Studies on the Control of Lipid Oxidation in Ground Fish by Some Polyphenolic Natural Products

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Oxidative rancidity leads to the qualitative deterioration of muscle foods, resulting in the production of unpalatable flavor and odor, thereby shortening shelf life. Lipid oxidation, measured by the production of thiobarbituric acid reactive substances (TBARS), proceeded to a greater extent in raw and cooked fish stored at 4 °C than in that stored at −20 °C over 3 weeks. Several antioxidants were used to study their effects on lipid oxidation in ground fish. On storage (4 °C) day 14, all of the antioxidants, except rutin (200 and 30 ppm) and α-tocopherol (30 ppm), were effective in inhibiting lipid oxidation in raw fish. L-Ascorbic acid acted as a prooxidant in steam- and microwave-cooked fish, as well as in the 1-week-stored (at either 4 or −20 °C) steam-cooked fish. The polyphenols quercetin (200 ppm), myricetin (200 ppm), tannic acid (30 and 200 ppm), and ellagic acid (30 and 200 ppm) were potent antioxidants under the same conditions.

INTRODUCTION

Lipid oxidation, defined as the oxidative deterioration of polyunsaturated fatty acids, is a free radical mediated phenomenon. It occurs via a chain reaction mechanism and can be initiated either enzymatically or nonenzymatically. The process can give rise to several degenerative conditions and can cause the qualitative deterioration of muscle foods producing off-flavor and off-odor effects from them (Olcott, 1962; Watts, 1962; Shahidi and Brooker, 1988).

Flavonoids are naturally occurring benzo-γ-pyrone derivatives, ubiquitous in photosynthesizing cells and accessible to animals through their diet. They can be found in fruits, vegetables, nuts, seeds, leaves, flowers, and bark (Wollenweber and Dietz, 1981). The human dietary consumption of these natural products is about 1 g/day of mixed flavonoids (Kuhnau, 1976).

Since the first report by Rusznyak and Szent-Gyorgi (1936), flavonoids have been known to exhibit a myriad of pharmacological effects on biological systems (Cody et al., 1986). Some flavonoids exhibit antioxidative effects and so are able to inhibit free radical mediated reactions (Kimuya et al., 1981; Younes and Siegers, 1981; Sorata et al., 1984; Valenzuela et al., 1985; Jha et al., 1985; Kappus and Lukacs, 1986; Ratty and Das, 1988). Flavonoids have been shown to exhibit quality-preserving and antioxidative effects on raw and cooked meat (Herrmann, 1976), on edible oils (Das and Pereira, 1990; Pereira and Das, 1990), and on milk fat and lard (Nelson, 1980).

Fish is more prone to lipid oxidation than meat, due to the high degree of unsaturation in fish lipids (Olcott, 1962) and to the high concentrations of metals in seafood (Sweet, 1973; Khayat and Schwall, 1983). The use of various antioxidants in controlling lipid oxidation in fish systems has been reported by several workers (Jurewicz and Salmonowicz, 1971; Benedict et al., 1975; Zama et al., 1979; Arai and Kinumaki, 1980; Ke et al., 1981).

Owing to the limited reports on the use of natural plant products for the control of lipid oxidation in meats, the present study was carried out to investigate the effects of several flavonoids, polyphenols, and other commonly used synthetic and natural antioxidants on lipid oxidation in raw and cooked ground fish (Ikan tinggeri or *Scomberomorus commersoni*) stored at either −20 or 4 °C. It also aimed to determine the possible use of some of these natural products as antioxidants in the storage of raw and cooked fish.

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MATERIALS AND METHODS

Materials. The flavonoids rutin, quercetin, morin, myricetin, and kaempferol and the polyphenols tannic acid and ellagic acid were obtained from Extrasynthese (Genay, France). The antioxidants L-ascorbic acid, α-tocopherol, and butylated hydroxytoluene were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of analytical grade and were obtained from either Sigma or E. Merck (Darmstadt, Germany).

Preparation of Fish. The fish (*Scomberomorus commersoni*) was obtained from the local wet market. After filleting, the fish was deskinned and deboned before grinding in a blender for 30 s at maximum speed. The ground fish was then divided into 10-g portions prior to the addition of the test compounds. The different samples of fish used in this study were analyzed for fat content according to the modified Folch method (Low and Ng, 1987).

Preparation of Test Compounds. Relative amounts of the test compounds were dissolved separately in 50% ethanol. Aliquots (0.5 mL) of this stock solution were then mixed well into the samples of ground fish (10 g) to obtain the final concentrations of either 30 or 200 ppm. Control samples either contained 50% ethanol (Tables I–III) or had no such additives (Figures 1 and 2). All of the samples were individually wrapped in aluminum foil, placed in a polyethylene bag, and stored at either −20 or 4 °C until analyzed.

Assay for Thiobarbituric Acid Reactive Substances (TBARS). The TBA test is commonly used as a measurement for the extent of lipid oxidation in muscle foods (Gray, 1978; Rhee, 1978). The procedure used was that described by Siu and Draper (1978). Briefly, each fish sample (10 g) was homogenized in 25 mL of distilled water using a polytron homogenizer (Kinematica AG) for 30 s at speed 3 (10 000 rpm). Twenty-five milliliters of 10% trichloroacetic acid (TCA) was then added to the homogenate, and the mixture was vortex mixed and filtered. One milliliter of thiobarbituric acid (TBA, 0.06 M) was added to 4-mL aliquots of the filtrate, which were then heated in a boiling water bath (10 min) for color development. The tubes were then cooled, and the absorbance was read at 532 nm using a Shimadzu dual-beam soectrophotometer (Model 160-A).

The extent of lipid oxidation was expressed either as (i) TBA number [mg of malonyldialdehyde (MDA)/kg of fish] calculated

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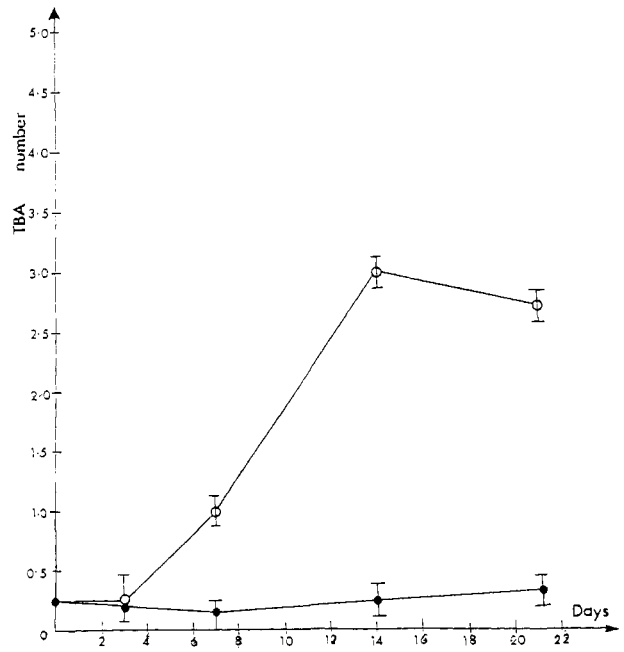

Figure 1. Effect of storage (4 and −20 °C) of raw fish on lipid oxidation: (●) raw fish stored at −20 °C; (○) raw fish stored at 4 °C. (Each point is the average value of four determinations, two different experiments.)

using the molar extinction coefficient for MDA, $\epsilon = 1.56 \times 10^5$ M^{-1} cm^{-1} (Sinnhuber and Yu, 1958), or as (ii) % TBARS, determined using $A_{532(test)}/A_{532(control)} \times 100\%$. (The extraction efficiency of TBARS was 77 ± 3%).

Treatment of Fish. *(A) Without Any Additives.* Duplicate packets of raw and steam-cooked fish samples were analyzed for TBARS at 0, 3, 7, 14, and 21 days after storage at either −20 or 4 °C.

(B) With Additives to Raw Fish. Weekly (over a 3-week period), duplicate packets of fish samples containing the various additives (at concentrations of either 30 or 200 ppm) were defrosted at ambient temperature and assayed for TBARS.

(C) With Additives to Precooked Fresh Fish. After addition of the various test compounds (at the concentration of 200 ppm), the raw fish samples were stored (24 h) at −20 °C. After this time, duplicate packets of the fish samples were defrosted at ambient temperature. Each sample (10 g) was spread evenly on a glass Petri dish and subjected to either steam (for 15 min in a closed boiling water bath) or microwave (for 30 s at 50% power in a Samsung RE-725 TC microwave oven) cooking. The cooked samples were cooled to ambient temperature before they were assayed for TBARS.

(D) With Additives to Precooked Stored Fish. Fish samples pretreated with the various test compounds were stored (24 h) at −20 °C and then defrosted at ambient temperature before they were transferred to glass Petri dishes and subjected to steam cooking. The cooked fish samples were then stored in glass Petri dishes at either −20 or 4 °C for 1 week before they were analyzed for TBARS.

Statistics. The results obtained from various experiments were analyzed using the unpaired Student's *t*-test (Snedecor and Cochran, 1971).

RESULTS AND DISCUSSION

Effect of Storage of Raw Fish. The extent of lipid oxidation in raw fish stored at −20 or 4 °C (common household storage temperatures) over a 3-week period, in the absence of any additives, is shown in Figure 1. The inherent lipid oxidation occurring in fresh raw ground fish can be observed from the data shown.

There was no significant lipid oxidation ($p < 0.05$) in raw fish stored at −20 °C over the storage period (Figure 1). This finding supports the report by Birch and Lindley (1986), who showed that biological materials undergo very little deterioration when stored at low temperatures. Therefore, it was felt unnecessary to investigate the effects of antioxidants in the control of lipid oxidation in raw fish stored at −20 °C. However, raw fish stored at 4 °C exhibited a gradual increase in the rate of lipid oxidation from day 0 to day 14, followed by a slight decrease on day 21 (Figure 1). The increase in the extent of lipid oxidation with temperature has been reported and attributed to the increase in the rate of propagation and decomposition of alkyl peroxides (Troller and Christian, 1978).

In view of this finding, we decided to investigate further the effects of various antioxidants on the control of lipid oxidation in raw fish stored at 4 °C. The results are presented in Table I. Each test compound was used at two concentration levels (200 and 30 ppm) to determine its effectiveness at either the low or the high addition levels. These two concentrations lie within the range of commercial antioxidants and have also been used in similar studies by other workers (Shahidi and Brooker, 1988; Benedict et al., 1975; Ke et al., 1981).

Table I. Effect of Antioxidants on Raw Fish Stored at 4 °C over 3 Weeks

		TBA number,[a] mg of MDA/kg			
compound	ppm	day 1	day 7	day 14	day 21
control (50% ethanol)		0.1	0.7	1.8	1.5
rutin	30	0.1	0.4b	1.0c	0.6d
	200	0.1	0.3b	0.9c	0.5d
quercetin	30	0.1	0.2b	0.2c	0.2d
	200	0.1	0.1b	0.1c	0.1d
morin	30	0.1	0.2b	0.4c	0.3d
	200	0.1	0.1b	0.2c	0.1d
myricetin	30	0.1	0.1b	0.1c	0.1d
	200	0.1	0.1b	0.1c	0.1d
kaempferol	30	0.1	0.1b	0.3c	0.3d
	200	0.1	0.1b	0.2c	0.2d
tannic acid	30	0.1	0.3b	0.3c	0.5d
	200	0.1	0.2b	0.2c	0.3d
ellagic acid	30	0.1	0.3b	0.6c	0.6d
	200	0.1	0.2b	0.4c	0.5d
L-ascorbic acid	30	0.1	0.2b	0.2c	0.3d
	200	0.1	0.2b	0.2c	0.2d
α-tocopherol	30	0.1	0.5b	0.9c	0.3d
	200	0.1	0.4b	0.6c	0.2d
butylated hydroxytoluene	30	0.2a	0.2b	0.2c	0.2d
	200	0.2a	0.2b	0.2c	0.2d

[a] Each value is the mean of four determinations (two different experiments). SD values varied between 10 and 20%. Values with the same suffix, within a column, are significantly different from the control ($p < 0.05$).

Effect of Storage of Antioxidant Pretreated Raw Fish. Raw fish pretreated with quercetin, morin, myricetin, kaempferol, tannic acid, ellagic acid, L-ascorbic acid, and BHT exhibited <50% TBA numbers compared to the control samples, throughout the storage period (Table I). However, on day 14, raw fish pretreated with rutin (200 and 30 ppm) and α-tocopherol (30 ppm) showed that these compounds were less effective than the other test compounds. They exhibited >50% TBA numbers compared to the control samples (Table I). On day 21, the TBA numbers of some samples decreased (rutin, morin, α-tocopherol, and control), while others increased (tannic acid and ellagic acid) and some remained unchanged (quercetin, myricetin, kaempferol, L-ascorbic acid, and BHT) (Table I). It is interesting to note that all of the antioxidants were generally more effective at the higher con-

Table II. Effect of Antioxidants (200 ppm) on Cooked Fish

compound	% TBARS[a] (A) steam-cooked	(B) microwave-cooked
control (50% ethanol)	100*	100**
rutin	37a	19b
quercetin	9a	2b
morin	25a	73b
myricetin	37a	20b
kaempferol	63a	26b
tannic acid	4a	2b
ellagic acid	3a	3b
L-ascorbic acid	205a	198b
α-tocopherol	123	85b
butylated hydroxytoluene	72a	11b

[a] Each value is the mean of four determinations (two different experiments). SD values varied between 10 and 20%. *, TBA no. = 2.03 ± 0.21. **, TBA no. = 3.29 ± 0.04. Values with the same suffix, within a column, are significantly different from the control ($p < 0.05$).

centration, with the exception of myricetin and BHT, which were equally effective at both concentrations.

Effect of Cooking. The effect of two different methods of cooking (steam and microwave) on lipid oxidation in antioxidant-pretreated ground fish was also studied. In Asian countries, fish is commonly steam-cooked; however, microwave cooking is gaining in popularity.

It is known that the extent of lipid oxidation is increased after cooking. The process probably disrupts the muscle membrane system, thereby exposing the lipid components to oxygen and other reaction catalysts such as iron (Sato and Hegarty, 1971; Love and Pearson, 1976; Igene et al., 1979). Thus, further studies were carried out to determine whether polyphenols could inhibit the increase in lipid oxidation caused by cooking (regardless of the mode of cooking).

Effect of Antioxidant-Treated Cooked Fish. L-Ascorbic acid acted as a prooxidant (>100% TBARS of the control samples) when ground fish was pretreated with this vitamin and subjected to either steam or microwave cooking (Table II). Other workers have also shown that L-ascorbic acid acted as a prooxidant in meat (Sato and Hegarty, 1971; Benedict et al., 1975; Igene et al., 1985).

The other test compounds used in this study acted as antioxidants (Table II) in pretreated cooked ground fish (regardless of the mode of cooking). Rutin, quercetin, myricetin, tannic acid, and ellagic acid were potent antioxidants (exhibiting <50% TBARS of the controls) in both steam- or microwave-cooked fish (Table II). α-Tocopherol, on the other hand, was not an effective antioxidant in ground fish that was subjected to either steam (123% of control) or microwave cooking (85% of control) (Table II). Morin was effective in pretreated steam-cooked fish (25% of control), while kaempferol and BHT were effective in pretreated microwave-cooked fish (26 and 11% of control, respectively) (Table II). From the data it is not possible to infer as to whether a test compound was more effective in inhibiting lipid oxidation in steam- or microwave-cooked fish, because the type of energy involved in the two modes of cooking is quite different.

Effect of Storage of Cooked Fish. The extent of lipid oxidation in steam-cooked fish stored at −20 °C, without pretreatment of additives, increased from day 0 to day 3 and then remained relatively unchanged ($p > 0.05$) from day 3 to day 21 (Figure 2). Steam-cooked fish stored at 4 °C, on the other hand, exhibited a significantly greater extent of lipid oxidation ($p < 0.05$) when compared to fish stored at −20 °C over the 3 weeks (Figure 2). These data

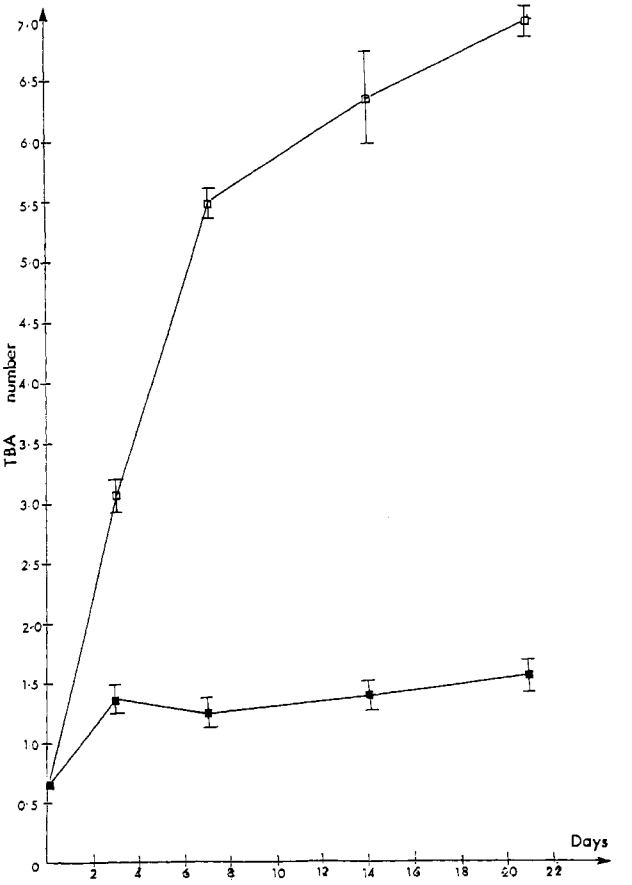

Figure 2. Effect of storage (4 and −20 °C) of steam-cooked fish on lipid oxidation: (■) steam-cooked fish stored at −20 °C; (□) steam-cooked fish stored at 4 °C. (Each point is the average value of four determinations, two different experiments.)

give an overview of the inherent lipid oxidation that is present in steam-cooked fish upon storage.

Effect of Storage of Antioxidant Pretreated Cooked Fish. L-Ascorbic acid (at both 30 and 200 ppm) added to precooked and stored (1 week at either −20 or 4 °C) ground fish still acted as a prooxidant (Table III). Steam-cooked ground fish pretreated with one of the following compounds (200 ppm), quercetin, morin, myricetin, kaempferol, tannic acid, and ellagic acid, all produced <50% TBARS at the end of 1 week of storage at either −20 or 4 °C (Table III). However, rutin and α-tocopherol (200 ppm) were less effective antioxidants (>50% TBARS of control samples) than the other test compounds under the same experimental conditions (Table III). The test compounds were generally more effective antioxidants at the higher concentration (200 ppm) than at the lower concentration (30 ppm).

None of the test compounds used in this study interfered with the TBA test (results not shown). It must also be noted that the different samples of fish used in this study did not vary significantly ($p < 0.05$) in their lipid content. Their lipid values ranged from 1.5 to 1.9%. Therefore, the varying antioxidant potencies observed for the test compounds cannot be attributed to the differences in the lipid content of the fish.

The present study indicated that the polyphenols rutin, quercetin, morin, myricetin, kaempferol, tannic acid, and ellagic acid were generally more effective antioxidants (at 200 ppm) than L-ascorbic acid or α-tocopherol. Several workers have reported that polyphenols possess molecular structural features that enable them to act as free radical acceptors as well as metal chelators (Simpson and

Table III. Effect of Antioxidants on Steam-Cooked Fish Stored for 1 Week at 4 and −20 °C

compound	ppm	% TBARS[a] (A) 4 °C	% TBARS[a] (B) −20 °C
control (50% ethanol)		100*	100**
rutin	30	79a	83b
	200	50a	56b
quercetin	30	63a	38b
	200	12a	16b
morin	30	72a	60b
	200	20a	23b
myricetin	30	22a	30b
	200	3a	11b
kaempferol	30	74a	81b
	200	35a	48b
tannic acid	30	6a	16b
	200	3a	13b
ellagic acid	30	5a	16b
	200	3a	15b
L-ascorbic acid	30	124a	373b
	200	128a	223b
α-tocopherol	30	73a	75b
	200	60a	50b
butylated hydroxytoluene	30	28a	65b
	200	26a	53b

[a] Each value is the mean of four determinations (two different experiments). SD values varied between 10 and 20%. *, TBA no. = 5.49 ± 0.13. **, TBA no. = 1.26 ± 0.01. Values with the same suffix, within a column, are significantly different from the control ($p < 0.05$).

Uri, 1956; Mehta and Seshardi, 1958; Crawford et al., 1960; Ratty et al., 1988). Heimann and Reiff (1953) showed that (a) the α,β-unsaturated ketone structure of the pyrone ring, (b) the free hydroxyl group on the C ring, and (c) the o-hydroxyl groups on the phenyl side ring increased the antioxidant effect of flavones in commercial ethyl linoleate.

Although the antioxidative action of BHT is comparable to that of some of the polyphenols used in this study, it is not desired for use as a food additive because it can act as a mutagenic and/or carcinogenic agent (Imaida et al., 1983; Chen et al., 1986). Polyphenols, on the other hand, are naturally occurring, nontoxic plant products present ubiquitously in vascular plants (Bate-Smith, 1954; Griffith et al., 1955; Booth and DeEds, 1958; Glick and Joslyn, 1969).

In view of our findings, the application of the natural plant products flavonoids and polyphenols as alternative preservatives in foods may be exploited and merits further investigation.

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Registry No. BHT, 128-37-0; rutin, 153-18-4; quercetin, 117-39-5; morin, 480-16-0; myricetin, 529-44-2; kaempferol, 520-18-3; ellagic acid, 476-66-4; L-ascorbic acid, 50-81-7; α-tocopherol, 59-02-9.