# Control of Lipid Oxidation in Fish Oil with Various Antioxidative Compounds

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**Samples of a commercial fish oil were separately treated with various chemical compounds and then studied for their susceptibility to rancidity by means of an accelerated oxidation test at 60°C. α-Tocopherol acetate and ascorbyl palmitate showed the lowest antioxidative effects among the group of seven chemicals. Anoxomer, a synthetic phenolic polymer, had an antioxidative power comparable to that of ethoxyquin, butylated hydroxytoluene or butylated hydroxyanisole when all were applied to the oil in the concentration of 0.02%. However, the most powerful antioxidant was tertiary-butylhydroquinone (TBHQ), with an antioxidant efficiency twice that of the above-mentioned phenolic compounds when used at only 0.01% concentration in the oil. Although TBHQ and Anoxomer proved to be potential compounds for preventing rancidity in fish oils, their use is still hindered by the limited acceptance from the appropriate authorities.**

KEY WORDS: Antioxidants, capelin, fish oil, oxidation.

Fish oils have been an important ingredient of edible fats and a source of energy in different formulations for animal feed. The world production of fish oils was about 1,400 metric tons in 1985 and was expected to rise to 1,540 metric tons by 1990, as reviewed by Mielke in 1987 (1).

As a source of nutritionally important ω3 fatty acids, marine oils are the most well-known. The two main components of this fatty acid family, eicosapentaenoic (20:5ω3, EPA) and docosahexaenoic (22:6ω3, DHA) acids, exist in high proportions in various fish oils (2,3). Although the importance of these acids is not yet completely understood, their essential nature indicates a bright future for fish oils. The high content of EPA, DHA and other unsaturated acids makes fish oils susceptible to oxidative deterioration. These oxidative changes result in the formation of low-molecular weight carbonyl compounds, which in turn give rise to the unpleasant off-flavor in rancid fish oils (4).

Rancidity of fish oils can be retarded in several ways. Phenolic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and organic esters of gallic acid (gallates) have been widely used as antioxidants in foods containing fish oils. Their safety, however, has been questioned after Ito (5) reported his findings showing BHA to be carcinogenic in animal experiments. Although the phenolic compounds are chemically related to each other, their physiological effects in humans may not be the same. For this reason the Joint FAO/WHO Expert Committee on Food Additives (JECFA) might consider each individual antioxidant separately rather than grouping them together (6). Nevertheless, the need for efficient and safe new antioxidants remains.

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The aim of this study was to compare the efficiency of some commonly used antioxidants to that of lesser known or less commonly used compounds in retarding oxidation in a selected commercial fish oil. The compounds consisted of α-tocopherol acetate, ascorbyl palmitate, BHA, BHT and propyl gallate as commonly used antioxidants. The other structures included ethoxyquin (EQ), tertiary-butylhydroquinone (TBHQ) and Anoxomer. Ethoxyquin is extensively used in fish meal production (7), whereas the use of TBHQ is currently authorized in just a few countries, including the U.S., Australia and Israel (8). Anoxomer is a synthetic nonabsorbable polymer with a phenolic nature. It is not yet approved for use in foods.

## MATERIALS AND METHODS

*Fish oil.* The fish oil used in these experiments was processed from capelin *(Mallotus villosus)* caught in Norwegian waters. The oil was sampled from a commercial process before addition of any antioxidant. The iodine value (IV), free fatty acids (FFA) and peroxide value (POV) were determined for the oil by AOAC-methods 28.020, 28.029 and 28.022 (9), respectively. In addition, the fatty acid composition of the oil was analyzed as described earlier (10).

*Antioxidants.* The antioxidants α-tocopherol acetate and ascorbyl palmitate were purchased from E. Merck (Darmstadt, Germany). BHA, BHT, propyl gallate (PG) and TBHQ were obtained from Eastman Chemical International (Zug, Switzerland). Ethoxyquin was donated by Rexolin Chemicals (Helsingborg, Denmark) and Anoxomer samples were obtained from the inventors (Dynapol, Palo Alto, CA). Antioxidants were separately dissolved in ethanol to give 5% (w/vol) solutions. These solutions were added to the fish-oil aliquots to produce the oil samples for the experiments. The percentage amounts of antioxidants in oil were selected according to the levels allowed in Finland, or they were based on the instructions of the suppliers. Moreover, several concentration levels ranging from 0.02% to 0.2% were studied for Anoxomer. The amounts of other antioxidants in oil were 0.02%, except for PG and TBHQ, which were used in concentrations of 0.01%, and α-tocopherol acetate, which was applied to the oil at the level of 0.03%.

*Accelerated oxidation test.* For the measurement of the rate of lipid oxidation the oil samples treated with antioxidants were placed in a forced hot-air oven at 60°C. The weights of oil samples were regularly recorded and sampling was made for the thiobarbituric acid (TBA)-value determinations. The sample weights after 6 h from the start of an experiment were used as the basis for calculations to avoid any error due to the evaporation of alcohol from the added antioxidant solution. The method has been described in detail by Kaitaranta and Ke (11).

*Thiobarbituric acid value.* The TBA values were determined by the direct spectrophotometric micromethod of Ke and Woyewoda (12). The TBA value was calculated

according to the molar absorptivity at 538 nm and expressed as mmol malonaldehyde/kg of oil.

## RESULTS

*Fish oil.* Gas chromatographic analysis on a free fatty acid phase (FFAP) capillary column revealed more than 40 components in the fatty acid analysis. Thirty-one identified components, ranging from 14 to 24 in carbon chain-length, were used in the quantitations. The major fatty acids and their relative amounts in the oil are listed in Table 1.

*Anoxomer in retarding rancidity.* The efficiency of Anoxomer in fish oils was unknown, and thus a practical concentration range was to be studied. Figure 1 shows the percentage weight gain of the fish oil mixed with varying amounts of Anoxomer as found in the accelerated oxidation test. The respective changes in the TBA values are shown in Figure 2.

*Relative efficiency of antioxidants.* The weight gains in the accelerated oxidation test of the fish oils treated with selected amounts of various antioxidants are shown in Figure 3. The TBA values were measured at the time of

TABLE 1

**The General Quality Parameters and the Major Fatty Acids in the Capelin Oil Used in the Accelerated Oxidation Tests**

| Fatty acid | Amount (%) | Fatty acid | Amount (%) |
|---|---|---|---|
| 14:0 | 6.9 | 22:1ω9 | 1.7 |
| 16:0 | 11.8 | 24:1 | 0.7 |
| i 17:0 | 0.6 | 18:2ω6 | 1.6 |
| i 18:0 | 0.8 | 18:3ω3 | 0.8 |
| 18:0 | 0.9 | 18:4ω3 | 4.2 |
| 16:1ω7 | 9.8 | 20:4ω3 | 0.5 |
| 18:1ω9 | 9.0 | 20:5ω3 | 9.1 |
| 18:1ω7 | 2.7 | 22:5ω3 | 0.6 |
| 18:1ω5 | 0.6 | 22:6ω3 | 6.5 |
| 20:1ω9 | 14.7 | Others | 2.9 |
| 20:1ω7 | 0.5 | | |
| 22:1ω11 | 13.1 | Total | 100 |
| Iodine value | 141 | | |
| Free fatty acids | 3.2 | | |
| Peroxide value | 6.4 | | |
| TBA value | 0.2 | | |

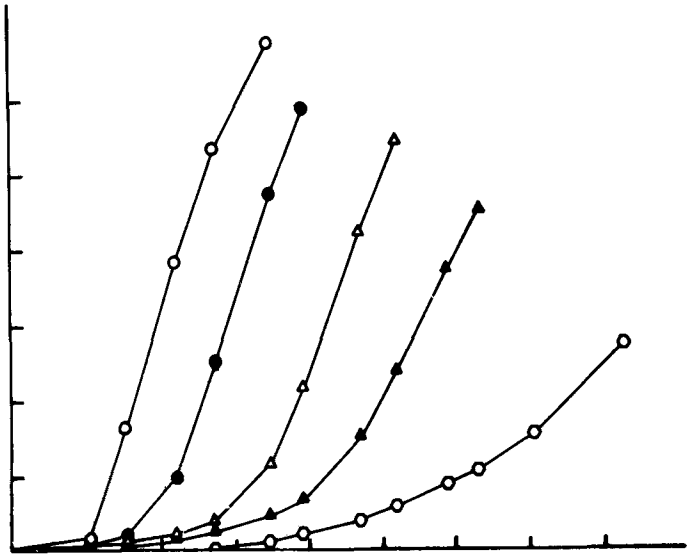

**FIG. 1. Development of weight gain in capelin oil treated with different amounts of Anoxomer during autoxidation at 60°C. Symbols: ○, Control fish oil without an antioxidant; ●, fish oil with 0.02% Anoxomer; △, fish oil with 0.05% Anoxomer; ▲, fish oil with 0.10% Anoxomer; and ○, fish oil with 0.20% Anoxomer.**

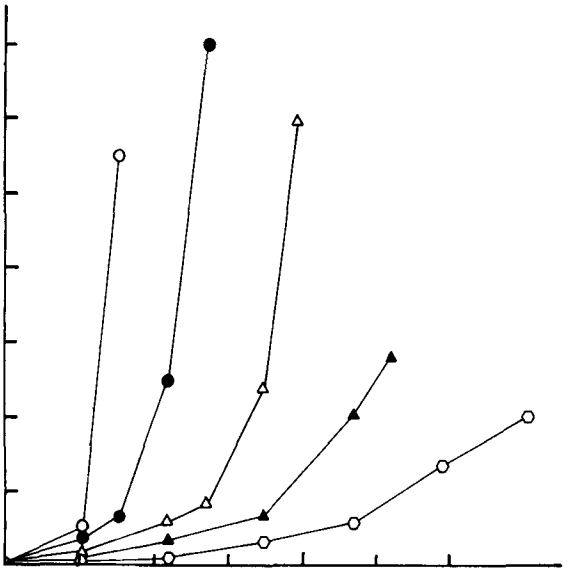

**FIG. 2. Development of TBA value (mmol/kg oil) in capelin oil treated with different amounts of Anoxomer. Symbols as in Figure 1.**

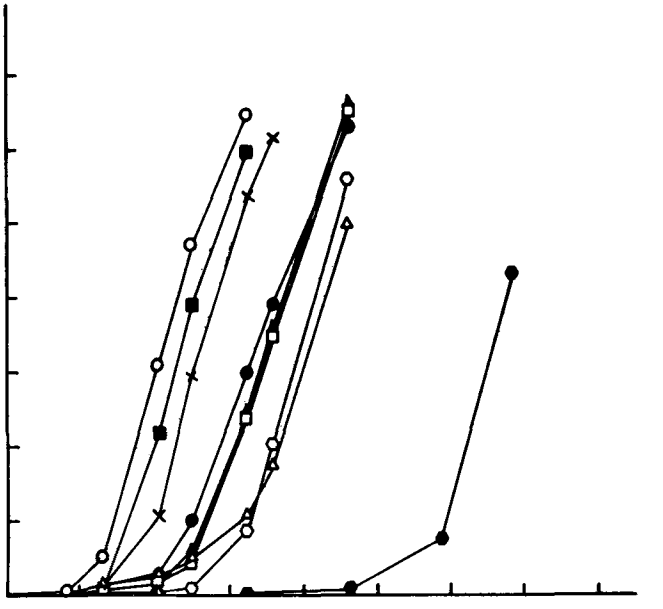

**FIG. 3. Development of weight gain in capelin oil treated with different antioxidative compounds during autoxidation at 60°C. Symbols: ○, Control fish oil without an antioxidant; ●, fish oil with 0.02% Anoxomer; △, fish oil with 0.02% BHA; ▲, fish oil with 0.02% BHT; ●, fish oil with 0.01% propyl gallate; ○, fish oil with 0.01% TBHQ; □, fish oil with 0.03% α-tocopherol acetate; ■, fish oil with 0.02% ethoxyquin; and ×, fish oil with 0.02% ascorbyl palmitate.**

TABLE 2

**TBA Values of Capelin Oil Treated with Various Antioxidants at the Time of 0.6% Weight Increase During the Accelerated Oxidation Test at 60°C**

| Antioxidant treatment | TBA value (mmol/kg) | Duration of the test (h) | Antioxidant efficiency |
|---|---|---|---|
| Control oil | 10.0 | 30 | 1 |
| α-Tocopherol acetate, 0.03% | 19.4 | 34 | 1.1 |
| Ascorbyl palmitate, 0.02% | 20.0 | 42 | 1.4 |
| Anoxomer, 0.02% | 15.0 | 52 | 1.7 |
| BHT, 0.02% | 11.0 | 55 | 1.8 |
| Ethoxyquin, 0.02% | 10.2 | 56 | 1.9 |
| Propyl gallate, 0.01% | 28.8 | 67 | 2.2 |
| BHA, 0.02% | 9.2 | 67 | 2.2 |
| TBHQ, 0.01% | 9.0 | 119 | 4.0 |

weight recording, and the results corresponding to the 0.6% weight gain are listed in Table 2.

## DISCUSSION

*Fish oil composition.* The high content of FFA, 3.2% of the total fatty acids, and the POV of 6.4 meq/kg oil suggest that there were ongoing oxidative reactions in the fish oil. The iodine value, 141, is proof of the high unsaturation of fatty acids, thus supporting the assumption about high susceptibility to oxidation. The low TBA value, however, refers only to minor progress in the secondary reactions of lipid oxidation and is still at a level reported earlier for fish oils (12).

The fatty acid analysis revealed that more than 75% of the acids were unsaturated, which is in good accordance with the high iodine value. The major components and their percentages, with almost one-third of the acids made up of monoenoic C20 and C22 chains, are comparable to those reported earlier (13) for the triglycerides of a commercially produced Canadian capelin oil.

*Anoxomer as an antioxidant in fish oil.* Anoxomer is a synthetic oil-soluble polymer formed by condensation of several phenolic compounds with divinylbenzene (14). This patented compound was developed to meet the needs of the food industry. It is claimed to be a nonabsorbable polymer with a molecular size too large to be digested and absorbed (14). To date, no reports have been produced dealing with the antioxidative potential of Anoxomer in fish oils. In vegetable oils its effectiveness has been studied within the concentration range from 0.01 to 1.0% (14). The lower end was selected in this study to provide a comparison with the amounts of other phenolic compounds typically used in various oils.

The oxidation test at 60°C involving weight gain and TBA measurements was applied in this study because it had been shown to be practical in following lipid oxidation in various fish oils (11,15). Even the lowest concentration of Anoxomer, 0.02%, showed a significant retardation in the oxidation rate (Fig. 1). The efficiency increased with the antioxidant concentration up to the highest amount used in this study. The same effect can be seen in the TBA values (Fig. 2).

In Germany, a 27% limit has been proposed for the content of polar artefacts in frying oils (16). This limit, as applied to fatty fish lipids in the accelerated oxidation test, corresponds to a 0.6% weight gain (11). The 0.6% weight increase was reached after 27 h of experiment for the control oil, and within 45–130 h for the oil containing 0.02–0.2% Anoxomer (Table 3). The retarding power for oxidation or the efficiency factor ranged from 1.7 to 4.8, as calculated from the oxidation rate in the control oil.

The TBA values obtained from Figure 2 at the point of 0.6% weight increase varied from 18.5 in the control oil to 8.5 in the oils with 0.1 or 0.2% Anoxomer (Table 3). These values fall into the range described as acceptable when compared to the rancidity indices proposed for frozen mackerel (4). That proposal suggests that the product is unacceptable if the TBA value in the oil exceeds 19. Thus, the control oil in this study at the point of 0.6% weight gain was already on the border line, especially if its TBA value is considered. The remarkably low TBA value of 8.5 meq/kg oil after a prolonged study in the oils with the highest Anoxomer contents may result partly from the escape of the volatile TBA-reactive compounds over the course of time.

*Retardation of fish oil oxidation.* Based on the progress of oxidation in the fish oil, as measured by the weight gain method, the selected antioxidants can be separated into three groups (Fig. 3). The first group is composed of α-tocopherol acetate and ascorbyl palmitate. The second group includes Anoxomer, EQ, BHT, BHA and PG, whereas only one compound, TBHQ, goes into the third group. The control oil without added antioxidant reached the 0.6% weight increase after 30 h at 60°C (Table 2). For the same weight gain, the oils with 0.03% α-tocopherol acetate or 0.02% ascorbyl palmitate needed 34 and 42 h, respectively. This delay in oxidation indicates that these antioxidants have efficiency values of 1.1 and 1.4, respectively. α-Tocopherol acetate and ascorbyl palmitate used in these concentrations provide poor protection against oxidation in fish oil. Thus, their use in fish oils cannot be justified, although their structural components exist as natural compounds.

The components in the second group were all used at 0.02% concentration, except for PG. The 0.01% concentration for PG was selected due to the limitations to the use of gallates in Finland. Anoxomer, EQ and BHT had similar behavior in retarding oxidation, and their efficiency readings varied from 1.7 to 1.9, whereas PG and

TABLE 3

**TBA Values of Capelin Oil Treated with Various Amounts of Anoxomer at the Time of 0.6% Weight Increase During the Accelerated Oxidation Test at 60°C**

| Percentage of Anoxomer in oil | TBA value (mmol/kg) | Duration of the test (h) | Antioxidant efficiency |
|---|---|---|---|
| Unoxidized oil | | 0 | |
| 0 (Control) | 18.5 | 27 | 1 |
| 0.02 | 14.8 | 45 | 1.7 |
| 0.05 | 13.0 | 70 | 2.6 |
| 0.10 | 8.5 | 88 | 3.3 |
| 0.20 | 8.5 | 130 | 4.8 |

BHA each had an efficiency reading of 2.2. As seen in Figure 2, the TBA method gives a good picture of the development of oxidation in fish oil. However, when comparing the TBA values at the time of 0.6% weight increase for oils with varying antioxidants (Table 2), a wide range, from 9.0 to 28.8 mmol/kg oil, is found. An earlier study (11) also showed that the correlation between polar lipid content and weight gain was linear, whereas a hyperbolic curve described the correlation between polar lipid content and the development of the TBA value. Therefore, we can conclude again that a single TBA value, like those given in Table 2, shows the amount of the secondary oxidation products at the time of measurement, but it does not really describe the overall quality of the oil. If used for following the development of oxidation (Fig. 2), the TBA value complements the information obtained by other methods.

The content of TBHQ in the oil was equal to that of PG. This selection was made after the preliminary experiments, which showed practically no measurable oxidation in the accelerated test if the oil contained more than 0.02% TBHQ. Despite the low content in the oil, an efficiency value of 4.0 was measured for TBHQ. This is more than double the value of EQ, which is commonly used in fish oils of the same origin, although EQ is usually applied at 0.04–0.05%. Recently, the Codex Committee on Fats and Oils amended the food additive provisions of all Codex Standards for Fats and Oils and established the following maximum levels in mg/kg: BHT, 75; PG, 100; TBHQ, 120; BHA, 175; tocopherols, 500 and ascorbyl palmitate, 500 (17). This decision shows the continuing awareness of the health aspects of antioxidants. Comparing these limits to those contents used in this study, we can see that $\alpha$-tocopherol acetate and ascorbyl palmitate, as well as PG and TBHQ, were within the limits, although BHA (and especially BHT) were used in excessively high concentrations.

The results in this study suggest that the use of TBHQ as an antioxidant in fish oil affords the best protection against oxidation. This effectiveness is obtained well within the limits set for this compound. Another interesting result is that Anoxomer, a synthetic polymer, proved to be equal in its antioxidative power to the commonly used BHT and EQ, and did not differ significantly from BHA. If the inventors can convince the authorities about the nonabsorbability of Anoxomer, it could become an important quality protector in fish oils.

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