Anti-oxidative Properties of Lipids Extracted from Rainbow Trout (*Oncorhynchus mykiss*) Fed with Carotenoids and Conjugated Linoleic Acid

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A commercial diet supplemented with carotenoids and conjugated linoleic acid was fed to rainbow trout (*Oncorhynchus mykiss*) for 8 weeks. To investigate the anti-oxidative properties of these compounds, lipids from the muscle and viscera of the fish were subjected to different assays. At 10 μg/mL, L-ascorbic acid exhibited 95% 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, while the tissue lipids showed little radical scavenging activity. At 50 and 100 μg/mL, the lipids of the muscles and viscera showed 11.7-22.6% and 11.3-24.9% DPPH radical-scavenging activity, respectively. A lipid peroxidation inhibitory assay using the ferric thiocyanate method was also performed in comparison with α-tocopherol at a concentration of 6.0 mg/mL. Our results indicate that the anti-oxidative property of the lipids in fish muscle, which was 85.2% compared to 85.3% for the visceral lipids, was stronger than that of α-tocopherol (74.3%) following 3 days of storage at 40°C.

Key words: Carotenoids, Conjugated linoleic acid (CLA), Antioxidants, Rainbow trout

Introduction

Cumulative oxidative stress by reactive oxygen radical species such as superoxide anion, hydrogen peroxide, hydroxyl radical, and lipid peroxide causes oxidative damage to lipids, proteins, and nucleic acids. Such oxidative damage leads to degenerative diseases, including diabetes mellitus, atherosclerosis, arthritis, and cancer (Calliste et al., 2001). Most organisms protect themselves from oxidaive stress through the enzymatic activities of superoxide dismutase, peroxidase, and catalase, and the quenching and scavenging action of low-moleular-weight compounds like α-tocopherol, ascorbic acid, and polyphenols (Mau et al., 2002). One of the most widely discussed roles of carotenoids is their interaction with free radicals that initiate harmful reactions such as lipid peroxidation, which is a chain reaction involving short-lived carbon-centered as well as oxygen-centered free radicals. During lipid peroxidation, carotenoids exert an anti-oxidative effect, as evidenced by their reaction with free peroxyl radicals in an additional chainbreaking process (Qian et al., 2000).

In mammals and birds, immune responses have been shown to increase with the addition of certain nutrients to the diet (Krinsky, 1991). Carotenoids are natural fat-soluble pigments that are widespread and structurally quite diverse. Some carotenoids, including β-carotene, are important sources of vitamin A for animals. Because of their role in intermediary meta-olism, carotenoids are considered to be essential nutrients in some aquatic animals (Olson, 1989). Carotenoids are also known to be indispensable cellular components in microorganisms, algae, higher plants, animals, and humans. Most expensive sea-foods, including shrimp, lobster, crab, crayfish, trout, salmon, redfish, red snapper, and tuna, have orange–red integuments and/or flesh containing carotenoid pigments (Haard, 1992).

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The relationships among diet, health, and lifestyle are now key focal points for consumers, researchers, and policy-makers alike given the widespread increase in obesity and rise of diet-related chronic diseases (Swinburn, 2009). Increasing the conjugated linoleic acid (CLA) content of food products (e.g., milk, meat, and fish) may increase their nutritional and therapeutic value. Such enhancement could favorably influence the marketing of these value-added designer foods. CLA isomers have been shown to possess anti-oxidative properties (Lee et al., 1994), inhibit carcinogen-DNA adduct formation (Josyula et al., 1998), induce apoptosis (Ip et al., 1999), modu-ate tissue fatty acid composition and eicosanoid metabolism (Sugano et al., 1998), and affect the expression and action of cytokines and growth factors (Turek et al., 1998). Most *in vitro* and experimental animal studies of CLA support its effectiveness in reducing the risk of cancer. CLA prevents cancer in a dose-dependent manner at levels up to 1%, with no further beneficial effect at levels exceeding 1% (Ip et al., 1991). When dietary CLA was fed to laboratory animals at 0.05, 0.1, 0.25, and 0.5% by weight, as little as 0.1% CLA was effective in reducing mammary tumors. Moreover, CLA was more effect-ive than β-carotene in inhibiting cellular proliferation. Although CLA's anticarcinogenic activity was once thought to be due to its anti-oxidative properties, recent data dispute this hypothesis (Zhang and Chen, 1997).

This study compared the anti-oxidative, free radical-scavenging activities, and reducing power of lipids from the muscles and viscera of rainbow trout fed natural carotenoids extracted from ascidian tunic and synthetic CLA for 8 weeks.

Materials and Methods

Chemicals

Hydrogen peroxide, ammonium thiocyanate, thiobarbituric acid, sodium dodecyl sulfate, butyl hydroxyl toluene, ferrous chloride, potassium ferricyanide [$K_3Fe(CN)_6$], ferrous sulfate chelate solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxyribose, linoleic acid, α-tocopherol, and butylated hydroxyanisole (BHA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All of the reagents were of analytical grade.

Preparation of CLA and carotenoids

Soybean oil (SO) was purchased from a local market (Jinju, Korea). CLA was chemically synthe-sized (56% purity) from SO by high-temperature

alkaline isomerization (Park et al., 2000); the mixture consisted of 46% c9,t11-CLA, 49% t10,c12-CLA, and 5% other CLA isomers (c9,c11-, c10,c12-, t9,t11-, and t10,t12-CLA) as shown by gas chromatography (GC 17A, Shimadzu Corp., Tokyo, Japan). Caro-tenoids were extracted from ascidian tunic with acetone (Choi et al., 1994). The total carotenoid content was found to be 2,400 at 450 nm, the $E^{1\%}_{1cm}$ value of diethyl ether.

Experimental diets and fish

Rainbow trout (*Oncorhynchus mykiss*) was obtained from an aquacultural farm in Yeongdong, Korea. The experimental diets were prepared by mixing appropriate amounts of CLA (1.0%) and ascidian tunic extract (0.4%) with a commercial feed (Woosung Feed Co., Nonsan, Korea). The test diets for the fish were stored in a freezer (GC-124AGF, LG Electronics, Seoul, Korea) at -30°C for 8 weeks until use. Twenty rainbow trout (200±10 g each) were placed in a round glass fiber aquarium (1 ton) equipped with a water circulation and biological filtration system. The diet composition and daily growth index were as reported previously (Guo et al., 2008).

Total lipid extraction

Total lipids were extracted by the method of Bligh and Dyer (1959). The muscles and viscera of rainbow trout cultured for 8 weeks were homogenized into pooled pates. Total lipids were extracted from homogenized tissue pates (50 g) in three volumes of chloroform/methanol (2:1, v/v) using an Ultra-Turrax tissue disrupter (T25, IKA Analysentechnik GmbH, Staufen, Germany). The lipid content was determined gravimetrically.

DPPH free radical-scavenging activity

The assay was carried out according to the method of Oyaizu (1986) with a slight modification. Briefly, 1 mL of sample (0.12%, w/v) was added to 2.0 mL of ethanol and 4.0 mL of 0.5 mM DPPH radical (Sigma-Aldrich Co.) and 2.0 mL of 0.1 M acetic acid buffer (pH 5.5). The mixture was then shaken vigorously and left at room temperature for 30 min before measuring the absorbance at 517 nm (UV-1700, Shimadzu Corp.). The DPPH scavenging percent was calculated as follows: DPPH scavenging activity (%)=[(Control absorbance−Sample absorbance)/ (Control absorbance)]×100.

Thiobarbituric acid-reactive substances

The oxidative rancidity of the fish lipids was mea-sured by a 2-thiobarbituric acid reactive substance

(TBARS) assay of malondialdehyde (MDA) as described by Kosugi et al. (1992). An ethanol solution (0.12 %, w/v) containing total lipids in a 12-mL glass bottle was incubated at 40°C in a darkened area. Three replicates of 0.1-mL aliquots of the reaction mixture containing the lipid sample (0.12 %, w/v) were combined with 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of acetate buffer (pH 3.5), 1.5 mL of 0.8% TBA in water, 50 μL of 0.8% BHT in acetic acid, and 0.7 mL of 5 mM ferric chloride. The mixture was kept at 5°C for 60 min then heated to 100°C for another 60 min. The red pigment was extracted with 1.0 mL of water and 5.0 mL of *n*-butanol-pyridine (15:1). After centrifugation at 3,000 rpm for 10 min, the absorbance of the upper layer was measured (UV-1700, Shimadzu Corp.) at 532 nm using the following equation: TBARS (μmol/g)= (Sample absorbance − Control absorbance) × 1.2393.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Briefly, the sample solution (0.12%, w/v) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1.0%) then incubated at 50°C for 30 min. Next, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%) and the absorbance was measured at 700 nm (UV-1700, Shimadzu Corp.).

Inhibition of linoleic acid autoxidation

The lipid peroxidation inhibitory activity of the lipids was measured in a linoleic acid emulsion system according to the method of Oyaizu (1986). A mixture of 0.5 mL of rainbow trout lipids (0.12%, w/v) in absolute ethanol, 0.5 mL of 2.51% (w/v) linoleic acid in 99.5% ethanol, 1.0 mL of 50 mM sodium phosphate buffer (pH 7.0), and 0.5 mL of distilled water were transferred to a screw-cap tube, which was then placed in an oven at 40±1°C for 6 days in a dark room. Next, to 0.1 mL of the reaction mixture, 9.7 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate, and 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloric acid were added. After a 3 min incubation, the absorbance of the solution (the presence of color indicated linoleic acid oxidation) was measured at 500 nm using a spectrophotometer. L-Ascorbic acid and α-tocopherol (a natural anti-oxidative agent) were used as a reference with 50 mM phosphate buffer (pH 7.0) as a

control. The anti-oxidative capacity for the inhibition of peroxide formation in the system was assessed as follows: Inhibition (%)=[1−(Sample absorbance / Control absorbance)] × 100.

Statistical analysis

All experiments were performed three times. The data were analyzed for the degree of variation with significant differences based on analysis of variance (ANOVA) with Tukey's pairwise comparison test ($P<0.05$) between treatment means using JMP statistical discovery software (SAS Institute Inc., Cary, NC, USA).

Results

CLA and carotenoid contents

Five different diets containing fish oil in combination with CLA and ascidian tunic carotenoids were fed to rainbow trout for 56 days. Feed analyses of the lipid and carotenoid contents performed at the start and end of the feeding periods produced similar results among the groups. The CP14 group, consisting of 1.0% CLA and 0.4% carotenoids, was used in a lipid oxidation stability experiment. The CLA and carotenoid contents in the muscles and viscera were found to be 3.72±0.9 and 8.35±0.9 g/100 g tissue and 10.2±0.4 and 15.3±2.3 mg/kg tissue, respectively.

DPPH free radical-scavenging activity

Proton radical scavenging is an important mechanism of anti-oxidation. DPPH was used to determine the proton radical-scavenging activity of the lipids because it possesses a proton-free radical and shows a characteristic absorption at 517 nm. The scavenging activities of the lipids and natural antioxidants α-tocopherol and L-ascorbic acid toward alkyl radicals are shown in Fig. 1. At 50 and 100 μg/mL, the activities of the lipids from muscle tissue and viscera were 11.7-22.6 and 11.3-24.9%, respectively. Thus, the scavenging activity of the DPPH radicals increased as the concentration of the extract was increased. In comparison to the commercial antioxidants, the concentrations needed to obtain 80% DPPH radical-scavenging activity for α-tocopherol and L-ascorbic acid were 10 and 50 μg/mL, respectively.

Thiobarbituric acid-reactive substances

Fish lipids are high in polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, C20: 5n-3) and docosahexaenoic acid (DHA, C22:6n-3),
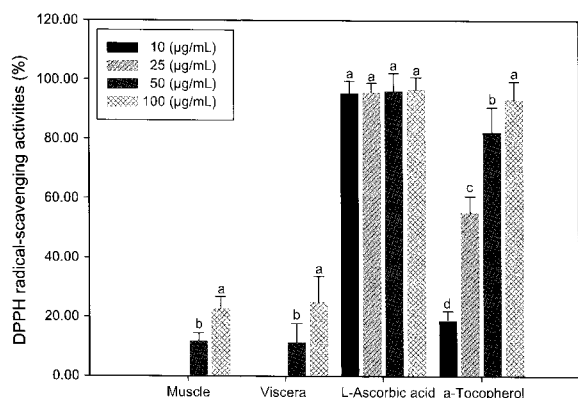
Fig. 1. DPPH radical-scavenging activities of muscle and viscera lipids extracted from rainbow trout. Columns with different superscript are significantly different ($P<0.05$).

which are fairly susceptible to oxidation. The TBARS test is based on the formation of colored products when TBA is reacted with MDA or other TBA-reactive substances presumed to be produced from oxidized PUFA lipids. Fig. 2 shows that the TBARS levels in the sample at 40°C increased from 0 to 48.6 μmol/g lipids with the control on day 4, whereas the TBARS values of the muscles and viscera reached 35.8 vs. 43.3 μmol/g lipids and 147.7 vs. 48.7 μmol/g lipids on days 5 and 6, respectively. After 6 days, the TBARS values of the samples increased rapidly to 334.6 vs. 243.8 μmol/g lipids and 768.8 vs. 635.8 μmol/g lipids for the muscle and viscera, respectively.
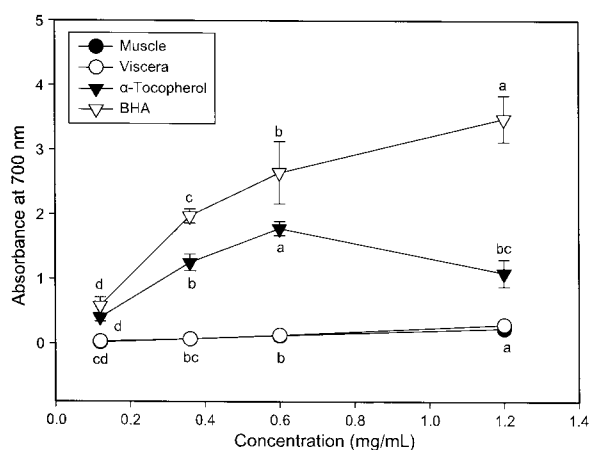

Fig. 2. Reducing power of muscle and viscera lipids extracted from rainbow trout. Lines having different superscript are significantly different ($P<0.05$).

Reducing power of the lipids

In the reducing power assay, the presence of reductants (antioxidants) in a sample results in the reduction of Fe^{3+}/ferricyanide complexes to the ferrous form. Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Fig. 2 shows the reducing powers of the lipids, α-tocopherol, and BHA. The reducing power was found to be 0.07 for the lipids of the muscles and viscera at a dose of 0.36 mg/mL versus 1.25 and 1.97 for α-tocopherol and BHA, respectively. In compareson, the reducing power was found to be 0.12 and 0.13, respectively, for the lipids of the muscles and viscera at a dose of 0.6 mg/mL vs. 1.78 for α-tocopherol, 2.65 for BHA, and 0.23 and 0.29 for the lipids of the muscles and viscera at 1.2 mg/mL. Thus, the reducing power of the lipids from rainbow trout was very low.
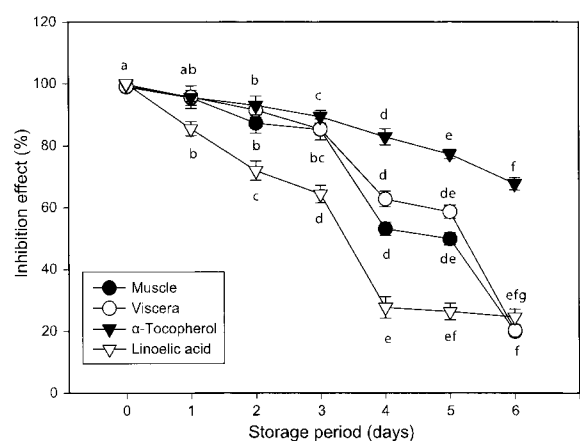

Fig. 3. Lipid peroxidation inhibition effect of muscle and viscera lipids extracted from rainbow trout stored at 40°C. Lines having different superscript are significantly different ($P<0.05$).

Inhibition of linoleic acid autoxidation

The peroxidation of lipids is a complex process that involves the formation and propagation of lipid radicals, with lipid hydroperoxides formed as primary oxidation products in the presence of oxygen. The anti-oxidative activity of the lipids from the muscles and viscera at 0.12% (w/v) against the peroxidation of linoleic acid was investigated and compared to that for α-tocopherol, a widely used natural anti-oxidative agent. As shown in Fig. 4, the control sample showed the lowest inhibition, while α-tocopherol had the greatest effect (about 77.3% inhibition). The inhibittory effect (58.6%) of visceral lipids obtained from rainbow trout fed an experimental diet consisting of CLA and carotenoids was higher than that of the muscle lipids on day 5 in our linoleic acid emulsion system.

Discussion

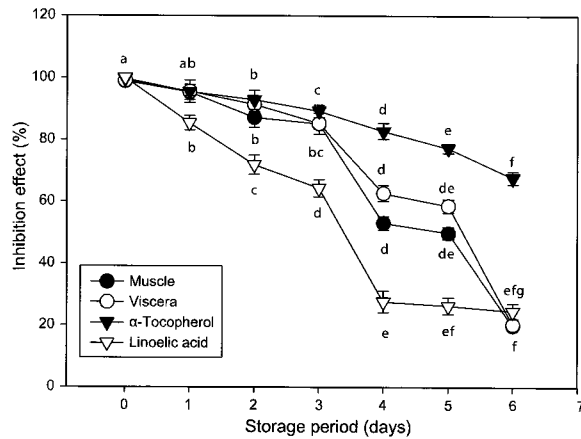To investigate the oxidative stability of CLA and

Fig. 4. TBARS activities of muscle and viscera lipids extracted from rainbow trout stored at 40°C. Columns with different superscript are significantly different (*P*<0.05).

carotenoids, these compounds were used to create five experimental diets. After 56 days of feeding, the muscles and viscera of the fish were tested for carotenoid deposition. Carotenoids were detected at 10.2 and 15.3 mg/kg of tissue (Table 1). A study of the effects of diet, including various natural carotenoid sources and synthetic astaxanthin, on the pigmentation and growth of rainbow trout (*O. mykiss*) was performed by Büyükçapar et al. (2007). At the end of their 60 day experiment, synthetic astaxanthin was found to have caused the greatest carotenoid accumulation in fish flesh (6.42 mg/kg) in all groups. Variations in the muscle carotenoid concentration may be explained by several factors, including the nature of the carotenoids used, the dietary carotenoid concentration, and the size or physiological status of the fish.

Table 1. CLA and carotenoids contents in muscle and viscera lipids extracted from rainbow trout fed for 8 weeks

	Tissues	Concentration
CLA (g/100 g)	Muscle	3.7 ± 0.6
	Viscera	8.4 ± 0.9
Carotenoids (mg/kg)	Muscle	10.2 ± 0.4
	Viscera	15.3 ± 2.3

Mean ± standard deviation of triplicate samples.

The role of free radical oxidative damage in the physiology of human disease, which is a topic of considerable current interest, has been suggested for a wide spectrum of clinical conditions, ranging from cancer to atherosclerosis, stroke, and several neuro-degenerative diseases (Halliwell and Gutteridge, 1990). Of major interest in the reaction of carotenoids with free radicals is whether carotenoids can provide

effective protection against diseases caused by oxidative stress. Burton and Ingold (1984) reported that carotenoids might act in lipid peroxidation as a prooxidant at a high oxygen pressure and high carotenoid concentration. In contrast, Chen and Djuric (2001) reported that carotenoids are degraded by free radicals but do not affect lipid peroxidation in unilamellar liposomes under different oxygen tensions. Under the conditions used in this study, the carotenoid and CLA contents in the lipid extracts were not significant at a level of 10 and 25 μg/mL; however, they increased the DPPH radical-scavenging activity with increasing concentration (Fig. 1). The concentration dependence of the DPPH radical-scavenging activity was obvious for α-tocopherol but not L-ascorbic acid. The reducing power of the tissue lipid extracts was lower than that for BHA and α-tocopherol (Fig. 2) because the reducing power of the samples showed a strong correlation with the total phenolic content of the samples (Chou et al., 2003).

The lipid peroxide retarding effect and TBARS value for the lipids from the muscles and viscera were similar after 6 days at 40°C (Figs. 3 and 4). Marine lipids contain a high percentage of long-chain PUFAs such as EPA and DHA. Because of their high degree of unsaturation, these PUFAs are much more susceptible to oxidation than linoleic acid, which is found in vegetable oils (Cho et al., 1987). However, combining CLA and carotenoids with the PUFAs in the tissue lipids made them more stable than linoleic acid (Fig. 3). The oxidation products of these PUFAs are responsible for the development of rancidity by the production of low-molecular-weight decomposed compounds that impart undesirable flavors. Lipid peroxidation is a latent problem in marine lipid consumption. Alternatively, the high levels of EPA and DHA in marine lipids imply the presence of a strong anti-oxidative system in marine animal tissues. The ascidian tunic extract used in the present study contained high percentages of EPA (3.51%) and DHA (8.30%), with 10.0% (w/w) of the extract being polar lipids (Choi et al., 1996). Phospholipids are generally useful as synergists in reinforcing the anti-oxidative activity of phenolic compounds such as α-tocopherol (Bandarra et al., 1999).

CLA is a collective term used to describe one or more positional and geometric isomers of linoleic acid, an essential fatty acid. The oxidation rate of CLA is temperature-dependent, but CLA in solution is more stable to oxidation (Yurawecz et al., 1995). No oxidation was detected for CLA in a polar solvent (methanol) or in a 90:10 (v/v) mixture of methanol and water. In an *in vivo* study, Ip et al. (1991) showed

that CLA was as effective as vitamin E in inhibiting the formation of TBARS in the mammary glands but not in the liver. Given the results of this experiment, the PUFAs in fish lipids are presumed to be rapidly oxidized during storage at 40°C (Fig. 4). However, the carotenoids and CLA contained in the lipids of the muscles and viscera showed reduced PUFA oxidation in the dark at 40°C. Therefore, rainbow trout fed an experimental diet fortified with ascidian tunic carotenoids and CLA may be used as a good source of functional lipids rich in EPA, DHA, carotenoids, and CLA. Given the continued rise in population size combined with consumer expectations, global demand for fish will continue to grow. Aquacultural methods are expected to develop significantly in the near future with the rise of fish as a functional food with anticancer, anti-oxidative, bioremediative, and immunoenhancing effects in the wake of the widespread consumption of functional foods now on the world market (Yalpani, 2002).

References

Bandarra NM, Campos RM, Batista I, Nunes ML and Empis JM. 1999. Antioxidant synergy of α-tocopherol and phospholipids. J Am Oil Chem Soc 76, 905-913.

Bligh EG and Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37, 911-917

Burton GW and Ingold KU. 1984. β-Carotene: an unusual type of lipid antioxidant. Science 224, 569-573.

Büyükçapar HM, Yanar M, Yanar Y. 2007. Pigmentation of rainbow trout (Oncorhynchus mykiss) with carotenoids from marigold flower (Tagetes erecta) and red pepper (Capsicum annum). Turkish J Veter Ani Sci 31, 7-12.

Calliste CA, Trouillas P, Allais DP, Simon A and Duroux JL. 2001. Free radical scavenging activities measured by electron spin resonance spectroscopy and B 16 cell antiproliferative behaviors of seven plants. J Agric Food Chem 49, 3321-3327.

Chen G and Djuric Z. 2001. Carotenoids are degraded by free radicals but do not affect lipid peroxidation in unilamellar liposomes under different oxygen tensions. FEBS Letters 505, 151-154.

Cho SY, Miyashita K, Miyazawa T, Fujimoto K and Kaneda T. 1987. Autooxidation of ethyl eicosapentaenoate and docosahexaenoate. J Am Oil Chem Soc 64, 876-879.

Choi BD, Kang SJ, Choi YJ, Youm MG and Lee KH. 1994. Utilization of ascidian (Halocynthia roretzi) tunic 5. Feeding effect of ascidian tunic extracts on liver lipid of rainbow trout, Oncorhynchus mykiss. J Korean Fish Soc 27, 445-453.

Choi BD, Kang SJ and Lee KH. 1996. Effect of ascidian tunic extracts on cholesterol accumulation and pigmentation of kuruma prawn, Penaeus japonicus. J Korean Fish Soc 29, 393-408.

Chou ST, Chao WW and Chung YC. 2003. Antioxidative activity and safety of 50% ethanolic red bean extract (Phaseolus radiatus L. var. Aurea). J Food Sci 68, 21-25.

Guo R, Jeong UC, Kang SJ, Choi YJ and Choi BD. 2008. Effect of dietary conjugated linoleic acid on growth, lipid class, and fatty acid composition in rainbow trout (Oncorhynchus mykiss) J Fish Sci Technol 11, 125-132.

Haard NF. 1992. Biochemistry and chemistry of color and color changes in seafood. In: Advances in Seafood Biochemistry, Composition and Quality. Flick GJ and Martin RE, eds. Technomic Publishing Co. Inc., Lancaster and Basel, USA, 305-310.

Halliwell B and Gutteridge JMC. 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Methods Enzymol 186, 1-85.

Ip C, Chin SF, Scimeca JA and Pariza MW. 1991. Mammary cancer prevention by conjugated dienoic derivatives of linoleic acid. Cancer Res 51, 6118-6124.

Ip MM, Masso-Welch PA, Shoemaker SF, Shea-Eaton WK and Ip C. 1999. Conjugated linoleic acid inhibits proliferation and induces apoptosis of normal rat mammary epithelial cells in primary culture. Exp Cell Res 250, 22-34.

Josyula S, He YH, Ruch RJ and Schut HAJ. 1998. Inhibition of DNA adducts formation of PhIP in female F344 rats by dietary conjugated linoleic acid. Nutr Cancer 32, 132-138.

Kosugi H, Kojima T, Yamaki S and Kikugawa K. 1992. Interpretation of the thiobarbituric acid reactivity of rat liver and brain homogenates in the presence of ferric ion and ethylenediaminetetraacetic acid. Anal Biochem 202, 249-255.

Krinsky NI. 1991. Effects of carotenoids in cellular and animal systems. Am J Clin Nutr 53, 238-246.

Lee KN, Kritchevsky D and Pariza MW. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 108, 19-25.

Mau JL, Lin HC and Song SF. 2002. Antioxidant properties of several specialty mushrooms. Food Res Int 35, 519-526.

Olson JA. 1989. Provitamin A function of carotenoids: the conversion of β-carotene into vitamin A. J Nutr 119, 105-108.

Oyaizu M. 1986. Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr 44, 307-

315.

Park WS, Kim SJ, Park SJ, Kim JO, Lim DG and Ha YL. 2000. Chemical synthesis of conjugated linoleic acid (CLA) derivatives with glycerol. J Korean Soc Food Sci Nutr 29, 389-394.

Qian SY, Wang HP, Schafer FQ and Buettner GR. 2000. EPR detection of lipid derived free radicals from PUFA, LDL, and cell oxidations. Free Radic Biol Med 29, 568-579.

SAS. 1986. SAS User's Guide. SAS Institute Inc., Cary, NC. USA.

Sugano M, Tsujita A, Yamasaki M, Noguchi M and Yamada K. 1998. Conjugated linoleic acid modulates tissue levels of chemical mediators and immuno-globulins in rats. Lipids 33, 521-527.

Swinburn B. 2009. Obesity prevention in children and adolescents. Child Adol Psychi Clin North America 18, 209-223.

Turek JJ, Li Y, Schoenlein IA, Allen KGD and Watkins BA. 1998. Modulation of macrophage cytokine production by conjugated linoelic acids in influenced by the dietary n-6:n-3 fatty acid ratio. J Nutr Biochem 9, 258-266.

Yalpani M. 2002. New technologies for health foods and nutraceuticals. Nutraceuti Foods 7, 222-229.

Yurawecz MP, Hood JK, Mossoba MM, Roach JAG. and Ku Y. 1995. Furan fatty acids determined as oxidation products of conjugated octadecadienoic acid. Lipids 30, 595-598.

Zhang A and Chen ZY. 1997. Oxidative stability of con-jugated linoleic acids relative to other polyunsaturated fatty acids. J Am Oil Chem Soc 74, 1611-1613.