Original Article

Antioxidative activity of hen egg ovalbumin hydrolysates

Mingsheng Xu PhD[1,2], Xinchen Shangguan PhD[2], Wenjun Wang PhD[2], Jinping Chen MD[1]

[1] *Department of Food Engineering, Shaanxi Normal University, Xi´an, China*
[2] *Department of Food Science, Jiangxi Agricultural University, Nanchang China*

To evaluate the antioxidative activity of the hydrolysates of ovalbumin, the antioxidative activities of the enzymatic extracts were evaluated using three different methodologies scavenging assays such as superoxide anion, hydroxyl radical, and inhibitory oxidation of linoleic acid in vitro, and the activities of SOD, GSH-PX, CAT and the level of MDA were determined in serum and liver of aged mice induced by G-gal. The results showed that the hydrolysates had a distinctly inhibitory action to superoxide anion ($O_2^{\cdot-}$) made by alkaline pyrogallic acid, HO• produced by Fenton reaction, the oxidation of linoleic acid in linoleic acid autoxidation system, and presented a positive correlation. The inhibition capacity of hydrolysates against $O_2^{\cdot-}$ and HO• were more than 45% and 56% respectively at the concentration 5 mg/mL. And the hydrolysates could significantly ($p< 0.01$) prevented the activities of SOD, GSH-Px, and CAT against reducing and all three concentrations could significantly ($p< 0.01$) decrease the MDA contents in the serum and liver of aged mice induced by G-gal. The antioxidative activity of high concentration was similar to that of control group.

Key Words: ovalbumin, hydrolysates, antioxidative activity, mice

Introduction

Oxidation of biomolecules has been identified as a free radical-mediated process, which causes many unfavorable impacts on food and biological systems. In aerobic organisms, harmful radicals that inevitably form during the metabolism of oxygen are associated with the occurrence of several disease conditions including atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders and some types of cancer as well as aging.[1,2] Cellular antioxidative enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and some food-derived nutritional antioxidants protect tissues from freeradical-mediated oxidative injuries.[3] Therefore, there is a growing interest in identifying antioxidative properties in many natural sources including some dietary protein compounds. Chen *et al*. has reported that enzymatically derived soybean peptides can act as potent antioxidants against lipid peroxidation and the primary structure of those peptides were critical for their activity.[4,5] Hu *et al*. has reported that hydrolysed of casein could raise activities of SOD and GSH-Px in plasma and liver of mice, reduced the level of malondialdehyde (MDA).[6] Recently, a research group reported the antioxidant activity of peptides produced by enzymatic hydrolysis of crude egg white with pepsin.[7]

To evaluate the antioxidant activity of the hydrolysates of egg white proteins, we determined in vitro radical scavenging activities by the auto-oxidation of pyrogallol, the α-deoxyribose oxidation method, and linoleic acid model system. Furthermore, the effect of hydrolysates on antioxidant enzyme activity and level MDA in serum and liver of mice were detected.

Materials and methods

Preparation of Ovalbumin Hydrolysates

Ovalbumin hydrolysates were prepared by hydrolysis of ovalbumin (grade V, Sigma) with pepsin (P7000, sigma). The ovalbumin was dissolved in water at a concentration of around 4% and heated at 90°C before enzyme digestion. Pepsin was used sequentially at pH 2 and 37 °C. The hydrolysis reaction was stopped after 6 h by heating to 90°C for 5 min. The hydrolysate fraction with a molecular weight lower than 3,000 Da was lyophilized for activity testing.

Superoxide anion scavenging activity assay

Superoxide anion scavenging activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a slightly modified method of Marklund and Marklund.[8] A sample solution (0.1 mL) and 2.8 mL of 50 mmol/L phosphate buffer (pH 8.2) were added into freshly prepared 0.1 mL of 0.6 mmol/L pyrogallol (dissolved in 10 mmol/L HCl). The inhibition rate of pyrogallol autooxidation was measured at 325 nm. Absorbance of each extract was recorded at every 0.5 min for 4 min and the increment of absorbance was calculated by the difference (the absorbance at 10 min—the absorbance at the starting time).

Corresponding Author: Professor Mingsheng Xu, Department of Food Science, Jiangxi Agricultural University, Nanchang China 330045
Tel: 86 791 3813 655; Fax: 86 791 3813 655
Email: xmsjy3418@163.com

Hydroxyl radical scavenging activity assay

Hydroxyl radical scavenging activity was determined according to a slightly modified method of the α- deoxyribose oxidation method. [9] Hydroxyl radical was generated by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. A reaction mixture containing each 0.2 mL of 10 mmol/L $FeSO_4$- EDTA and 0.5 mL of 10 mmol/L α-deoxyribose was mixed with 0.2 mL of the extract solution was added into the reaction mixture until the total volume reached 1.8 mL. Then 0.2 mL of 10 mmol/L H_2O_2 was added into the reaction mixture and incubated at 37°C for 1 h. After incubation, each 1 mL of 2.8% trichloroacetic acid (TCA) and 1.0% thiobarbituric acid (TBA) were added. Then, the mixture was placed in a boiling water bath for 15 min. Absorbance was measured at 532 nm.

Lipid peroxidation inhibition assay

Linoleic acid was oxidized in a linoleic acid model system to measure the antioxidative activity following the method of Osawa and Namiki with slight modifications. [10] Briefly, the sample was dissolved in 5 mL of 50 mmol/L phosphate buffer (pH 7.0) and added into a mixture of 99.5% ethanol (5 mL) and linoleic acid (0.065 mL) in which the final volume was adjusted to 12.5 mL with distilled water. The mixed solution in a slightly sealed screw-cap conical tube was incubated at 40℃ in the dark. The degree of linoleic acid oxidation was measured at 24-h intervals by ferric thiocyanate method described by Mitsuda *et al*. [11] Aliquot (0.1 mL) of reaction mixture was mixed with 75% ethanol (4.7 mL), 30% ammonium thiocyanate (0.1 mL) and 0.02 mol/L ferrous chloride (0.1 mL) in 3.5% HCl. After 3 min, degree of color development that represented linoleic acid oxidation was measured spectrophotometrically at 500 nm.

Animals and experimental design

180 healthy Kunming male mice were selected and divided into 6 groups randomly, one as control group (NC), the other five groups were aged mice group induced by D-galactose (D-gal), ovalbumin group (Ova), low dose of hydrolysates group (L), medium dose of hydrolysates group (M), high dose of hydrolysates group (H), each group with two repeats. The mice were supplemented with 0.25 mL normal saline for NC, and other groups administrated with 150 mg/kg D-gal for 30-d. After that, NC and D-gal groups were administrated with 0.25 mL/d distilled water, and Ova, L, M, H groups were supplemented 10 mg/mL ovalbumin, 4, 10 and 16 mg/mL by 0.25 mL/d respectively. 40-d later, all mice were euthanatized, and the activities of SOD, GSH-Px, CAT and the level of MDA were determined. Meanwhile, before mice were euthanatized, all the mice were fasted for 12 h, and blood was sampled from eyepit, then centrifugalized at 3000 g for 10 min to isolate the serum, and stored the serum at –20℃ for later use. After death, all livers were collected and washed with cooled saline three times, eliminated fat and connective tissue, dried the surface moisture with filter paper and weighted, then mashed the live and diluted it with cooled saline at an end concentration of 10 %, centrifugalized at 3000 g, 15 min, the suspension was collected and stored at 4℃ for later use.

Superoxide Dismutase Assay

The nitroblue tetrazolium (NBT) method of Beauchamp and Fridovich,[12] which is based on the inhibition of NBT reduction by SOD, was used for the determination of SOD activities. Briefly, 2.5 mL of 0.05 mol sodium carbonate buffer (pH 10) was mixed with 0.1 mL of 3 mmol/L EDTA, 3 mmol/L xanthine, 1.5 mg/mL bovine serum albumin, 0.75 mmol/L NBT, and the serum and homogenates of liver containing SOD. Reaction was initiated by adding 0.1 mL of 56 mU/mL xanthine oxidase. After 30 min of incubation, the reaction was terminated by mixing 6 mmol/L $CuCl_2$ and was centrifugalized at 350 *g* for 10 min. Absorbance of blue formazan was recorded at 560 nm and 25 °C. The relative absorbance was then converted into unit of SOD activity per mL or per mg protein, where one unit of SOD activity was equivalent to the quantity of SOD that caused a 50% reduction in the background rate of NBT reduction.

Glutathione Peroxidase Assay

The method of Wendel,[13] with some modifications, was employed to quantitate the activity of GSH-Px. Into 1 mg of NADPH, 9.2 mL of 1 mmol/L sodium azide solution (in 50 mmol/L sodium phosphate buffer with 0.4 mmol/L EDTA), 0.1 mL of glutathione reductase enzyme solution (100 U/mL), and 0.05 mL of glutathione reduced (GSH) were added and mixed by inversion. Then, 3 mL of the mixture 0.05 mL of the serum and homogenates of liver containing GSH-Px was added. It was vortexed and incubated for 5 min at room temperature. After the incubation, 0.05 mL of H_2O_2 was immediately mixed by inversion and the spectrophotometric measurements were recorded kinetically at 340 nm after every 30 s over a period of 5 min. GSH-Px activity was calculated from the change in optical density per minute in the maximum linear rate range using a molar extinction coefficient for NADPH of 6.22×10^3 μmol and assuming 2 mol of GSH formed for each mole of NADPH consumed. One unit activity was defined as 1μmol/L NADPH oxidized per minute.

Catalase Assay

Catalase activity was determined according to the method of Beers *et al*.,[14] by following the decomposition of H_2O_2 at 240 nm and 25°C. Hydrogen peroxide solution (10 mmol/L) in 50 mmol/L potassium phosphate buffer (pH 7.0) with 0.1 mL of the serum and homogenates of liver containing catalase was mixed with inversion, and decrease of absorbance every 30 s over a period of 3 min was recorded kinetically using a spectrophotometer. Changes in the rate of absorbance were converted into unit of catalase/mg protein using a conversion factor (3.45), which corresponds to the decomposition of 3.45 micromoles of hydrogen peroxide in a reaction mixture producing a decrease in the absorbance from 0.45 to 0.40 unit.

MDA assay

This assay was used to determine malondialdehyde (MDA) levels as described by Ohkawa *et al*. [15] 200 μL of the serum or homogenates of liver was added into 50 μL of 8.1% sodium dodecyl sulfate (SDS), vortexed, and was incubated for 10 minutes at room temperature. 375 μL of

Table 1. Levels of MDA and activities of SOD, GSH-Px and CAT in tested mice serum and liver

		NC	D-gal	Ova	H	M	L
Serum	SOD	412±66.8^{aA}	285±55.2^{eC}	336±44.6^{cdB}	394±26.5^{abA}	368±42.6^{bcAB}	328±55.3^{dBC}
	GSH-PX	480±46.6^{aA}	237±25.6^{eD}	272±53.2^{dD}	402±72.3^{bB}	372±36.8^{bB}	317±24.6^{cC}
	CAT	7.90±1.14^{aA}	3.57±0.68^{eD}	4.27±0.75^{dD}	7.09±0.89^{bAB}	6.58±0.94^{bcBC}	6.21±0.72^{cC}
	MDA	8.88±1.47^{cD}	14.2±2.18^{aA}	11.8±1.81^{bB}	9.30±1.22^{cD}	9.59±1.04^{cCD}	11.1±2.02^{bBC}
Liver	SOD	654±68.3^{aA}	356±46.8^{dD}	379±52.1^{dCD}	488±73.6^{bB}	423±70.2^{cC}	391±23.7^{cdCD}
	GSH-PX	51.6±4.92^{aA}	38.5±5.33^{dC}	40.6±3.51^{cdC}	45.8±3.86^{bB}	42.2±2.02^{cC}	41.6±1.20^{cC}
	CAT	2.17±0.23^{aA}	1.36±0.25^{dD}	1.51±0.16^{dCD}	1.87±0.10^{bB}	1.73±0.31^{bcB}	1.68±0.24^{cBC}
	MDA	2.47±0.51^{dD}	6.43±1.44^{aA}	5.24±0.87^{bB}	3.26±0.74^{cCD}	3.60±0.23^{cC}	3.82±0.81^{cC}

Note: Values expressed as Mean± SD, n=15. Different capital letter in the same row means greatly significantly (p<0.01), different lowercase means significant difference (p<0.05).

20% acetic acid (pH 3.5) and 375 μL of thiobarbituric acid (0.6%) were added and placed in a boiling water bath for 60 min. The samples were allowed to cool at room temperature, then 1.25 mL of butanol: pyridine (15:1) was added, vortexed and centrifuged at 1000 g for 5 minutes. 500 μL of the colored layer was measured at 532 nm using 1,1,3,3−tetraethoxy propane as a standard.

Statistical analysis

Values were presented as means±standard deviation (*SD*) when appropriate. Differences between groups were evaluated by one-way ANOVA, followed by a least-significant difference (LSD). All analyses were performed with the statistical software DPS8.0.

Results and discussion

Antioxidative activities of ovalbuin hydrolysates in vivo

Antioxidative activities of ovalbuin hydrolysates in vivo were investigated by aged mice model, which was induced by injected daily with D-galactos.

The activities of SOD, GHS-Px, CAT and concentration of MDA in treatment group mice were shown in Table 1. From table 1 we can see, the SOD, GSH-Px, and CAT activities decreased significantly, and the MDA level increased in the liver and serum of aged mice induced by continuously injected with D-gal, and all the parameters were significantly different to the control group (p<0.01). After being dealt with hen egg ovalbumin hydrolysates, the SOD, GSH-Px, and CAT activities were lower than those of control group (p<0.01), and the H group was the highest, respectively; to the MDA level, all the experimental groups were higher than that of control group (p<0.05), meanwhile, the SOD, GSH-Px, and CAT activities were higher than those of aged group (p<0.01), and the MDA level was lower than that of control group (p<0.05).

It was reported that injection of a low dose of D-galactose into mice could induce changes which resembled accelerated aging. The aging model showed neurological impairment, decreased activity of anti-oxidant enzymes, and poor immune responses.[16] Anti-aging action of ovalbumin hydrolysates was evaluated by aged mice model, which was induced by injected daily with D-galactos.

Nutritional antioxidants play an important role in cellular antioxidative defense mechanisms. In addition, antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) act to protect tissues from oxidative injury generated by oxygen free radicals e.g., superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2).[17,18]

Free radicals are the source of lipid peroxidation derived from oxygen, and the first line of defense against them is SOD. SOD is an important eliminated enzyme for, and plays key role on balancing between production and removal of endogenous ROS and other pro-oxidants in the body, it can catalyze superoxide anion occurs disproportional reaction, thereby clears the $O_2^{\cdot-}$ and superoxide free radicals to protect cell from damage. The activity of SOD in the body decreased with aging, hen egg ovalbumin hydrolysates could significantly prevented the SOD activity against reducing in the serum and liver of the mice that induced with D-gal, which indicates that hen egg ovalbumin hydrolysates processing antiaging function. In this experiment, of all three concentrations groups, H group possessed higher SOD level in serum and liver, but serum is slightly lower than liver, it suggests that hydrolysates play an important role in the antioxidative defense status of serum, but increases it in liver, which consisted with the other study in rat with fish protein.[19] Also, this suggests that the antioxidative of hydrolysates is dose-dependent.

GSH-Px is one of the anti-oxidation enzymes, and its level could be regarded as an important index for anti-oxidation. GSH-Px can clear the free radicals in the body which produced during the metabolism, prevents the lipid oxidation and decomposes H_2O_2, so that the harmful products can not be formed and accumulated. In our experiment, hydrolysates could increase the GSH-Px activity both in serum and liver, and the serum was much

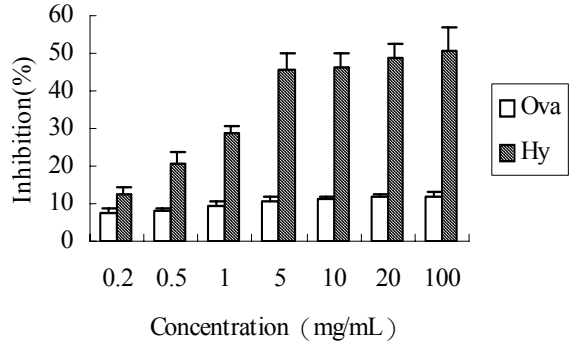

Figure 1. Effects of hydrolysates from ovalbumin on inhibited the auto-oxidation of pyrogallol

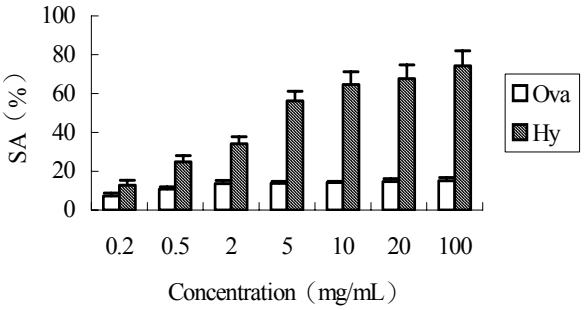

Figure 2. Effects of hydrolysates from ovalbumin on hydroxyl radical scavenging activity

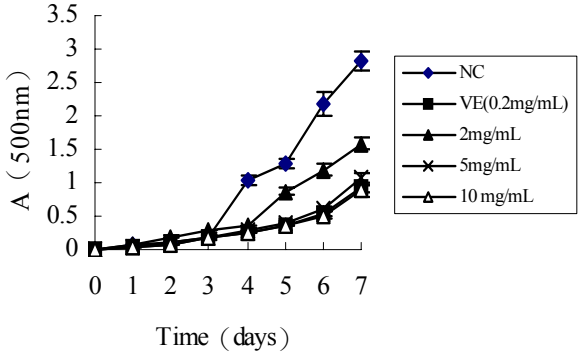

Figure 3. Effects of hydrolysates from ovalbumin on antioxidative activities in linoleic acid autoxidation system

higher than that of liver, which indicated that ovalbumin hydrolysates functioned mainly in serum. This differs with the anti-oxidation activity of VE, which functions indirectly through serum or the oxidases in the tissue.[20] Hen egg ovalbumin hydrolysates could significantly inhibit the GSH-Px activity reducing in the serum and liver of the mice that induced with D-gal, which indicates that hydrolysates processing antiaging function.

H_2O_2 is an important semi-finished product during the free radical metabolism. In common, superoxide anion free radicals could be catalyzed by SOD and produce H_2O_2, and H_2O_2 could be cleared by catalase immediately, and when the catalase is insufficiency, H_2O_2 can penetrate cellular membrane and damage the cell structure. High activity of SOD and CAT can prevent the lipid peroxidation, main the integration of the cellular membrane. Activities of CAT in experimental groups were higher than that of D-gal group both in serum and liver, and H group was the highest. Elevation of CAT and SOD could be an indication of increased antioxidant protection of the tissues.[21]

Aging causes prominent oxidative damage to various cellular components, especially in tissues with high oxidative capacity.[22,,23] Therefore, it is not surprising that lipid peroxidation and protein oxidation in the aged heart and oxidative muscle fibers were intensified, as demonstrated by the higher levels of MDA and carbonyl formation. MDA is the superoxide products of polyunsaturated fatty acids in the body induced by O_2^-, and its level can reflect the free radical contents, thereby the damaged degree can be deduced indirectly. In the present study, significantly lower serum and liver concentrations of MDA were observed in mice receiving hydrolysates supplementation. The plausible reason for this is that hydrolysates attenuate free radical production. It may directly prevent the production of free radicals or it may facilitate the scavenging of free radicals. [24]

Radicals-scavenging activity

Free radicals with the major species of reactive oxygen species (ROS) are unstable, and react readily with other groups or substances in the body, resulting in cell damaging and human disease. Especially, the chemical activity of hydroxyl radical is the strongest among the ROS. It easily reacts with biomolecules such as amino acids, proteins, and DNA. Therefore, the removal of hydroxyl radical and superoxide anion is probably one of the most effective defences of a living body against various diseases. The resultant hydrolysates were tested for their scavenging activities on two different radicals, superoxide, and hydroxyl radical using the pyrogallol auto-oxidation system , and Fenton reaction ($Fe^{2+}+ H_2O_2 \rightarrow Fe^{3+} + O^- + HO\bullet$), respectively. We could observe that ovalbumin hydrolysates exert potential scavenging effects on two radicals in different capacities. Figure 1 presents the inhibitory rate of the superoxide productivity in concentrations of hydrolysates. The hydrolysates of ovalbumin exhibited antioxidant activity, and the antioxidant activity increased with increasing concentrations, and more significantly ($p < 0.01$) than the antioxidant activity of ovalbumin with the same concentrations. Superoxide anion (O_2^-) is formed in viable cells during several biochemical reactions[16] and its effect can be magnified because it produces other types of free radicals and oxidizing agent that can induce cell damage.[25] In our research, the inhibition capacity of hydrolysates against O_2^- was more than 45% at the concentration of 5 mg/mL.

The cell damaging action of hydroxyl radical is well known, as it is the strongest among free radicals. [26] Some hydrolysates have exhibited positive effects on hydroxyl radical. [27] As shown in figure 2, each various concentrations tested possessed hydroxyl radical scavenging effects and more significantly ($p< 0.01$) than the antioxidant activity of ovalbumin with the same concentration. At the concentration of 5 mg/mL, the inhibition capacity reached around 56%.

Inhibitory effects of ovalbumin hydrolysates on lipid peroxidation

To assess the inhibitory effects of Ovalbmin hydrolysates on lipid peroxidation, linoleic acid was oxidized using a

linoleic acid model system where transition metal ion, Fe^{2+}, accelerated lipid peroxidation (Fig 3). Several studies have already observed that some hydrolysates derived from different protein sources possess antioxidative effects.[28,29] As observed in this research, the hydrolysates could retard lipid perxidation efficiently than that of control. Among concentrations of hydrolysates, the concentration of 5 mg/mL, and 10 mg/mL exhibited the highest activities and their antioxidative patterns were closer to that of α-tocopherol with 0.2 mg/mL concentration during 7 days of oxidative reaction.

In summary, ovalbumin hydrolysates supplementation mice decreased oxidant production and age-related oxidative damage. Elevated SOD and GPX activities may partially explain these protective effects in mice, whereas the mechanism benefiting the aged mice are currently unknown. Future studies should be conducted to verify this effect and elucidating the mechanisms underlying the protection.

References

1. Kovatcheva EG, Koleva II, Ilieva M, Pavlov A, Mincheva M, Konushlieva M. Antioxidant activity of extracts from Lavandula vera MM cell culture. Food Chem. 2001; 7: 1069-1077.
2. Ruberto G, Baratta MT, Biondi DM, Amico V. Antioxidant activity of extracts of the marine algal genus Cystoseira in a micellar model system. J. Appl. Phycol. 2001;13: 403-407.
3. Chow CK. Interrelationships of cellular antioxidant defense systems. In Cellular Antioxidant Defense Mechanisms; Chow, C. K., Ed.; CRC Press: Boca Raton, FL, 1988; Vol. II, pp 217-237.
4. Chen HM, Muramoto K, Yamauchi F, Nokihara K. Antioxidant activity of design peptides based on the antioxidative peptide isolated from digests of a soybean protein. J. Agric. Food Chem. 1996; 44: 2619-2623.
5. Chen HM, Muramoto K, Yamauchi F, Fujimoto K, Nokihara K. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. J. Agric. Food Chem. 1998; 46: 49-53.
6. HU Wen-qin, WANG Tian, ZHOU Yan-min, min ، LIN Gui-juan. Effects of hydrolysed casein on antioxodative enzymes in mice. Acta Nutrimenta Sinica 2005; 27:54-57.
7. Davalos A, Miguel BM. Bartolome B, Lopez-fandino R. Antioxidant Activity of Peptides Derived from Egg White Proteins by Enzymatic Hydrolysis. Journal of Food Protection, 2004; 67: 1939-1944.
8. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and convenient assay for superoxide dismutase. Eur. J. Biochem. 1974;47: 469-474.
9. Chung SK, Osawa T, Kawakishi S. Hydroxyl radical scavenging effects of spices and scavengers from Brown Mustard (Brassica nigra). Biosci. Biotechnol. Biochem. 1997; 61: 118-123.
10. Osawa T, Namiki M. Natural antioxidnts isolated from eucalyptus leaf waxes. J. Agric. Food Chem. 2001; 49: 2992-2997.
11. Mitsuda H, Yasumoto K, Iwami K. Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyoto Shokuryo 1966; 19: 210-214.
12. Beauchamp C, Fridovich I. Assays of superoxide dismutase. Anal. Biochem. 1971; 44: 276-287.
13. Wendel A. Glutathione peroxidase. In Enzymatic Basis of Detoxification; Academic Press: New York, 1980; pp 333-336.
14. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 1952; 195:133-140.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reationl. Anal Biochem. 1979; 95: 351-8.
16. Sonz X, Bao M, Li D. Advanced glycation in D-galactose induced mouse aging model. Mech Ageing Dev 1999;108:239-251.
17. Bray TM, and Taylor CG. Tissue glutathione, nutrition, and oxidative stress. Can. J. Physiol. Pharmacol. 1993; 71: 746-751.
18. Pence BC. Dietary selenium and antioxidant status: toxic effects of 1,2-dimethylhydrazine in rats. J. Nutr. 1991;121: 138-144.
19. Dalila Ait Yahia, Sihem Madani, Emmanuelle Prost, Josiane Prost, Malika Bouchenak and Jacques Belleville. Tissue Antioxidant Status Differs in Spontaneously Hypertensive Rats Fed Fish Protein or Casein. J. Nutr. 2003;133: 479-482.
20. Maggi-Capeyron MF, Cases J, Badia E. A diet high in cholesterol and deficient in vitaminE induce lipid peroxidation but does not enhance antioxidant enzyme expression in rat liver. J. Nutr. Biochem. 2002;13: 296-301.
21. Yih-Fwu Lin, Hsiu-Ling Tsai, Yi-Chun Lee, and Sue-Joan Chang. Maternal Vitamin E Supplementation Affects the Antioxidant Capability and Oxidative Status of Hatching Chicks. J. Nutr. 2005;135: 2457-2461.
22. Yu BP. Cellular defense against damage from reactive oxygen species. Physiol. Rev. 1994; 74: 139-162.
23. Ames BN, Shigenaga MK, and Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. U.S.A. 1993; 90: 7915-7921.
24. Sahin N, Onderci M, Sahin K, Cikim G, and Kucuk O. Magnesium Proteinate Is More Protective than Magnesium Oxide in Heat-Stressed Quail. 2005; 135: 1372-1378.
25. Fridovich I. Superoxide dismutases. Adv. Enzym. 1974; 41:35-97.
26. Lui F, Ng TB. Antioxidative and free radical scavenging activities of selected medicinal herbs. J. Life Sci. 1999; 66: 725-735.
27. Koppenaol WH, Liebman J.F. The oxidizing nature of the hydroxyl radical. A comparison with the ferryl ion (FeO_2^+). J. Phys. Chem. 1984;88:99-101.
28. Eresha Mendis, Niranjan Rajapakse, Se-Kwon Kim. Antioxidant Properties of a Radical-Scavenging Peptide Purified from Enzymatically Prepared Fish Skin Gelatin Hydrolysate. J. Agric. Food Chem. 2005;53:581-587.
29. Rajapakse N, Mendis E, Jung WK, Je JY, Kim SK. Purification of radical scavenging peptide from fermented mussel sauce and its antioxidant properties. Food Res Int 2005; 38:175-82.