Shelf-Life Dating of Fish Meats in Terms of Oxidative Rancidity as Measured by Chemiluminescence

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Chemiluminescence of fresh minced meats of six fish species was measured in order to examine the applicability of chemiluminescence in the prediction of shelf life. The chemiluminescence intensity of fresh fish meats was increased in proportion to the measuring temperature and differed significantly among fish meats. The shelf life of fish meats was estimated based on oxidative deterioration as measured by peroxide, carbonyl and thiobarbituric acid values, as well as sensory evaluation during storage of the fish meats at 0°C. It was observed that the shelf life of minced fish meats as judged by oxidative deterioration significantly correlated with chemiluminescence intensity of the fresh meats. The chemiluminescence method was shown to be very available for the prediction of the shelf life of fish meat.

KEY WORDS:Chemiluminescence, fish meats, lipid peroxidation, oxidative rancidity, shelf-life dating.

A very important problem in the field of food chemistry has been to predict the shelf life of oils and oily foods. Although aging tests, such as the oven test, generally have been used for prediction of meat and fish as well as edible oil, determination of the shelf life is time consuming (1). Several new techniques for predicting the shelf life of oily foods such as fresh meat and fish have been reported. Rhee *et al.* (2) have reported that lipid peroxidation in beef muscle was correlated with myoglobin and pigment contents, and proposed heme compounds as an important factor in the oxidative stability of meats. Kajimoto (3) has recommended that the degree of oxidative deterioration of lipids in foods should be used as an index of the shelf life of oily foods. Ke *et al.* (4) have suggested that the actual frozen shelf life of fishes might be determined by the thiobarbituric acid (TBA) value based on the oven test for lipids extracted from fish meats. Kurade and Baranowski (5) have also reported that the shelf life of frozen and minced fish meats might be predicted by measuring TBA value, iron, myogobin and hemoglobin levels. However, speedier and easier methods for predicting the shelf life of foods have been sought.

On the other hand, it is known that chemiluminescence can be detected in foods and biomaterials containing fats and oils exposed to air (6,7). Chemiluminescence is thought to be generated by active oxygen species such as singlet oxygen and electrically exited states provoked by free radical reaction and lipid peroxidation (8). We have previously reported that the oxidative deterioration of edible oils and fried foods (9) and the activity of antioxidative compounds (10) might be determined by measuring chemiluminescence intensity.

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In this study, we used fish meats as typical oily foods and tried to develop a chemiluminescence method for predicting their shelf life, because the chemiluminescence method is both versatile and speedy to operate.

MATERIALS AND METHODS

Materials. Six species of fresh fishes, sardine (*Sardinops melanostictus*), red sea bream (*Pagrus major*), tuna (*Thunnus orientalis*), Kichiji (*Sebastolobus macrochir*), mackerel (*Scomber japonicus*) and blue sprat (*Spratelloides gracilis*) were purchased from a fish store in Sendai, Japan. All fish meats were minced after removing the head, tail, skin and internal organs from each fish. All samples were stored at -25°C until analysis.

Storage test. Ten grams of minced fish meats were put in a polyethylene bag and stored at 0°C. Chemical characteristics such as peroxide (PV), carbonyl (CV) and TBA values, as well as sensory evaluation, were estimated daily.

Analysis. Chemiluminescence intensity of each fresh fish meat was measured with a Chemiluminescence Analyzer OX-3A (Tohoku Electronic Industries Co., Sendai, Japan) at 25, 35 and 45°C after five grams of fresh minced fish meats was placed in a stainless steel cell (53 mm in a diameter), as described in our previous papers (9,10). Chemiluminescence was also measured for minced fish meats after storage at 0°C. For sardine meat, chemiluminescence was also measured at 35°C after tert-butylhydroxyanisole (BHA), as a typical antioxidant, was added to it. Chemiluminescence intensity of fish meats was represented as counts per 30 seconds.

Lipid content was gravimetrically determined after extraction from fresh minced fish meats with a mixture of chloroform and methanol (2:1) (11). Fatty acid composition was estimated by gas liquid chromatography.

Sensory evaluation of stored fish meats was made by twelve panelists in our laboratory. Off-flavor generated in fish meats was judged by all panelists during storage. Shelf life of each fish meat was determined to be the period until the day when half of the panelists judged it inedible.

TBA values of stored fish meats were measured by the method described by Yu and Sinnhuber (12), and the malondialdehyde (MDA) level was represented as the TBA value. PV and CV were estimated by the colorimetric method (13) and Kumazawa and Oyama's method (14), respectively, after lipids were extracted from stored fish meats with a chloroform and methanol (2:1) mixture.

RESULTS AND DISCUSSION

A typical chemiluminescence pattern of fresh minced fish meats is in Figure 1. Chemiluminescence intensity of fish meats generally increased with time at 35°C. Blue sprat, sardine and mackerel meats showed higher chemilumi-

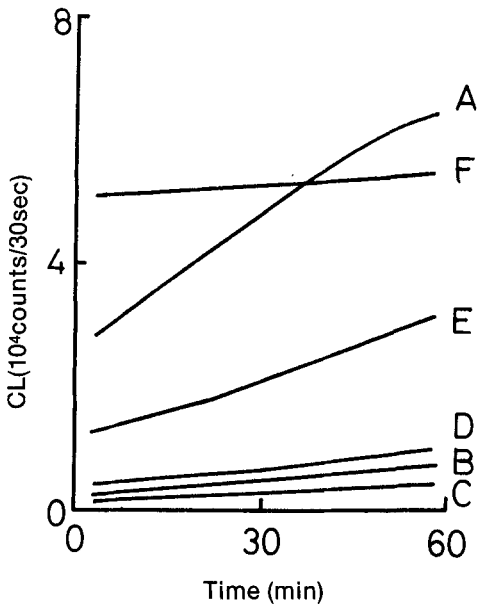

FIG. 1. Changes in chemiluminescence (CL) in fresh fish meats measured at 35°C. A, sardine; B, red sea bream; C, tuna; D, kichiji; E, mackerel; and F, blue sprat.

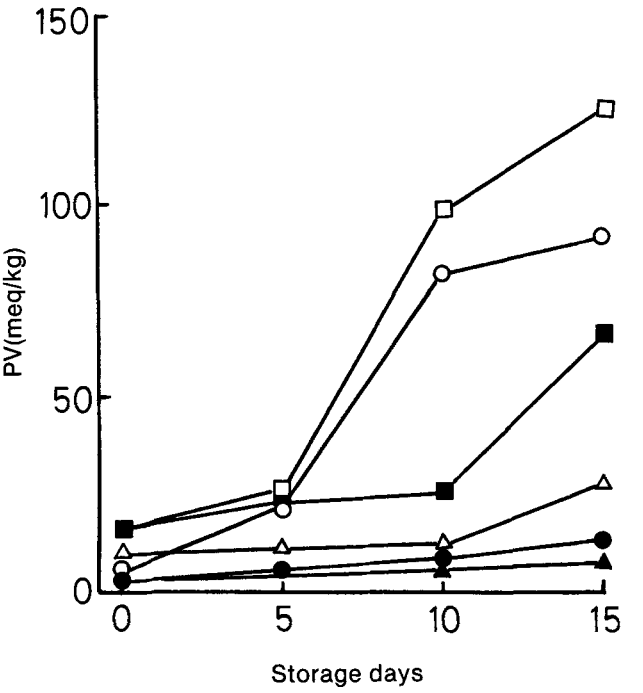

FIG. 3. Changes in peroxide value (PV) of total lipids in fish meats during storage at 0°C. ○, sardine; ●, red sea bream; △, tuna; ▲, kichiji; □, mackerel; and ■, blue sprat.

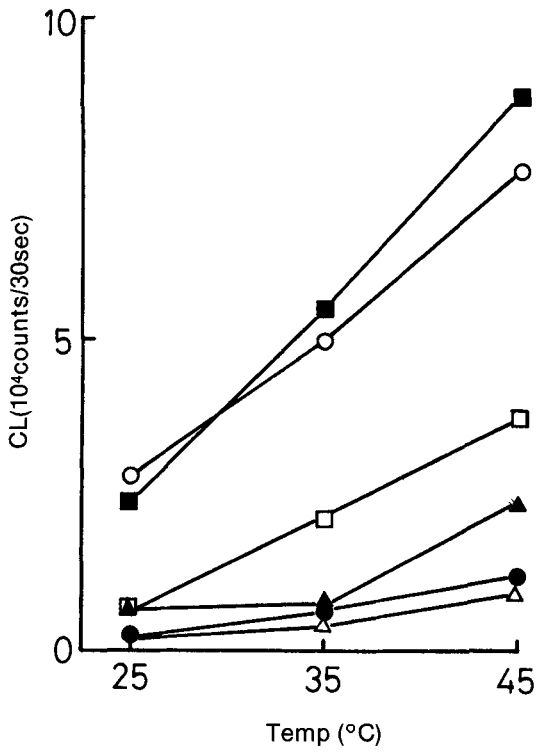

FIG. 2. Effect of sample temperatures on chemiluminescence (CL) in fresh fish meats. CL intensity was estimated after incubation for 30 min at 25, 35 and 45°C. ○, sardine; ●, red sea bream; △, tuna; ▲, kichiji; □, mackerel; ■, blue sprat.

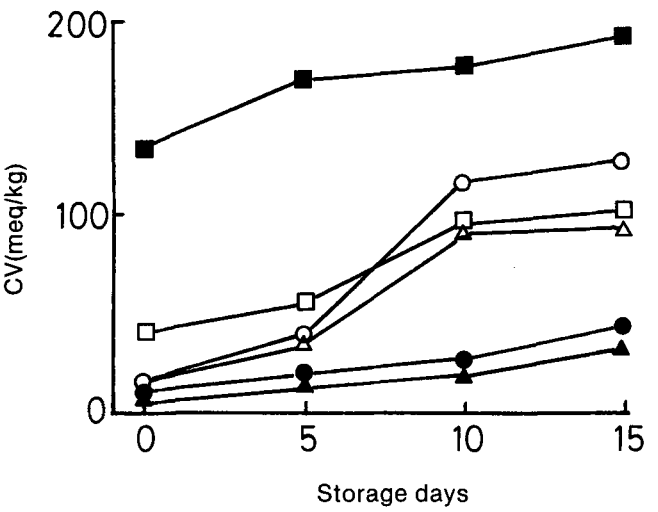

FIG. 4. Changes in carbonyl value (CV) of total lipids in fish meats during storage at 0°C. ○, sardine; ●, red sea bream; △, tuna; ▲, kichiji; □, mackerel; and ■,blue sprat.

nescence intensities among the fish meats tested. In particular, blue sprat showed the highes initial chemiluminescence intensity of all at 35°C, while the ratio of increase in chemiluminescence intensity of sardine and mackerel meats during the 1 hr-measurement was the most remarkable. On the other hand, there were low chemiluminescence intensities in red sea bream, tuna and kichiji meats. The effects of measurement temperatures on chemiluminescence generated in fresh minced fish meats are shown in Figure 2, where the chemilumi-

TABLE 1

Relationship Between Chemiluminescence Intensity, Lipid, Eicosapentaenoic (EPA) and Docosahexaenoic Acid (DHA) Contents and Shelf Life of Fish Meats

	Sardine	Red sea bream	Tuna	Kichiji	Mackerel	Blue sprat
Chemiluminescence intensity[a] (100 counts/30 sec)						
After 30 min	478	47	24	57	196	526
Total amounts (0–30 min)	22779	2180	1060	2958	9197	31081
Lipid content (%)	16.1	1.7	1.6	17.8	12.1	1.5
Fatty acid content (%)						
EPA (20:5)	12.0	2.6	4.3	8.6	8.6	11.1
DHA (22:6)	13.0	12.0	18.2	5.1	19.1	21.4
Shelf Life[b] (days)	4.4	13.0	13.2	10.0	5.5	1.8

[a]Chemiluminescence was measured for 60 min at 35°C.

[b]Shelf life was the storage period until the day when half of the panelists judged the fish meat inedible.

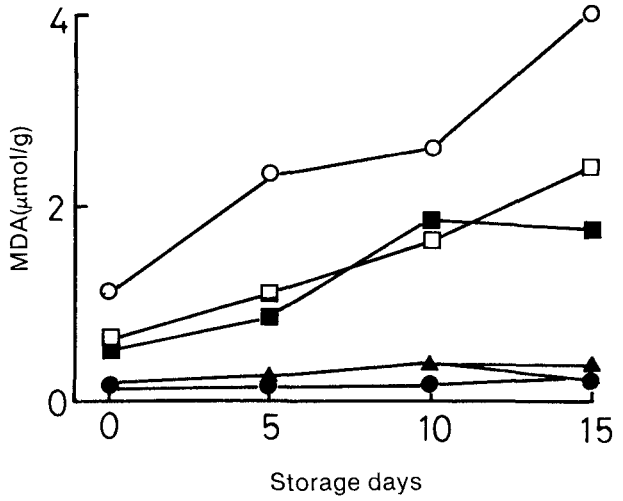

FIG. 5. Changes in thiobarubituric acid (TBA) value of fish meats during storage at 0°C. TBA value was converted into malondialdehyde (MDA) level. ○, sardine; ●, red sea bream: △, tuna; ▲, kichiji; □, mackerel; and ■, blue sprat.

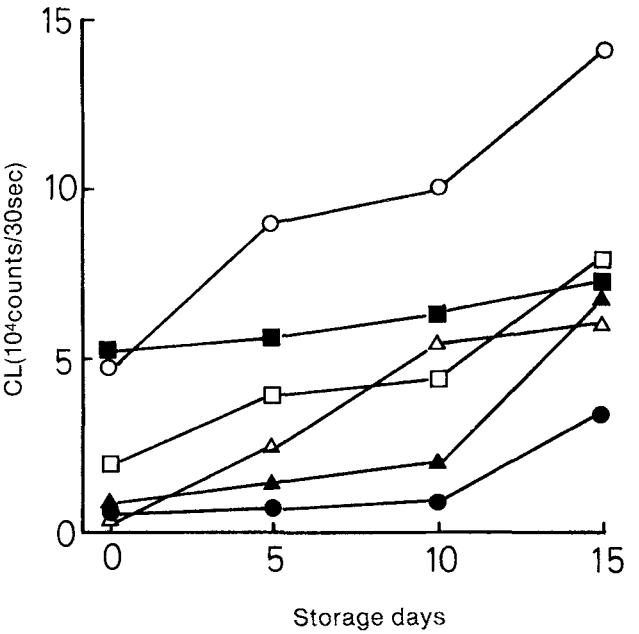

FIG. 6. Changes in chemiluminescense (CL) of minced fish meats during storage at 0°C. CL intensity of six fish species was estimated after incubating the sample meats at 35°C for 30 min, after minced meats had been stored at 0°C for 0 to 15 days. ○, sardine; ●, red sea bream; △, tuna; ▲, kichiji; □, mackerel; and ■, blue sprat.

nescence intensity of fresh fish meats are given after incubation for 30 min at 25, 35 and 45°C. A similar pattern was observed in chemiluminescence emission among all fish meats tested. It was observed that in all fish meats chemiluminescence intensity was increased in proportion to the sample temperature. Among them, blue sprat meat showed lower chemiluminescence intensity than sardine meat when measured at 25°C, although the blue sprat showed higher chemiluminescence intensity than the sardine at 35 and 45°C. The lowest chemiluminescence intensity was observed for red sea bream and tuna meats at all temperatures examined. Chemiluminescence generated from fish meats depended on fish species and sample temperature.

Chemical characteristics and sensory evaluation were investigated for minced meats of six fish species during storage at 0°C, to determine their oxidative deterioration. Changes in PV, CV and TBA values of minced fish meats during storage at 0°C are shown in Figure 3, 4 and 5. Sardine and mackerel meats showed very high PV after 10 days of storage (Fig. 3). The PV of blue sprat meat was also significantly increased after 15 days of storage. Among all fish meats examined the highest CV was observed in blue sprat meat (Fig. 4). Sardine, mackerel and tuna meats also showed relatively higher CV during storage, as compared with red sea bream and kichiji. Moreover, large increases in TBA value were also observed in sardine, mackerel and blue sprat meats (Fig. 5). From these results, lipids contained in blue sprat, sardine and mackerel meats were found to be highly susceptible to oxidative derioration during storage at 0°C. Lipids in kichiji and red sea bream meats, however, seem to be rather stable against oxidative deterioration, because all their chemi-

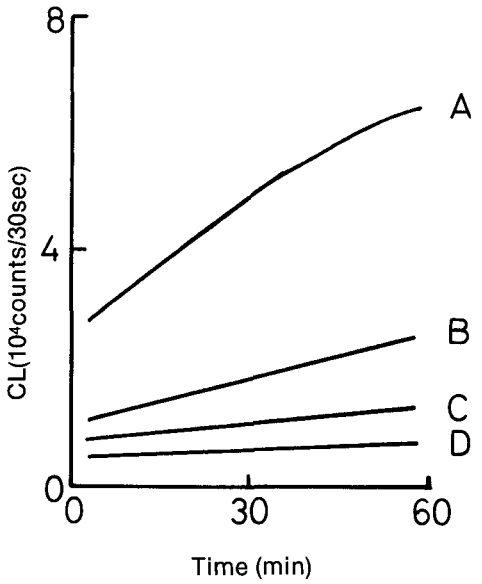

FIG. 7. Effects of antioxidants on chemiluminescence (CL) of minced sardine meats. CL was measured at 35°C, after BHA was added to minced sardine meats at levels of 0 to 0.02%. A, control; B, 0.002% BHA; C, 0.01% BHA; and D, 0.02% BHA.

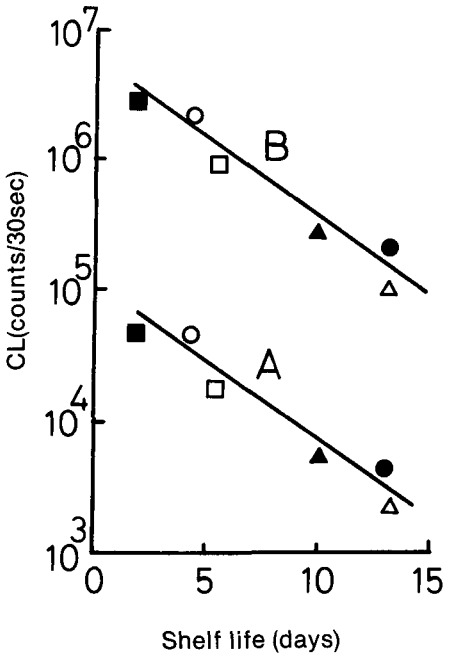

FIG. 8. Correlation between chemiluminescence (CL) and shelf life of minced fish meats. A, CL measured after 30 min-incubation at 35°C; B, total CL integrated during incubation from 0 to 30 min at 35°C; ○, sardine: ●, red sea bream; △, tuna; ▲, kichiji; □, mackerel; and ■, blue sprat.

cal characteristics were low even after storage. These results were also in accordance with the sensory evaluation of stored fish meats. Strong oxidative and deteriorative flavor was observed in blue sprat, sardine and mackerel meats rather than in kichiji and red sea bream meats, even in the early storage stage at 0°C. Lipid peroxidation in fish meats was recognized as a major factor in determining the shelf life of fish meats.

The relationship between chemiluminescence, lipid content and fatty acid composition of fish meats was investigated because the chemiluminescence observed in high fat-containing foods is thought to originate mainly from lipid peroxidation, and because many fish meats contain high levels of eicosapentaeonic (EPA) and docosahexaenoic acids (DHA) which are susceptible to oxidation. Table 1 shows lipid, EPA and DHA levels and chemiluminescence intensities, as well as shelf life of fish meats. Sardine and mackerel meats showing intense chemiluminescences contained large amounts of lipids, while red sea bream and tuna meats showing lower chemiluminescence intensity contained fewer lipids. However, no correlation was observed between lipid content and chemiluminescence intensity, because blue sprat meat showed very high chemiluminescence intensity in spite of a lower lipid content, and kichiji meat showed low chemiluminescence intensity in spite of a high lipid content. On the other hand, it was an interesting observation that EPA and DHA contents, and particularly EPA content, in fish meats were correlated with their strong chemiluminescence to some extent. In fact, blue sprat and sardine meats that showed very high chemiluminescence intensity had high levels of EPA, as shown in Table 1. However, chemiluminescence of fish meats could not be easily explained by EPA level alone because metals, natural antioxidants, oxidative and reductive enzymes etc., might affect chemiluminescence in addition to oxidative deterioration in fish meats.

On the other hand, it was confirmed that oxidative deterioration contributed to chemiluminescence observed in fish meats since the chemiluminescence intensity was increased in all fish meats in accordance with storage period (Fig. 6). Sardine, blue sprat and mackerel meats always showed intense chemiluminescence during storage at 0°C, and tuna, kichiji and red sea bream meats also showed much higher chemiluminescence emission after 10 days of storage at 0°C than before storage. Moreover, as shown in Figure 7, the observation that the chemiluminescence of minced sardine meats could be reduced remarkably by addition of an antioxidant (free radical scavenger) such as BHA also demonstrated that chemiluminescence was derived from lipid peroxidation in fish meats. Therefore, we concluded that measurement of chemiluminescence in minced fish meats may provide a means of predicting shelf life based on oxidative deterioration. Table 1 also shows actual shelf life as determined by sensory evaluation of minced fish meats during storage at 0°C (in days): blue sprat, 1.8; sardine, 4.4; mackerel, 5.5; kichiji, 10.0; red sea bream, 13.0; and tuna, 13.2. The coefficient of correlation between the chemiluminescence intensity measured after incubating the sample meats at 35°C for 30 min and the shelf life, and between the integrated chemiluminescence counts measured for 0 to 30 min at 35°C and the shelf life were -0.970 and -0.976, respectively (Fig. 8). There was a significant correlation between the chemiluminescence and the shelf life of fish meats, although the shelf life of fish meats could not be determined by lipid and highly unsaturated fatty acid levels alone. These results suggest that the chemiluminescence method may be of value in the prediction of shelf life of fish meats.

Measurement of TBA value in lipids extracted from fish meats (4) and levels of minor components, such as heme

compounds and iron (5), have been established as tests to predict shelf life of minced fish meats. However, these methods require a long time and involve lengthy procedures as do the conventional aging tests. On the other hand, the chemiluminescence method that we have developed is so versatile and speedy to operate that it should be of practical use for predicting the shelf life of various minced fish meats. We expect that the chemiluminescence method can also be applied for the prediction of shelf life of other products such as cereals, fried snack foods, fresh meat, frozen meat, seafood, coffee, tea, etc.

REFERENCES

1. Labuza, T.P., in *Shelf-Life Dating of Foods*, Food & Nutrition Press Inc., Westport, CT, 1982, p. 41.
2. Rhee, K.-S., S.C. Seideman and H.R. Cross, *J. Agric. Food Chem. 34*:308 (1986).
3. Kajimoto, G., *J. Jpn. Cook Sci.* 9:199 (1976).
4. Ke, P.J., B.A. Linke and B. Smith-Lall, *Lebensm.-Wiss. u.-Technol.* 15:203 (1982).
5. Kurade, S.A., and J.D. Baranowski, *J. Food Sci. 52*:300 (1987).
6. Miyazawa, T., R. Usuki and T. Kaneda, *Agric. Biol. Chem. 46*:1671 (1982).
7. Usuki, R., and T. Miyazawa, *J. Antibact. Antifung. Agents 11*:475 (1983).
8. Miyazawa, T., K. Fujimoto and T. Kaneda, in *Lipid Peroxidation in Biological Systems*, edited by A. Sevanian, American Oil Chemists Society, Champaign, IL, 1988, p. 1.
9. Usuki, R., T. Kaneda, A. Yamagishi, C. Takyu and H. Inaba, *J. Food Sci. 44*:1573 (1979).
10. Usuki, R., Y. Endo and T. Kaneda, *Nippon Shokuhin Kogyo Gakkaishi 28*:583 (1981).
11. Folch, J., M. Lee and G.H. Sloane Stanley, *J. Biol. Chem. 226*:497 (1957).
12. Yu, T.C., and R.O. Sinnhuber, *J. Am. Oil Chem. Soc. 44*:256 (1967).
13. Asakawa, T., and S. Matsushita, *Lipids 15*:965 (1980).
14. Kumazawa, H., and T. Oyama, *Yukagaku 14*:167 (1965).

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