peptide fragments was chosen as a model for the characterization of antioxidative properties. Chemical syntheses of 28 structurally related peptides were carried out to investigate the residue−activity relationship. Antioxidative activities of these peptides were compared by the ferric thiocyanate method, and the synergistic effects of the synthetic peptides and soybean protein hydrolysates with nonpeptidic antioxidants were examined. Additionally, the ferric thiocyanate method was evaluated by reversed-phase high-performance liquid chromatography (HPLC).

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## MATERIALS AND METHODS

**Materials.** Soybean proteins were prepared from *Glycine max* by the method of Iwabuchi and Yamauchi (1987) for glycinin and $\beta$-conglycinin, and the method of Asano et al. (1993) for basic 7S globulin. Protease S from *Bacillus* sp. was purchased from Amano Seiyaku Company (Nagoya, Japan). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) from Tokyo Kasei Company (Tokyo, Japan), *d*-$\delta$-tocopherol (>86%) from Eisai Company (Tokyo, Japan), and linoleic acid (~99%) from Sigma Chemical (St. Louis, MO) were used as received. Amino acid derivatives, which were in the L-configuration except for L-Pro-D-His-L-His ($P^{D}HH$), coupling reagents, and resins for peptide-assembly were SynProPep reagents (Shimadzu Corporation, Kyoto, Japan). All other reagents were of analytical grade from Nakarai Tesque (Kyoto, Japan) or Wako Pure Chemical Industries (Osaka, Japan).

Peptides were prepared by the fluorenylmethoxycarbonyl (Fmoc)-strategy using a simultaneous multiple peptide synthesizer (model PSSM-8, Shimadzu) as described by Nokihara et al. (1992). The sidechain of His was protected with a trityl group. After cleavage, high quality peptides were obtained. These peptides were confirmed by reversed-phase HPLC with a SynPropep RPC18 column (4.6 × 150 mm; Shimadzu) with a linear gradient elution with 0.01 N HCl and acetonitrile at a flow rate of 1.0 mL/min and monitored at 210 nm; and by mass spectrometry on a Shimadzu-Kratos Kompact III (Manchester, UK).

**Enzymatic Hydrolysis.** $\beta$-Conglycinin (1 g) was dissolved in distilled water (33 mL) and adjusted to pH 8.0 with 0.1 N

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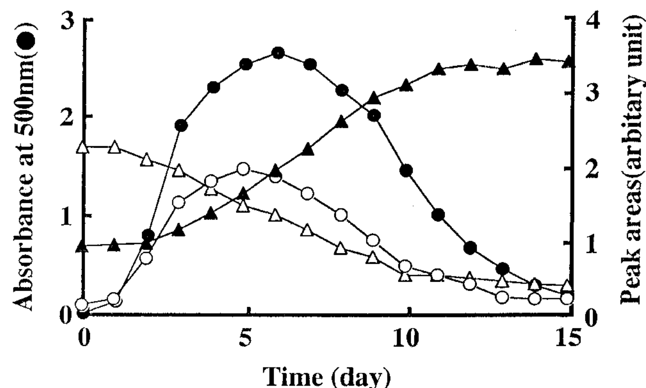


**Figure 1.** Monitoring of the autoxidation of linoleic acid by the ferric thiocyanate and the reversed-phase HPLC methods. Data represent the mean of triplicate determinations. Key: (●) absorbance at 500 nm (△) linoleic acid; (○) linoleic acid hydroperoxides; (▲) secondary oxidation products. The autoxidation conditions were the same as described in the text.

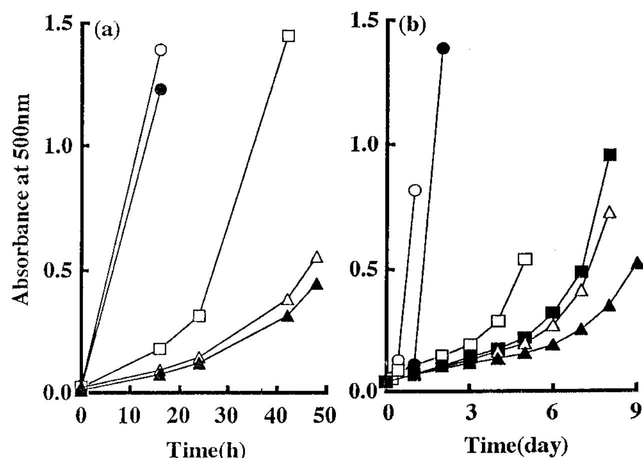


**Figure 2.** Effects of pH of 0.1 M phosphate buffer on autoxidation of linoleic acid in aqueous system in the absence (**a**) and the presence (**b**) of a soybean protein hydrolysate (5 mg). Test tubes were kept at 60 °C, and the stage of oxidation was measured by the ferric thiocyanate method. Key: (**a**) (○) pH 6.5; (●) pH 6.0; (□) pH 7.0; (△) pH 7.5; (▲) pH 8.0; (**b**) (○) pH 6.5; (●) pH 6.6; (□) pH 6.7; (■) pH 6.8; (△) pH 6.9; (▲) pH 7.0. The autoxidation conditions were the same as described in the text.

NaOH. After addition of protease S (30 mg), the mixture was incubated at 70 °C for 1 h. The resulting hydrolysate was heated in boiling water for 3 min to inactivate the protease, neutralized, and centrifuged (10 min at 20000*g*). The supernatant was lyophilized and stored in a desiccator at room temperature. The hydrolysates of glycinin and basic 7S globulin were also prepared in the same manner.

**Measurement of Antioxidative Activity.** For oxidation, 1.0 mL of 0.1 M sodium phosphate buffer (pH 7.0), 0.5 mL of distilled water, and 1.0 mL of 50 mM linoleic acid in ethanol (99.5%) were mixed in a glass test tube (5-mL volume). Test samples were added with the aforementioned buffer or ethanol by keeping the total volume. The tubes were sealed tightly with silicon rubber caps and kept at 60 °C in the dark. Aliquots of the reaction mixtures were withdrawn at intervals with a microsyringe for the measurement of antioxidative activity by the reversed-phase HPLC method or the ferric thiocyanate method according to Mitsuda et al. (1966) with a slight modification.

The reaction mixture (20 $\mu$L) was injected into a reversed-phase HPLC column to determine the residual linoleic acid, linoleic acid hydroperoxides, and secondary oxidation products. Analyses were performed on a TSK ODS 80TM column (5 $\mu$m, 4.6 × 150 mm, Tosoh, Tokyo, Japan) with a linear gradient of acetonitrile (0→95% in 60 min) in 0.1% trifluoroacetic acid (TFA) as eluents, at 35 °C, at a flow rate of 1.0 mL/min, and monitoring at 215 nm. The peak areas were calculated with an integrator (model C-R6A, Shimadzu).

The ferric thiocyanate analysis was performed as follows: To the reaction mixture (50 $\mu$L) were added 75% ethanol (2.35 mL), 30% ammonium thiocyanate (50 $\mu$L), and 20 mM ferrous chloride solution in 3.5% HCl (50 $\mu$L). After 3 min, the absorbance of the colored solution was measured at 500 nm in a 1-cm cuvette with a Jasco model Ubest 30 spectrophotometer (Tokyo, Japan). The number of days taken to attain the absorbance of 0.3 was defined as the induction period. The relative antioxidative activity was calculated by dividing the induction period of test samples by that of the control.

## RESULTS AND DISCUSSION

**Evaluation of the Method for the Antioxidative Activity Measurement.** In the present study, the ferric thiocyanate method was routinely used for the measurement of the antioxidative activity. The autoxidation process of linoleic acid in the ethanol−0.1 M sodium phosphate buffer (pH 7.0) system was examined by both HPLC and the ferric thiocyanate method. As shown in Figure 1, the oxidation of linoleic acid generates linoleic acid hydroperoxide, which decomposed to many secondary oxidation products. The change in the peak area of linoleic acid hydroperoxide correlated well with the change in the absorbances of the ferric thiocyanate method (i.e., the absorbance increased to a maximum and then decreased gradually). In the course of this experiment, we observed that the pH and the concentration of sodium phosphate buffer influenced the autoxidation of linoleic acid. The autoxidation proceeded more rapidly in concert with a decrease of pH in the presence and absence of protein hydrolysates (Figure 2). Phosphate is known to inhibit the autoxidation by chelating metal ions (Tims and Watts, 1958). Thus, the pH-sensitivity of the chelating activity must have influenced the autoxidation. Although the mechanism of the antioxidative activity of protein hydrolysates has not been elucidated, the activity was also pH-sensitive (see Figure 2). Higher concentration of the phosphate buffer slowed down the oxidation rate, which resulted in a longer induction period in a dose-dependent manner (data not shown). Hence, the pH and concentration of the buffer were carefully controlled for the measurement of the antioxidative activity.

**Antioxidative Activity of Synthetic Peptides.** The antioxidative activity of synthetic LLPHH, which was originally isolated from the hydrolysate of soybean $\beta$-conglycinin as an antioxidative peptide, was compared with the activities of BHA and BHT in the range of 4.0 × $10^{-4}$ to 8.0 × $10^{-7}$ M (Figure 3). At these concentrations, LLPHH showed higher antioxidative activity than BHA. Although the peptide was much less active than BHT above 0.5 × $10^{-4}$ M, it was more potent than BHT below 0.5 × $10^{-4}$ M. It should be noted that this comparison is valid only for the conditions stated, because the activities of the peptides are expected to differ in different media, such as in aqueous and lipid phases and at the interface. The deletion of the N-terminal Leu of LLPHH did not affect the antioxidative activity. On the other hand, the deletion of the C-terminal His of LLPHH caused loss of the activity.

To explore the residue−activity relationship of LL-PHH, 28 structurally related peptides were synthesized and their activities were compared and summarized in Figure 4. The segment HH in LLPHH was important to the antioxidative activity of the peptide. Addition of one His residue did not significantly change the activity.

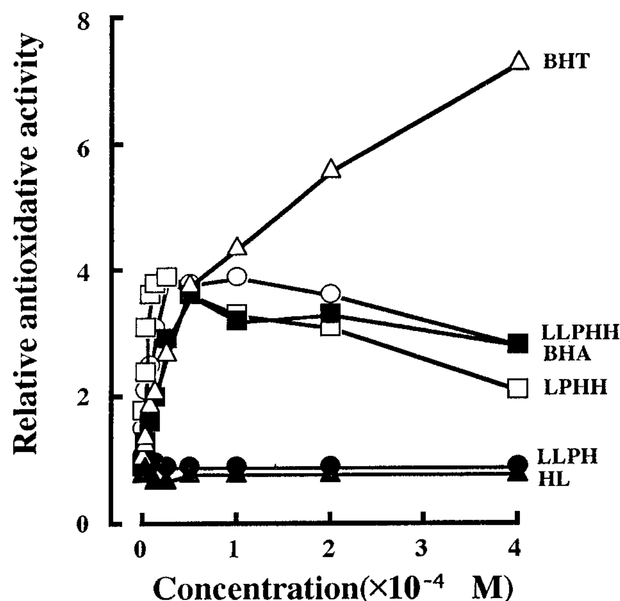


**Figure 3.** Antioxidative activity of synthetic peptides and nonpeptidic antioxidants. Key: (○) Leu-Leu-Pro-His-His; (□) Leu-Pro-His-His; (●) Leu-Leu-Pro-His; (▲) His-Leu; (■) BHA; (△) BHT.

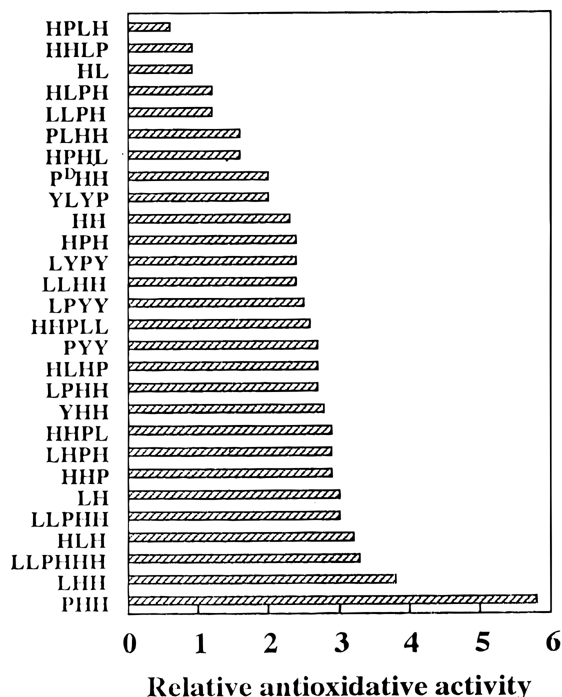


**Figure 4.** Antioxidative activities of Leu-Leu-Pro-His-His and its structurally related peptides. The peptide concentration was $4.0 \times 10^{-5}$ M. Data represent the mean of three replications. Key: (H) His; (P) Pro; (L) Leu; (Y) Tyr; (DH) D-His.

In contrast, addition of Pro to the N-terminus of HH dramatically increased activity. The addition of Pro to the C-terminus or the addition of two additional amino acid residues to the N-terminus of HH resulted only in a marginal positive effect. Further addition of Leu or Leu-Leu to the C-terminus of HHP showed no significant effect on the activity. The addition of Leu to the N-terminus of HH resulted in an increase in the activity, but to a less extent than that from the addition of Pro. Addition of Leu or Pro to the N-terminus of LHH decreased the activity. A similar negative effect was observed for the addition of Leu to the N-terminus of PHH.

Although two His residues adjacent to one another were preferred for the antioxidative activity, the insertion of Pro or Leu between HH did not reduce the activity, whereas the insertion of PL or LP greatly decreased the activity. The addition of Leu or Pro to the C-terminus of HPH or HLH, respectively, slightly decreased the activity. However, the addition of Leu to the N-terminus of HPH gave a small positive effect. These results indicate that the antioxidative activity of the peptides depends on their amino acid sequences as well as the constituent amino acids. A typical example is observed for HHPL or LH, where activity is lost by shuffling the amino acid to HHLP or HL.

PHH was the most active sequence in this study. The configuration of the peptide seemed to be very important for the activity, because substitution for the second L-His with D-His diminished the activity. On the basis of this result, it is apparent that the correct positioning of imidazole groups is essential for the antioxidative activity. Tyr is known to be an antioxidant itself; therefore, the Pro or His of PHH was replaced with Tyr. Both YHH and PYY were not as active as PHH. Other Tyr-containing peptides, such as LPYY, LYPY, and YLYP, were also less active than the corresponding His peptides.

Although the structure–activity relationship of antioxidative His-containing peptides has not been well defined yet, the activity must be attributed to hydrogen-donating ability, lipid peroxyradical trapping, and/or the metal ion-chelating ability of the imidazole group (Chan and Decker, 1994). The differences in the activity of individual peptides are due to the environment surrounding the imidazole group, as indicated by various observations. Murase et al. (1993) found that *N*-(long-chain-acyl) histidine-containing compounds suppressed the oxidation of phosphatidylcoline liposomes and methyl linoleate. The hydrophobicity of the compounds was important for the accessibility to the hydrophobic targets. Uchida and Kawakishi (1992) investigated the oxidation of Asp-Arg-Val-Tyr-Ile-His-Pro-Phe mediated by copper (II)/ascorbate. The N-terminal Asp-Arg-Val-Tyr sequence contributed significantly to the reactivity of the His residue, which was converted to the 2-imidazolone derivative upon oxidation. The His residue in Ile-His-Pro-Phe showed no reactivity against the oxidation without the N-terminal segment. Furthermore, Tsuge et al. (1991) reported the isolation of a potent antioxidative peptide Ala-His-Lys from the eggwhite albumin hydrolysate, in which neither His-Lys nor a constituent amino acid mixture had any activity, but Ala-His was as potent as the parent peptide.

**Synergistic Antioxidative Effect of Peptides with Nonpeptidic Antioxidants.** The synergistic antioxidative effects of synthetic peptides with BHA, BHT, and *d*-δ-tocopherol has been studied. As summarized in Table 1, most of the synthetic peptides showed synergistic effects on combined use with BHA. In particular, HPLH and HHLP, which had no antioxidative activity, showed large synergistic effects. On the other hand, highly active peptides, such as PHH and LHH, showed only moderate effects. LLPH did not show any synergistic effect and BHT had very little effect. The antioxidative activities of HPLH, HHLP, PLHH, and HPHL were comparable to that of PHH in the combined use of BHT. In the case of tocopherol, HPLH, HHLP, PLHH, and HPHL were synergistic, although LLPH, LLPHH, and LLPHHH were not.

**Table 1. Synergistic Effects of Synthetic Peptides on the Antioxidative Activity of Nonpeptidic Antioxidants**[a]

| | antioxidative activity | | | |
|---|---|---|---|---|
| | peptide | +BHA | +BHT | +tocopherol |
| | Antioxidant | | | |
| | 1.0 | | | |
| BHA | | 2.4 | | |
| BHT | | | 1.9 | |
| tocopherol | | | | 1.3 |
| | Synthetic Peptide | | | |
| HPLH | 0.6 | 13.1 (4.4) | 5.7 (2.3) | 7.2 (3.8) |
| HHLP | 0.9 | 16.1 (4.9) | 5.7 (2.0) | 9.4 (4.3) |
| HL | 0.9 | 7.4 (2.2) | 3.2 (1.1) | 3.9 (1.8) |
| HLPH | 1.2 | 9.3 (2.6) | 3.4 (1.1) | 3.9 (1.6) |
| LLPH | 1.2 | 2.5 (0.7) | 1.4 (0.5) | 0.9 (0.4) |
| PLHH | 1.6 | 10.3 (2.6) | 6.9 (2.0) | 10.1 (3.5) |
| HPHL | 1.6 | 14.3 (3.6) | 6.2 (1.8) | 10.1 (3.5) |
| HH | 2.3 | 13.6 (2.9) | 5.9 (1.4) | 8.2 (2.3) |
| LLHH | 2.4 | 14.0 (2.9) | 6.5 (1.5) | 9.1 (2.5) |
| HHPLL | 2.6 | 14.2 (2.8) | 5.7 (1.3) | 9.2 (2.4) |
| HLHP | 2.7 | 11.9 (2.3) | 4.5 (1.0) | 6.4 (1.6) |
| LPHH | 2.7 | 11.4 (2.2) | 6.2 (1.3) | 7.6 (1.9) |
| HHPL | 2.9 | 14.2 (2.7) | 6.4 (1.3) | 8.1 (1.9) |
| LHPH | 2.9 | 15.0 (s.8) | 6.3 (1.3) | 9.8 (2.3) |
| LH | 3.0 | 12.1 (2.2) | 6.1 (1.2) | 9.2 (2.1) |
| LLPHH | 3.0 | 9.3 (1.7) | 4.8 (1.0) | 3.1 (0.7) |
| HLH | 3.2 | 14.0 (2.5) | 4.3 (0.8) | 6.5 (1.4) |
| LLPHHH | 3.3 | 12.4 (2.2) | 5.8 (1.1) | 5.1 (1.1) |
| LHH | 3.8 | 14.0 (2.3) | 6.3 (1.1) | 8.9 (1.7) |
| PHH | 5.8 | 16.4 (2.0) | 6.6 (0.9) | 9.4 (1.3) |

[a] Antioxidative activity was evaluated by the ferric thiocyanate method. The assay was performed with 40 $\mu$M peptides, 100 $\mu$M BHA and BHT, and 10 $\mu$M tocopherol. The number in parentheses is the magnitude of synergistic effect: (activity of peptide + antioxidant)/(activity of peptide) + (activity of antioxidant). Data represent the mean of three replications.

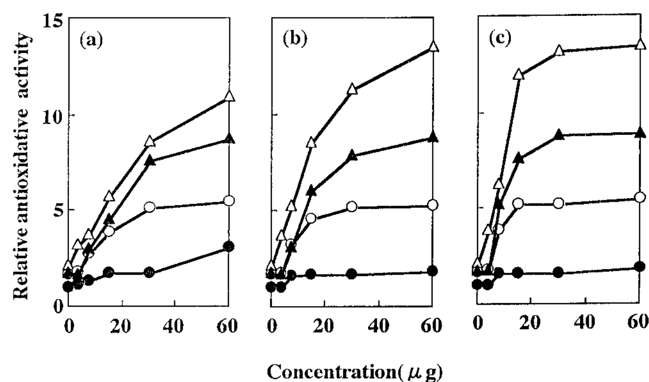


**Figure 5.** Synergistic effects of soybean protein hydrolysates on the antioxidative activity of nonpeptidic antioxidants (data represent the mean of three replications): (**a**) $\beta$-conglycinin hydrolysate; (**b**) glycinin hydrolysate; and (**c**) basic 7S globulin hydrolysate. Key: (●) hydrolysate itself; (△) hydrolysate + BHA; (○) hydrolysate + BHT; (▲) hydrolysate + tocopherol.

The synergistic effects of several soybean protein hydrolysates on the antioxidative activities with nonpeptidic antioxidants are shown in Figure 5. In the presence of BHA, BHT, and *d*-$\delta$-tocopherol, each hydrolysate derived from $\beta$-conglycinin, glycinin, and basic 7S globulin exhibited synergistic effects. Without the antioxidants, the hydrolysates had very little antioxidative activity in the concentration range tested, whereas the activity was strongly enhanced by increasing the concentrations of the hydrolysate in the presence of antioxidants. Sixty micrograms of the hydrolysates correspond to ~20 $\mu$M, as calculated from the degree of hydrolysis. Each protein hydrolysate had a different synergistic effect. The hydrolysate from basic 7S globulin showed a high synergistic effect even at a low concentration. As with the synthetic peptides, the synergistic effect increased in the order BHA, tocopherol, and BHT.

The synergistic effects of nonpeptidic antioxidants on the antioxidative activity have been demonstrated with the hydrolysates of a vegetable protein and yeast protein (Bishov and Henick, 1972, 1975), and bovine serum albumin (Hatate et al., 1990). Soybean protein hydrolysates were also shown to be synergistic to the antioxidative activity of *d*-$\delta$-tocopherol (Yamaguchi et al., 1975a, 1980). The results of the present study indicate that the synergistic effect of nonpeptidic antioxidants on the antioxidative activity of protein hydrolysates has nothing to do with the antioxidative activity of the constituted peptides, as demonstrated with a series of synthetic peptides.

In conclusion, His and Pro play important roles in the antioxidant activity of synthetic peptides, which were designed based on an antioxidative peptide (Leu-Leu-Pro-His-His), in an aqueous system. Nonpeptidic antioxidants, such as tocopherol, BHA, and BHT, potentiated the antioxidative activities of the peptides. The magnitude of the synergism had no relationship with the antioxidative activities of the peptides.

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## LITERATURE CITED

Asano, M.; Endo, I.; Yamauchi, F. Soybean Basic 7S Globulin which was detected from okara and its isolation methods. *Nippon Shokuhin Kogyo Gakkaishi* **1993**, *40*, 323–330.

Bishov, S. J.; Henick, A. S. Antioxidant effect of protein hydrolyzates in a freeze-dried model system. *J. Food Sci.* **1972**, *37*, 873–875.

Bishov, S. J.; Henick, A. S. Antioxidant effect of protein hydrolyzates in freeze–dried model system. Synergistic action with a series of phenolic antioxidants. *J. Food Sci.* **1975**, *40*, 345–348.

Chan, K. M.; Decker, E. A. Endogenous skeletal muscle antioxidants. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 403–426.

Chen, H.-M.; Muramoto, K.; Yamauchi, F. Structural analysis of antioxidative peptides from soybean $\beta$-conglycinin. *J. Agric. Food Chem.* **1995**, *43*, 574–578.

Hatate, H.; Nagata, Y.; Kochi, M. Antioxidative effect of bovine serum albumin hydrolyzates and their synergistic effect with antioxidants. *Yukagaku* **1990**, *39*, 42–46.

Iwabuchi, S.; Yamauchi, F. Determination of glycinin and $\beta$-conglycinin in soybean proteins by immunological methods. *J. Agric. Food Chem.* **1987**, *35*, 200–205.

Karel, M.; Tannenbaum, S. R.; Wallace, D. H.; Maloney, H. Autoxidation of methyl linoleate in freeze-dried model systems. III. Effects of added amino acids. *J. Food Sci.* **1966**, *31*, 892–896.

Kawashima, K.; Itoh, H.; Miyoshi, M.; Chibata, I. Antioxidant properties of branched-chain amino acid derivatives. *Chem. Pharm. Bull.* **1979**, *27*, 1912–1916.

Marcuse, R. Antioxidative effect of amino-acids. *Nature* **1960**, *186*, 886–887.

Marcuse, R. The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. *J. Am. Oil Chem. Soc.* **1962**, *39*, 97–103.

Mitsuda, H.; Yasumoto, K.; Iwami, K. Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Syokuryo* **1966**, *19*, 210–214.

Murase, H.; Nagao, A.; Terao, J. Antioxidant and emulsifying activity of N-(long-chain-acyl) histidine and N-(long-chain-acyl) carnosine. *J. Agric. Food Chem.* **1993**, *41*, 1601–1604.

Nokihara, K.; Yamamoto, R.; Hazama, M.; Wakizawa, O.; Nakamura, S. Design and applications of a novel simulta-

neous multi solid-phase peptide synthesizer. In *Innovation and Perspectives in Solid-Phase Synthesis 1992*; Epton, R., Ed.; Intercept Limited: Andover, U.K., 1992; pp 445−448.

Tims, M. J.; Watts, B. M. Protection of cooked meats with phosphate. *Food Technol.* **1958**, *12*, 240−243.

Tsuge, N.; Eikawa, Y.; Nomura, Y.; Yamamoto, M.; Sugisawa, K. Antioxidative activity of peptides prepared by enzymatic hydrolysis of egg-white albumin. *Nippon Nogei Kagaku Kaishi* **1991**, *65*, 1635−1641.

Uchida, K.; Kawakishi, S. Sequence-dependent reactivity of histidine-containing peptides with copper (II)/ascorbate. *J. Agric. Biol. Chem.* **1992**, *40*, 13−16.

Yamaguchi, N. Studies on antioxidative activities of amino compounds on fats and oils. Part I. Oxidation of methionine during course of autoxidation of linoleic acid. *Nippon Shokuhin Kogyo Gakkaishi* **1971**, *18*, 313−318.

Yamaguchi, N.; Yokoo, Y.; Fujimaki, M. Studies on antioxidative activities of amino compounds on fats and oils. Part II. Antioxidative activities of dipeptides and their synergistic effects of tocopherol. *Nippon Shokuhin Kogyo Gakkaishi* **1975a**, *22*, 425−430.

Yamaguchi, N.; Yokoo, Y.; Fujimaki, M. Studies on antioxidative activities of amino compounds on fats and oils. Part III. Antioxidative activities of soybean protein hydrolyzates and synergistic effect of hydrolyzate on tocopherol. *Nippon Shokuhin Kogyo Gakkaishi* **1975b**, *22*, 431−435.

Yamaguchi, N.; Yokoo, Y.; Fujimaki, M. Antioxidative activities of protein hydrolyzates. *Nippon Shokuhin Kogyo Gakkaishi* **1979**, *26*, 65−70.

Yamaguchi, N.; Naito, S.; Yokoo, Y.; Fujimaki, M. Oxidative stability of dried model food consisted of soybean protein hydrolyzate and lard. *Nippon Shokuhin Kogyo Gakkaishi* **1980**, *27*, 51−55.

Yamashoji, S.; Kajimoto, G. Antioxidant effect of Gly-Gly-His on Cu(II)-catalyzed autoxidation and photosensitized oxidation of lipids. *Agric. Biol. Chem.* **1980**, *44*, 2735−2736.

Yee, J. J.; Shipe, W. F.; Kinsella, J. E. Antioxidant effects of soy protein hydrolysates on copper-catalyzed methyl linoleate oxidation. *J. Food Sci.* **1980**, *45*, 1082−1083.

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