Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 86 (2004) 99–103
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system

Senji Sakanaka [a,*], Yumi Tachibana [a], Noriyuki Ishihara [b], Lekh Raj Juneja [b]

[a] School of Bioresources, Hiroshima Prefectural University, 562 Nanatsuka, Shobara, Hiroshima 727-0023, Japan
[b] Central Research Laboratories, Taiyo Kagaku Co., Ltd., 1-3 Takaramachi, Yokkaichi, Mie 510-0844, Japan

Received 7 March 2003; received in revised form 14 August 2003; accepted 14 August 2003

Abstract

The antioxidant activities of egg-yolk protein hydrolysates in a linoleic acid system were investigated. Egg-yolk protein hydrolysates were prepared by enzymic hydrolysis of fat-free egg-yolk protein, which led to the main peak of the molecular mass distribution of lower than 1000. Egg-yolk protein hydrolysates showed strong antioxidant activity in a linoleic acid oxidation system as compared with the egg-yolk protein or amino acids mixture in which egg-yolk protein hydrolysates were constituted. The effect of the sample was concentration-dependent. Egg-yolk protein hydrolysates also showed strong antioxidant activities on cookies containing linoleic acid. These results suggest that egg-yolk protein hydrolysates could be a suitable natural antioxidant for preventing the oxidation of polyunsaturated fatty acids and related food ingredients.

Keywords: Antioxidant activity; Egg-yolk protein hydrolysates; Linoleic acid; Peroxide value

1. Introduction

Lipid oxidation is of great concern to the food industry because it results in subsequent development of undesirable off-flavours, odours, dark colours and potentially toxic reaction products (Lin & Liang, 2002; Wang, Pace, Dessai, Bovell-Benjamin, & Phillips, 2002). Therefore, the control of lipid oxidation in food products is desirable. Synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, may be added to food products to retard lipid oxidation. However, the demand for natural antioxidants has recently increased because of questions about the long-term safety and negative consumer perception of synthetic antioxidants (Yu et al., 2002).

Many antioxidative substances have been and are being isolated from natural materials, including foods. Antioxidative action and the structure of those 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). Water-soluble antioxidants, such as amino acids and proteins, have been reported, because of their chelating effects of metal ions (Lu & Baker, 1986). Furthermore, some protein hydrolysates from animal and plant sources have also been found to possess antioxidant activity (Amarowicz & Shahidi, 1997; Pena-Ramos & Xiong, 2002). These antioxidants have been investigated mainly in 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 triacylglycerols, phospholipids, proteins, and carbohydrates. Among these, triacylglycerols and phospholipids are mainly used as food- or cosmetic-grade yolk-lecithin. Egg-yolk protein is produced as the residue of egg yolk (after extraction to give yolk-lecithin by organic solvent). Egg-yolk protein hydrolysates are prepared by the enzymic hydrolysis of the yolk protein, and the hydrolysates are water-soluble and have high nutritional value.

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

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.08.014

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) 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 but to a limited extent. In the present investigation, the antioxidant activities of egg-yolk protein hydrolysates in a linoleic acid oxidation system and cookies containing linoleic acid were investigated and were compared with those of egg-yolk protein and amino acids.

2. Materials and methods

2.1. Chemicals

Linoleic acid was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Amino acids, ammonium thiocyanate, and ferrous chloride were from Waco Pure Chemical Industries (Osaka, Japan). The other chemicals were of analytical grade.

2.2. Preparation of egg-yolk protein hydrolysates

Eggs from hens were collected and 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 Bio-industry, Osaka, Japan) and protease (EC 3.4.11.12; Amano Enzyme, Nagoya, Japan) were used sequentially at pH 10 and 50 °C. The hydrolysis reaction was stopped after 6 h by heating to 90 °C for 5 min. The soluble fraction was then filtered and spray-dried. The powder was used as egg-yolk protein hydrolysates.

2.3. Analytical method for egg-yolk protein hydrolysates

The amino acid content of egg-yolk protein hydrolysates was determined by the method described previously (Gutierrez et al., 1998). The molecular mass distribution of egg-yolk protein hydrolysates was estimated by gel permeation chromatography (Superdex Peptide HR 10/30, Amersham Biosciences Corp., NJ, USA) with UV detection at an optical density of 210 nm. The mean number of amino acids of egg-yolk protein hydrolysates was determined by the HCl hydrolysis method described by Nakano, Shimatani, Murakami, Sato, and Idota (1994).

2.4. Measurement of antioxidant activity

Each sample which was dissolved in 1.5 ml of 0.1 M phosphate buffer (pH 7.0) and 1.0 ml of 50 mM linoleic acid in ethanol (99.5%) was mixed in test tubes (5 ml volume). The tubes were sealed tightly with silicon rubber caps and kept at 40 °C in the dark. At regular intervals, aliquots of the reaction mixtures were withdrawn with a micro-syringe for measurement of the oxidation, using the ferric thiocyanate method with a slight modification (Mitsuda, Yasumoto, & Iwami, 1966; Chen, Muramoto, & Yamauchi, 1995).

2.5. Ferric thiocyanate method

To 50 μl of the reaction mixture were added 2.35 ml of 75% ethanol, 50 μl of 30% ammonium thiocyanate and 50 μl of 20 mM ferrous chloride solution in 3.5% HCl. After 3 min, absorbance of the coloured solution at 500 nm was measured in a 1 cm cuvette with a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan).

2.6. Cookie formula

Cookies were formulated with a simplified recipe with flour (100 g), linoleic acid (20 g), water (20 g) and each sample (10 g) to avoid the influence of other ingredients (Yamamoto, Sogo, Iwao, & Miyamoto, 1991). Flour was sifted and added to the linoleic acid, water and each sample. The doughs were formatted to 3 cm diameter discs and wrapped with a clear-plastic wrap. They were kept for 1 h in the freezer. The dough was formatted to 3 cm diameter and 7 mm thick cookies using a cutter. The cookies were baked in an oven at 180 °C for 15 min. The cookies were allowed to cool at room temperature for 2 h. They were kept at 40 °C to evaluate the oxidative stability.

2.7. Analysis of oxidative stability of cookies

The antioxidant activity of the egg-yolk protein hydrolysates was evaluated by measuring the peroxide value (POV) in the solvent extracts of cookies (Hattori, Yamaji, Kumagai, Feng, & Takahashi, 1998; Takano, 2000). The POV was measured by putting the sample (1 g) into an Erlenmeyer flask (100 ml) and adding an acetic acid/chloroform (3:2) solution (25 ml). After nitrogen gas was flushed into the Erlenmeyer flask, a saturated potassium iodide solution (1 ml) was added. The flask was immediately sealed, shaken gently for 1 min, and allowed to stand in the dark for 5 min. After distilled water (75 ml) was added with vigorous stirring, the solution was titrated with 0.01 N sodium thiosulfate solution, using a 1% starch solution (400 μl) as the indicator.

3. Results and discussion

3.1. Characteristics of egg-yolk protein hydrolysates

Fig. 1 shows the gel permeation chromatography profiles of egg-yolk protein hydrolysates. The main peak of the molecular mass distribution of egg-yolk protein hydrolysates was lower than 1000. The average chain length of the egg-yolk protein hydrolysates was 2.6. The amino acid compositions of egg-yolk protein hydrolysates and egg-yolk protein are shown in Table 1. The amino acid mixtures used in this study were prepared, based on this composition, in order to compare it with the egg-yolk protein hydrolysates.

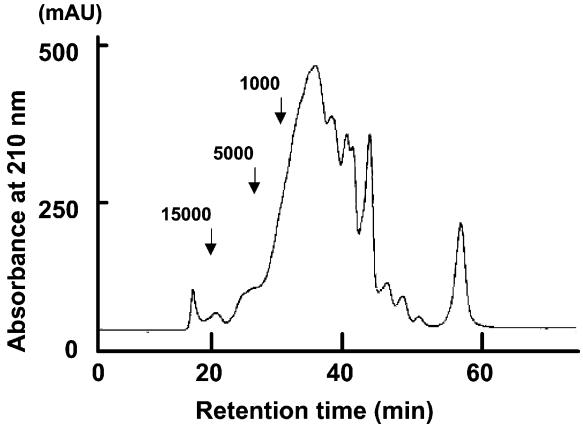

Fig. 1. Gel filtration patterns of egg-yolk protein hydrolysates. The arrows indicate the elution times of molecular mass markers.

Table 1
Amino acid composition of egg-yolk protein hydrolysates and egg-yolk protein (mg/g protein)

Amino acid	Egg-yolk protein hydrolysates	Egg-yolk protein
Threonine	54	48
Tyrosine	41	42
Phenylalanine	42	43
Cystine	15	20
Methionine	26	26
Valine	62	56
Isoleucine	51	50
Leucine	90	84
Lysine	75	71
Tryptophan	11	15
Histidine	27	25
Aspartic acid[a]	102	91
Serine	85	79
Glutamic acid[b]	134	119
Proline	41	39
Glycine	33	30
Alanine	59	50
Arginine	70	69

[a] Asparagine + aspartic acid.
[b] Glutamine + glutamic acid.

3.2. Antioxidant activity of egg-yolk protein hydrolysates

Egg-yolk protein hydrolysates showed stronger antioxidant activity than the control. The control was rapidly oxidized after 5-days storage at 40 °C, but the hydrolysates completely inhibited the oxidation of linoleic acid at the concentration of 0.0125% (Fig. 2). The egg-yolk protein, which was a material of the hydrolysates, also showed more effective activity than the control. The activity of the protein was not so strong as that of the hydrolysates; it inhibited the oxidation at concentrations of more than 0.025% (Fig. 3). The amino acid mixture showed a relatively weak antioxidant effect in comparison with both the hydrolysates and the protein (Fig. 4). The antioxidant activity was increased with increasing concentration of the hydrolysates.

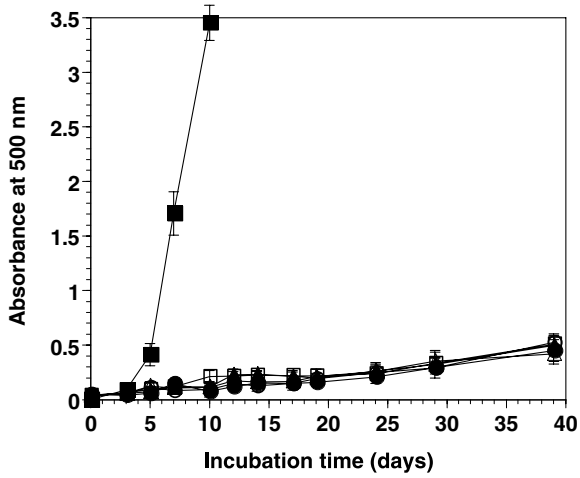

Fig. 2. Effect of egg-yolk protein hydrolysates on oxidation of linoleic acid at 40 °C. Control (■), 0.2% (●), 0.1% (▲), 0.05% (□), 0.025% (○), 0.0125% (△).Values are means ± SD ($n = 3$).

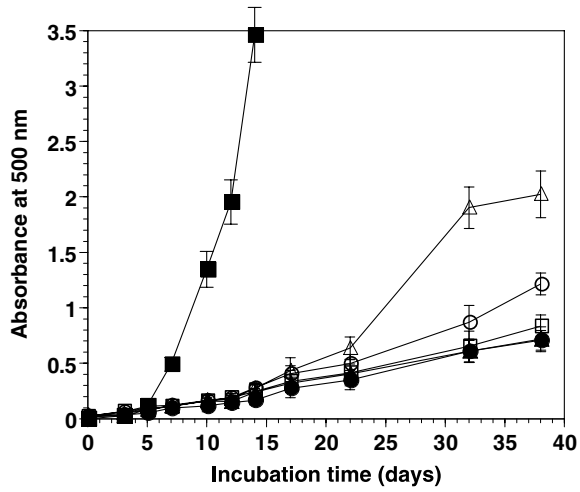

Fig. 3. Effect of egg-yolk protein on oxidation of linoleic acid at 40 °C. Control (■), 0.2% (●), 0.1% (▲), 0.05% (□), 0.025% (○), 0.0125% (△).Values are means ± SD ($n = 3$).

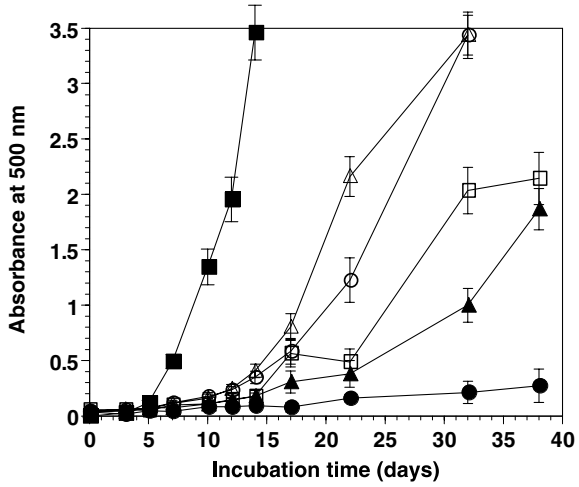

Fig. 4. Effect of amino acids mixture on oxidation of linoleic acid at 40 °C. Control (■), 0.2% (●), 0.1% (▲), 0.05% (□), 0.025% (○), 0.0125% (△).Values are means ± SD ($n = 3$).

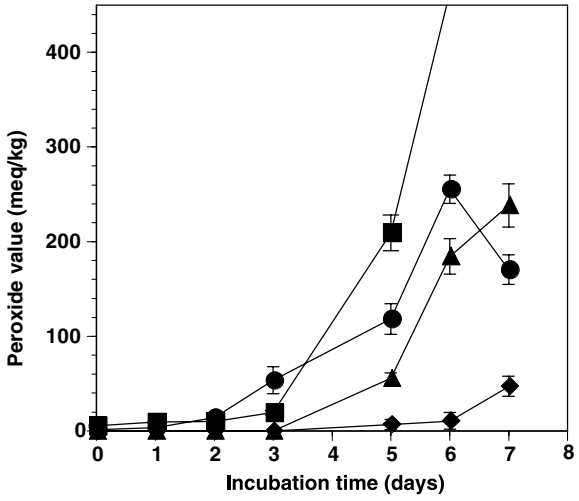

Fig. 5. Antioxidant activities of egg-yolk protein, egg-yolk protein hydrolysates and amino acids mixture on cookies containing linoleic acid at 40 °C. Control (■), egg-yolk protein (●), egg-yolk protein hydrolysates (◆), amino acids mixture (▲). Values are means ± SD ($n = 3$).

3.3. Antioxidant effect of egg-yolk protein hydrolysates on oxidation of cookies containing linoleic acid

The POV of cookies containing linoleic acid was rapidly increased after 3 days storage at 40 °C (Fig. 5). This method was simple and effective in comparing the antioxidant activities of the samples. Cookies containing linoleic acid with egg-yolk protein or amino acids mixture showed the increase of POV, but it increased slowly in comparison with that of the control. Cookies with added egg-yolk protein hydrolysates showed strong antioxidant activity on linoleic acid and the increase of POV was not observed during this experimental period. Their activities, in order of their strengths, were egg-yolk protein hydrolysates, amino acids mixture, and egg-yolk protein. These results showed that egg-yolk protein hydrolysates were more effective on the antioxidation of linoleic acid than egg-yolk protein or amino acids mixture, which were the material or components of the hydrolysates.

Amino acids are known to be effective, both as primary antioxidants and as synergists (Bishov & Henick, 1975). In this study, amino acids inhibited the oxidation of linoleic acid. However, their ability was not as strong as the egg-yolk protein hydrolysates. The egg-yolk protein hydrolysates could be used as antioxidants in foods, because other natural products have been proposed for use as antioxidants, including amino acids, protein hydrolysates and proteins (Kawashima, Itoh, & Chibata, 1979; Taylor, Richardson, & Jasensky, 1981).

Antioxidant peptides have previously been isolated from lecithin-free egg yolk (Park et al., 2001). The isolated peptides were composed of 10 and 15 amino acid residues. As shown in this study, the egg-yolk hydrolysates, in which the mean number of amino acids was 2.6, showed the antioxidant activity. Furthermore, the protein hydrolysates inhibited the lipid oxidation in cookies. The antioxidant activity of the hydrolysates is thought to be related to their amino acid sequence. It is well known that nutritional values of egg protein and egg peptides (hydrolysates) have been extensively evaluated (Gutierrez et al., 1998; Sakanaka, Kitahata, Mitsuya, Gutierrez, & Juneja, 2000). Their high nutritional values have been used for the nutritional enhancement of several kinds of foods. The antioxidant activity is a new functional characteristic of egg-yolk protein hydrolysates and this characteristic will expand the use of egg-yolk protein hydrolysates as a food material.

Our results suggest that egg-yolk protein hydrolysates could be a suitable natural antioxidant in preventing the oxidation of polyunsaturated oils and related food ingredients, which are susceptible to oxidation.

References

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

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

Chen, H. M., Muramoto, K., & Yamauchi, F. (1995). Structure analysis of antioxidative peptides from soybean β-conglycinin. *Journal of Agricultural and Food Chemistry, 43*, 574–578.

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

Hattori, M., Yamaji, T. K., Kumagai, H., Feng, Y., & Takahashi, K. (1998). Antioxidative activity of soluble elastin peptides. *Journal of Agricultural and Food Chemistry, 46*, 2167–2170.

Kawashima, K., Itoh, H., & Chibata, I. (1979). Synergistic ternary antioxidant compositions comprising tocopherol, partially hydrolyzate of gelatin and organic acid. *Agricultural and Biological Chemistry, 43*, 827–831.

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

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 phospholipid 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.

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

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

Nakano, T., Shimatani, M., Murakami, M., Sato, N., & Idota, T. (1994). Digestibility and absorption of enzymatically hydrolyzed whey protein. *Japanese Journal of Food and Nutrition, 47*, 195–201.

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 the 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., Kitahata, K., Mitsuya, T., Gutierrez, M. A., & Juneja, L. R. (2000). Protein quality determination of delipidated egg-yolk. *Journal of Food Composition and Analysis, 13*, 773–781.

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

Takano, K. (2000). Peroxide lipid. In T. Sugahara & A. Maekawa (Eds.), *Handbook of foods analysis* (pp. 100–102). Tokyo: Kenpakusya Co., Ltd.

Taylor, M. J., Richardson, T., & Jasensky, R. D. (1981). Antioxidant activity of amino acids bound to Trolox-C. *Journal of the American Oil Chemists' Society, 58*, 622–626.

Wang, B., Pace, R. D., Dessai, A. P., Bovell-Benjamin, A., & Phillips, 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.

Yamamoto, Y., Sogo, N., Iwao, R., & Miyamoto, T. (1991). Antioxidant activity of egg yolk during baking and storage of cookies. *Nippon Shokuhin Kogyo Gakkaishi, 38*, 184–188.

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.