J Sci Food Agric 85:1119–1124 (2005)
DOI: 10.1002/jsfa.2077

Retardation of lipid oxidation in blue sprat by hot water tea extracts

Yoshie Seto,[1]* Chih-Cheng Lin,[2] Yasushi Endo[3] and Kenshiro Fujimoto[3]

[1]Faculty of Home Economics, Kyoritsu Women's University, Tokyo 101-8433, Japan
[2]Department of Food Science, Yuan-Pei University of Science and Technology, Hsinchu 300, Taiwan
[3]Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

Abstract: The blue sprat is a small fish belonging to the family Clupeidae. Deterioration of its freshness after catching is so rapid that the fish is cooked in simple ways and consumed locally. The prooxidant activity of the skin, dark meat and ordinary meat was much higher than that of other fishes such jack mackerel. Suppression of lipid peroxidation in blue sprat was attempted using hot-water extracts of five types of Taiwanese tea with different degrees of fermentation, ie Longjing-type green tea, Shy Jih Chuen oolong tea, Tungting oolong tea, Pouchong tea and black tea. All the tea extracts showed antioxidant activity on oxidation of linoleic acid induced by the extracts of blue sprat's tissues. The antioxidant activity of each tea extract showed a positive correlation with the contents of total catechins, especially with that of epigallocatechin gallate, but a poor correlation with the contents of total polyphenols. The Tungting oolong tea together with Chinese green tea effectively suppressed the prooxidant activities of the dark meat and skin of blue sprat. Impregnation of blue sprat in the extract of the Tungting oolong tea retarded the lipid oxidation in fish meat assessed by peroxide value and carbonyl value during refrigeration.

Keywords: blue sprat; catechin; fish; lipid peroxidation; tea

INTRODUCTION

In China, various teas with different degrees of fermentation are produced, each having a characteristic flavor and color. It is well known that tea extracts show high antioxidative activities, mainly due to polyphenols such as catechins.[1] When tea is fermented, catechins are oxidized by various oxidases, such as polyphenol oxidase, to form orange to red substances, ie theaflavins and thearubigins.[2] In fermented teas, the concentration of unoxidized catechins decreases, and the amount of oxidative polymerization products of catechins increases, but antioxidative activity remains. For instance, oolong tea, which is a semi-fermented tea, has higher free radical-scavenging activity than green tea and black tea.[3] It has also been reported that theaflavins, which are responsible for the red color of black tea, have high scavenger activity of lipid hydroperoxyl radicals.[4,5]

The blue sprat, *Spratelloides gracilis* (Temminck and Schlegel) is a small fish of the family Clupeidae, and deterioration of its freshness after catching is extremely rapid. Since long-term storage of blue sprat is impossible due to degradation of taste even in a freezer, the fish is cooked in simple ways, and consumed locally. To evaluate the perishability of blue sprat, we compared the lipid oxidation of blue sprat in a buffer with that of Japanese jack mackerel. In blue sprat samples, the lipid peroxidation activity of the skin, dark meat, ordinary meat and viscera was higher than in Japanese jack mackerel samples.[6] This may be the cause of the lower preservability of blue sprat.

We hypothesized that antioxidative activities of teas would suppress lipid peroxidation in blue sprat and examined the suppressive effects of hot-water tea extracts on lipid peroxidation in blue sprat using green tea, oolong tea, Pouchong tea and black tea with different degrees of fermentation and different kinds of tea leaves, all of which were produced in Taiwan. We also examined the effects of impregnation of blue sprat samples in tea extracts on deterioration by lipid oxidation.

MATERIALS AND METHODS

Fish, tea and reagents

Blue sprat, with a length of 9–11 cm and a weight of 5–8 g, were purchased from a fresh fish store in Nagasaki, Japan, immediately after catching between May and July. Mucosa on the surface of the fish was removed using absorbent cotton, and the skin, viscera, ordinary meat and dark meat were separated.

* Correspondence to: Yoshie Seto, Faculty of Home Economics, Kyoritsu Women's University, 2-2-1 Hitotsubashi, Chiyoda-ku, Tokyo 101-8433, Japan
E-mail: seto_ys@ybb.ne.jp
(*Received 22 October 2003; revised version received 8 September 2004; accepted 11 October 2004*)
Published online 26 January 2005

Five types of Taiwanese tea were purchased from Taiwan Tea Experimental Station in Taipei, Taiwan. Table 1 shows the five types of tea used in this study. The reagents used were of the reagent grade (Nacalai Tesque, Kyoto, Japan).

Extraction of teas and analysis of polyphenols

Tea leaves (5 g) were added to 500 ml distilled water at 90 °C and, after keeping at 90 °C for 12 min, the solution was filtered through a membrane filter (porosity size 0.45 μm, Millipore, Bedford, MA, USA). The total amount of polyphenols was measured by the Folin–Denis method.[7] The composition of catechins was analyzed by HPLC using a Jasco 801-SC apparatus (Jasco, Tokyo, Japan) combined with a UV–visible detector (Jasco). The mobile phase contained 2.5% acetic acid(solvent A) and acetonitrile(solvent B), with a linear gradient starting with A:B(87:13), changing to A:B(60:40) in 60 min with a flow rate of 1 ml min^{-1}. Authentic catechins were purchased from Sigma(St Louis, MO, USA).

Color of the tea extracts

The color was measured using 1 ml filtered hot water extracts within 1 h after extraction. Hunter's *L*, *a* and *b* values were measured using a color difference meter model TC-1800MK2(Tokyo Denshoku, Tokyo, Japan). The Hunter colorimeter is commonly used for color measurement of tea extract in both Taiwan and Japan.

Preparation of water extracts from blue sprat

The water extracts from various tissues of blue sprat were prepared according to the method used for the isolation of lipid prooxidants from the skin of the Japanese sardine.[8] Portions (1 g) were weighed from the separated tissues, homogenized in 2.0 ml of 0.05 M sodium phosphate buffer (pH 7.0) for 1 min using an Ultra-Turrax homogenizer (T-25, IKA-Labortechnik, Staufen, Germany), extracted and centrifuged at 10 000 × *g* for 15 min. The precipitate was dissolved in 0.05 M sodium phosphate buffer and extracted again by the above method. The supernatant was combined with the first supernatant, brought to 5.0 ml and used as the water extract sample. All procedures were conducted at temperatures lower than 5 °C. The extraction was performed using at least three tissue samples.

Measurement of lipid peroxidation activity in the blue sprat tissues

Pretreatment of linoleic acid

A very small amount of oxidation products contained in commercially available linoleic acid (Nacalai Tesque, Kyoto, Japan) was removed by passage through a Sep-Pak silica cartridge (Waters, Milford, MA, USA) followed by elution with diethyl ether/hexane (1:9, v/v). After treatment, the purity was confirmed by thin-layer chromatography with Silica Gel G (Merck, Darmstadt, Germany) as the absorbent, hexane–diethyl ether–acetic acid (80:20:1, v/v) as the solvent, and 30% sulfuric acid for detection. The purified linoleic acid was stored at −25 °C as ethanol solution after column treatment.

Measurement of lipid peroxidation activity

The lipid peroxidation activity in the tissues of blue sprat was assessed principally by the procedure used for assay of lipoxygenase in plant.[9]

Aliquots of 0.05 M sodium phosphate buffer (2.5 ml; pH 7.0) containing the water extract equivalent to 50 mg fish tissues were added to 5.0 ml of 0.2 M sodium phosphate buffer (pH 7.0) containing 5 mM linoleic acid and 0.12% Tween20. After addition of 50 μl of tea extracts at a concentration (as tea leaves) of 1, 1.5 or 5 mg (or 2.5 mg), the reaction mixture was incubated at 25 °C for 20 min. Immediately after addition of nine parts of 60% ethanol, the amount of conjugated diene derived from hydroperoxide was determined before and after incubation by measuring the absorbance at 234 nm (A_{234}) using a UV spectrophotometer (V-520, Jasco). The activity was expressed as the changes in A_{234} g^{-1} protein measured in a 1 cm cell over 20 min. Protein was quantified using the phenol reagent.[10] The samples without tea extracts were also examined by the same procedures. Measurement was performed at least three times.

Effects of the tea extracts on the oxidative stability of refrigerated blue sprat

Impregnation in the tea extract

According to the standard tea-extracting procedure,[11] 3 g Tungting oolong tea leaves per 100 ml were added to distilled water at 97 °C, kept at the temperature for 3 min, and filtered. Blue sprat (100 g) was impregnated in 110 ml tea extract at 5 °C for 30 min, put into a container (17 × 12 cm, polypropylene) without wiping off the solution, and stored in a refrigerator at 5 °C for 1, 4 and 6 days. The same weight of blue sprat was put

Table 1. List of teas used for the experiments

Tea sample	Type	Variety	Cultivation area	Harvesting season
Longjing-type green tea	Non-fermented	Gan Tzy	Taipei County	Winter
Shy Jih Chuen oolong tea	Semi-fermented	Shy Jih Chuen	Nantou	Winter
Tungting oolong tea	Semi-fermented	Taiwan TTES no 12	Nantou	Winter
Pouchong tea	Semi-fermented	Taiwan TTES no 13	Taipei County	Winter
Black tea	Fermented	Taiwan TTES no 8	Nantou, Yuchi	Winter

into another container without impregnation in the tea extract, and stored.

Extraction of lipids
Lipids were extracted from about 10 g tissue samples in 120 ml chloroform–methanol (2:1, v/v) containing butylatedhydroxytoluene (Nacalai Tesque, Kyoto) at a concentration of 0.002% by Folch's method.[12]

Moisture
The moisture of samples was measured by the thermal dry method according to the official procedure of the Japanese Society of Food Technology.[13]

Evaluation of oxidative deterioration
The peroxide value (POV) and carbonyl value (CV) were determined by the method of the Japanese Oil Chemists' Society[14] and the 2,4-dinitrophenylhydrazine method, with pretreatment of reduction with triphenylphosphine[15] to avoid disturbance of peroxides.

Statistical analyses
One-way analysis of variance using repeated measures was conducted. When significant differences ($p \leq 0.05$) were observed, they were tested by Bonferroni's multiple comparison test.[16]

RESULTS AND DISCUSSION
Color and polyphenols in the tea extracts
Table 2 shows the color of the tea extracts and the content of total polyphenols and Table 3 shows the catechin contents.

Table 2. Color of the tea extracts

	Color		
Tea sample	*L* value	*a* value	*b* value
Longjing-type green tea	79.5	2.8	24.5
Shy Jih Chuen oolong tea	86.8	−2.0	13.6
Tungting oolong tea	83.1	−1.8	18.4
Pouchong tea	84.8	−1.2	12.2
Black tea	52.1	31.9	31.9

Teas are classified into non-fermented, semi-fermented and fermented groups according to their manufacturing processes.[17] Green tea is produced without fermentation by inactivation of enzymes by heating in the first stage of processing. Therefore, the tea leaves remain green. However the color of Longjing-type green tea was not typically green, but rather yellowish brown as recognized from the positive *a* value in Table 2. This is because, unlike Japanese green tea which is produced by steaming, Longjing-type green tea is produced by roasting. To produce oolong tea, collected fresh leaves are wilted, and slightly fermented by enzymes with occasional stirring in the shade until leaves become slightly brown and fragrant. Fermentation is terminated midway by the pot-roasting method. Pouchong tea is produced by almost the same method as oolong tea, but both wilting and fermentation are very weak. These teas are called semi-fermented teas and gave a similar color pattern as shown in Table 2, in which the light brown color is suggested by larger *L* values and smaller absolute *a* and *b* values compared with the black tea. Black tea, which is classified in the fermented group, is produced by fermenting for 1–2 h at 25 °C at a humidity of 90% after rubbing treatment, and drying after appropriate color and fragrance are obtained. The black tea extract gave a typical red color and was darker than other teas, as shown in Table 2.

The major polyphenol contained in tea leaves is catechin[18] and oxidative polymerization products of catechins are contained in fermented teas. As shown in Table 3, the total content of polyphenols was the lowest in the Shy Jih Chuen oolong tea and the highest in the Chinese green tea. The difference in the catechin content among the tea samples was even larger, being high in the Chinese green tea and Tungting oolong tea and the lowest in the black tea (Table 3). Tungting oolong tea is produced from tea leaves cultivated in a high-mountain area and graded high-class because of its fragrance. As shown in Table 3, the content of catechins was lower, and that of oxidized polyphenols was higher in the black tea compared with the Longjing-type green tea. In black tea, most of catechins have been converted into theaflavins and thearubigins by the enzymatic oxidation. Therefore, the ratio of catechins–total polyphenol in the black tea was the lowest. In the oolong teas and Pouchong tea in

Table 3. Content of total polyphenols and composition of catechins in the tea extracts

			Catechins					
Tea sample	Total polyphenols	Total catechins	GC	EGC	C	EGCG	ECG	Polyphenols other than catechins
			Content (g kg^{-1} of tea leaves)					
Longjing-type green tea	139.3	84.0	2.0	45.8	5.5	24.4	6.3	55.3
Shy Jih Chuen oolong tea	84.3	43.5	0.2	6.1	1.3	31.9	4.0	40.8
Tungting oolong tea	95.0	83.9	0.4	15.8	2.9	59.7	5.1	11.1
Pouchong tea	96.2	48.1	0.1	21.7	0.9	23.0	2.4	48.1
Black tea	114.0	12.0	1.6	7.8	0.1	1.7	0.8	102.0

GC, gallocatechin; EGC, epigallocatechin; C, catechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate.

the semi-fermented group, the decrease in the content of catechins was small, because the fermentation during wilting and loss of greenness are mild, without destruction of tissues. The catechins in Longjing-type green tea were suggested to be thermally oxidized during roasting instead of enzymatic oxidation.

Effects of the tea extracts on lipid peroxidation induced by the extracts of blue sprat

We examined the suppressive effects of five types of tea extracts on the prooxidants extracted from the tissues of blue sprat and summarize the results in Fig 1 and Table 4. In the absence of the tea extracts, the lipid peroxidation activity was the highest in the dark meat followed by the skin, while the activities of ordinary meat and viscera were weak (Fig 1). These results agreed well with our previous study.[6] All the tea extracts examined suppressed lipid peroxidation in all tissue samples (Fig 1).

Judging from LI_{50}, the concentration of the tea extracts that suppressed the prooxidant activity to 50% of the original extract, the antioxidant activity of the tea extracts for skin was in the following order: Tungting oolong tea > Longjing-type green tea = Shy Jih Chuen oolong tea > Pouchong tea > black tea. Similar results were obtained in the cases of other tissues, and no specificity was observed on the protective effects of tea extracts on the different tissues of blue sprat (Table 4).

Table 4. The inhibitory activity of hot water tea extracts on the prooxidant activity of the blue sprat tissue extracts

Tea sample	Skin	Dark meat	Viscera	Ordinary meat
	LI_{50} of tea extracts[a]			
Longjing-type green tea	9	10	9	11
Shy Jih Chuen oolong tea	9	10	10	12
Tungting oolong tea	5	4	9	5
Pouchong tea	1	9	10	13
Black tea	19	10	17	21

[a] LI_{50}, Concentration of the tea extract (g tea leaves l-of water) that suppressed the lipid peroxidation activity to 50% of the original tissue extract.

We analyzed the relationship between the suppressive effects of tea extracts on peroxidation in blue sprat and the content of total polyphenols and

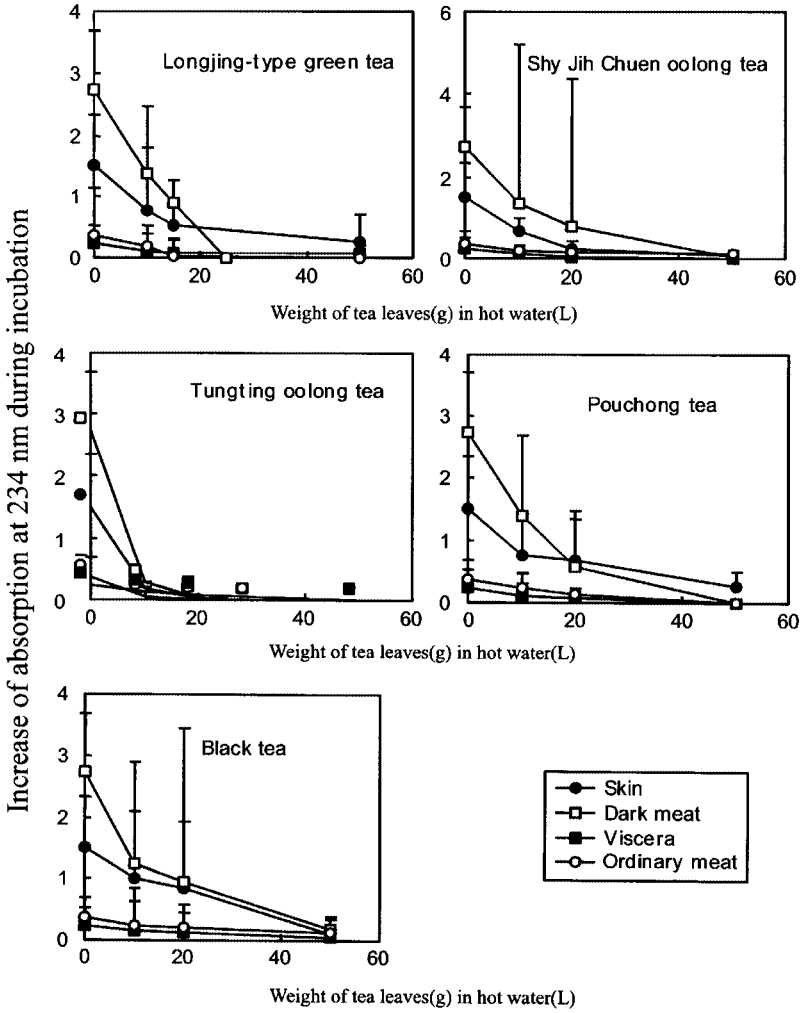

Figure 1. Effects of tea extracts on lipid peroxidation induced by the tissue extracts of blue sprat. Lipid peroxidation activity of tissue extracts was represented by increase of absorbance at 234 nm during incubation for 20 min. The protein content in the tissue extracts of blue sprat was adjusted to 1 g.

catechins. The antioxidative effects showed a positive correlation with the content of catechins in the tea extracts, ie Longjing-type green tea ≈ Tungting oolong tea ≫ Pouchong tea > Shy Jih Chuen oolong tea ≫ black tea, and also agreed well with the total catechins–total polyphenols ratio, but were not correlated with the content of total polyphenols (Tables 3 and 4). These results suggested that major antioxidative substances responsible for the retardation of lipid oxidation induced in blue sprat are catechins. The Tungting oolong tea was the most effective in suppressing the lipid peroxidation in blue sprat, which may be due to >2-fold higher epigallocatechin gallate (EGCG) content in the Tungting oolong tea than in Longjing-type green tea (Table 3), suggesting the importance of EGCG for the suppression of lipid peroxidation. High antioxidative activity of EGCG has already been reported.[19] The oxidatively polymerized polyphenols such as thearubigins, large amounts of which are contained in black tea, were suggested to be ineffective in preventing oxidation in blue sprat.

Evaluation of the prevention of lipid oxidation in blue sprat

Since the Tungting oolong tea was the most effective in retardation of lipid peroxidation induced by the blue sprat extracts in the above experiments, we assessed the suppressive effects of impregnation of blue sprat in the Tungting oolong tea extract on lipid peroxidation. In both raw and impregnated blue sprats, the water content was 78.3%. Fig 2 shows the effects of impregnation in the tea extract on the POV of refrigerated blue sprat, and Fig 3 shows the CV of refrigerated blue sprat. As shown in Fig 2, there were significant differences in the POV of the samples stored in a refrigerator between those on day 0 and on days

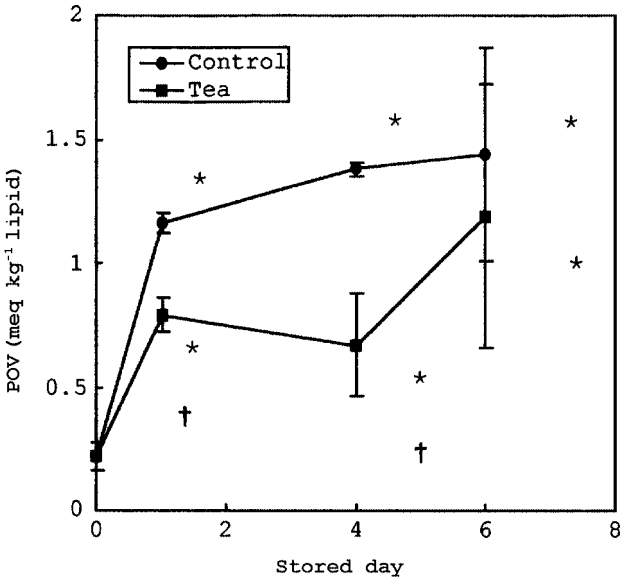

Figure 2. Effects of impregnation with the hot water extract of the Tungting oolong tea on the POV of the lipids extracted from refrigerated blue sprat. *Significantly different from 0 days ($p < 0.05$). † Significantly different from control ($p < 0.05$).

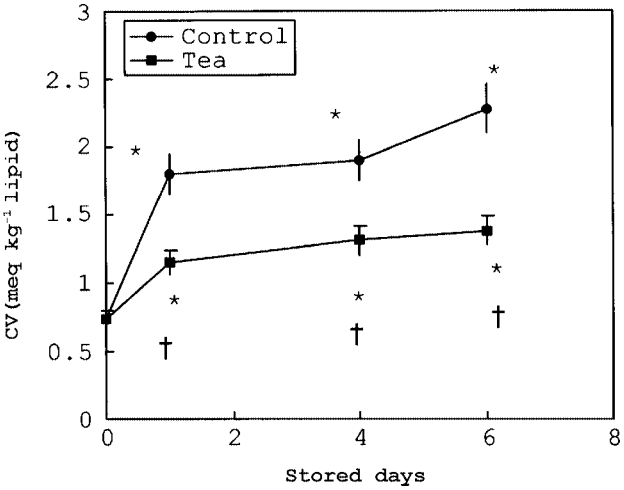

Figure 3. Effects of impregnation with the hot water extract of the Tungting oolong tea on the CV of the lipids extracted from refrigerated blue sprat. *Significantly different from 0 days ($p < 0.05$). † Significantly different from control ($p < 0.05$).

1, 4 and 6, but the POV of the samples impregnated in the tea extract was much less elevated between days 1 and 4. There were significant differences between the impregnation and control groups on days 1 and 4. The CV showed a tendency similar to that of the POV (Fig 3). The CV in the control group was gradually elevated during the storage period while, in the impregnation group, lipid oxidation was suppressed. There were significant differences in the CV between the control and impregnation groups on all storage days, 1, 4 and 6.

The POV and CV obtained in this study indicated that impregnation of blue sprat by the tea extract was effective in suppressing deterioration by lipid oxidation during storage in a refrigerator. As shown in our previous study,[6] the activity of lipid peroxidation in the water extract of blue sprat tissues was highest in the dark meat, followed by the viscera and skin. Since the lipid peroxidation activity in the dark meat is thermally stable, it may have been due to myoglobin, which is present at a high percentage. Since the skin is the outermost layer in contact with the atmosphere, it is likely to be most susceptible to lipid peroxidation when fish is stored intact,[20] but the lipid peroxidation is easily inactivated by heating, suggesting the involvement of lipoxygenase-like enzymes.[6]

Catechins and their gallates, which are good free radical inhibitors, are effective on autoxidation of fats and oils[1] and suppressing lipid oxidation induced by the skin of Japanese sardines and various other fishes.[21] As shown in this study, catechins effectively suppressed the lipid oxidation induced by the water extracts of blue sprat tissues.[22] In Japan, tea extracts have been considered to suppress fishy smell, and used in cooking of boiled fish. The effects are reported to be mainly due to decrease in volatile amines.[23] As the volatile aldehydes derived from lipid oxidation are also responsible for fishy odor,[24] tea extracts may

be involved in deodorization by their antioxidative activity. The present study demonstrated that tea extracts suppressed lipid oxidation of blue sprat, which is very susceptive to oxidative deterioration.

CONCLUSIONS

Among five types of Taiwanese teas, the Tungting tea was the most antioxidative on prooxidative extracts of blue sprat followed by Longjing-type green tea, Shy Jih Chuen oolong tea and Pouchong tea, and the effect of black tea was the smallest. The antioxidant activity was positively correlated with the content of catechins, especially with that of EGCG, but not with the content of total polyphenols. The impregnation of blue sprat in the extract of Tungting oolong tea was effective in retarding the lipid peroxidation during storage in a refrigerator.

REFERENCES

1 Matsuzaki T and Hara Y, Antioxidative activity of tea leaf catechins. *Nippon Nogeikagaku Kaishi* **59**:129–134 (1985).

2 Roberts EAM and Myers M, The phenolic substances of manufactured tea. VI. The preparation of theaflavin and of theaflavin gallate. *J Sci Food Agric* **10**:176–179 (1959).

3 Von GA, Joubert E and Hansmann CF, Comparison of the antioxidant activity of rooibos tea with green, oolong and black tea. *Food Chem* **60**:73–77 (1997).

4 Terao J, Piskula M and Yao Q, Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. *Arch Biochem Biophys* **308**:278–284 (1994).

5 Yoshino K, Hara Y, Sano M and Tomita I, Antioxidative effects of black tea theaflavins and thearubigin on lipid peroxidation of rat liver homogenates induced by tert-butyl hydroperoxide. *Biol Pharm Bull* **17**:146–149 (1994).

6 Seto Y, Endo Y and Fujimoto K, Characterization of prooxidants in tissues of blue sprat. *Nippon Shokuhin Kagaku Kogaku Kaishi* **49**:703–711 (2002).

7 Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents. *Am J Enol Vitic* **16**:144–158 (1965).

8 Mohri S, Cho S-Y, Endo Y and Fujimoto K, Linoleate 13 (S)-lipoxygenase in sardine skin. *J Agric Food Chem* **40**:573–576 (1992).

9 Surrey K, Spectrophotometric method for determination of lipoxidase activity. *Plant Physiol* **39**:65–70 (1964).

10 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**:265–275 (1951).

11 Yamanishi T, *Science of Tea.* Shokabo, Tokyo, pp 159–160 (1999).

12 Folch J, Lees M and Sloane-Stanley GH, A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**:497–509 (1957).

13 Japanese Society of Food Technology, *Analytical Methods of Foods*, pp 4–8 (1982).

14 Japan Oil Chemists' Society, *Standard Methods for Fats, Oils and Related Materials*, Vol 1, p 2.5.2.1 (1996).

15 Chiba T, Takazawa M and Fujimoto K, A simple method for estimating carbonyl content in peroxide-containing oils. *J Am Oil Chem Soc* **66**:1588–1592 (1989).

16 Ishimura S, *How to Use Analysis of Variance.* Tokyo Tosho, Tokyo, pp 152–155 (2001).

17 Yamamoto M, Types of tea and their manufacturing processes, in *Health Science of Tea*, Ed by Muramatsu K, Oguni K, Isemura M, Sugiyama K and Yamamoto M. Japan Scientific Societies Press, Tokyo, pp 17–23 (2002).

18 Yamamoto M, Physiological modulative functions of tea. *Nippon Shokuhin Kagaku Kogaku Kaishi* **43**:653–662 (1996).

19 Iwai K, Sato M and Matsue H, Relationship between antioxidative activity estimated by XYZ-dish method and catechin concentration in commercially available tea drinks. *Nippon Shokuhin Kagaku Kogaku Kaishi* **47**:120–129 (2000).

20 Yamaguchi K, Nakamura T and Toyomizu M, Preferential lipid oxidation in the skin of round fish. *Nippon Suisan Gakkaishi* **50**:869–874 (1984).

21 Mohri S, Tokuori K, Endo Y and Fujimoto K, Prooxidant activities in fish skin extracts and effects of some antioxidants and inhibitors on their activities. *Fish Sci* **65**:269–273 (1999).

22 Nida S, Miller N, Paganga G, Tijburg L, Bolwell GP and Rice-Evans C, Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch Biochem Biophys* **322**:339–346 (1995).

23 Hatae K, Sato T and Yoshimatsu F, Effect of green tea infusions on tenderness of bones and intensity of odors in cooked fish. *J Home Econom Jpn* **31**:88–93 (1980).

24 Fujimoto K, Flavor chemistry of fish oils, in *Flavor Chemistry of Lipid Foods*, Ed by Min DB and Smouse TH. American Oil Chemists' Society, Champaign, IL, pp 190–195 (1989).