Action of Tocopherol-Type Compounds in Directing Reactions Forming Flavor Compounds in Autoxidizing Fish Oils

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Menhaden and cod liver oils without additives or containing either α-tocopherol (670 ppm) or Trolox C (1000 ppm) with or without copper (II) cations (20 ppm) were oxidized under air at 65°C, and their headspace volatiles were quantitatively measured by capillary gas chromatography. Samples prepared without additives contained very little 1,5-octadien-3-one. Fish oils with Trolox C had distinct metallic, vinyl-ketone aromas, while those with α-tocopherol exhibited extremely fishy, cod liver-like aromas. High levels of 1,5-octadien-3-one and 1,5-octadien-3-ol and moderate levels of 2,4,7-decatrienals were found in samples with Trolox C, while moderate levels of the 8-carbon compounds and high levels of the 2,4,7-decatrienals were present in the α-tocopherol samples. Copper (II) greatly accelerated the overall rate of oxidation in samples, and in the presence of Trolox C it induced the formation of very high levels of 1,5-octadien-3-ol.

Oxidations of long-chain, highly unsaturated n-3 fatty acids found in marine oils notably develop distinct fishy flavors and aromas (1–4). Because these flavors are objectionable to consumers, there is considerable interest in developing antioxidant systems that will suppress or direct reactions away from the formation of fishy compounds.

α-Tocopherol and related compounds have often been suggested as antioxidants for fish oils because of their effective H-donor capability (5), but tocopherols can also act as prooxidants when high concentrations are added to oxidizing lipid systems (6–10). Studies evaluating the antioxidant effect of α, β, and γ-tocopherols have shown γ-tocopherol as the most effective, and α-tocopherol as the least effective antioxidant in oils (11,12). Of the tocopherol isomers, α-tocopherol is the most unstable because of its highly oxidizable nature, and this characteristic contributes to its low antioxidant capability (13).

Recently, studies evaluating the antioxidant properties of α-tocopherol in model systems containing polyunsaturated fatty acids have shown that α-tocopherol selectively affects the ratio of isomeric hydroperoxides in unsaturated fatty acids. Porter et al. (14) have outlined the mechanism responsible for the formation of cis-trans and trans-trans hydroperoxide isomers in autoxidizing fatty acids, and the reactions proceed as a series of reversible oxygenation and deoxygenation steps involving pentadienal radicals. Conditions allowing reversible reactions to proceed unhindered permit rotations of carbon-carbon bonds which yield both cis-trans and trans-trans hydroperoxides. The distribution of isomers is dependent upon the concentration and H-donating ability of the tocopherol-type compound in the system.

Peers et al. (15) have noted that only the cis-trans hydroperoxide isomers were formed when 5% α-tocopherol was present. When lower α-tocopherol concentrations were present, some trans-trans isomers were formed, although their concentrations diminished as the level of α-tocopherol increased. Thus, α-tocopherol quenched peroxy free radicals by readily donating a hydrogen atom, and this rapid quenching of peroxo radicals apparently stabilized the carbon-carbon backbone, which prevented formation of trans-trans hydroperoxides. These results have been confirmed by Koskas et al. (13) and Terao and Matsushita (16).

α-Tocopherol also inhibits the formation of diperoxides through its H-donating mechanism, and hydroperoxides thus stabilized are prevented from forming peroxo radicals that cyclize to the diperoxides (17,18). Inner rather than outer hydroperoxides are selectively formed in the pentadiene system of polyunsaturated fatty acids (17). In the presence of α-tocopherol, compounds from inner, cis-trans monohydroperoxides of n-3 fatty acids are favored among decomposition products.

Studies on fishy-metallic off-flavors in tainted butters which contain low levels of long-chain n-3 fatty acids have led to conclusions that α-tocopherol and copper were required in model systems for these off-flavors to form (19,20). Metallic taints in butters were attributed to 1,5-octadien-3-one (20,21). Low concentrations (<10 ppb) of this compound have been found to contribute green, fresh-like flavors and aromas to fresh fish (22) and fish oils (1). Neat preparations of 1,5-octadien-3-one exhibit heavy green, geranium leaf-like aroma qualities (21,22). Previous research (17,19) has indicated that 1,5-octadien-3-one can result from the directed oxidation of long-chain n-3 fatty acids in the presence of α-tocopherol through an inner, cis-trans hydroperoxide.

Trolox C is a synthetic antioxidant containing a chroman ring that has been proposed as a food antioxidant (23,24), but its use as a food additive has not yet been approved in the U.S. Trolox C is structurally-related to the tocopherols, and it exhibits some unique structural features that make it useful for studies of the antioxidant mechanism of α-tocopherol. Particularly, the Trolox C molecule lacks the long alkyl side chain of α-tocopherol, and it forms a stable oxidized quinone derivative more readily than the tocopherols (25,23).

Additional information on the mechanism of directing reactions in oxidizing fish oils should accelerate the development of antioxidant strategies that will minimize the formation of pronounced off-flavors in these oils. Thus, the purpose of this research was to investigate the effect of combinations of α-tocopherol or Trolox C with copper (II) on the production of volatile oxidation products in fish oils, and to relate these observations to the mechanisms of reactions that lead to the formation of characterizing fish compounds in n-3 oils.

MATERIALS AND METHODS

Preparation of steam deodorized fish oils. Commercially refined menhaden oil (20) (Zapata Hayne Corp., Reedville, VA) and cod liver oil (McKesson Corp., Dublin, CA) were low-temperature deodorized using a batch-type, vacuum laboratory apparatus (1,27) operated at 130°C at 4 mm Hg for 2 hr. Before initiating deodorizations, each 300 ml
batch of oil was blended with 60 ml of water for 5 sec using a Waring Blender (Dynamic Corp., New Hartford, CT). Steam for deodorization was generated from distilled water for menhaden oil, and from dilute acetic acid solution (0.01N; J.T. Baker Chemical Co., Phillipsburg, NJ) for cod liver oil (1).

Preparation of model systems of fish oils. Samples composed of 20 ml portions of menhaden or cod liver oils were placed in 60 ml open glass (85 mm × 30 mm) bottles, and either 1000 ppm of Trolox C® (6,hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid; Hoffman-La Roche, Inc., Nutley, NJ) or 670 ppm of d-a-tocopherol (670 mg/g soybean oil; 0.5%-2% non-a isomers; Sigma Chemical Co., St. Louis, MO), or 20 ppm of cupric palmitate (Pfaltz and Bauer, Inc., Waterbury, CT) was added to each sample. Samples were also prepared with combinations of either cupric palmitate plus a-tocopherol or cupric palmitate plus Trolox C®. Control samples for each series were prepared without antioxidants. Uncapped bottles were then placed in an incubator (Blue M, Blue Island, IL) in the dark at 65°C, and held for up to 72 hr with samples periodically taken for analysis.

Analysis of headspace volatiles from oils. Volatile compounds in fish oil were quantitatively measured using the dynamic headspace procedure described by Olafsdottir et al. (28), with modifications. Aliquots of oil (15 ml) were added to cylindrical glass 30 ml reservoirs (3 cm × 10 cm) constructed with 24/40 ST glass joints (female), and assembled with a purging head described in the earlier procedure. Ethyl heptanoate was added as the internal standard at a level of 2.08 ppm of the oil. Headspace volatiles were purged from the oil by introducing nitrogen (270 ml/min for 3 hr at 75 ± 5°C) below the surface of the sample, and volatiles were entrained onto Tenax GC® (60-80 mesh, ENKA N.V., Holland). Volatile compounds were eluted from Tenax GC® traps with ca. 0.5 ml of redistilled diethyl ether (Fisher Scientific, Fairlawn, NJ), and extracts were then concentrated under a slow stream of nitrogen to about 30 µl at room temperature (21°C).

Volatile compounds were separated by capillary column gas chromatography using a Varian 3700 gas chromatograph (Varian Associates, Inc., Sunnyvale, CA) equipped with an on-column injector system and FID detector. A Carbowax 20M (60 m × 0.25 mm) fused silica capillary column (J&W Scientific, Inc., Rancho Cordova, CA) operated with helium as the carrier gas was employed. A program rate of 50°C (1 min hold) to 220°C at 4°C/min was used. Chromatographic data were processed with a computing integrator (Model 4200, Spectra Physics, San Jose, CA).

Mass spectra were obtained using a Finnegan 4500 mass spectrometer fitted with the same Carbowax 20M capillary column and temperature program rate. Identification of peaks was achieved by matching electron impact (70/ev) mass spectral data to those published in “EPA/NIH Mass Spectral Data Base” (29,30), or those of authentic compounds. Coincidence of retention indices of unknown compounds (Ip) (31) with authentic compounds was also employed for compound identification.

Assessments of fish oils. Oil samples were assessed by the authors for odor after equilibration to room temperature (21°C), and the samples were also assessed for flavor quality by placing a small drop of oil on the tip of the tongue (to prevent coating of the lips) (4). Samples were expectorated immediately after tasting, and lukewarm rinse water was available.

Determination of hydroperoxides. Hydroperoxides were determined by the method of Buege and Aust (32), and the mean of duplicate analyses were expressed as micromoles of hydroperoxide per mg of oil.

RESULTS AND DISCUSSION

Antioxidant behavior of a-tocopherol and Trolox C in fish oils. Characteristic data for hydroperoxide concentrations in oxidizing fish oil containing either Trolox C (1000 ppm) or a-tocopherol (670 ppm) are shown in Figure 1, and the data show that the two compounds behave differently with regard to antioxidant capability in fish oils. It has been well established that levels of a-tocopherol in excess of 1% (10,000 ppm; wt/wt basis) in lipid/water systems (6-9,17,33), and >0.1% (1000 ppm) in pure lipid systems (34) promote lipid autoxidation. In the present study, a-tocopherol (670 ppm) also accelerated the rate of hydroperoxide formation in menhaden oil, whereas the higher level of Trolox C (1000 ppm) exhibited an antioxidant effect. Similar prooxidant effects for a-tocopherol and antioxidant effects for Trolox C were observed for cod liver oil (Table 1).

The prooxidant activity of a-tocopherol (Fig. 2) apparently occurs because chroman ring free radicals formed by reactions with lipid radicals abstract hydrogen atoms from either oxidized methylene carbon of unsaturated fatty acids or existing hydroperoxide (16,34). Thus, instead of retarding oxidation rates, a-tocopherol at high concentrations actually participates in the propagation of autoxidation. However, high concentrations of Trolox C suppress hydroperoxide formation because its chroman radical does not readily abstract hydrogen from hydroperoxide groups or unsaturated lipids, and it can scavenge a hydrogen radical from the medium to form a quinone (Fig. 3) (23,24). The carboxyl group in the 2-position on Trolox C provides an electron withdrawing group that

FIG. 1. Hydroperoxide concentrations in oxidizing menhaden oils held under air at 65°C for 72 hr. The control sample did not contain additives.