Solubilization of Vitamin C in Fish Oil and Synergistic Effect with Vitamin E in Retarding Oxidation

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Antioxidative effects of ascorbic acid and δ-tocopherol on the oxidation of sardine oil stored at 30°C in the dark has been investigated. It was found from phase diagrams and peroxide values of fish oil/lecithin/water systems that the desirable levels of lecithin and water to solubilize ascorbic acid in the oil were 0.3% (w/w) and 0.1% (w/w), respectively. When ascorbic acid (0.02%) and δ-tocopherol (0.4%) were used together, the induction period of fish oil could be lengthened 22-fold, due to their synergism. They could inhibit the production of carbonyl and volatile compounds and oxidative polymerization.

KEY WORDS: Antioxidation, ascorbic acid, fish oil, tocopherol, water/oil microemulsion.

Since Dyerberg and Bang (1) reported the beneficial effect of fish oil to human health from an epidemiological study, a number of investigations on nutritional and medicinal roles of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have demonstrated that EPA has a potential pharmaceutical effect on the prevention of the diseases associated with the heart and circulatory systems (2) and that DHA is an important component of biological tissues, such as brain, retina and sperm (3,4).

With the growing recognition by consumers and industry of the beneficial uses of fish oil, the oil and its fatty acid concentrate products have recently become available in retail shops (5). Almost all of the products are soft capsules because polyunsaturated fatty acids contained in fish oil become rancid easily and are thus encapsulated to prevent the oil from coming into contact with oxygen.

In order to develop new products and to utilize fish oil in a wide variety of applications, protection of omega-3 fatty acids from oxidation may be the highest hurdle for food industries. The simplest method of retarding oxidation is to use antioxidant. Synthetic antioxidants have been in common use to tackle the problem (6). However, consumers are increasingly reluctant to accept synthetically derived additives in their foods. Moreover, according to legislation in some countries such as Korea and Japan, incorporation of synthetic antioxidants to EPA and DHA products is not permitted. Natural antioxidant is a natural answer in this regard.

Recently we reported that ascorbic acid can be solubilized in oils *via* reversed micelles, and that it markedly inhibited the autoxidation of fats and oils when used in mixtures with δ-tocopherol (7,8). To add understanding to the previous publications, the phase diagram of the fish oil/lecithin/water system, the peroxide value (POV) as a function of lecithin and water contents, and the antioxidative effect of ascorbic acid and δ-tocopherol are described in this paper. Their effect on the quality of the stored oil is also included.

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MATERIALS AND METHODS

Materials. Refined sardine oil, obtained from E-Hwa Oil & Fat Ind. Co. (Korea), was bleached with activated clay and charcoal and then deodorized for 1 hr at 160°C under 200 micron Hg. Refined, bleached and deodorized (RBD) oil contains 32.6% saturated fatty acids, 33.6% monoenes, 2.2% dienes, 30.3% polyunsaturated fatty acids and 1.1% unknown components. Some chemical characteristics of RBD oil are as follows: POV, 0.1 meq/Kg; acid value, 0.1; iodine value, 148; color measured by Lovibond (Tintometer Ltd., U.K.), 0.2 red and 2.4 yellow. Ascorbic acid (ACS reagent) and δ-tocopherol (*ca.* 90%) were purchased from Sigma Chemical Co. (St. Louis, MO). Lecithin (Centrol 1F-UB, Central Soya Co., Chicago, IL) was used as a surfactant for preparing water/oil (w/o) microemulsion. All the other reagents were of the highest grade available.

Preparation of oil samples with antioxidants. Solubilization of ascorbic acid was carried out according to the procedure of Han *et al.* (7). For the phase diagram of oil/lecithin/water, lecithin was solubilized in fish oil under nitrogen gas, and then distilled and demineralized water was injected. The mixture was stirred vigorously for 10 min. The amounts of lecithin and water were varied in the range of 0–0.8% (w/w). Ten grams of the prepared samples were weighed into serum vials (internal volume, 34 mL), and stored at 30°C in the dark after seal-capping. After eight days, the solubilization of water in fish oil was checked by the naked eye. If the appearance of the oil phase becomes even slightly turbid upon vigorous shaking, it was determined that the three components do not form a single phase at the concentrations of the corresponding vial.

To add ascorbic acid to fish oil, it was first solubilized in water, and then the ascorbic acid solution was injected into the oil containing lecithin. The concentration of ascorbic acid in the oil was not controlled by adding different amounts of the same ascorbic acid solution, but by adding the same amount of the solutions containing different amounts of ascorbic acid. This means that the water content in fish oil was always 0.1%, regardless of the ascorbic acid concentration in the oil. δ-Tocopherol was directly mixed with oil under nitrogen gas. The order of adding tocopherol and ascorbic acid solution to the oil does not affect their antioxidative activities. The contents of any compounds were always calculated and expressed on a weight basis.

Assessment of stability. For the assessment of the storage stability of oil samples with and without antioxidants, 10 g of oil in covered petri dishes (87 mm dia × 13 mm h) were stored in a dark incubator. To mitigate thermal oxidation of the oil and thermal degradation of ascorbic acid, the storage temperature was controlled at 30°C. After a certain storage time, petri dishes were withdrawn from the incubator for the analyses of POV, carbonyl value and fatty acid composition.

For the analysis of volatile compounds, 10 g of the prepared samples were weighed into serum vials and stored at 30°C in the dark after seal-capping.

Analytical methods. POV and carbonyl values were measured according to the procedures of AOCS Method Cd 8-53 (9) and JOCS Official Method 2.4.22-73 (10), respectively. For the determination of total volatiles in the headspace of serum vials, 1.0 mL gas sample was injected directly into a Hewlett-Packard gas chromatograph, model 5890 (Hewlett-Packard, Palo Alto, CA). A stainless-steel column packed with 10% PPE-20 on Tenax GC (Supelco Inc., Bellefonte, PA) was operated isothermally at 120°C. Total area count from the flame ionization detector was used as an index of total volatiles. Fatty acid composition was analyzed as described previously (11).

RESULTS AND DISCUSSION

Is it possible to solubilize ascorbic acid in fats and oils? Amphiphilic molecules, when dissolved in organic solvent, form spherical or ellipsoidal aggregates. In these systems, often referred to as reversed micelles, hydrophobic carbon chains of the surfactants are arranged toward the organic solvent, and hydrophilic groups are localized in the interior of the aggregates (12). Several polar solvents, including water, can be solubilized in this polar core. With the help of those polar solvents, hydrophilic compounds such as ascorbic acid can also be incorporated into that polar core.

Since the solubilization of ascorbic acid in oil is accomplished through formation of a w/o microemulsion (we prefer this general terminology to "reversed micelles" because the structure of oil/lecithin/water system is not defined), a number of surfactants that are soluble in nonpolar media and have water-holding power can be used to aid the solubilization of ascorbic acid. Preliminary experiments have shown that larger amounts of water could be solubilized in oils when dioctyl sodium sulfosuccinate and cetyltrimethylammonium bromide are used as surfactants, instead of lecithin. On the other hand, Span (TM) and Polysorbate series could not form a stable w/o microemulsion. Previously, phosphatidylcholine was used because of its practical application in the field of edible fat and oil technology. However, lecithin looks more useful because it has antioxidative properties as well as nutritive value, as well as being available on a large scale.

To determine the lecithin and water levels that form stable w/o microemulsion, various amounts of them were added to fish oil, and then the occurrence of precipitation was checked after standing eight days. The phase diagram of the fish oil/lecithin/water system is shown in Figure 1, in which the slashed region indicates where clear w/o microemulsion is maintained during the experimental period. The solubility of water in fish oil generally increases with increasing lecithin, and then, after reaching a maximum at lecithin content of around 0.1%, it decreases on further increase of lecithin. When a similar experiment was carried out with fatty acids of fish oil, the two phase diagrams were nearly superimposable (data not shown). Considering that the solvent power of fatty acids is stronger than that of the corresponding fat or oil, the data suggest that the precipitation does not result from the limited solubility of the individual components of lecithin and water but from their interaction. As is evident from the degumming process, which is used to remove lecithin from crude vegetable oil during the refining process, lecithin will bind water added to oil to cause the formation of oil-insoluble lecithin-water residue.

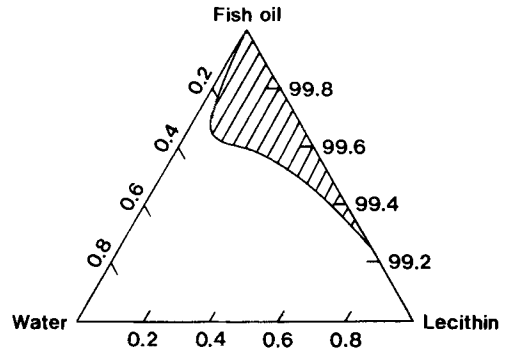

FIG. 1. Phase diagram of fish oil/lecithin/water system. Units of each component are expressed in weight percent.

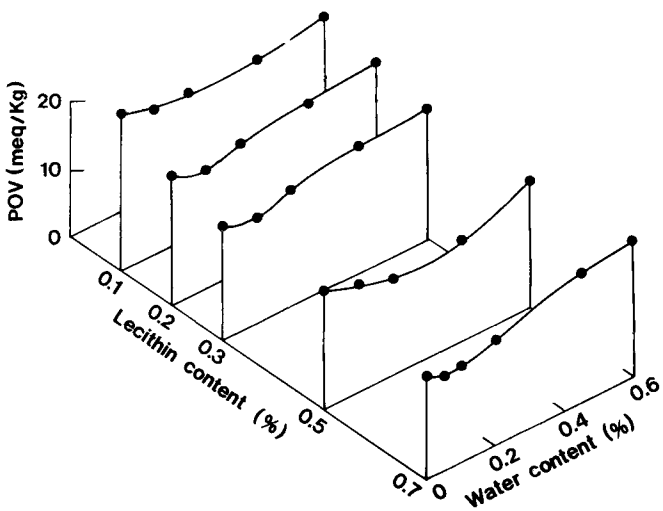

FIG. 2. Peroxide value of fish oil as a function of lecithin and water contents. POV of the oil samples in serum vials were determined after eight days of storage at 30°C in the dark.

Lecithin can act as an antioxidant in inhibiting autoxidation of polyunsaturated fatty acids. The effect of lecithin and water contents on the oil stability was analyzed from POV determinations of oil samples after eight days of storage. POV of a control (fish oil stored without antioxidant) is 24.9 meq/Kg. As shown in Figure 2, the oil stability is improved with the addition of lecithin. Its effect improves as the amount of lecithin is increased. The antioxidative property of lecithin was largely ascribed to phosphatidylethanolamine among the three major phospholipids of lecithin (13). The POV profile of the oil samples as a function of water content looks slightly concave with a minimum value at around 0.1% water. All subsequent experiments have been performed at a lecithin content of 0.3% and at a water content of 0.1%.

Some antioxidants exhibit synergistic effects when used together. The combined use of ascorbic acid and tocopherol attracts much interest because not only can they inhibit the deterioration of fats and oil *in vitro*, but also are believed to cure biological oxidation (14,15). The

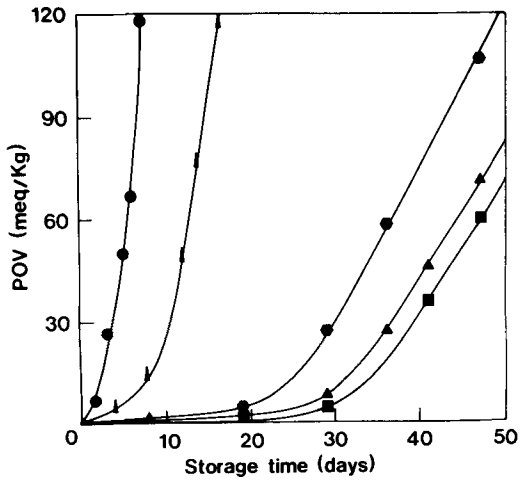

FIG. 3. Effect of ascorbic acid contents on POV of fish oil at a fixed content of δ-tocopherol (0.2%, w/w). The contents of ascorbic acid of oil samples containing 0.3% lecithin and 0.2% δ-tocopherol are: ●, 0.01%; ▲, 0.02%; ■, 0.03%. ● is control and ▌ is fish oil stabilized with 0.3% lecithin and 0.02% ascorbic acid.

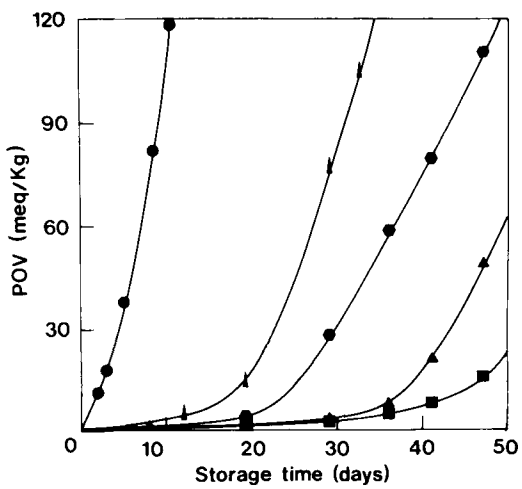

FIG. 4. Effect of δ-tocophrol contents on POV of fish oil at a fixed content of ascorbic acid (0.02%). The tocopherol contents in oil samples containing 0.3% lecithin and 0.02% ascorbic acid are: ▌, 0.05%; ●, 0.1%; ▲, 0.3%; ■, 0.4%. The symbol ● indicates fish oil stabilized with 0.2% δ-tocopherol alone.

individual and combined effects of both antioxidants are shown in Figures 3 and 4. When ascorbic acid or tocopherol was used alone, both had a weak retarding effect on autoxidation. The effect of δ-tocopherol is less than that of ascorbic acid, although the concentration of the former is higher.

When the contents of ascorbic acid were varied at a fixed content of δ-tocopherol of 0.2%, the induction period, defined as the time to reach the intersect of two tangents of lag and exponential periods in the oxidation curve, is roughly proportional to the amount of ascorbic acid. The induction period of the oil stabilized with 0.02% ascorbic acid and 0.2% δ-tocopherol (29.8 days) is increased by 14-fold, as compared to that of a control (2.1 days). In addition, when the δ-tocopherol content was varied from 0.05% to 0.4% at a fixed content of ascorbic acid of 0.02%, a similar trend was observed. The effect of δ-tocopherol was steadily enhanced with increasing concentration. The induction period of the oil sample with 0.02% ascorbic acid and 0.4% δ-tocopherol is 46.4 days.

The induction period of fish oil stabilized with both antioxidants is always longer than the sum of the individual induction periods of oils stabilized with ascorbic acid or δ-tocopherol alone. Their combination, therefore, prolongs the shelf life of fish oil much more efficiently than synthetic antioxidants. In an earlier work, for example, butylated hydroxytoluene and butylated hydroxyanisole were reported to have little effect on the oxidative stability of fish oil (16). Above data indicate that the vitamins are synergistic in their antioxidative properties (17). The research groups of Niki (18) and Yamauchi (19) have reported that a free-radical interaction between ascorbic acid and tocopherol may result in the synergism. Additional investigations on their synergistic effects in a w/o microemulsion, which more closely mimicks biological systems than aqueous emulsions or alcoholic solutions frequently used for that purpose, may be published later (O.S. Yi, D. Han and H.-K. Shin, unpublished data).

Figure 5 shows the changes of carbonyl values for a control and an oil sample with antioxidants. The carbonyl value of the control increased exponentially after a certain lag period, with storage time, reaching 45 after 36 days of storage. For the oil with ascorbic acid and δ-tocopherol, the carbonyl value remained around 1.0 during the same period. When comparing the carbonyl values of oil samples with the same POV, the level of the oil with antioxidants is lower than that of control, indicating that the decomposition of hydroperoxide to carbonyl com-

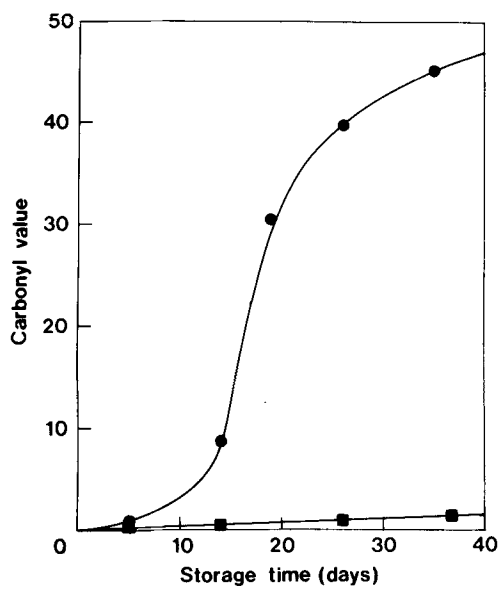

FIG. 5. Combined effects of ascorbic acid and δ-tocopherol on the carbonyl value of fish oil stored in petri dishes. Circles are for the control and squares represent fish oil stabilized with 0.3% lecithin, 0.02% ascorbic acid and 0.2% δ-tocopherol.

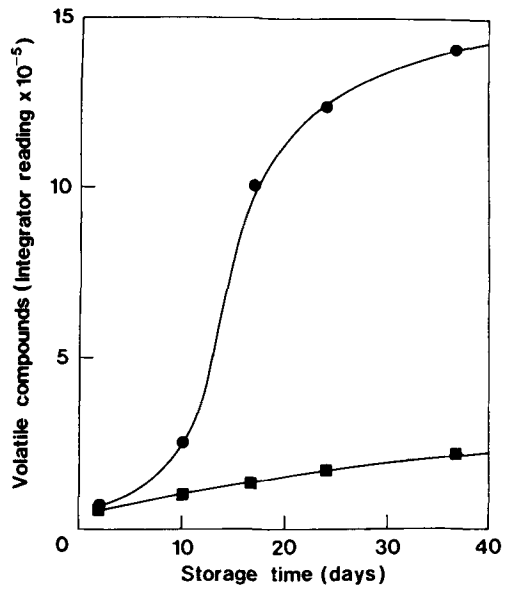

FIG. 6. Combined effects of ascorbic acid and δ-tocopherol on volatile compound formation of fish oil stored in serum vials. Circles are for the control and squares represent fish oil stabilized with 0.3% lecithin, 0.02% ascorbic acid and 0.2% δ-tocopherol.

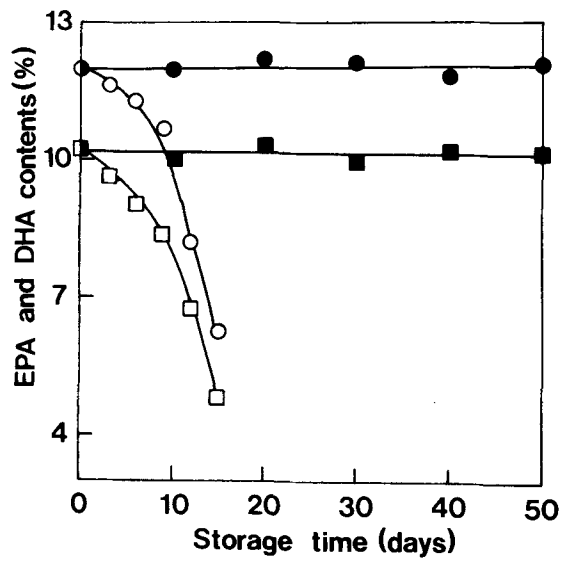

FIG. 7. Changes of EPA and DHA composition of fish oil stored in petri dishes. Circles and squares indicate EPA and DHA content, respectively. Open symbols are control and closed symbols are fish oil stabilized with 0.3% lecithin, 0.02% ascorbic acid and 0.2% δ-tocopherol.

pounds could be suppressed by the addition of both antioxidants.

The effects of ascorbic acid and δ-tocopherol on volatile compound formation of the oils stored in serum vials were examined. Integrator readings of the headspace gases show that the combined use of these antioxidants inhibits the occurrence of rancid flavor of fish oil, as seen in Figure 6. While the volatile compounds of the control increase quickly to reach an integrator reading of 1.4×10^5 after 36 days of storage, the oil stabilized with the two antioxidants produced one tenth of the level of the control. This effect may be ascribed to minimal formation of low molecular weight compounds, such as aldehydes and ketones (Fig. 5). It is well known that as the volatile compounds in soybean oil increase, the flavor quality decreases (20). The characteristic objectionable rancid odor of fish oil in a control was intense within nine days, whereas we could barely sense a discernible flavor from the oil with both antioxidants, even after 36 days.

Changes of fatty acid composition in the stored oils are shown in Figure 7. The initial contents of EPA (12.1%) and DHA (10.0%) in the control were reduced to *ca.* one half of the initial level within 15 days. Those of a stabilized oil (0.3% lecithin, 0.02% ascorbic acid and 0.2% δ-tocopherol), however, remained unchanged over 50 days. This result suggests that the combined use of both antioxidants also inhibits the polymerization of unsaturated fatty acids.

The present study shows that formation of a w/o microemulsion in oils with the help of lecithin and water is a powerful way to solubilize hydrophilic compounds in oils. The system has opened a new opportunity to investigate the effect of hydrophilic antioxidants, in combination with lipophilic antioxidants if necessary, on fat and oil stability against oxidation. The combined addition of ascorbic acid and tocopherol appears practical for stabilizing edible oil and vegetable/fish oil blends.

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