SHORT COMMUNICATIONS

Synergistic Antioxidative Effects of Tocopherol and Ascorbic Acid in Fish Oil/Lecithin/Water System

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Individual and combined effects of ascorbic acid and δ-tocopherol on the autoxidation of fish oil have been evaluated with the induction period monitored by Rancimat. The antioxidative efficiency of them was found to increase with increasing concentration. δ-Tocopherol and ascorbic acid acted highly synergistic with each other. When δ-tocopherol content was varied at a fixed content of ascorbic acid, the synergistic efficiency was generally 100% or more. On the other hand, when ascorbic acid content was varied at a fixed content of δ-tocopherol, the synergistic efficiency rose sigmoidally with increasing concentration. We concluded that at least 0.01∼0.02% ascorbic acid is required to obtain a considerable synergistic effect with δ-tocopherol in stabilizing fish oil.

KEY WORDS: **Antioxidation, ascorbic acid, fish oil, synergism, tocopherol, w/o microemulsion.**

During the last decade, interest in the dietary effects of ω-3 polyunsaturated fatty acids and especially of EPA ($C_{20:5}$, eicosapentaenoic acid) and DHA ($C_{22:6}$, docosahexaenoic acid) has increased because their antithrombotic and hypocholesterolemic actions associated with coronary heart disease have become accepted widely (1–3). Practical use of EPA and DHA for the preventive purpose is, however, limited because these fatty acids are highly susceptible to autoxidation.

It is generally accepted that the principal route of deterioration of lipids is through rancidity resulting from oxidation that takes place at the double bond sites in the triglyceride molecules. Therefore, the higher the degree of unsaturation, the more susceptible the fatty acid is to oxidative deterioration. This is true for fish oil, which contains abundant quantities of unsaturated fatty acids such as EPA and DHA.

To overcome the liability of fish oil to become rancid, the rate of the free-radical chain reaction has to be slowed down by removing oxygen, sequestering metal ions and sensitizers, stabilizing radicals and/or by adding antioxidants. When applying antioxidant to fats and oils, it is valuable to use several antioxidants in combination because no single antioxidant offers a panacea for all lipid products, and the combined use of antioxidants usually results in a synergistic effect. Tocopherol and ascorbic acid have frequently been reported as synergistic in their antioxidative properties, in which ascorbic acid acts not only as a primary antioxidant but also as a hydrogen donor for the tocopherol radical (4).

To date, the evaluation of the synergism of ascorbic acid and tocopherol has been studied in alcoholic solution (5), which is impractical, or in aqueous emulsion systems (6) because their solubilities are incompatible. Recently, we introduced oil/lecithin/water w/o microemulsions to solve the solubilization problem (7). The system proved to be powerful for solubilizing hydrophilic antioxidants into fats and oils. This paper deals with the antioxidative and synergistic effects of δ-tocopherol and ascorbic acid on the autoxidation of fish oil. The dependency of synergistic efficiency on the concentration is described on the basis of the induction period derived from the oxidation curve.

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MATERIALS AND METHODS

Materials. Refined sardine oil obtained from E-Hwa Oil & Fat Ind. Co. (Seoul, Korea) was bleached with activated clay and charcoal, and deodorized at 160°C for 1 hr under 200 micron Hg before use. The fatty acid composition of the oil was determined according to AOCS Official Method Ce 2-66: 32.6% saturated fatty acids; 35.6% monoenoic fatty acids; 30.3% polyenoic fatty acids (10.3% EPA and 13.9% DHA).

Ascorbic acid and δ-tocopherol were purchased from Sigma (St. Louis, MO), and lecithin (Centrol 1FUB) was a gift from Central Soya Co., Fort Wayne, IN.

Preparation of oil samples containing δ-tocopherol and/or ascorbic acid. Ascorbic acid was solubilized in fish oil *via* w/o microemulsion as described previously (7). The concentration of ascorbic acid in fish oil was controlled by injecting the same amount of ascorbic acid solution with different concentrations. Thus, the water content in the fish oil was always the same when ascorbic acid was added. δ-Tocopherol was directly mixed with the oil.

Determination of the induction period. Oxidation of oil samples was monitored with a Rancimat 679 (Metrohm CH-9100, Herisau, Switzerland). The oil samples (2.5 g) were heated to 80°C, and the flow rate of air was 15L/hr. After oxidation curves, based on the conductometric recording of volatile degradation products, were obtained from the Rancimat, the induction period was determined by determining the time to reach the intercept of the two tangents of induction and exponential periods during oxidation (8). All the data are reproducible within ± 10% experimental error if fish oil from a single batch is used.

The synergistic efficiency between two antioxidants was calculated from the following equation:

$$\text{synergistic efficiency (\%)} = 100 \times [(AB - C) - \{(A - C) + (B - C)\}] / [(A - C) + (B - C)] \quad [1]$$

where C is the induction period of the control, A and B are the induction periods of the oil samples stabilized with antioxidants A and B, respectively, and AB is the induction period of the oil samples stabilized with both antioxidants. That is, synergistic efficiency of 100% means that the increment of the induction period due to the combined effect is twice as much as the sum of the increments of induction period due to the individual antioxidants. On the other hand, the increment represents the induction period of the oil samples with antioxidant(s) minus the induction period of a control.

RESULTS AND DISCUSSION

Individual effects of ascorbic acid and δ-tocopherol. Figure 1 shows the induction period of fish oil as a function of ascorbic acid content. The oil samples contained 0.1% lecithin as a surfactant to aid the solubilization of ascorbic acid in the oil *via* w/o microemulsion. The induction period is increasing linearly with increasing ascorbic acid content up to 0.04%. The induction period of the oil containing 0.04% ascorbic acid is *ca.* 12 hr. It is 3 times longer than that of fish oil (4.0 hr). In a control, fish oil with 0.1% lecithin, the induction period was 4.4 hr, indicating that lecithin itself has a weak antioxidative effect.

The effect of δ-tocopherol on the oxidative stability of fish oil is shown in Figure 2. This shows that the addition of δ-tocopherol extends the induction period of the oil sample rapidly at low concentrations. Comparison of the induction periods of the oil samples with the same amount of ascorbic acid or δ-tocopherol shows that ascorbic acid is better than δ-tocopherol in stabilizing the oil.

Because lecithin acted not only as a primary antioxidant but also as a synergist of tocopherol (9), its presence for solubilizing ascorbic acid makes it difficult to interpret the synergistic property of ascorbic acid and δ-tocopherol. Therefore, the induction period of the oil samples as a function of δ-tocopherol at a fixed concentration of lecithin (0.1%) was examined to obtain the base data needed for studying synergism between ascorbic acid and δ-tocopherol. The induction periods shown in Figure 3 indicate that the combined effect of lecithin and δ-tocopherol is higher than the sum of the individual effects (Fig. 3). Their synergistic efficiency rose rapidly up to 50% at a δ-tocopherol content of 0.1%. Above that, however, it remained essentially unchanged. A similar effect was observed earlier when the synergistic efficiency was evaluated as a function of lecithin at a fixed concentration of δ-tocopherol (10).

Synergism of ascorbic acid and δ-tocopherol. δ-Tocopherol showed a marked synergistic activity with ascorbic acid. As shown in Figure 4, the increment of the induction period resulting from the combination was two

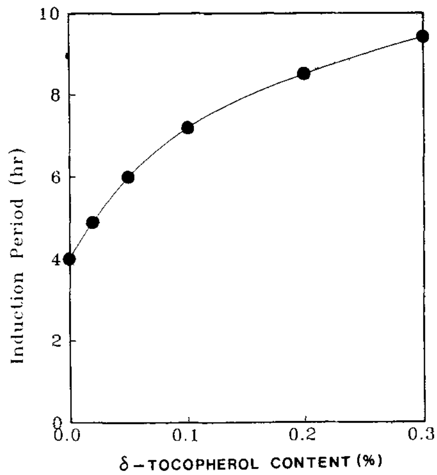

FIG. 2. Effect of δ-tocopherol content on the induction period (Rancimat, 80°C) of fish oil.

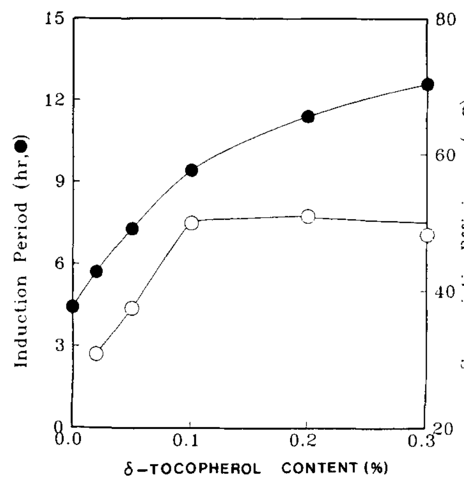

FIG. 3. Effect of δ-tocopherol content on the induction period (Rancimat, 80°C) of fish oil with 0.1% lecithin and change of the synergistic efficiency.

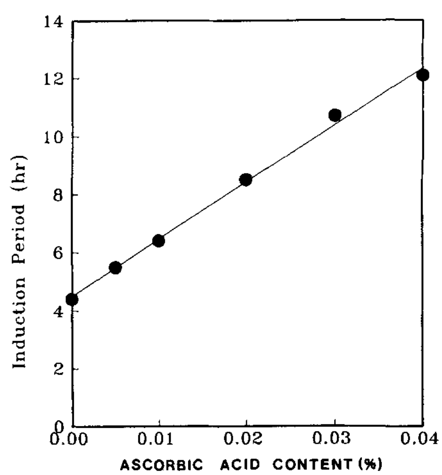

FIG. 1. Effect of ascorbic acid content on the induction period (Rancimat, 80°C) of fish oil. Lecithin (0.1%) and water (0.1%) were used to solubilize ascorbic acid.

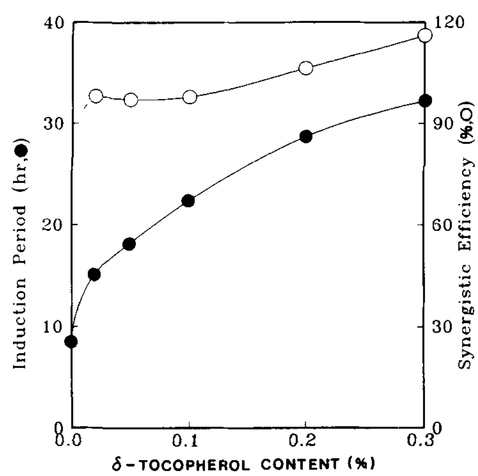

FIG. 4. Dependence of the induction period (Rancimat, 80°C) of fish oil and synergistic efficiency on δ-tocopherol content in the presence of 0.02% ascorbic acid.

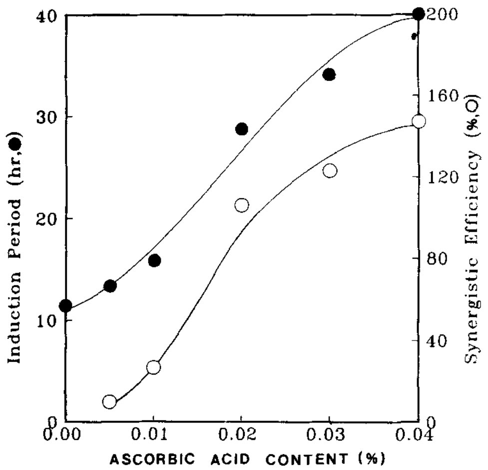

FIG. 5. Dependence of the induction period (Rancimat, 80°C) of fish oil and synergistic efficiency on ascorbic acid content in the presence of 0.2% δ-tocopherol.

times longer than the sum of the induction period increments resulting from individual antioxidants. For example, when 0.2% δ-tocopherol and 0.02% ascorbic acid are used, the increment of the induction period is 23.1 hr (27.5 hr − 4.4 hr = 23.1 hr). However, the sum of the increments due to individual addition is 11.0 hr [(8.5 hr − 4.4 hr) + (11.3 hr − 4.4 hr) = 11.0 hr]. This means that the synergistic efficiency is more than 100%, and indicates that δ-tocopherol acts as an excellent synergist of ascorbic acid, independent of addition level.

When ascorbic acid content was varied from 0.0 to 0.4% at a fixed content of δ-tocopherol (0.2%), the induction period rose sigmoidally with increasing amount of ascorbic acid (Fig. 5). The induction period of the oil sample stabilized with 0.04% ascorbic acid and 0.2% δ-tocopherol is 40 hr, which is *ca.* 9-fold longer than that of a control. When a similar experiment was carried out at 30°C, the extension of the induction period reached 24-fold (8). Therefore, the combined effect in this study was found to be lower, probably due to the thermal destruction of ascorbic acid at 80°C (11).

Synergistic efficiency rose sigmoidally from 0 to 148% as ascorbic acid content increased from 0.0 to 0.04%. The curve shows that at least 0.01∼0.02% ascorbic acid is required to obtain a considerable synergistic effect with δ-tocopherol in stabilizing fish oil against oxidation.

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