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Antioxidant Properties of a Dihydromyricetin-Rich Extract from Vine Tea (Ampelopsis grossedentata) in Menhaden Oil

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Research Article

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Abbreviations: Vine tea extract (VTE); Rosemary extract (RME); Green tea extract (GTE); Grape seed extract (GSE); Ascorbyl palmitate (AP); Citric acid (CA); long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs); 20:5n-3 (EPA); 22:6n-3 (DHA); Fish oil (FO); Fatty acid methyl esters (FAMEs); Equivalent chain lengths (ECL); Menhaden oil (MO); Butylatedhydroxyanisole (BHA); Dihydromyricetin (DHM); Quercetin (QC); Mixed tocopherols (MT); Anisidine Value (AV).

ABSTRACT

Preventing oxidative deterioration of fish oil is a significant challenge for the food industry. Natural antioxidants are widely incorporated into foods to prevent oxidation and extend shelf life. Vine tea (*Ampelopsis grossedentata*) extract has been shown to have antioxidant activity in vegetable oils, but the efficacy in fish oil is unknown. The goal of our study was to test the antioxidant activity of vine tea extract (VTE) and compare it with other natural antioxidants (rosemary extract - RME, green tea extract - GTE, grape seed extract - GSE, ascorbyl palmitate - AP, and citric acid - CA) in tocopherol stabilized menhaden oil. Baseline levels of alpha, gamma, and delta tocopherols were 0.18mg/g, 0.37mg/g, and 0.14mg/g, respectively. VTE delayed menhaden oil oxidation when stored at 40°C for eight days as determined by primary and secondary oxidation products. Mixtures of VTE and RME and their combinations with AP and CA more effectively improved stability of menhaden oil containing tocopherols than VTE alone. Our results show that VTE is an effective natural antioxidant in menhaden oil, especially in combination with RME.

INTRODUCTION

The long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) 20:5n-3 (EPA) and 22:6n-3 (DHA) are associated with promoting good health, including protection against cardiovascular disease, immunity improvements, brain function development, memory and vision improvements, reduction of depression and inflammation diseases and cancer prevention [1,2]. Marine fish products, including fish oil (FO), are the major dietary sources of EPA and DHA, and there has been tremendous interest in these fatty acids since the late 1970s, when they were shown to have cardioprotective effects [3,4]. Research has focused on FO supplements and food products modified to incorporate FO, including eggs, dairy foods, snacks, infant formulas, and juices to produce nutraceutical/functional foods that enhance health [5,6]. However, fish oils contain highly unsaturated fatty acids with 4, 5 and 6 double bonds, and this makes them highly susceptible to oxidation in the presence of light, oxygen, transition metals or enzymes. Oxidation greatly reduces the shelf-life of FO supplements and FO enriched foods. There is also a loss of nutrients and toxic compounds may be formed from the decomposition of hydroperoxides [7,8].

Preventing oxidative deterioration in foods has been a significant challenge for the food industry. Natural antioxidants are

widely incorporated into foods and oils to prevent oxidation, improving stability and shelf life. These natural antioxidants are used due to consumer preferences for clean food labels. Herbs and other spices, especially rosemary extracts, have become popular additives to improve stability and extend shelf life because of their demonstrated ability to retard oxidation [9,10].

Antioxidants can be classified based on their mode of action. Primary antioxidants donate hydrogen and scavenge alkoxy and preoxy radicals to form poorly reactive (more stable) antioxidant radicals; this breaks the propagation cycle. Secondary antioxidants, such as metal chelating agents, singlet oxygen quenchers, oxygen scavengers, and antioxidant regenerators, prevent the formation of volatile and toxic compounds and convert hydroperoxides to more stable radicals and to non-radical products [8,11].

Ampelopsis grossedentata is a vine that grows wild in the southern mountainous areas of China. It has been used medicinally for centuries and contains high concentrations of the flavanonol, dihydromyricetin, also known as ampelopsin [12,13]. It has been shown to have antioxidant activity in lard and soybean oil [14,15]. Recent experiments have shown positive effects of dihydromyricetin on skeletal muscle insulin resistance and protection of endothelial cells against hydrogen peroxide induced oxidative stress [16,17].

Tocopherols are commonly added to foods as natural antioxidants [8,11]. However, use of a single antioxidant is less effective for FO compared to combinations of antioxidants [18,19]. Tocopherols can have synergistic antioxidant properties when they are added with other antioxidants in FO [8]. As a result of the good synergistic antioxidant properties of tocopherols, greater antioxidant effectiveness in FO was observed when primary antioxidants and secondary antioxidants were formulated with tocopherols [8,20,21].

The purpose of this study was to investigate the activity of vine tea extract in menhaden oil, to compare activity to other natural antioxdants, and to develop optimum formulations to control oxidation of menhaden oil. The specific objectives were: 1) To determine baseline tocopherol composition and content in menhaden oil using extraction and HPLC analysis, 2) To determine antioxidant activities of natural compounds (green tea, grape seed, vine tea, rosemary extracts, ascorbyl palmitate and citric acid), added individually to menhaden oil containing mixed tocopherols, 3) To evaluate oxidative stability of menhaden oil containing mixed tocopherols and mixtures of vine tea and rosemary extracts, 4) To evaluate oxidative stability of menhaden oil containing mixed tocopherols and mixtures of ascorbyl palmitate, citric acid, vine tea and rosemary extracts. Oxidation was determined by measuring primary and secondary oxidation products after storage at 40°C for eight days.

MATERIALS AND METHODS

Fatty acid analysis

HPLC grade isooctane and toluene were purchased from Fisher Scientific (St. Louis, MO). BF3-Methanol (12%) was purchased from Supelco (Bellefonte, PA). Tricosanoic acid-methyl ester was obtained from Crescent Chemical Company (Islandia, NY). Sodium hydroxide and sodium chloride were purchased from Fischer Scientific. Menhaden oil (OmegaPure®) containing natural mixed tocopherols (Covi-ox®T-50) was donated by Omega Protein (Reedville, VA).

The fatty acid composition of menhaden oil was determined by using the AOCS official method Ce 1b-89 [22]. Samples were manually injected (1 µl) into a Shimadzu QP5050 GCMS (Kyoto, Japan) equipped with a Zebron ZB-WAX plus capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness, Phenomenex, Torrance CA) with a split ratio of 1:77. Ultra-pure helium was used as a carrier gas at 30 cm/sec linear flow velocity (1.3ml/min column flow). The oven temperature program started at 135°C, was increased to 235°C by 1°C/min, and was held at 235°C for 20 minutes. The total run time was 120 minutes. Injector temperature was 250°C and GCMS interface temperature at 230°C. Fatty acids methyl esters (FAMEs) were identified by using MS fragmentation patterns and a Wiley 2002 library, and identifications were confirmed by calculation of equivalent chain lengths (ECL) as described by Ackman, and ECL data compared to published values [23,24]. The amounts of EPA and DHA were calculated as mg per gram of menhaden oil. Area percentages of the FAMEs were also calculated. Experiments were conducted in duplicate.

Baseline tocopherol analysis of Menhaden Oil (Mo)

MO containing natural mixed tocopherols from Covi-ox®T-50 was donated by Omega Protein (Reedville, VA). HPLC grade n-hexane and isopropanol were obtained from Fisher Scientific. Polysorbate 20 (Tween 20), butylatedhydroxyanisole (BHA), dl-α-tocopherol, dl-γ-tocopherol, dl-δ-tocopherol and anhydrous magnesium sulfate were obtained from Sigma Aldrich (St. Louis, MO). Butylatedhydroxyanisole (BHA, 50ppm, Fisher Scientific) was added and dissolved in n-hexane to prepare the hexane/BHA solution.

The α-, γ- and δ- tocopherol contents of MO were determined by using a normal phase HPLC analysis originally reported for Vitamin E in margarine and reduced fat products [25,26]. The three tocopherols were quantified using external standard procedures (0-0.1 mg/ml curves). Tocopherol standard solutions were prepared in 25 ml of volumetric flask containing n-hexane and BHA (0.05 mg/ml).

The extracted samples and standard solutions were used to determine tocopherol contents in the MO. An Agilent 1200 Infinity Series HPLC was used (Agilent Technology, Richmond, VA) with diode array and fluorescence detectors in series. A Macherey-Nagel silica (25 cm x 0.46 cm i.d.) normal phase column was used at ambient temperature. The autosampler was held

at 4°C and protected from light. The mobile phase consisted of n-hexane and isopropanol (99.1:0.9 v/v) with a flow rate at 1.0ml/min. The injection volume was 20 µl, fluorimeter excitation wavelength was 290 nm and emission wavelength was 330 nm. Total run time was 20 min. The retention times and UV absorbance spectra were used to identify alpha, gamma and delta tocopherol contents in the sample.

MO (5.0 g) was weighed into a 125 Erlenmeyer flask and 40 ml of a hexane/BHA solution was added to dissolve the MO. Aluminum foil was wrapped outside the flask in order to avoid light exposure during the extraction. The remaining oil residue inside of the flask was rinsed with the n-hexane and BHA solution. Tween 20 (3 drops) and anhydrous magnesium sulfate (3 g) were added to the flask, which was flushed with nitrogen and capped tight. The oil solution was mixed thoroughly and let stand in refrigerator at 3°C for two hours. After two hours, the solution was filtered through Whatman no. 1 filter paper into a 100 ml volumetric flask. The remaining residue solution in the flask was rinsed with 10 ml of the hexane/BHA solution, and the 100ml volumetric flask brought to volume. The extracts were prepared in duplicate.

Antioxidant extraction and analysis

Three replicate vine tea samples were obtained from retail tea stores in Zhangjiajie, China. Dihydromyrectin (DHM) was purchased from ChromaDex (Irvine, CA). Japanese green tea (Bancha) and grape seed extract (Nature's Plus Herbal, Melville, NY) were purchased from local natural food stores. Ethanol (95.5% aq) was obtained from Ricca Chemical Company (Arlington, TX). Acetic acid, acetonitrile and methanol were supplied by Fisher Scientific. Gallic acid and 2 N Folin-Ciocalteau's reagents were obtained by Sigma-Aldrich (St. Louis, MO).

Natural antioxidants: Preparation

Ascorbyl palmitate was obtained from Spectrum Chemical (Gardena, CA). Quercetin dehydrate (97%) was purchased from Alfa Aesar (Heysham, England). Citric acid, anhydrous, was obtained from Archer Daniels Midland Co. (Decatur, IL). Rosemary extract oil (Herbalox Seasoning, Type HT-25, NS) was obtained from Kalsec (Kalamazoo, MI). Vine tea extract was prepared from vine tea. Mixed tocopherol standard was obtained from Sigma Aldrich. Starch indicator (1%) was supplied from Ricca Chemical Company (Arlington, TX). Acetic acid, chloroform, and isooctane of HPLC grade, 0.1 N sodium thiosulfate, and p-anisidine were purchased from Sigma Aldrich (St. Louis, MO). Activated charcoal was purchased from Bio-Rad Laboratories (Richmond, CA).

Vine tea extract, green tea extract, and grape seed extract were prepared for natural antioxidants' inhibitory activities on lipid oxidation of the menhaden oil. Total phenolic values of each crude extract sample were evaluated using the Folin-Phenol method with gallic acid standard solutions using the method described by Spanos et al., [27]. The phenolic content of the vine tea extract sample was compared to commercial dihydromyricetin (DHM). DHM content in vine tea extract sample was quantified using HPLC methods with DHM standard solution [15]. The extraction and lyophilization of vine and green tea extracts followed the method described by Ye et al., [15]. The commercial grape seed extract was purified using the extraction procedure described by Gokogluet al., [26].

Vine tea (10 g) was extracted using 200 ml of 74 % (v/v) aqueous ethanol with shaking at 65 °C for 30 minutes at 100 rpm in a water bath (360 Orbital Shaker, Precision Scientific, Chicago, IL). A stomacher bag was used for filtering the extracts into 1000 ml round bottom flasks. For evaporating ethanol in the extracts, a Buchi Rotavapor R-3000 (Buchi Laboratory Equipment, Flawil, Switzerland) was operated at 60°C until little ethanol remained in the flask. The extracts in the round bottom flask were transferred to a crystallizing dish (70mm x 50mm), and 50 ml of distilled water was added to the round bottom flask, rinsing remaining extracts. This was combined with the first extract in the crystallizing dish. The extract in the crystallizing dish was covered with parafilm to prevent contamination, the extract was frozen completely at - 50°C, and then freeze dried at - 50 °C for about 72 hours in a Labconco Freezone18 Freeze Dryer (Labconco, Kansas City, MO) with cheesecloth on the top of the crystallizing dishes. The freeze dried extracts were weighed on analytical balance scale and stored at - 50 °C. Triplicate vine tea extracts were prepared. Japanese Bancha green tea (10g) was extracted with 200 ml of 70% aqueous ethanol following the extraction procedure described for vine tea [15].

Commercial grape seed extract (10 g) was dissolved completely in 200 ml of 20% (v/v) aqueous ethanol and extracted for four hours by shaking at 40°C at 160 rpm in a Precision Scientific 360 Orbital Shaker (Precision Scientific Inc., Chicago, IL). The extracts were filtered through Whatman no. 4 paper and transferred to a 1000 ml round bottom flask. The filtered grape seed extract was concentrated at 60°C until most of ethanol was removed by using a Buchi Rotavapor R-3000 (Buchi Laboratory Equipment, Flawil, Switzerland). The concentrated extract was transferred into a crystallizing dish (70 mm x 50 mm) and 50 ml of distilled water added to wash residual extracts in the round bottom flask. The extract in the crystalizing dish was frozen at -50°C then freeze dried at -50°C for 72 hours with cheesecloth cover in a Labconco Freezone 18 Freeze Dryer (Labconco, Kansas City, MO). The dried extract was weighed on analytical balance and kept frozen at -50°C until use. The grape seed extracts were prepared in triplicate.

DHM quantification in the vine tea extract by HPLC analysis

DHM in the vine tea extract was quantified by using an Agilent 1260 Infinity Series HPLC (Agilent Technologies, Richmond, VA). An Agilent Porshell 120 EC-C18 column with 2.7 µm particle size (5 cm x 0.46 cm) was operated at ambient temperature. The mobile phase consisted of solvent (A) 90% distilled water containing 0.1% v/v acetic acid and solvent (B) 10% v/v acetonitrile aq.

containing 0.1% acetic acid (v/v). The flow rate was 0.5 mL/min; the injection volume was 5.0 µL. The detection wavelength was 290 nm and absorbance was recorded from 190-400 nm. A mobile phase gradient was run with 90% A and 10% B from 0-4 min, a linear increase to 70% A and 30 % B from 4-8 min, for an 8 minute run time. The initial solvent conditions were run thereafter for 10 minutes to regenerate the column. Triplicate extract samples were prepared in methanol (1 mg/ml) and injected into the HPLC. The DHM peak was identified by comparing retention times and UV absorbance spectra with authentic standard. The DHM content in the extract was determined by using an external standard procedure (0-1 mg/ml DHM in methanol).

Total phenolic contents

Total phenolic concentration in the vine tea extract, green tea extract, grape seed extract and DHM were measured by using the Folin-Ciocalteu reagent with gallic acid (0-0.5 mg/ml) as the phenolic standard [27]. The reaction mixture contained 100 µL of each extract solution (0.5 mg/ml) or the gallic acid standard solutions, 900 µL distilled water and 2.5 mL of 0.2 N Folin-Ciocalteau reagent and were mixed on a Fisher Vortex Genie2 mixer (Fisher Scientific, Pittsburgh, PA). Saturated sodium carbonate (2 ml) was added to each test tube and mixed on the vortex. After standing for two hours at room temperature, the absorbance was measured at 765 nm using a Shimadzu UV-2550 UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). The estimated total phenolic value of each extract was recorded as milligrams of gallic acid equivalents per grams of the extract. Experiments were run in triplicate.

Natural antioxidant activity on lipid oxidation of Menhaden Oil (MO)

MO samples were prepared with each antioxidant treatment at three different concentrations in triplicate. We first focused on evaluating efficacy of each antioxidant in MO samples because some antioxidants act as peroxidants at high concentration. Twenty seven treatment samples were prepared in triplicate and stored in screw capped glass bottles in an incubator (Blue M, Blue Island, IL) at a temperature of 40°C for eight days. Triplicate samples of each treatment were taken randomly from the incubator on Days 0, 2, 4, 6, 8 and evaluated for peroxide value, headspace oxygen content (primary oxidation products) and anisidine value for the secondary oxidation product.

Single antioxidant activity on oxidation of Menhaden Oil (MO)

Three different concentrations of ascobyl palmitate (AP) (200 ppm AP, 500 ppm AP, 1000 ppm AP) and citric acid (CA) (50 ppm CA, 100 ppm CA and 150 ppm CA) were prepared respectively in 10 ml Teflon-lined, screw cap test tubes. Methanol was used to help dissolve different concentrations of AP and CA antioxidant treatments. After methanol was removed by using a nitrogen stream, MO (3 g) was added to the test tubes flushed with nitrogen and capped tight. AP and CA in MO were dispersed using vortex mixing for one minute. Each test tube containing different concentration of AP or CA was then placed in a Fisher FS20 ultrasonic bath (Fisher Scientific, Pittsburgh, PA) for three minutes to assist the dispersing of AP and CA. Theses vortex and ultrasonication processes were repeated three times until AP or CA were dispersed completely. The mixture of AP or CA and MO in the test tubes was transferred to 250 ml Erlenmeyer flasks, additional amounts of MO were added to prepare MO samples with 200 ppm AP, 500 ppm AP, 1000 ppm AP, 50 ppm CA, 100 ppm CA and 150 ppm CA.

MO (3g) was added to test tubes containing each antioxidant treatment and flushed with nitrogen. Each extract and QC treatment with MO in test tube was mixed on vortex for one minute and placed in ultrasonic bath for three minutes. The processes of vortex and ultrasonication bath were repeated until the extracts and QC were mixed well with MO. The extracts and QC with MO in the test tubes were transferred to 250 ml Erlenmeyer flasks, and additional MO was added to prepare 200 ppm QC, 500 ppm QC, 1000 ppm QC, 500 ppm GTE, 1000ppm GTE, 2000ppm GTE, 200 ppm GSE, 500 ppm GSE, 1000 ppm GSE, 200 ppm VTE, 500 ppm VTE, 1000 ppm VTE in each flask. The flasks were flushed with nitrogen and capped tight.

Different concentration of mixed tocopherols (MT) and rosemary extract (RME) were directly added into MO in Erlenmeyer flasks due to good solubility of MT and RME in MO. MO samples with 200 ppm MT, 500 ppm MT, 1000 ppm MT, 500 ppm RME, 1000 ppm RME and 2000 ppm RME were prepared in triplicate. MO samples with MT and RME were flushed with nitrogen.

Each flask containing MO with different antioxidants treatments was placed in the ultrasonic bath for five minutes followed by mixing on the stir plate for ten minutes. Each sample 6g was transferred to glass vials in triplicate and capped tight and randomly distributed in the incubator without light exposure at 40°C for up to eight day storage.

Preparation of Mixtures of Vine Tea Extract (VTE) and Rosemary Extract (RME) with Menhaden Oil (MO)

In the evaluation of single antioxidant activity, 200 ppm, 500 ppm and 1000 ppm VTE showed good antioxidant inhibitory activity on lipid oxidation of menhaden oil compared to other antioxidants and control. To examine combined effects, 1000 ppm or 2000 ppm RME were combined with 200 ppm VTE or 500 ppm VTE in MO. Nine treatments were prepared: control without any antioxidants, 200 ppm VTE, 500 ppm VTE, 1000 ppm RME, 2000 ppm RME, mixtures of 200 ppm or 500 ppm VTE and 1000 ppm RME and mixtures of 200 ppm or 500 ppm VTE and 2000 ppm RME. Each sample (6 g) was prepared in glass vials and capped tight; nine treatments were prepared in triplicate. They were distributed randomly and stored in the dark incubator set temperature at 40°C for eight days.

Preparation of mixtures of Ascorbyl Palmiate (AP) and Citric Acid (CA), Rosemary Extract (RME) and Vine Tea Extract (VTE) in Menhaden Oil (MO)

All samples contained AP (200 ppm) and CA (100 ppm). The MO samples with two levels of RME (1000 or 2000 ppm) and two levels of VTE (500 ppm or 1000 ppm) were prepared. All MO samples (6 g) were transferred to glass vials and capped tight. Triplicate samples were distributed randomly in the dark incubator set temperature at 40°C for up to eight day storage.

Peroxide value determination

Peroxide values were determined by the American Oil Chemists' Society procedure [28].

Head space oxygen content

Headspace oxygen contents in MO samples were evaluated using an OxySense 4000B (OxySense, Dallas, TX). Fluorescent O2xyDots® were attached to the inside of glass vials by using a silicon-based translucent adhesive glue (RTV 108, Momentive Performance Materials, Albany, NY). The OxySense 4000B was calibrated by using a 0% oxygen standard containing 1% sodium sulfite and a 21% oxygen standard containing 1 ml of distilled water. The samples (5 g) of each treatment were added to the vials that already had the fluorescent O2xyDots® on the inside walls. Tubes were capped tightly and distributed in an incubator for up to eight days storage at 40°C. Every two days (0, 2, 4, 6, 8 days), samples were removed from the incubator in order to measure headspace oxygen content. The headspace oxygen data for each treatment was recorded directly in an excel work sheet of the instrument.

Anisidine Value (AV) determination

The AV primarily measures aldehydes, especially unsaturated aldehydes. The p-anisidine was recrystallized before use using the following procedure. The p-anisidine (20 g) was dissolved in 500 ml of distilled water (75°C) in a 1L beaker containing stirring bar, sodium sulfite (1 g) and activated carbon (10 g) with stirring for five minutes. The solution was filtered through Whatman no. 1 filter paper twice. The filtered solution was stored in the refrigerator 4°C overnight. The white crystallized p-anisidine was filtered using vacuum filtration and washed with 10ml of cold distilled water. After the crystallized p-anisidine was completely dry, it was transferred to the glass vials and stored under darkness in a refrigerator at 4°C [29].

Each MO sample between 0.5 and 0.8 g was weighed in 50 ml centrifuge tubes. Isooctane (25 ml) was added to dissolve and dilute each MO sample. Isooctane with reagent was used to zero the spectrophotometer. The absorbance of each MO sample (Ab) was measured at 350 nm using a Shimadzu UV-2550 UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Samples (5ml) and isooctane were added to 1 ml of the p-anisidine solution (2.5 g/L), which was prepared with acetic acid [29]. The tubes were capped tight and swirled. After standing at room temperature for 10 min, absorbance (As) was measured at 350nm using isooctane blank and and p-anisidine mixture as a reference.The p-anisidine values of each sample were determined by the formula: p-AV = 25 * (1.2 As - Ab)/m where as was the absorbance of the reacted solution, Ab is the absorbance of the diluted menhaden oil sample, m is the weight of the menhaden oil sample (g) [29].

Statistical analysis

The test results for total phenolic contents (mg GAE/g) and peroxide values (meq/kg), headspace oxygen content (%) and anisidine values of the samples at 0, 2, 4, 6, 8 days were analyzed by one-way analysis of variance. The significant differences between mean values for total phenolic contents of the extract samples were determined by using Tukey's test at $p<0.05$ in JMP (Version 9.0.0, 2010 JMP, Cary, NC) and for the peroxide values, headspace oxygen contents and anisidine values were differentiated by using Tukey's test at $p<0.05$ in SAS (Version 9.1.3, 2003, SAS, Cary, NC). The headspace oxygen content experiment was a repeated measures experiment and was analyzed as such.

RESULTS AND DISCUSSION

Fatty acid analysis

Concentrations of fatty acids in MO were determined using an internal standard, tricosanoic acid (C23:0) methyl ester and as area %. Peak areas higher than 1% were 14:0, 16:0, and 16:1n-7, 18:0, 18:n1-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, and 22:6n-3. Moffat et al., reported that marine oils contain eight major fatty acids: tetradecanoic acid (14:0, myristic acid), hexadecanoic acid (16:0, palmitic acid), cis-9-hexadecenoic acid (16:1n-7, palmitoleic acid), cis-9-octadecenoic acid (18:1n-9, oleic acid), cis-9-eicosenoic acid (20:1n-11), cis-11-docosenoic acid (22:1n-11), cis-5,8,11,14,17-eicosapentaenoic acid (20:5n-3, EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (22:6n-3, DHA) [30]. Weight percentages of the fatty acids based on the internal standard showed similar fatty acid profiles to the OmegaPure®E reference data and Moffat et al., [30]. In most fish oils, the percentage of EPA is usually higher than DHA, but there is an exception with mackerel oil, which contains greater amounts of DHA than EPA [30,31].

The typical summed weight percent of EPA and DHA in marine fish fatty acids is around 12 and 34 %, depending fish types, seasons, sex and geographical locations [32]. The amount of EPA and DHA were 73.4mg/g and 66.8mg/g, respectively. The area percent of EPA+DHA (%) was 24.6 %, which was within the range (23-27%) of OmegaPure®E reference data as well as Moffatet

al., [30]. EPA and DHA are important PUFAs and provide protection against cardiovascular diseases and other health concerns [3,4].

Extraction and analysis of vine tea extract

Vine tea extract (3.78 g), green tea (3.24 g) and grape seed extract (4.20 g) were obtained by the extraction and freeze drying procedure (means, n=3). The DHM in vine tea was 26.2% w/w (dry basis). The Teng Cha extract contained 69.3 % w/w DHM (mean n=3). This was similar to the DHM content (64.7% w/w, mean n=3) reported by Ye et al., [15].

Total Phenolic Content (TPC) of natural antioxidants

Since total phenolic content contributes to overall antioxidant capacity, the total phenolic content of each extract was determined by using a Folin-Phenol assay [27]. The phenolic contents means (n=3) were: Vine tea extract (310 mg GAE/ g), commercial DHM (370 mg GAE/ g), green tea extract (180 mg GAE/g) and grape seed extract (380 mg GAE/g). The grape seed extract had the highest TPC among the samples. The TPC of the samples were significantly different by using Tukey's test ($p<0.05$).

Grape seeds, skins and grape pulps have different polyphenol compositions, depending on the extraction method and use solvents. Use of ethanol as an extraction solvent resulted in a high TPC [33]. Dihydromyricetin (DHM) is the major bioactive compound in vine tea, and was reported to have good antioxidant capacity [34]. The total phenolic content (324 mg GAE /g) reported by Ye et al., was agreed with our finding (310 mg GAE/g) [15].

The green tea extract was found to have the lowest total phenolic content among the extracts, 180 mg GAE/g. The phenolic concentration of the green tea extract was higher than the range 65.8-106.2 mg GAE/ g from Khojhar et al., [35]. They pointed out it varies depends on extraction/fractionation methods and growing regions.

Baseline vitamin E analysis in menhaden oil

The hexane extraction and direct HPLC analysis of Ye et al. was a simple and efficient method to determine tocopherol contents in menhaden oil, because the method avoided saponification processes, which often result in low recoveries [25]. Menhaden oil from OmegaPure® was formulated with Covi-ox®T-50, which is natural mixed tocopherol derived from edible vegetable oil. Approximately 1000ppm of mixed tocopherols was added to the menhaden oil for preventing oxidation during processing, handling and shelf-life provided by Cognis Corporation [36]. The typical mixed tocopherols in soybean oil are composed of 14 % of α-tocopherol, 2% ß-tocopherol, 60% γ-tocopherol, and 24% δ-tocopherol, from the Covi-ox®T-50 reference [36]. Alpha tocopherol, gamma tocophol, and delta tocopherol were found at 0.18 mg/g, 0.37 mg/g, and 0.14 mg/g, respectively, on average of duplicate samples by using the external standard procedures. The direct extraction and HPLC analysis used for tocopherol analysis was simple and rapid, although it did require the use of a fluorimeter [25]. Fluorescence detection for tocopherol analysis provides more sensitivity and specificity compared to UV detection [20].

Antioxidant activity of single antioxidants on oxidation of menhaden oil head space oxygen content

Loss of headspace oxygen was evaluated by measuring headspace oxygen contents. Oxygen levels of MO samples decrease as oxygen is removed from the headspace through oxidation in the samples. The headspace oxygen contents of each MO sample were determined by day 0, day 2, day 4, day 6 and day 8. At 0 day, headspace oxygen levels of the samples were ~20 %. Oxygen levels of control, AP (200, 500, 1000 ppm), CA (50, 100, 150 ppm), GSE (200, 500, 1000 ppm), GTE (500, 1000, 2000 ppm), MT (200, 500, 1000 ppm), QC (200, 500, 1000 ppm) and RME (500 and 1000 ppm) were dramatically decreased at 40°C storage for eight days. Although the oxygen contents of 200 ppm, 500 ppm and 1000 ppm of GSE and VTE, 500, 1000, 2000 ppm of GTE and RME for four days storage at 40°C were between 16.3 % and 18.1%, and 200 ppm, 500 ppm and 1000 ppm of VTE and 2000 ppm RME were 17.4%, 16.7%, 16.8% and 15.9%, respectively, the oxygen levels of other treatments were below 11% after six days storage at 40°C. Oxygen levels of 200 ppm, 500 ppm, 1000 ppm VTE and 2000 ppm RME in MO samples were 11.8%, 13.2 %, 14.4% and 10.4%, respectively, which contained higher oxygen levels than other treatments at 40°C storage after eight days. The 1000 ppm VTE sample had the highest oxygen content, followed by 500 ppm VTE, 200 ppm VTE and 2000 ppm RME. The VTE treatments inhibited the formation of primary oxygen products and loss of headspace oxygen in MO. These results were in agreement with antioxidant inhibitory effects of DHM and 200 ppm VTE on oxidation of soybean oil at 60° storage for 15 days [15].

Anisidine values (AV)

Aldehyde levels of the MO samples were evaluated for nonvolatile secondary oxidation products. Samples with 200 ppm, 500 ppm and 1000 ppm AP and 200 ppm, 500 ppm and 1000 ppm QC had higher AV values than the control. In 200 ppm, 500 ppm and 1000 ppm AP and 200 ppm, 500 ppm and 1000 ppm QC of samples, the headspace oxygen contents were low. This shows they did not effectively inhibit primary oxidation products and may have acted as prooxidants in MO [19]. VTE, GSE, GTE and MT had lower AV than control at eight days. It is important to measure both primary and secondary oxidation products for effectiveness antioxidants in lipid system [37]. In some cases, secondary oxidation products measured by AV can be low while primary oxidation products are increased. For example, peroxide values may be high in the presence of tocopherols, because tocopherols donate hydrogen to peroxy radicals to form stable peroxides whereas secondary oxidation products maybe formed at low rates in processes of tocopherols [37]. Looking at measurements of both primary and secondary oxidation products, 200 ppm, 500 ppm, and 1000 ppm VTE significantly improved oxidative stability and delayed oxidation of MO at 40°C storage for eight days

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since the VTE treatments had much higher oxygen contents and lower AV than control and other antioxidant treatments.

Antioxidant activity of mixtures of antioxidants on Oxidation of Menhaden Oil (MO) peroxide values

The treatments containing VTE inhibited oxidative degradation of MO at 40°C storage during eight days **(Table 1).** Mixtures of VTE and RME resulted in much lower peroxide values than VTE or RME alone. On eight day, peroxide value of control and 1000 ppm RME were dramatically increased and exceeded 35 meq/kg, whereas peroxide value of combinations in 500 ppm VTE and 2000 ppm RME treatment sample (10.7 meq/kg) had the lowest peroxide value in all samples at 40°C storage during eight days. 200 ppm VTE and 2000 ppm RME showed slightly less effective protection against formation of primary oxidation products. The peroxide values of 200 ppm and 500 ppm VTE samples with addition of same concentration of RME were not significantly different, but mixture of VTE and RME samples with addition of different levels of RME concentrations (1000 ppm or 2000 ppm) were significantly different in rate of primary oxidation product formation ($p<0.05$). Thus, RME can possibly provide additive antioxidant inhibitory effects that assisted in delaying oxidative degradation of MO more efficiently in combination with VTE.

Table 1. Peroxide values (meq/kg) of mixtures of VTE and RME in menhaden oil stored for 0-8 days at 40°C.Values are reported as means ± standard deviation of peroxide value (meq/kg) on each test day and different superscript letters indicate significant differences using Tukey's mean separation ($p<0.05$).

Treatment (ppm)	Peroxide Value (meq/kg)				
	0 day	2 days	4 days	6 days	8 days
Control	1 ± 0.2^{a}	4.2 ± 0.8^{a}	16.6 ± 1.6^{a}	29.4 ± 0.18^{a}	37.4 ± 1.3^{a}
200VTE	0.9 ± 0.1^{a}	2.9 ± 0.3^{ab}	6.9 ± 1.5^{bc}	15.3 ± 1.4^{c}	27.5 ± 0.7^{b}
500VTE	1.7 ± 1^{a}	3.0 ± 1.2^{ab}	9.5 ± 1.3^{b}	13.3 ± 0.8^{cd}	24.8 ± 2.2^{bc}
1000REX	1.1 ± 0.21^{a}	3.2 ± 0.8^{ab}	9.3 ± 1.4^{b}	24.6 ± 1.9^{b}	35.7 ± 1.3^{a}
1000RME+200VTE	1.1 ± 0.5^{a}	2.2 ± 0.2^{b}	6.1 ± 1.1^{c}	9.7 ± 0.5^{de}	21.9 ± 3.5^{cd}
1000RME+500VTE	1.1 ± 0.1^{a}	2.7 ± 0.4^{ab}	6.2 ± 0.5^{c}	11.1 ± 0.6^{d}	16.3 ± 0.2^{de}
2000RME	1.3 ± 0.4^{a}	2.2 ± 0.2^{b}	5.6 ± 0.1^{c}	10.7 ± 1.9^{d}	19.9 ± 3.2^{cde}
2000RME+200VTE	1.1 ± 0.3^{a}	2.1 ± 0.1^{b}	6.3 ± 1.7^{c}	10.0 ± 0.6^{d}	14.8 ± 1.5^{ef}
2000RME+500VTE	1.3 ± 0.1^{a}	1.9 ± 0.4^{b}	4.3 ± 1.0^{c}	6.2 ± 1.2^{e}	10.7 ± 1.3^{f}

Head space oxygen content

Measuring primary oxidation products of the samples by head space oxygen content **(Table 2)** test showed similar results as peroxide values **(Table 1).** During eight day storage at 40°C, headspace oxygen contents differed significantly ($p<0.05$) **(Table 2).** Mixture of 500 ppm VTE and 2000 ppm RME (14.5 %) had highest oxygen percentage followed by mixtures of 500 ppm VTE and 1000 ppm RME (14.2 %). The treatments containing VTE had much higher oxygen contents than control or RME alone. In combination of VTE and RME in MO samples, higher oxygen levels were observed than for VTE alone. RME assisted in protection against oxidation, since addition of RME increased effectiveness of VTE.

Table 2. Headspace oxygen content (%) of mixtures of VTE and RME in menhaden oil stored for 0-8 days at 40°C.Values are reported as means ± standard deviation of headspace oxygen content (%) on each test day and different superscript letters indicate significantly differences using Tukey's mean separation ($p<0.05$).

Treatment (ppm)	Oxygen content %				
	0 day	2 days	4 days	6 days	8 days
Control	20.2 ± 0.1^{ab}	18.4 ± 0.2^{c}	11.9 ± 0.0^{f}	7.4 ± 0.1^{g}	5.1 ± 0^{i}
200VTE	20.2 ± 0.2^{ab}	18.5 ± 0.2^{c}	17.6 ± 0.1^{e}	13.5 ± 0^{f}	10.7 ± 0^{f}
500VTE	20.2 ± 0.1^{ab}	18.6 ± 0.2^{c}	17.8 ± 0.1^{de}	14.8 ± 0^{d}	11.5 ± 0^{e}
1000REX	20.2 ± 0.1^{ab}	19.5 ± 0.1^{ab}	18 ± 0.1^{cd}	14.7 ± 0.1^{d}	8.4 ± 0.1^{h}
1000RME+200VTE	20.3 ± 0.5^{a}	19.3 ± 0.1^{b}	17.9 ± 0.1^{cde}	15.2 ± 0.1^{c}	11.9 ± 0.1^{d}
1000RME+500VTE	20.1 ± 0.1^{ab}	19.5 ± 0.1^{ab}	18 ± 0.1^{bcd}	17.8 ± 0.1^{a}	14.2 ± 0.1^{b}
2000RME	19.6 ± 0.08^{b}	19.3 ± 0.1^{b}	18.3 ± 0.1^{abc}	14.4 ± 0.1^{e}	10.1 ± 0.01^{g}
2000RME+200VTE	20.1 ± 0.3^{ab}	19.4 ± 0.1^{b}	18.4 ± 0.1^{ab}	17.3 ± 0.1^{b}	13.9 ± 0.02^{c}
2000RME+500VTE	20.1 ± 0.1^{ab}	19.8 ± 0.04^{a}	18.6 ± 0.1^{a}	17.9 ± 0.06^{a}	14.5 ± 0.03^{a}

Anisidine Value (AV)

The secondary oxidation products that include aldehydes causing rancid food flavors and odors were measured by the p-anisidine test. The AVs of the samples agreed with peroxide values and headspace oxygen content evaluations. The mixture of 500 ppm VTE and 2000 ppm RME produced the lowest AV (11.2) at 40°C storage for eight days. Combined 500 ppm VTE and RME (1000 ppm or 2000 ppm) were significantly better than VTE alone. The 1000 and 2000 ppm RME samples had improved stability in the mixture formulations. However, the AV of 1000 ppm RME alone (24.6) was higher than the control (21.2). Once RME, which was stabilized in MO, started degrading, the rate of oxidative degradation was increased because the effectiveness of RME was lost. The results of AV agreed with peroxide value and headspace oxygen contents, since the most effective antioxidant formulation in MO was mixture of 500 ppm VTE and 2000 ppm RME with the lowest AV (11.2) **(Table 3),** the lowest peroxide value (10.7 meq/kg) and highest headspace oxygen content (14.5%).

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Table 3. Anisidine values of mixtures of VTE and RME in menhaden oil stored for 0-8 days at 40°C.Values are reported as means ± standard deviation of anisidine value on each test day and different superscript letters are indicate significant differences using Tukey's mean separation (p<0.05).

Treatment (ppm)	Anisidine value				
	0 day	2 days	4 days	6 days	8 days
Control	7.7 ± 0.2^{a}	9.7 ± 0.7^{a}	13 ± 1^{a}	18.1 ± 0.4^{a}	21.2 ± 0.6^{b}
200VTE	7.5 ± 0.2^{a}	8.3 ± 0.1^{b}	10.6 ± 0.4^{ab}	13.4 ± 0.4^{c}	15.2 ± 0.4^{c}
500VTE	7.7 ± 0.4^{a}	9.5 ± 0.2^{ab}	11 ± 0.3^{ab}	14.3 ± 0.5^{c}	15.5 ± 0.3^{c}
1000REX	7.5 ± 0.2^{a}	9.1 ± 0.2^{ab}	10.3 ± 1.0^{ab}	16.5 ± 0.8^{b}	24.6 ± 0.2^{a}
1000RME+200VTE	7.4 ± 0.4^{a}	9.5 ± 0.4^{ab}	9.5 ± 0.3^{b}	11.4 ± 0.4^{de}	15.6 ± 0.7^{c}
1000RME+500VTE	6.4 ± 0.6^{b}	8.8 ± 0.2^{ab}	11.1 ± 3.2^{ab}	11.8 ± 1.3^{d}	13.5 ± 1.4^{d}
2000RME	8.3 ± 0.2^{a}	9.5 ± 0.2^{ab}	9.8 ± 0.28^{ab}	10.5 ± 1.2^{e}	14.9 ± 3.2^{c}
2000RME+200VTE	7.6 ± 0.4^{a}	9.2 ± 0.2^{ab}	10.6 ± 1^{ab}	11 ± 0.4^{de}	13.2 ± 0.7^{d}
2000RME+500VTE	7.9 ± 0.1^{a}	9.1 ± 0.1^{ab}	9.3 ± 0.4^{b}	10.4 ± 0.2^{e}	11.2 ± 1.3^{e}

Ascorbyl Palmitate (AP) and Citric Acid (CA) with Mixtures of Vine Tea (VTE) and Rosemary Extract (RME)

peroxide value

Peroxide values of each treatment and control were determined at 0 day, 2 day, 4 day, 6 day and 8 day at 40°C storage **(Table 4).** Control (35 meq/kg) and 200 ppm AP + 100 ppm CA (35.3 meq/kg) was had the highest peroxide values among the samples. Like previous peroxide value results, treatments containing VTE had lowest peroxide values. The mixtures of 200 ppm AP + 100 ppm CA + 2000 ppm RME and 1000 ppm or 500 ppm had VTE inhibited formation of primary oxidation products.

Table 4. Peroxide value (meq/kg) of mixtures of AP, CA, VTE and RME in menhaden oil stored for 0-8 days at 40°C.Values are reported as means ± standard deviation of peroxide value (meq/kg) on each test day and different superscript letters indicate significant differences using Tukey's mean separation (p<0.05).

Treatment (ppm)	Peroxide value (meq/kg) (p<0.05)				
	0 day	2 days	4 days	6 days	8 days
Control	1.7 ± 0.4^{ab}	3.1 ± 0.8^{a}	18.1 ± 1.3^{a}	26.9 ± 0.6^{a}	35 ± 0.6^{a}
200AP+100CA	1.3 ± 0.5^{ab}	3.1 ± 0.1^{a}	13.9 ± 1.1^{b}	25 ± 0.5^{b}	35.3 ± 2.2^{a}
200AP+100CA+500VTE	1.2 ± 1.0^{ab}	3.5 ± 0.2^{a}	5.1 ± 1^{c}	7.8 ± 0.4^{de}	11.8 ± 0.3^{d}
200AP+100CA+1000VTE	1.5 ± 0.6^{ab}	3 ± 1.7^{a}	5.88 ± 0.9^{c}	7.3 ± 0.4^{de}	9.8 ± 0.4^{de}
200AP+100CA+1000RME	1.3 ± 0.2^{ab}	2.9 ± 0.2^{a}	5.8 ± 0.2^{c}	11 ± 0.5^{c}	22.7 ± 1.1^{b}
200AP+100CA+1000RME+500VTE	1.8 ± 0.2^{a}	3.8 ± 0.3^{a}	6 ± 0.7^{c}	8.1 ± 0.9^{d}	10 ± 0.9^{de}
200AP+100CA+1000RME+1000VTE	1.4 ± 0.2^{ab}	2.7 ± 0.2^{a}	5.4 ± 0.6^{c}	6.3 ± 0.7^{ef}	7.3 ± 0.1^{ef}
200AP+100CA+2000RME	1.3 ± 0.2^{ab}	2.7 ± 0.5^{a}	5.2 ± 0.5^{c}	11.3 ± 1.0^{c}	16.6 ± 1.2^{c}
200AP+100CA+2000RME+500VTE	0.9 ± 0.1^{ab}	3.4 ± 0.2^{a}	4.8 ± 1.1^{c}	6.2 ± 0.1^{ef}	7.6 ± 0.2^{ef}
200AP+100CA+200RME+1000VTE	0.4 ± 0.7^{b}	3 ± 0.8^{a}	3.8 ± 0.1^{c}	5.3 ± 0.2^{f}	6.7 ± 0.2^{f}

Head space oxygen

Oxygen contents of the samples were determined at 0 day, 2 day, 4 day, 6 day and 8 day during storage at 40°C **(Table 5).** Lower oxygen values of the samples resulted from oxidation, development of hydroperoxides, as more oxygen was absorbed by the oil samples. Oxygen contents of control and 200 ppm AP + 100 ppm CA treatment in MO were significantly decreased whereas mixtures of 200 ppm AP+ 100 ppm CA + 2000 ppm RME+ 1000 ppm VTE or 500 ppm VTE had the highest oxygen contents after storage at 40°C for eight days. The headspace oxygen content evaluation carried out similar results with the peroxide value determination. The treatment containing 1000 ppm VTE inhibited on formation of primary oxidation products effectively. AP+CA+RME decreased delay oxidation of MO more effectively than VTE alone.

Table 5. Headspace oxygen content (%) of mixtures of AP, CA, VTE and RME in menhaden oil stored for 0-8 days at 40°C.Values are reported as means ± standard deviation of head space oxygen content (%) on each test day and different superscript letters are indicated significantly different using Tukey 's mean separation (p<0.05).

Treatment (ppm)	Oxygen % (p<0.05)				
	0 day	2 days	4 days	6 days	8 days
Control	19.8 ± 0.2^{ab}	18.9 ± 0.1^{a}	14.3 ± 0.02^{f}	9.1 ± 0.01^{h}	6 ± 0.01^{g}
200AP+100CA	19.6 ± 0.1^{b}	18 ± 0.1^{d}	13.2 ± 0.2^{g}	8.9 ± 0.01^{i}	5.9 ± 0^{g}
200AP+100CA+500VTE	19.9 ± 0.1^{ab}	18.6 ± 0.2^{abc}	17.7 ± 0.1^{bc}	16.3 ± 0.02^{e}	13.9 ± 0.01^{d}
200AP+100CA+1000VTE	19.9 ± 0.1^{ab}	18.3 ± 0.1^{cd}	17.6 ± 0.03^{bc}	17.6 ± 0.04^{bc}	15.3 ± 0.01^{b}
200AP+100CA+1000RME	20 ± 0.7^{ab}	18.3 ± 0.1^{cd}	15 ± 0.01^{e}	11.3 ± 0.01^{g}	7.9 ± 0.01^{f}
200AP+100CA+1000RME+500VTE	20.5 ± 0^{a}	18.3 ± 0.2^{bcd}	17.4 ± 0.01^{c}	16.6 ± 0.02^{d}	14.7 ± 0.3^{c}
200AP+100CA+1000RME+1000VTE	20.3 ± 0.03^{ab}	18.6 ± 0.1^{abc}	18.5 ± 0.2^{a}	17.7 ± 0.1^{b}	15.5 ± 0.02^{b}
200AP+100CA+2000RME	20.1 ± 0.3^{ab}	18.3 ± 0.1^{bcd}	16.6 ± 0.03^{d}	14.4 ± 0.1^{f}	9.80 ± 0^{e}
200AP+100CA+2000RME+500VTE	20.4 ± 0.1^{ab}	18.4 ± 0.3^{cd}	18 ± 0.02^{b}	17.4 ± 0.03^{c}	15.3 ± 0.02^{b}
200AP+100CA+200RME+1000VTE	20.3 ± 0.1^{ab}	18.3 ± 0.03^{bcd}	18.5 ± 0.2^{a}	18.1 ± 0.2^{a}	16.4 ± 0.04^{a}

Anisidine value

Anisidine value can use an indication of secondary oxidation products that provide undesirable odors after decomposition of the hydroperoxides [38]. The AV values of control and 200 ppm AP + 100 ppm CA were steadily increased and had the highest anisidine values among the samples at 40°C storage after eight days. However, the mixture of 200 ppm AP+ 100 ppm CA + 2000 ppm RME + 1000ppm VTE or 500 ppm VTE has much lower AV than control and 200 ppm AP + 100 ppm CA during eight days storage at 40°C. Combinations of 200 ppm AP, 100 ppm CA, VTE (500 and 1000 ppm) and RME (1000ppm and 2000ppm) inhibited oxidation and improved stability of MO containing mixed tocopherols at 40°C storage for eight days **(Table 6).**

Table 6. Anisidine values of mixtures of AP, CA, VTE and RME in menhaden oil stored for 0-8 days at 40°C. Values are reported as means ± standard deviation of anisidine on each test day and different superscript letters are indicate significant differences using Tukey's mean separation ($p<0.05$).

Treatment (ppm)	Anisidine Value				
	0 day	2 days	4 days	6 days	8 days
Control	7.6 ± 0.1^{abc}	8.7 ± 0.6^{b}	12.5 ± 0.3^{a}	16.9 ± 0.3^{a}	23.5 ± 1.3^{a}
200AP+100CA	7.2 ± 0.2^{bc}	10.7 ± 0.2^{a}	11.5 ± 0.3^{a}	16.3 ± 0.5^{a}	24.7 ± 1.7^{a}
200AP+100CA+500VTE	7 ± 0.3^{c}	8.6 ± 0.6^{b}	9.2 ± 0.2^{b}	10.6 ± 0.2^{c}	11.5 ± 1^{cde}
200AP+100CA+1000VTE	7 ± 0.1^{c}	8.4 ± 0.2^{bc}	9.1 ± 0.4^{b}	10.7 ± 0.3^{c}	12.1 ± 0.5^{bcd}
200AP+100CA+1000RME	7.8 ± 0.1^{ab}	8.8 ± 0.5^{b}	8.7 ± 0.2^{b}	11.8 ± 0.2^{b}	13.9 ± 0.2^{b}
200AP+100CA+1000RME+500VTE	8 ± 0.1^{a}	9.1 ± 0.2^{b}	9.7 ± 1^{b}	10.4 ± 0.3^{cd}	10.8 ± 0.4^{cde}
200AP+100CA+1000RME+1000VTE	7.5 ± 0.4^{abc}	8.2 ± 0.2^{bcd}	9.2 ± 0.2^{b}	9.6 ± 0.2^{de}	9.8 ± 0.2^{de}
200AP+100CA+2000RME	7.4 ± 0.2^{bc}	7.2 ± 0.4^{d}	9.4 ± 0.9^{b}	10.9 ± 0.4^{c}	12.7 ± 0.2^{bc}
200AP+100CA+2000RME+500VTE	7.5 ± 0.1^{abc}	7.5 ± 0.2^{cd}	9.3 ± 2.3^{b}	9.30 ± 0.5^{e}	10 ± 0.4^{de}
200AP+100CA+200RME+1000VTE	7 ± 0.1^{c}	7.5 ± 0.2^{cd}	9.5 ± 3.3^{b}	9.10 ± 0.5^{e}	9.4 ± 0.2^{e}

Mixtures of mixed tocopherols (γ-, or δ tocopherol and low concentration of α-tocopherol), AP, RME and CA efficiently improve stability of bulk fish oil rich in PUFAs at ambient temperature [18]. Because AP helps regenerating tocopherols, the stability of fish oil was improved by AP addition [39,40]. AP protects against α-tocopherol degradation during storage [21]. However, ascorbic acid or AP could be changed to a prooxidant if transition metals were present in fish oil [8]. Addition of CA as metal chelating agent helped increase stability for the fish oil formulation containing tocopherols, RME and AP by reduction of primary oxidation products, and sunflower oil blending with RME, α-tocopherol and AP delayed oxidative deterioration as well [18,21]. Although AP and CA possibly helped delay oxidative degradation of MO mixture formulations, the stability of the MO formulations was not improved by addition of AP and CA in our study.

CONCLUSIONS

The 200 ppm, 500 ppm and 1000 ppm vine tea extract (VTE) treatments effectively delayed oxidation of MO compared to control at 40°C storage for eight days. The combination of VTE and RME more effectively improved stability of MO containing mixed tocopherols at 40°C storage for eight days. The mixture of 500 ppm VTE and 2000 ppm RME was the most effective combination examined on lipid oxidation of the MO during the storage at 40°C. RME had an additive antioxidant effect in the mixed formulations as measured by peroxide value, headspace oxygen and anisidine value, since the combination of VTE and RME inhibited on oxidation more than VTE itself. Our results show that VTE is an effective natural antioxidant for menhaden oil, especially in combination with RME and tocopherols.

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REFERENCES

1. Gogus U and Smith C. n-3 Omega fatty acids: a review of current knowledge. International Journal of Food Science & Technology. (2010);45(3):417-436.
2. Ruxton CH, et al. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. See comment in PubMed Commons below J Hum Nutr Diet. (2004);17:449-459.
3. Wang C, et al. n-3 Fatty acids from fish or fish-oil supplements, but not a-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. American Journal of Clinical Nutrition. (2006);84:5-17.
4. Kris-Etherton PM, et al. Nutrition Committee Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol. (2003);23:e20-30.
5. Lee CM, et al. Global Trends in Marine Nutraceuticals In Food Technology 65: 23-31. Institute of Food Technologist. 525 W. Van Buren St., Suite 1000, Chicago, IL, U.S.A (2011).

6. Pazos M, et al. Hydroxytyrosol prevents oxidative deterioration in foodstuffs rich in fish lipids. J Agric Food Chem. (2008);56:3334-3340.

7. Frankel EN (1998) Lipid Oxidation 10: 185-221. Oily Press.

8. Indrasena WM and Barrow CJ. Oxidation and Stability of Food-Grade Fish Oil: Role of Antioxidants. In Handbook of Seafood Quality, Safety and Health Applications. (2010);317-334. Wiley-Blackwell.

9. Keokamnerd T, et al. Effect of commercial rosemary oleoresin preparations on ground chicken thigh meat quality packaged in a high-oxygen atmosphere. Poultry Science. (2008);87:170-179.

10. Yanishlieva NV, et al. Natural antioxidants from herbs and spices. European Journal of Lipid Science and Technology. (2006);108:776-793.

11. Berdahl D, Nahas R, Barren J, Decker E, Elias R, et al. (2010) Synthetic and natural antioxidant additives in food stabilization: current applications and future research. Oxidation in Foods and Beverages and Antioxidant Applications. Volume 1: Understanding Mechanisms of Oxidation and Antioxidant Activity: 272-320.

12. Liu B, Du J, et al. Characterization and antioxidant activity of dihydromyricetin–lecithin complex. European Food Research and Technology. (2009);230:325-331.

13. Huang H, et al. Dihydromyricetin suppresses the proliferation of hepatocellular carcinoma cells by inducing G2/M arrest through the Chk1/Chk2/Cdc25C pathway. Oncol Rep. (2013);30:2467-2475.

14. Wang W, et al. The antioxidation effects of the extracts of Ampelopsis grossedenta. Chienses Wild Plant Resources. (2004);23:46-47.

15. Ye L, et al. Antioxidant activities of vine tea (Ampelopsis grossedentata) extract and its major component dihydromyricetin in soybean oil and cooked ground beef. Food Chemistry. (2015);172:416-422.

16. Shi Li, et al. Dihydromyricetin improves skeletal muscle insulin resistance by inducing autophagy via the AMPK signaling pathway. Molecular and Cellular Endocrinology. (2015);409:92-102

17. Hou X, et al. Dihydromyricetin protects endothelial cells from hydrogen peroxide-induced oxidative stress damage by regulating mitochondrial pathways. Life sciences. (2015);130:38-46.

18. Drusch S, et al. Efficient stabilization of bulk fish oil rich in long-chain polyunsaturated fatty acids. European Journal of Lipid Science and Technology. (2008);110:351-359.

19. Hamilton RJ, et al. Chemistry of free radicals in lipids. Food Chemistry. (1997);60:193-199.

20. Let MB, et al. Ascorbyl palmitate, gamma-tocopherol, and EDTA affect lipid oxidation in fish oil enriched salad dressing differently. See comment in PubMed Commons below J Agric Food Chem. (2007);55:2369-2375.

21. Yi J, et al. Interactions between tocopherols, tocotrienols and carotenoids during autoxidation of mixed palm olein and fish oil. Food Chemistry. (2011);127:1792-1797.

22. AOCS. AOCS Official Method Ce 1b-89 Fatty Acid Compostion (marine oils) by GLC (5 ed.), AOCS: The American Oil Chemists' Soceity, Washington,DC (1999).

23. Ackman RG (1969) Gas-liquid chromatography of fatty acids and esters. In M. L. John (Ed.), Methods in Enzymology 14 :329-381 Academic Press.

24. Schulte E. Gas chromatography of acylglycerols and fatty acids with capillary columns. Analysis of Lipids. CRC Handbook of Chromatography (1993).

25. Ye L, et al. Vitamin E content of margarine and reduced fat products using a simplified extraction procedure and HPLC determination. Journal of Liquid Chromatography & Related Technologies. (1998);21:1227-1238.

26. Gokoglu N and Yerlikaya P. Inhibition effects of grape seed extracts on melanosis formation in shrimp (Parapenaeus longirostris). International Journal of Food Science & Technology. (2008);43:1004-1008.

27. Spanos GA and Wrolstad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural and Food Chemistry. (1990);38:1565-1571.

28. AOCS. Peroxide Value Acetic Acid-Chloroform Method. (5 ed.), AOCS: The American Oil Chemists' Society, Washington, DC (1998).

29. IUPAC Determination of the p-Anisidine Value, Method 2.504. (7 ed.), Oxford. Alden Press (1987).

30. Moffat CF and McGill AS. Variability of the composition of fish oils: significance for the diet. See comment in PubMed Commons below Proc Nutr Soc. (1993);52:441-456.

31. Rossell JB. Fish oils. Oxford: Wiley-Blackwell (2009).

32. Hardy R and Keay J. Seasonal variations in the chemical composition of Cornish mackerel, Scomber scombrus (L), with detailed reference to the lipids. International Journal of Food Science & Technology. (1972);7:125-137.

33. Nawaz H, et al. Extraction of polyphenols fromgrape seeds and concentration by ultrafiltration. Separation and Purification Technology. (2006);48:76-181.

34. Reische DW, Lillard DA, Eitenmiller RR 15 Antioxidants. Food Lipids: Chemistry, Nutrition, and Biotechnology 409 (2008).

35. Khokhar S1, Magnusdottir SG (2002) Total phenol, catechin, and caffeine contents of teas commonly consumed in the United kingdom. See comment in PubMed Commons below J Agric Food Chem 50: 565-570.

36. Cognis. Nutritional & Health Covi-ox®T-50. In C. T.-s. pdf. (Ed.). Cognis. 4900 Este Avenue Cincinnati, OH (2006).

37. Decker EA, et al. Measuring antioxidant effectiveness in food. See comment in PubMed Commons below J Agric Food Chem. (2005);53:4303-4310.

38. O'Sullivan A, et al. Use of natural antioxidants to stabilize fish oil systems. Journal of Aquatic Food Product Technology. (2005);14:75-94.

39. Han D, et al. Solubilization of vitamin C in fish oil and synergistic effect with vitamin E in retarding oxidation. Journal of the American Oil Chemists' Society. (1991);68:740-743.

40. Kul E and Ackman R. Protection of a-tocopherol in nonpurified and purified fish oil. Journal of the American Oil Chemists' Society. (2001);78:197-203.