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Trapping Effects of Green and Black Tea Extracts on Peroxidation-Derived Carbonyl Substances of Seal Blubber Oil

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Green and black tea extracts were employed to stabilize seal blubber oil at 60 °C for 140 h. On the basis of the headspace SPME-GC-MS analysis, with the addition of green/black tea extracts, the contents of acetaldehyde, acrolein, malondialdehyde, and propanal, four major lipid peroxidation products, were reduced. The inhibition rates of acrolein formation by green tea and black tea extracts were 98.40 and 96.41% respectively, and were 99.17 and 98.16% for malondialdehyde, respectively, much higher than the inhibition of the formation of acetaldehyde and propanal. Because malondialdehyde and acrolein are reactive carbonyl species (RCS) and recent studies have suggested that phenolics can directly trap RCS, this study also investigated whether green tea polyphenols can trap acrolein or not. Acrolein was reduced by 90.30% in 3 h of incubation with (−)-epigallocatechin-3-gallate (EGCG). Subsequent LC-MS analysis revealed the formation of new adducts of equal molars of acrolein and EGCG. The reaction site for acrolein was elucidated to be the A ring of EGCG as evidenced by LC-MS/MS analysis and by testing of the acrolein-trapping capacities of the analogous individual A, B, and C rings of EGCG. Thus, EGCG's direct trapping of RCS may also contribute to the significant reduction of acrolein and other aldehydes in the peroxidation of seal blubber oil.

KEYWORDS: Tea extracts; seal blubber oil; SPME; acrolein; EGCG

INTRODUCTION

The intake of ω-3 polyunsaturated fatty acids (PUFAs) has been positively related to human health. Mounting epidemiological and interventional trials showed the beneficial effects of ω-3 PUFAs on the prevention and treatment of cardiovascular diseases, psychiatric disorders, immune deficiency, and cancers (*1*). Dietary marine oils are considered to be a rich source of ω-3 fatty acids, including eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). Many countries have allowed formulating them in food products to achieve an adequate intake of ω-3 PUFAs (*2*). As a result, a wider variety of food products such as bread, milk, ice cream, and others that usually are not associated with marine origins are fortified with ω-3 PUFAs (*3*). However, ω-3 PUFAs of marine origin, such as fish and algae oils, are susceptible to rapid oxidative deterioration during storage and processing with the generation of undesirable rancid flavors and reduced nutritional quality (*4*). The stability of PUFAs varies widely according to their degree of unsaturation, position of PUFAs in the triacylglycerol molecule, and composition of minor components (*5*, *6*).

Many strategies have been employed to avoid the potential pro-oxidation factors that may initiate and/or accelerate lipid peroxidation. Addition of antioxidants is one of the effective ways to inhibit lipid peroxidation and preserve food quality. Synthetic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG), have commonly been used in several countries (*7*). However, with the trend toward the elimination of the utilization of synthetic food additives, considerable interest has been focused on natural antioxidants, which commonly exist in plants, microorganisms, fungi, or even animal tissues (*8*). The application of some plant polyphenols, such as phenolic compounds from grape and olive oil, as inhibitors of lipid peroxidation has been well documented (*9, 10*). It is reasonable to employ tea polyphenols, which are demon-

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strated to be effective antioxidants for scavenging of reactive oxygen species in vitro and in vivo, in the preservation of unsaturated lipid-containing foods (*11*).

In this study, we adopted headspace solid-phase microextraction (SPME) coupled with GC-MS (*12*) to monitor the major oxidation end products from ω-3 fatty acids in peroxidized seal blubber oil and evaluated the protective effects of green and black tea extracts against peroxidation of PUFAs. Compared to traditional analytical methods, SPME presents an attractive alternative for its solvent-free process, which combines sampling, extraction, concentration, and instrument introduction into a single step, eliminating complicated sample-preparation procedures (*13*). This method is sensitive and precise for the differentiation of various aldehydes according to their retention times and molecular weights.

Unsaturated aldehydes such as 4-hydroxy-*trans*-2-nonenal (HNE), malondialdehyde (MDA), and acrolein (ACR) are released from the peroxidation of PUFAs. They not only contribute to the aroma of oxidized oils but also are regarded as causative agents in the cytotoxic process initiated by the exposure of biological systems to oxidizing agents or free radicals (*14*). These simple aldehydes produced from the breakdown of PUFAs are termed as harmful reactive carbonyl species (RCS), and so far, a few inhibitors have been evaluated to quench them before they react with protein, DNA, to form advanced lipoxidation end products (ALEs) (*15*). However, little information is available on whether phenolic compounds can be potential candidates of RCS-sequestering agents or not. In this study, tea polyphenols' capacity to directly trap ACR (one RCS released from the peroxidation of seal blubber oil) was also tested.

MATERIALS AND METHODS

Chemicals and General Procedures. Acetaldehyde, propanal, acrolein, 1,1,3,3-tetramethoxypropane, *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA), corn oil, hexane, phosphate-buffered saline (PBS), pH 7.4, 1,3,5-trihydroxybenzene, 1,2,3-trihydroxybenzene, and methyl gallate were purchased from Sigma-Aldrich (St. Louis, MO). Poly(dimethylsiloxane)/divinylbenzene SPME fiber, fiber holder, and crimp-top amber glass vials were all purchased from Supelco (Bellefonte, PA). All analytical GC and HPLC grade solvents used were obtained from BDH Laboratory Supplies (Poole, U.K.). Malondialdehyde standard solution (10 mM) was prepared by dissolving 160 μL of 1,1,3,3-tetramethoxypropane into 5 mL of 1 M HCl and heated in a water bath at 95 °C for 3 min. Seal blubber oil was a Newfoundland product obtained from Atlantis Marine Inc. (St. John's, NL, Canada). Green tea and black extracts were gifts from Shenzhen Bannerbio Inc. (Shenzhen, China).

SPME On-Fiber Derivatization. PFBHA solution (1 mL, 17 mg/mL) was placed into 4 mL amber Teflon-capped vials with a 1-cm stirring bar. A SPME fiber was inserted into the vial headspace for 2 min to adsorb the volatile PFBHA. The fiber was then inserted into the headspace of each seal blubber oil-containing vial for 5 min. During the process, the PFBHA and sample solutions were stirred at 600 rpm. The fiber was then removed and inserted into GC.

GC-MS Analysis of PFBHA Oximes. An Agilent 6890 gas chromatographic analysis system equipped with an Agilent 5973 MS detector (EI mode) and an HP-5 MS 5% phenylmethylsiloxane column (30.0 m $\times$ 250 μm $\times$ 0.25 μm) was used. The injection port was kept at 250 °C. The oven temperature was held at 45 °C for 1 min, then increased to 200 at 10 °C/min, and held there for 8.5 min. The MS detector was operated in the electron ionization mode. The ionization voltage was held at 70 eV, and the ion temperature was 280 °C. *n*-Dodecane was used as an internal standard to adjust the peak area.

Effect of Solvent on Propanal On-Fiber Derivatization. Hexane, methanol, or ethanol (200 μL) was mixed with 2.5 ppm of propanal in 5 mL of corn oil, respectively. After loading PFBHA, the SPME fiber was inserted into the headspace of each vial for 2 min and then removed and inserted into the GC.

Seal Blubber Oil Purification and Aging Study. The oil (100 mL) was added into a separation funnel and mixed with a suitable amount of sodium hydroxide solution to remove free fatty acids. The oil was rinsed with 80 mL of distilled water three times. The oil layer was dried with anhydrous sodium sulfate and further purified by passing through a silica gel column. The oil collection process was accelerated by vacuum. The purified oil was transferred to an amber glass bottle, and the headspace was flushed with nitrogen before storing at −21 °C for further analysis. Aging studies were conducted at 60 °C. Purified seal oil (5 mL) was placed into a 10-mL crimp top amber glass vial with a TFE starburst stirring head of 9.5-mm diameter. Aldehydes formed from seal blubber oil peroxidation were collected every 24 h from different batches of samples.

Effect of Antioxidant Mixtures on Aldehyde Formation. Two hundred microliters of antioxidant mixture (1% lecithin, 0.25% green or black tea extract) was dissolved in 200 μL of hexane and added into 5-mL seal oil samples. The aldehydes formed from seal blubber oil were monitored by SPME-GC-MS with on-fiber derivatization.

Trapping Effects of EGCG and Three Simple Phenolic Compounds on Acrolein. This experiment was designed and conducted in a buffer system. ACR and EGCG were dissolved in pH 7.4 phosphate buffer (0.01 mol/L). The buffer (5 mL) containing 1 mM EGCG and 0.5 mM ACR was added to the amber crimp-top vial with a TFE starburst stirring head (diameter = 9.5 mm) and was incubated in a 37 °C water bath with shaking at 120 rpm. The amount of ACR in the samples was measured using the SPME-GC-MS or SPME-GC method with on-fiber derivatization every 1.5 h using different batches of samples and controls (each sample/control is measured only one time). The effects of 1,3,5-trihydroxybenzene, 1,2,3-trihydroxybenzene, and methyl gallate on ACR were evaluated under the same conditions as EGCG.

LC-MS Analysis of Reaction Products. Samples were analyzed on an LC-MS/MS instrument equipped with an electrospray ionization source interfaced to an Applied Biosystems Q-trap LC-MS/MS mass spectrometer. Liquid chromatography was carried out on an Agilent HPLC system with a degasser (G1379A), a quaternary pump (G1311A), a thermostated autosampler (G1329A), and a diode array detector (G1315B). Separation of reaction products was carried out on a Varian Inertsil ODS C-18 column (3 μm, 15 $\times$ 4.6 mm). The mobile phase was composed of 0.1% formic acid (solvent A) and acetonitrile (solvent B) with the following gradients: 0 min, 5% B/95% A; 25 min, 35% B/65% A; 28 min, 80% B/20% A; and 30 min, 5% B/95% A. Effluent from the UV detector was split 4:1, and only one part (200 μL/min) was directed to the MS for spectrometric analysis with the remaining discharged as waste. The MS operation parameters were as follows: negative ion mode; spray voltage, 4 kV; scan range, 200−1000 Da; capillary temperature, 300 °C. LC-MS/MS conditions: negative ion mode; precursor ion *m*/*z* 513; collision energy, 40 eV.

Statistical Analysis. Statistical analyses were performed with the SPSS statistical package (SPSS Inc., Chicago, IL). Paired sample *t* test was applied to determine whether there was significant difference.

RESULTS AND DISCUSSION

To evaluate the protective effects of green/black tea extracts against peroxidation of PUFAs, a sensitive analytical method was first to be established. In the present study, four major aldehydes, including acetaldehyde, propanal, acrolein, and malondialdehyde, were identified and quantified as their PFBHA carbonyl-oxime isomers with the application of headspace SPME-GC-MS. A derivatization reagent was used to ensure binding of aldehydes to fiber because without a strong chromophore, volatile polar aldehydes are difficult to analyze directly (*16*) and the binding affinity of short-chain aldehyde to SPME fiber is low. PFBHA is an excellent choice for derivatization of aldehydes under mild reaction conditions with good performance in this study.

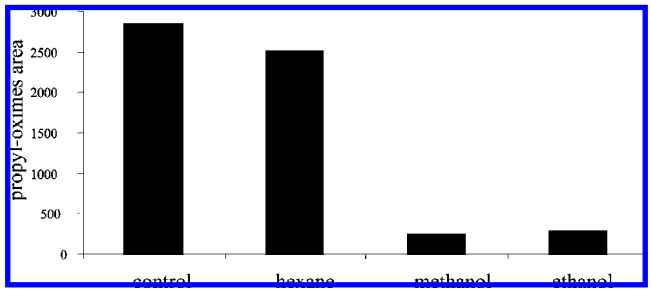

Figure 1. Effect of different solvents on propyl-oxime formation measured by SPME-GC-MS (2.5 ppm of propanal and 200 μL of solvent were added into 5 mL of corn oil).

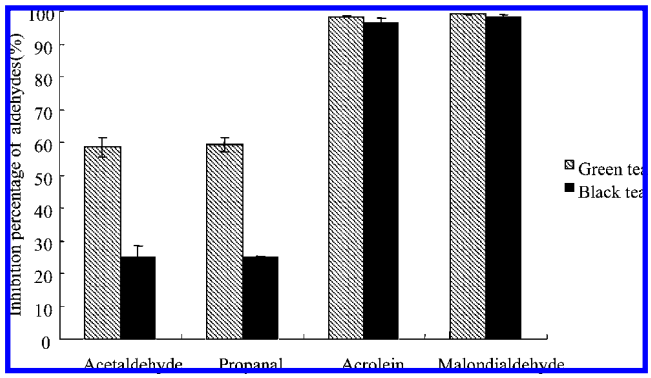

Figure 2. Inhibition percentage of aldehydes in the vial headspace of seal blubber oil incubated with/without tea extracts at 60 °C for 140 h. Each value is expressed as mean ± standard error of three replications.

The direct addition of plant extracts into the oil was not practical as the phenolic extracts used in this research are hydrophilic compounds, whereas oils are hydrophobic. Some dispersing solvents have to be used, which may affect the accuracy of aldehyde analysis by SPME-GC. When methanol, ethanol, and hexane were evaluated as dispersing reagents with propanal as the aldehyde, it was found that the formation of propyl-oximes in methanol- or ethanol-containing samples was reduced to 8.77 and 19.30%, respectively, compared with the control, whereas in hexane it was much better (**Figure 1**). This might be addressed by the fact that the high concentration of polar solvent in the headspace of sample vials interfered with the binging rate of propanal to PFBHA. In addition, methanol or ethanol could not act as a good dispersing reagent, and the plant extracts tend to remain at the surface of the oil. To address this concern, green or black tea extract was first dispersed into the oil using soy lecithin and hexane to achieve best dispersion of hydrophilic antioxidants into the oil and to minimize the solvent effect.

Compared with the control, after incubation at 60 °C for 140 h, the addition of green tea and black tea extracts in purified seal blubber oil exhibited strong inhibitory effects on the formation of aldehydes. The inhibition rates of ACR by green tea and black tea extracts were 98.40 and 96.41%, respectively, and those for MDA were 99.17 and 98.16%, respectively (**Figure 2**). A significant reduction in the formation of secondary oxidation products by tea catechins of seal blubber oil at 65 °C for 144 h, measured by TBARS method, has already been reported (*17*). However, the TBA test is intrinsically nonspecific for MDA because some MDA-like substances can interfere with the test (*18*). It has been found that non-lipid-related materials as well as fat peroxide-derived decomposition products other than MDA, such as 2-alkenals, 2,4-alkadienals, 4-hydroxyalk-

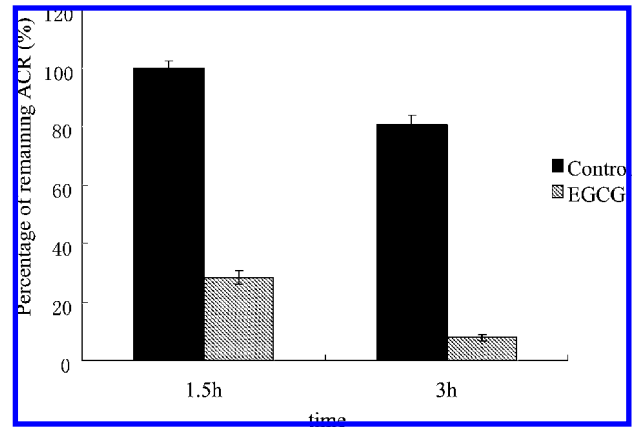

Figure 3. SPME-GC quantification of decreased ACR with the co-incubation of EGCG for 1.5 and 3 h with ACR. Each value is expressed as mean ± standard error of three replications. The remaining amount of ACR after both 1.5 and 3 h of incubation was significantly different from control ($P < 0.01$).

enals, or protein-bound MDA are TBA positive (*19*). The on-fiber derivatization SPME-GC-MS analysis of headspace volatile compounds provides a more specific analysis for the separation and identification of four major aldehydes in this study. There were also decreases in the amount of propanal and acetaldehyde by 58.44 and 59.40%, respectively, by green tea compared with corresponding values of 24.81 and 24.76% by black tea. This could be explained by the higher antioxidant power of green tea extract compared with black tea extract as we showed previously (*20*).

The primary purpose of adding green or black tea extract to seal blubber oil was to evaluate their effects on delaying the onset of lipid peroxidation and accumulation of peroxidation products. The reduced accumulation of aldehydes can mainly be explained by the polyphenols' capability to scavenge free radicals and their metal-chelating activities (*21*, *22*). By trapping free radicals in different stages of lipid peroxidation, polyphenols spare PUFAs from deterioration. However, it was interesting to find the inhibitory ratios by tea extracts for these four aldehydes were different. Green/black tea polyphenols could extensively decrease the amount of ACR and MDA, whereas the inhibitory activity on propanal and acetaldehyde was much lower. Alternative mechanisms other than antioxidant activity might, in part, be responsible for the significant reduction of ACR and MDA released to the headspace. Green/black tea polyphenols might directly trap ACR and MDA as direct trapping of methylglyoxal (also a kind of RCS) by green tea and black tea polyphenols has recently been reported (*23*, *24*). To further clarify the relationship between polyphenols and reactive aldehydes, the direct co-incubation of ACR and EGCG was studied, and significant decreases in the amount of ACR of up to 71.60% in 1.5 h and 90.30% in 3 h compared to the control were observed (**Figure 3**). In previous studies, ACR was proven to be a potent electrophile with high reactivity toward nucleophiles such as glutathione and amino groups through Michael addition reaction (*25*). As to its addition to polyphenols, the LC-MS analysis of the products of ACR and EGCG indicated the existence of significant amounts of potential adducts with predicted molecular ion peak $[M - H]^-$ at 513, which could be the direct combination products of ACR and EGCG at a mole ratio of 1:1. There are peaks of stereoisomers ranging from the retention time of 15.91 to 17.99 min on the HPLC chromatogram (**Figure 4**). For detailed positions of the electrophilic substitution, structural elucidation of the adducts

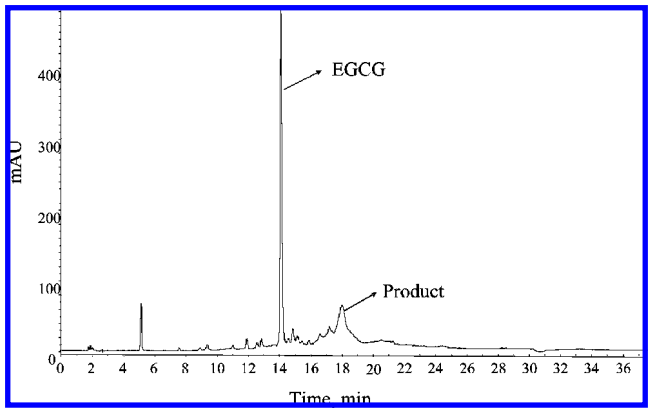

Figure 4. HPLC chromatogram of the reaction products of ACR and EGCG detected at the wavelength of 254 nm. The peak at 14.12 min is the remaining EGCG. The peaks at 15.91−17.99 min are possible new products from the reaction.

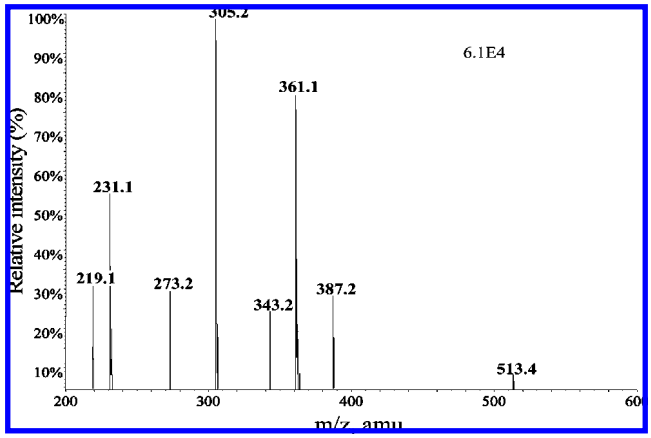

Figure 5. LC-MS/MS analysis of the precursor ion of *m/z* [M − H]$^-$ at 513.4.

was achieved using LC-MS/MS with collisionally activated dissociation (CAD) of the parent ion *m/z* [M − H]$^-$ 513 (**Figure 5**). The daughter ion of *m/z* 387 [M − 126 − H]$^-$ could be generated from the loss of a pyrogallol moiety of EGCG's B or C ring. The daughter ion of *m/z* 361 [M − 152 − H]$^-$ suggested the typical loss of a galloyl moiety of EGCG's C ring. Therefore, the conjugation of ACR to EGCG most likely involved the A ring of EGCG at the C-8 or C-6 position, but not the less nucleophilic rings B and C. This is in agreement with the findings that C-8 and C-6 positions of the EGCG A ring could be active sites to react with RCS such as methylglyoxal (MGO) and glyoxal (GO) (*23*, *24*). In other studies, it was reported that certain flavonoids, especially green tea catechins, could react with some of the aldehydes including acetaldehyde and glyoxylic acid to form (+)-catechin−aldehyde condensation products, leading to bridged dimers in a winelike model solution. The nucleophilic substitution also occurred at C-6 or C-8 of the A ring of monomeric flavanols (*26*, *27*).

To further demonstrate the trapping sites of EGCG on ACR, three simple phenolic compounds, 1,3,5-trihydroxybenzene, 1,2,3-trihydroxybenzene, and methyl gallate, were employed as the analogous individual A, B, and C rings of EGCG to evaluate their effects on ACR trapping, and SPME-GC/MS was used to quantify ACR. It was discovered that 1,2,3-trihydroxybenzene and methyl gallate showed a low activity, whereas 1,3,5-trihydroxybenzene was very active with only 13.20% ACR remaining after 1.5 h of incubation (**Figure 6**). The possible new product of ACR and 1,3,5-trihydroxybenzene was identified

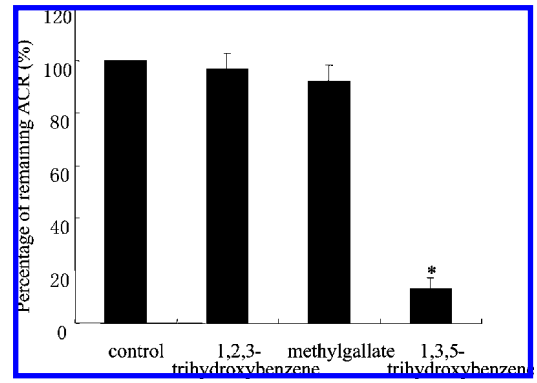

Figure 6. SPME-GC-MS quantification of decreased ACR with the co-incubation of 1,2,3-trihydroxybenzene, methyl gallate, and 1,3,5-trihydroxybenzene for 1.5 h with ACR. Each value is expressed as mean ± standard error of three replications. The remaining amount of ACR incubation of ACR with 1,3,5-trihydroxybenzene was significantly different from control ($P < 0.01$).

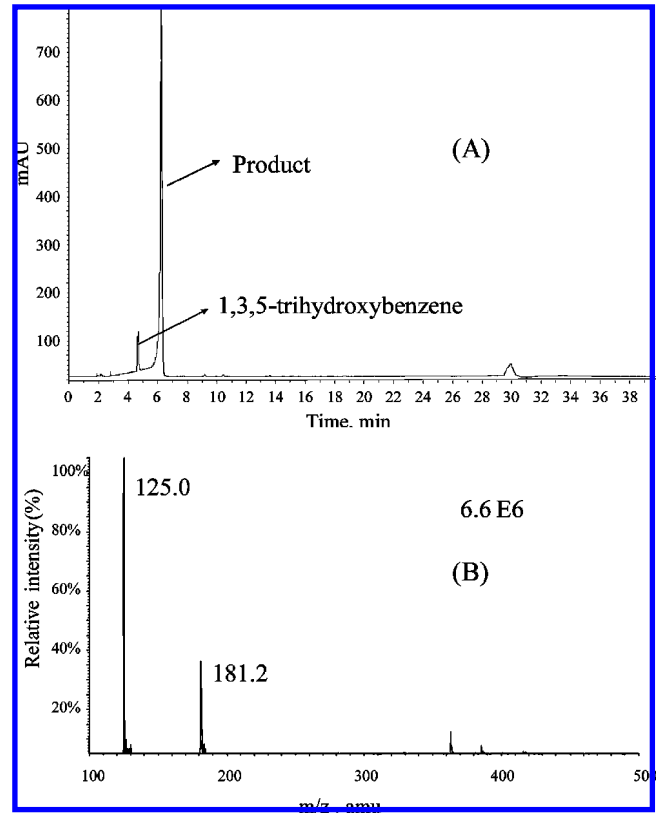

Figure 7. (**A**) HPLC chromatogram of the reaction products of ACR and 1,3,5-trihydroxybezene detected at the wavelength of 254 nm. (**B**) Mass spectrum of the peak at 6.15 min, a possible new product from the reaction, and its molecular weight is 182 with [M − H]$^-$ at 181.2.

using LC-MS, which showed one molecule of ACR (MW 56) was attached to 1,3,5-trihydroxybenzene (MW 126), and a final product of MW 182 (*m/z* [M − H]$^-$ at 181) was easily identified (**Figure 7**). This result further supported the hypothesis that the C-8 or C-6 position of the EGCG A ring could serve as the trapping site for ACR. This can be explained by three electron-donating hydroxyl groups in the meta configuration on a benzene ring of 1,3,5-trihydroxybenzene, which activate the ortho and para positions and result in a pronounced reactivity of the benzene ring for electrophilic aromatic substitution reactions at the three unsubstituted carbon sites (*28*).

From a physiological point of view, there are aldehyde-sequestering agents (such as aminoguanidine, pyridoxamine, OPB-9195) that deactivate or degrade cytotoxic carbonyls by behaving as sacrificial nucleophiles (*29*). The discovery of the ACR-quenching capability of EGCG could justify adding this polyphenol as a possible intervention agent for the inhibition of RCS.

Overall, this study demonstrated the inhibitory effects of tea extracts on ω-3 PUFA peroxidation of seal blubber oil by targeting the major aldehydes formed. EGCG, a major phenolic component in green tea, could also act as a nucleophile to trap ACR. A close examination of the reactive sites of EGCG in trapping reaction was unraveled by LC-MS/MS analysis and by using analogous individual A, B, and C rings of EGCG. However, the complicated relationship between natural phenolic compounds and lipid peroxidation requires further investigation. Beyond traditional consideration of tea polyphenols as antioxidants in lipid peroxidation systems, their potential as direct eliminating agents of lipid peroxidation-derived RCS should also be taken into consideration.

ABBREVIATIONS USED

ACR, acrolein; EGCG, (−)-epigallocatechin-3-gallate; MDA, malondialdehyde; PBS, phosphate-buffered saline; PFBHA, *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride; PUFAs, polyunsaturated fatty acids; RCS, reactive carbonyl species; SPME, solid-phase microextraction.

LITERATURE CITED

(1) Simopoulos, A. P. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr*. **1991**, *54*, 438–463.

(2) Sioen, I.; De Henauw, S.; Verbeke, W.; Verdonck, F.; Willems, J. L.; Van Camp, J. Fish consumption is a safe solution to increase the intake of long-chain n-3 fatty acids. *Public Health Nutr*. **2008**, 1–10.

(3) Perez-Mateos, M.; Boyd, L.; Lanier, T. Stability of omega-3 fatty acids in fortified surimi seafoods during chilled storage. *J. Agric. Food Chem*. **2004**, *52*, 7944–7949.

(4) Decker, E. A.; Warner, K.; Richards, M. P.; Shahidi, F. Measuring antioxidant effectiveness in food. *J. Agric. Food Chem*. **2005**, *53*, 4303–4310.

(5) Senanayake, S. P. J. N.; Shahidi, F. Oxidative stability of structured lipids produced from borage (*Borago officinals* L.) and evening primrose (*Oenothera biennis* L.) oils in the docosahexaenoic acid. *J. Am. Oil Chem. Soc*. **2002**, *79*, 1003–1014.

(6) Shahidi, F.; Shukla, V. K. S. Nontriacylglycerol constituent of fats, oils. *Int. News Fats, Oils Relat. Mater*. **1996**, *7*, 1227–1232.

(7) Yanishlieva-Maslarova, N. Inhibiting oxidation In *Antioxidants in Food: Practical Applications*; Pokorny, J., Yanishlieva, N., Gordon, M., Eds.; Woodhead Publishing: Cambridge, U.K., 2001; pp 22−70.

(8) Pokorny, J. Antioxidants in food preservation. In *Handbook of Food Preservation*; Rahman, M. S., Ed.; Dekker: New York, 1999; pp 309−338.

(9) Medina, I.; Satue-Gracia, M. T.; German, J. B.; Frankel, E. N. Comparison of natural polyphenol antioxidants from extra virgin olive oil with synthetic antioxidants in tuna lipids during thermal oxidation. *J. Agric. Food Chem*. **1999**, *47*, 4873–4879.

(10) Pazos, M.; Alonso, A.; Sanchez, I.; Medina, I. Hydroxytyrosol prevents oxidative deterioration in foodstuffs rich in fish lipids. *J. Agric. Food Chem*. **2008**, *56*, 3334–3340.

(11) Higdon, J. V.; Frei, B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr*. **2003**, *43*, 89–143.

(12) Liang, C.-P. Wang, M.; Simon, J. E.; Shahidi, F.; Ho, C.-T. Method development for monitoring seal blubber oil oxidation based on propanal and malondialdehyde formation. In *Antioxidant Measurement and Applications*; Shahidi, F., Ho, C.-T., Eds.; Oxford University Press: Oxford, U.K., 2007; pp 125−139.

(13) Eriksson, M.; Faldt, J.; Dalhammar, G.; Borg-Karlson, A. K. Determination of hydrocarbons in old creosote contaminated soil using headspace solid phase microextraction and GC-MS. *Chemosphere* **2001**, *44*, 1641–1648.

(14) Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med*. **1991**, *11*, 81–128.

(15) Negre-Salvayre, A.; Coatrieux, C.; Ingueneau, C.; Salvayre, R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br. J. Pharmacol*. **2008**, *153*, 6–20.

(16) Li, Z.; Jacobus, L. K.; Wuelfing, W. P.; Golden, M.; Martin, G. P.; Reed, R. A. Detection and quantification of low-molecular-weight aldehydes in pharmaceutical excipients by headspace gas chromatography. *J. Chromatogr., A* **2006**, *1104*, 1–10.

(17) Wanasundara, U.; Shahidi, F. Stabilization of seal blubber and menhaden oils with green tea catechins. *J. Am. Oil Chem. Soc*. **1996**, *73*, 1183–1190.

(18) Janero, D. R. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biol. Med*. **1990**, *9*, 515–540.

(19) Kikugawa, K.; Kojima, T.; Kosugi, H. Major thiobarbituric acid-reactive substances of liver homogenate are alkadienals. *Free Radical Res. Commun*. **1990**, *8*, 107–113.

(20) Liang, C. P.; Wang, M.; Simon, J. E.; Ho, C. T. Antioxidant activity of plant extracts on the inhibition of citral off-odor formation. *Mol. Nutr. Food Res*. **2004**, *48*, 308–317.

(21) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev*. **1998**, *56*, 317–333.

(22) Balentine, D. A.; Wiseman, S. A.; Bouwens, L. C. The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr*. **1997**, *37*, 693–704.

(23) Lo, C. Y.; Li, S.; Tan, D.; Pan, M. H.; Sang, S.; Ho, C. T. Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. *Mol. Nutr. Food Res*. **2006**, *50*, 1118–1128.

(24) Sang, S.; Shao, X.; Bai, N.; Lo, C. Y.; Yang, C. S.; Ho, C. T. Tea polyphenol (−)-epigallocatechin-3-gallate: a new trapping agent of reactive dicarbonyl species. *Chem. Res. Toxicol*. **2007**, *20*, 1862–1870.

(25) Witz, G. Biological interactions of α,β-unsaturated aldehydes. *Free Radical Biol. Med*. **1989**, *7*, 333–349.

(26) Saucier, C.; Bourgeois, G.; Vitry, C.; Roux, D.; Glories, Y. Characterization of (+)-catechin−acetaldehyde polymers: a model for colloidal state of wine polyphenols. *J. Agric. Food Chem*. **1997**, *45*, 1045–1049.

(27) Drinkine, J.; Glories, Y.; Saucier, C. (+)-Catechin−aldehyde condensations: competition between acetaldehyde and glyoxylic acid. *J. Agric. Food Chem*. **2005**, *53*, 7552–7558.

(28) Noda, Y.; Peterson, D. G. Structure−reactivity relationships of flavan-3-ols on product generation in aqueous glucose/glycine model systems. *J. Agric. Food Chem*. **2007**, *55*, 3686–3691.

(29) Burcham, P. C.; Kaminskas, L. M.; Fontaine, F. R.; Petersen, D. R.; Pyke, S. M. Aldehyde-sequestering drugs: tools for studying protein damage by lipid peroxidation products. *Toxicology* **2002**, *181−182*, 229–236.

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