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Active oxygen scavenging activity of egg-yolk protein hydrolysates and their effects on lipid oxidation in beef and tuna homogenates

Senji Sakanaka *, Yumi Tachibana

School of Bioresources, Hiroshima Prefectural University, 562 Nanatsuka, Shobara, Hiroshima 727-0023, Japan

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Abstract

Egg-yolk protein hydrolysates were prepared by the enzymic hydrolysis of fat-free egg-yolk protein. The active oxygen scavenging activity, of egg-yolk protein hydrolysates, was investigated using several methods. Egg-yolk protein hydrolysates suppressed discoloration of β-carotene, strongly as compared with the control in β-carotene bleaching method. Superoxide-scavenging activity of egg-yolk protein hydrolysates was measured using the xanthine–xanthine oxidase system. Egg-yolk protein hydrolysates exhibited superoxide-scavenging activity in a dose-dependent manner. Egg-yolk protein hydrolysates also showed 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and hydroxyl radical-scavenging activity. At 0.5% of the hydrolysates, DPPH and hydroxyl radical-scavenging activities were 74.2% and 91.7%, respectively. In food model systems, egg-yolk protein hydrolysates effectively inhibited thiobarbituric acid reactive substances (TBARS) formation from ground beef and tuna homogenates. These results suggest that egg-yolk protein hydrolysates are good source of natural antioxidants.

Keywords: Egg-yolk protein hydrolysates; Radical-scavenging; Lipid oxidation; Beef; Tuna

1. Introduction

Lipid oxidation in foods is a serious problem to the food industry because it results in subsequent development of undesirable off-flavors, odors, dark colors and potentially toxic reaction products (Lin & Liang, 2002; Wang, Pace, Dessai, Bovel-Benjamin, & Philips, 2002). Therefore, the control of lipid oxidation in food products is desirable and the benefits of antioxidants in food storage have been studied by many researchers (Kikuzaki & Nakatani, 1993; Kim & Godber, 2001; Shin & Daigle, 2003). Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate have been commonly added to food products to retard lipid oxidation. However, the demand for natural antioxidants have recently increased because of questions and negative perceptions of consumers about the long-term safety of synthetic antioxidants (Yu et al., 2002).

Many antioxidative substances have been and are being isolated from natural materials including foods. The antioxidative action and the structure of these compounds have been reported by many researchers and several antioxidants have already been developed (Bishov & Henick, 1975; Nagai, Inoue, Inoue, & Suzuki, 2003; Shahidi & Wanasundara, 1992). Amino acids and proteins have been reported as water-soluble antioxidants because of this chelating effect on metal ions (Cervato, Cazzola, & Cestaro, 1999; Lu & Baker, 1986). Furthermore, some protein hydrolysates from animal

* Corresponding author. Tel./fax: +81 824 74 1779.
E-mail address: sakanaka@bio.hiroshima-pu.ac.jp (S. Sakanaka).

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and plant sources have also been found to possess antioxidant activity (Amarowicz & Shahidi, 1997; Pena-Ramos & Xiong, 2002). Use of these antioxidants have been investigated primarily for the prevention of lipid oxidation in foods.

Egg-yolk is widely used as a functional and nutritional ingredient in food products. Main components of egg-yolk are phospholipids, triacylglycerols and proteins. Among them, phospholipids and triacylglycerols are mainly used as food or cosmetic grade yolk lecithin. Egg-yolk protein constitutes about 30% of dried egg-yolk, and it is produced as the by-product of egg-yolk lecithin production. Egg-yolk protein hydrolysates are prepared by the enzymic hydrolysis of the yolk protein, and the hydrolysates are water-soluble and have high nutritional values (Gutierrez et al., 1998).

Egg-yolk has been recognized to contain antioxidant activity in a linoleate emulsion (Yamamoto, Sogo, Iwao, & Miyamoto, 1990). It is reported that egg-yolk phospholipids (King, Boyd, & Sheldon, 1992; Sugino et al., 1997) and egg-yolk phosvitin (Lee, Han, & Decker, 2002; Lu & Baker, 1987) have antioxidant activities, and significant studies on the use of protein hydrolysates of lecithin-free egg-yolk as an antioxidant (Park, Jung, Nam, Shahidi, & Kim, 2001) have been performed only to a limited extent. We also reported the antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system (Sakanaka, Tachibana, Ishihara, & Juneja, 2004). In the present study, we evaluated the action of egg-yolk protein hydrolysates against β-carotene bleaching and free radicals such as superoxide radicals, DPPH radicals, and hydroxyl radicals. In addition, we investigated effects of egg-yolk protein hydrolysates on lipid oxidation in ground beef and tuna homogenates.

2. Materials and methods

2.1. Materials

Linoleic acid, β-carotene, Tween 40, nitro-blue tetrazolium salt, xanthine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, xanthine oxidase (from buttermilk, 0.049 U/ml), sodium dodecyl sulfate (SDS), trichloroacetic acid, 2-thiobarbituric acid, HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) and 1,1,3,3-tetramethoxypropane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ground beef (containing 15.8% fat) and tuna (containing 22.5% fat) were purchased from a local supermarket. All other reagents were of analytical grade.

2.2. Preparation of egg-yolk protein hydrolysates

Egg-yolk protein hydrolysates were prepared as described previously (Sakanaka et al., 2004). In brief, they were prepared as following: eggs from hens were collected, broken and the yolks were separated from the albumen. Yolks were defatted with ethanol at 40 °C under slow agitation. Then the yolk protein fraction was filtered and dried under reduced pressure. Egg-yolk protein hydrolysates were prepared by hydrolysis of the yolk protein by food-grade proteinase from *Bacillus* sp. The yolk protein was dissolved in water at a concentration of around 20% and heat treated at 90 °C before enzyme digestion. Orientase (EC 3.4.21.62; Hankyu Bioindustry, Osaka, Japan) and protease (EC 3.4.11.12; Amano Enzyme Inc., Nagoya, Japan) were used sequentially at pH 10 and 50 °C. The hydrolysis reaction was stopped after 6 h by heating at 90 °C for 10 min. The soluble fraction was then filtered and spray-dried. The powder was used as egg-yolk protein hydrolysates. The molecular mass of the main peak of the hydrolysates measured by gel permeation chromatography (Superdex Peptide HR 10/30 column, Amersham Bioscience Corp., NJ, USA) was lower than 1000.

2.3. Determination of anti-autooxidant activity using the β-carotene bleaching method

Anti-autooxidant activity was assayed using the β-carotene bleaching method (Kauer & Kapper, 2002; Miller, Rice-Evans, Davies, Gopinathan, & Miller, 1993; Wanasundara, Amarowicz, & Shahidi, 1994). β-carotene (2 mg) was dissolved in 20 ml of chloroform. A 4-ml aliquot of the solution was added to a conical flask with 40 mg linoleic acid and 400 mg Tween 40. Chloroform was removed using a rotary evaporator at 50 °C. Distilled water (100 ml) was added to the β-carotene emulsion and mixed, and aliquots (3 ml) of the β-carotene emulsion and 0.2 ml of the sample solution were placed in capped culture tubes and mixed well. The tubes were immediately placed in a water bath and incubated at 50 °C. Oxidation of the carotene emulsion was monitored, taking absorbance at 20 min intervals at 470 nm for 100 min. A control consisted of 0.2 ml of distilled water instead of the sample solution.

2.4. Superoxide radical-scavenging activity

Superoxide radicals were generated in vitro by the xanthine oxidase. The scavenging activity of the egg-yolk protein hydrolysates was determined using the nitro-blue tetrazolium (NBT) reduction method. In this method, O_2^- reduces the yellow dye (NBT^{2+}) to produce the blue formazan, which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation (Cos et al., 1998; Parejo et al., 2002). The capacity of the extracts to scavenge the superoxide radicals was assayed as follows: The reaction mixture contained 0.5 ml of 0.8 mM xanthine in 0.1 mM

phosphate buffer (pH 8.0), 0.48 mM NBT in 0.1 mM phosphate buffer (pH 8.0) and 0.1 ml of the sample solution. After heating to 37 °C for 5 min, the reaction was initiated by adding 1.0 ml of XOD (0.049 U/ml) and carried out at 37 °C for 20 min, the reaction was stopped by adding 2.0 ml of 69 mM SDS. The absorbance of the reaction mixture was measured at 560 nm. The results were calculated as the percentage inhibition according to the following formula:

$$\% \text{ inhibition} = [\{(C - CB) - (S - SB)\}/(C - CB)] \times 100,$$

where S, SB, C, and CB are the absorbance of the sample, the blank sample, the control, and the blank control, respectively.

2.5. DPPH radical-scavenging activity

The assay mixture contained 0.3 ml of the 1.0-mM DPPH radical solution, 2.4 ml of ethanol and 0.3 ml of the sample solution. The solution was rapidly mixed and after standing for 30 min at room temperature, the absorbance of the mixture was measured at 517 nm (Nagai et al., 2003). The results were calculated as the percentage inhibition according to the following formula:

$$\% \text{ inhibition} = [\{(C - CB) - (S - SB)\}/(C - CB)] \times 100,$$

where S, SB, C, and CB are the absorbance of the sample, the blank sample, the control, and the blank control, respectively.

2.6. Hydroxyl radical-scavenging activity

The effect of hydroxyl radicals was assayed using the 2-deoxyribose oxidation method (Chung, Osawa, & Kawakishi, 1997). 2-Deoxyribose is oxidized by hydroxyl radicals formed by the Fenton reaction and degrades to malondialdehyde (Gutteridge, 1984, 1987). The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.4), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM $FeSO_4$–EDTA, 0.15 ml of 10 mM hydrogen peroxide, 0.525 ml of distilled water, and 0.075 ml of the sample solution in a tube. The reaction was started by the addition of hydrogen peroxide. After incubation at 37 °C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% trichloroacetic acid and 0.75 ml of 1.0% thiobarbituric acid. The mixture was boiled for 10 min, cooled in ice, and then measured at 520 nm. Hydroxyl radical-scavenging ability was evaluated as the inhibition rate of 2-deoxyribose oxidation by hydroxyl radicals. The results were calculated as the percentage inhibition according to the following formula:

$$\% \text{ inhibition} = [\{(C - CB) - (S - SB)\}/(C - CB)] \times 100,$$

where S, SB, C, and CB are the absorbance of the sample, the blank sample, the control, and the blank control, respectively.

2.7. Antioxidant activity against lipid peroxidation in ground beef and tuna homogenates

Ground beef (containing 15.8% fat) and tuna (containing 22.5% fat) were used in this experiment. The compositions of fatty acids (saturated fatty acids/monounsaturated fatty acids/polyunsaturated fatty acids) of beef and tuna were 43/55/2 and 25/45/30, respectively. Fatty tuna is known to have high contents of polyunsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid (Kagawa, 2004).

Ground beef and tuna were homogenized (20% w/v and 10% w/v) in 50 mM HEPES buffer (pH 7.0) using a homogenizer (Nihon Seiki Seisakusyo Co., Tokyo, Japan) for 5 min. Buffered systems have been widely used to study oxidation reduction reactions in food model systems (Lee & Hendricks, 1997). The mixture containing 0.8 ml of homogenate (beef or tuna) and 0.2 ml of either the HEPES buffer or one of the test solutions was incubated at 37 °C for 60 min. After incubation, the mixture was tested for the formation of thiobarbituric acid reactive-substances (TBARS).

TBARS were determined by modifying the procedure as shown below (Buege & Aust, 1978; Lee & Hendricks, 1997; Macdonald & Hultin, 1987).On the day of use, a trichloroacetic acid (TCA/TBA) stock solution was prepared consisting of 15% TCA (w/v) and 0.375% TBA (w/v) in 0.25 M HCl. After mild heating and agitation to dissolve the components, 3 ml of 2% butylated hydroxytoluene (BHT) in absolute ethanol was added per 100 ml of the TCA/TBA stock solution. At appropriate intervals, 1.0 ml of aliquot of the sample medium was added to the TCA/TBA stock solution in a test tube and immediately mixed thoroughly with a Vortex mixer. The sample was then heated in a boiling water bath for 10 min and cooled to room temperature, and it was centrifuged at 1710*g* for 10 min. The absorbance of the supernatant was measured at 532 nm using a UNIDEC-50 spectrophotometer (JASCO Corporation, Tokyo, Japan). TBARS were calculated from a standard curve of malonaldehyde (MDA), a breakdown product of tetraethoxypropane (TEP).

2.8. Statistical analysis

Values represent means of triplicate analysis and are given with standard deviations. Differences among experimental data were analyzed by Tukey's studentized

range test, and those at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Antioxidant effect with the β-carotene bleaching method

Egg-yolk protein hydrolysates were analyzed for their antioxidant activity by the β-carotene bleaching assay, because β-carotene shows strong biological activity and is a physiologically important compound (Kumazawa et al., 2002; Sarkar, Bishayee, & Chatterjee, 1995). Furthermore, β-carotene is used as a coloring agent for beverages, and its discoloration would markedly reduce the quality of these products.

The decrease in the absorbance of β-carotene with or without the egg-yolk protein hydrolysates was recorded as a function of time (Fig. 1). Egg-yolk protein hydrolysates strongly suppressed discoloration of β-carotene compared with the control. The absorbance of the control dropped at a faster rate 0.4–0.5 after 60 min, whereas at 0.1% egg-yolk protein hydrolysates, the rate was slower, maintaining an absorbance of 0.75 after 60 min. Thus, it is apparent that egg-yolk protein hydrolysates have strong effects against the discoloration of β-carotene.

3.2. Superoxide radical-scavenging activity

Superoxide radicals have been observed to kill cells, inactivate enzymes and degrade DNA, cell membranes and polysaccharides (Fridovich, 1978). These radicals may also play an important role in the peroxidation of unsaturated fatty acids and possibly other susceptible substances (Nice & Robinson, 1992). Therefore, studying the scavenging effects of egg-yolk protein hydrolysates on superoxide radicals is one of the most important ways of clarifying the mechanism of antioxidant activity.

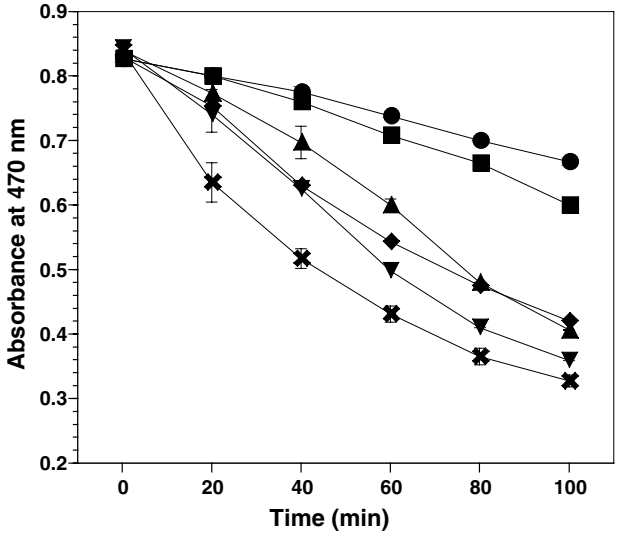

Fig. 1. Antioxidant activity of egg-yolk protein hydrolysates (EYPH) measured using β-carotene bleaching method. Control, ×; 0.1% of EYPH, ●; 0.05% of EYPH, ■; 0.025% of EYPH, ▲; 0.0125% of EYPH, ◆; 0.00625% of EYPH, ▼. Data represent the means ±SD of three determinations.

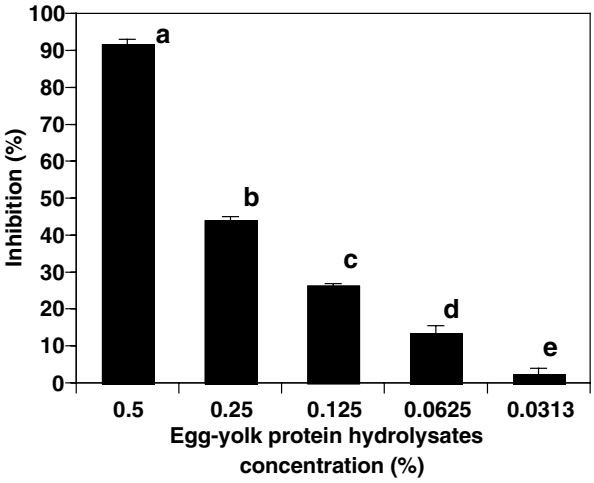

Fig. 2. Superoxide radical-scavenging activity of egg-yolk protein hydrolysates. Data represent the means ± SD of three determinations. Bars with different letter are significantly different ($p < 0.05$).

The superoxide-scavenging activity of egg-yolk protein hydrolysates was measured using the xanthine–xanthine oxidase system, and the results were indicated as the inhibition rate of superoxide activity. Egg-yolk protein hydrolysates exhibited superoxide-scavenging activity and these activities were dose-dependent (Fig. 2). These results show that egg-yolk protein hydrolysates have strong superoxide-radical-scavenging effects.

3.3. DPPH radical-scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Benvenuti, Pellati, Melegari, & Bertelli, 2004; Hatano, Takagi, Ito, & Yoshida, 1997; Shimoji et al., 2002). Fig. 3 shows the scavenging activity of egg-yolk protein hydrolysates on DPPH radicals at various concentrations. The scavenging activity of egg-yolk protein hydrolysates on DPPH radicals increased with increasing concentrations (0 to 1%). According to Fig. 3, egg-yolk protein hydrolysates (0.5%) exhibited 83.5% scavenging activity. The result shows that egg-yolk protein hydrolysates is also a free radical scavenger, particularly of the peroxyl radical, which is the major propagator of the oxidation chain of fat, thereby terminating the chain reaction (Frankel, 1991; Yen, Chang, & Chen, 2002).

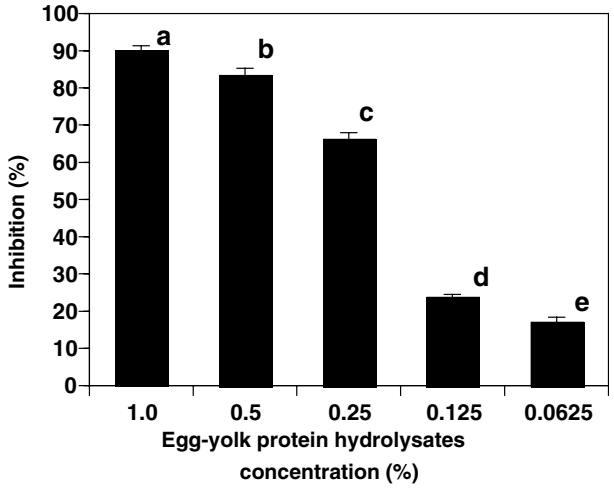

Fig. 3. DPPH radical-scavenging activity of egg-yolk protein hydrolysates. Data represent the means ± SD of three determinations. Bars with different letter are significantly different ($p < 0.05$).

3.4. Hydroxyl radical-scavenging activity

Among the oxygen radicals, the hydroxyl radical is the most reactive and severely damages adjacent biomolecules. The scavenging effect against hydroxyl radicals was investigated by using the Fenton reaction. Fig. 4 shows the hydroxyl radical-scavenging effects of egg-yolk protein hydrolysates using the 2-deoxyribose oxidation method. The results are shown as the inhibition rate. Egg-yolk protein hydrolysates showed hydroxyl radical-scavenging activity and its activity increased with an increasing concentration of the sample. A 0.5% concentration of egg-yolk protein hydrolysates exhibited 74.2% scavenging activity.

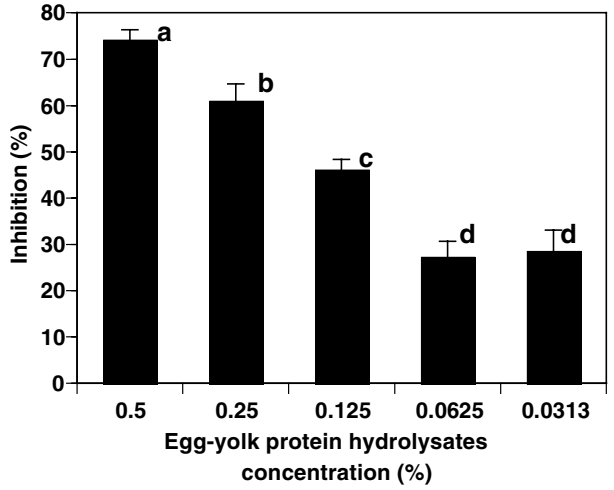

Fig. 4. Hydroxyl radical-scavenging activity of egg-yolk protein hydrolysates. Data represent the means ± SD of three determinations. Bars with different letter are significantly different ($p < 0.05$).

3.5. Antioxidant effects in ground beef and tuna homogenates

As mentioned above, egg-yolk protein hydrolysates showed antioxidant activity with four different in vitro methods. In addition, their activity was tested using food model systems. The lipid contents of the ground beef and the raw tuna were 15.8% and 22.5%, respectively. Egg-yolk protein hydrolysates were added to the homogenates at different concentrations, and lipid oxidation in the homogenates was evaluated. The reaction was measured by monitoring TBARS, and the results are shown in Figs. 5 and 6. In ground beef (20% w/v) homogenized with 50 mM HEPES buffer at pH 7.0, egg-yolk protein hydrolysates effectively inhibited the formation of TBARS in a dose-dependent manner (Fig. 5). With 0.5% of egg-protein hydrolysates, the inhibition rate was 91.7%.

The tuna used in this experiment was raw fatty tuna, and it contained 22.5% fat. Tuna is known to have high contents of polyunsaturated fatty acids, and it is well known to be oxidized easily. Egg-protein hydrolysates was inhibited the formation of TBARS in the tuna homogenates (Fig. 6). With 1% and 2% of egg-protein hydrolysates, inhibitions were 43.8% and 65.7%, respectively.

The antioxidant activity was assayed using several different test systems. Recent investigations show differences between the test systems in determining antioxidant activity (Gahler, Otto, & Böhm, 2003; Schlesier, Harwat, Böhm, & Bitsch, 2002). Using at least two methods is recommended. In this study, we used several methods showing different sensitivity and using different systems. Egg-yolk protein hydrolysates showed antioxidant activity in all of the different in vitro assay systems.

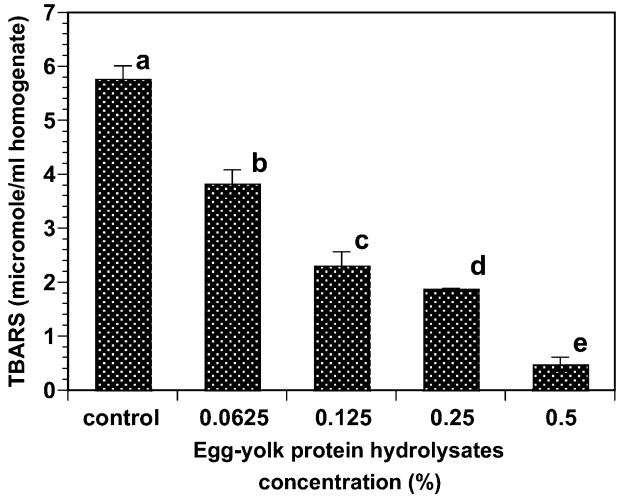

Fig. 5. Effects of egg-yolk protein hydrolysates on the formation of TBARS in ground beef homogenates. Data represent the means ± SD of three determinations. Bars with different letter are significantly different ($p < 0.05$).

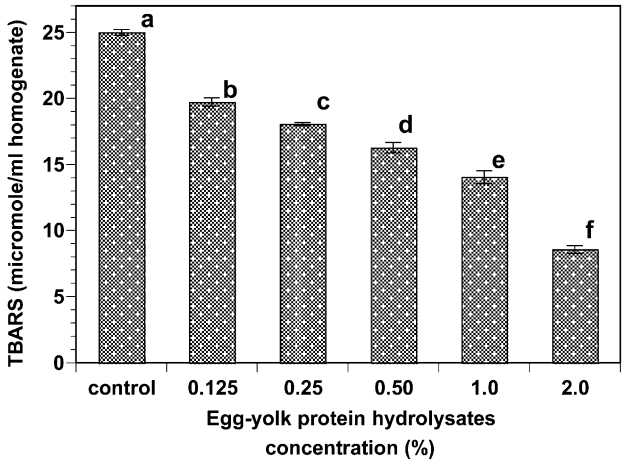

Fig. 6. Effects of egg-yolk protein hydrolysates on the formation of TBARS in tuna homogenates. Data represent the means ± SD of three determinations. Bars with different letter are significantly different ($p < 0.05$).

These results suggest that egg-yolk protein hydrolysates are a good source of natural antioxidant. Further work on the characterization of antioxidant compounds in the egg-yolk protein hydrolysates is in progress to establish the connection between antioxidant activity and chemical composition.

4. Conclusions

Egg-yolk protein hydrolysates were found to be effective antioxidants in different in vitro assay systems. When incorporated into beef and fatty tuna homogenates, egg-yolk protein hydrolysates effectively inhibit lipid oxidation in both homogenates. Egg-yolk protein hydrolysates may be as useful in meat and fish processing as other naturally occurring antioxidants, helping to prevent the formation of off-flavor in meat and fish and their products and increasing shelf life.

References

Amarowicz, R., & Shahidi, F. (1997). Antioxidant activity of peptide fraction of capelin protein hydrolysates. *Food Chemistry, 58*, 355–359.

Benvenuti, S., Pellati, F., Melegari, M., & Bertelli, D. (2004). Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus*, *Ribes*, and *Aronia*. *Journal of Food Science, 69*, FCT164–FCT169.

Bishov, S. J., & Henick, A. S. (1975). Antioxidant effect of protein hydrolysates in freeze-dried model system. *Journal of Food Science, 40*, 345–348.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology, 52*, 302–304.

Cervato, G., Cazzola, R., & Cestaro, B. (1999). Studies on the antioxidant activity of milk caseins. *International Journal of Food Science and Nutrition, 50*, 291–296.

Chung, S. K., Osawa, T., & Kawakishi, S. (1997). Hydroxyl radical-scavenging effects of species and scavengers from brown mustard (*Brassica nigra*). *Bioscience, Biotechnology and Biochemistry, 61*, 118–123.

Cos, P., Ying, L. Y., Calomme, M., Hu, J. H., Cimanga, K., Van Poel, B., Pieters, L., Vlietinck, A. J., & Berghe, D. V. (1998). Structure-activity relationships and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *Journal of Natural Products, 61*, 71–76.

Frankel, E. N. (1991). Recent advances in lipid oxidation. *Journal of the Science of Food and Agriculture, 54*, 495–511.

Fridovich, I. (1978). The biology of oxygen radicals. *Science, 201*, 875–880.

Gahler, S., Otto, K., & Böhm, V. (2003). Alterations of vitamin C, total polyphenols, and antioxidant capacity as affected by processing tomatoes to different products. *Journal of Agricultural and Food Chemistry, 51*, 7962–7968.

Gutierrez, M. A., Mitsuya, T., Hatta, H., Koketsu, M., Kobayashi, R., Juneja, L. R., et al. (1998). Comparison of egg-yolk protein hydrolysates and soybean protein hydrolysates in terms of nitrogen utilization. *British Journal of Nutrition, 80*, 477–484.

Gutteridge, J. M. (1984). Reactivity of hydroxyl and hydroxyl-like radicals discriminated by release of thiobarbituric acid-reactive material from deoxy sugars, nucleosides and benzoate. *Biochemical Journal, 224*, 761–767.

Gutteridge, J. M. (1987). Ferrous-salt-promoted damage to deoxyribose and benzoate. The increased effectiveness of hydroxyl-radical scavengers in the presence of EDTA. *Biochemical Journal, 243*, 709–714.

Hatano, T., Takagi, M., Ito, H., & Yoshida, T. (1997). Phenolic constituents of liquorice. VII. A new calcone with a potent radical scavenging activity and accompanying phenols. *Chemical and Pharmaceutical Bulletin, 45*, 1485–1492.

Kagawa, Y. (2004). *Standard tables of food composition in Japan*. Tokyo, Japan: Kagawa Nutrition University Publishing Division.

Kauer, C., & Kapper, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology, 37*, 153–161.

Kikuzaki, H., & Nakatani, N. (1993). Antioxidant effects of some ginger constituents. *Journal of Food Science, 58*, 1407–1410.

Kim, J. S., & Godber, J. S. (2001). Oxidative stability and vitamin E levels increased in restructured beef roasts with added rice bran oil. *Journal of Food Quality, 24*, 17–26.

King, M. F., Boyd, L. C., & Sheldon, B. W. (1992). Antioxidant properties of individual phospholipids in a salmon oil model system. *Journal of American Oil Chemists' Society, 69*, 545–551.

Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M. S., & Nakayama, T. (2002). Antioxidant activity of polyphenols in carob pods. *Journal of Agricultural and Food Chemistry, 50*, 373–377.

Lee, B. J., & Hendricks, D. G. (1997). Antioxidant effects of L-carnosine on liposomes and beef homogenates. *Journal of Food Science, 62*, 931–934.

Lee, S. K., Han, J. H., & Decker, E. A. (2002). Antioxidant activity of phosvitin in phosphatidylcholine liposomes and meat model systems. *Journal of Food Science, 67*, 37–41.

Lin, C. C., & Liang, J. H. (2002). Effect of antioxidants on the oxidative stability of chicken breast meat in a dispersion system. *Journal of Food Science, 67*, 530–533.

Lu, C. L., & Baker, R. C. (1986). Characteristic of egg yolk phosvitin as an antioxidant for inhibiting metal-catalyzed phospholipids oxidations. *Poultry Science, 65*, 2065–2070.

Lu, C. L., & Baker, R. C. (1987). Effect of pH and food ingredients on the stability of egg yolk phospholipids and the metal-chelator antioxidant activity of phosvitin. *Journal of Food Science, 52*, 613–616.

Macdonald, R. E., & Hultin, H. O. (1987). Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. *Journal of Food Science, 52*, 15–27.

Miller, N. J., Rice-Evans, C. A., Davies, M. J., Gopinathan, V., & Miller, A. (1993). A novel method of measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science, 84*, 407–412.

Nagai, T., Inoue, R., Inoue, H., & Suzuki, N. (2003). Preparation and antioxidant properties of water extract of propolis. *Food Chemistry, 80*, 29–33.

Nice, D. J., & Robinson, D. S. (1992). Inhibition of lipid autoxidation by bovine superoxide dismutase. *Food Chemistry, 45*, 99–103.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J., et al. (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *Journal of Agricultural and Food Chemistry, 50*, 6882–6890.

Park, P. J., Jung, W. K., Nam, K. S., Shahidi, F., & Kim, S. K. (2001). Purification and characterization of antioxidative peptides from protein hydrolysates of lecithin-free egg yolk. *Journal of American Oil Chemists' Society, 78*, 651–656.

Pena-Ramos, E. A., & Xiong, Y. L. (2002). Antioxidant activity of soy protein hydrolysates in a liposomal system. *Journal of Food Science, 67*, 2952–2956.

Sakanaka, S., Tachibana, Y., Ishihara, N., & Juneja, L. R. (2004). Antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system. *Food Chemistry, 86*, 99–103.

Sarkar, A., Bishayee, A., & Chatterjee, M. (1995). β-Carotene prevents lipid peroxidation and red blood cell membrane protein damage in experimental hepato carcinogenesis. *Cancer Biochemistry Biophysics, 15*, 111–125.

Schlesier, K., Harwat, M., Böhm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research, 36*, 177–187.

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Review in Food Science and Nutrition, 32*, 67–103.

Shimoji, Y., Tamura, Y., Nakamura, Y., Nanda, K., Nishidai, S., Nishikawa, Y., et al. (2002). Isolation and identification of DPPH radical scavenging compounds in kurosu (Japanese unpolished rice vinegar). *Journal of Agricultural and Food Chemistry, 50*, 6501–6503.

Shin, F. F., & Daigle, K. W. (2003). Antioxidant properties of milled-rice co-products and their effects on lipid oxidation in ground beef. *Journal of Food Science, 68*, 2672–2675.

Sugino, H., Ishikawa, M., Nitoda, T., Koketsu, M., Juneja, L. R., Kim, M., et al. (1997). Antioxidative activity of egg yolk phospholipids. *Journal of Agricultural and Food Chemistry, 45*, 551–554.

Wanasundara, U., Amarowicz, R., & Shahidi, F. (1994). Isolation and identification of an antioxidative component in canola meal. *Journal of Agricultural and Food Chemistry, 42*, 1285–1290.

Wang, B., Pace, R. D., Dessai, A. P., Bovel-Benjamin, A., & Philips, B. (2002). Modified extraction method for determining 2-thiobarbituric acid values in meat with increased specificity and simplicity. *Journal of Food Science, 67*, 2833–2836.

Yamamoto, Y., Sogo, N., Iwao, R., & Miyamoto, T. (1990). Antioxidant effect of egg yolk on linoleate in emulsion. *Agricultural and Biological Chemistry, 54*, 3099–3104.

Yen, G. C., Chang, Y. C., & Chen, J. P. (2002). Antioxidant activity of mycelia from *Aspergillus candidus*. *Journal of Food Science, 67*, 567–572.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., & Qian, M. (2002). Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry, 50*, 1619–1624.