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# Lipophilized Epigallocatechin Gallate (EGCG) Derivatives as Novel Antioxidants

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**ABSTRACT:** Epigallocatechin gallate (EGCG) is the major polyphenol in green tea and known to render many health benefits associated with tea consumption. EGCG was modified structurally to improve its lipophilicity, expand its application in lipophilic media, and enhance its cellular absorption in vivo. Esterification of the water-soluble EGCG with selected long-chain saturated and unsaturated fatty acids was carried out, followed by a purification process. Ester derivatives of EGCG with stearic acid (SA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were prepared, and their enhanced lipophilicity was confirmed by octanol−water partition coefficient. The chemical structures of the EGCG derivatives, determined by HPLC-MS and $^{1}$H and $^{13}$C NMR, were EGCG-3′,5′,3′′,5′′-*O*-tetraesters of SA, EPA, and DHA (compounds **1**, **2**, and **3**, respectively). The lipophilized EGCG derivatives exhibited greater antioxidant activity in scavenging the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical than EGCG itself. The results suggest that EGCG derivatives may be used as potential lipophilic antioxidants in the food, cosmetic, and medicinal industries.

**KEYWORDS:** epigallocatechin gallate (EGCG), polyunsaturated fatty acids (PUFA), esterification, lipophilization, antioxidant

## INTRODUCTION

Tea, a product made from the leaves and buds of the plant *Camellia sinensis*, is the second most popular beverage worldwide, after water, and a major source of dietary polyphenols that are known to render a myriad of health benefits.[1] There has been strong evidence demonstrating that green tea polyphenols (GTP) play a protective role in the risk and pathogenesis of several chronic ailments, especially cardiovascular disease and cancer.[2,3] The dry leaves of tea contain approximately 30% (by weight) of polyphenols, the majority of which are catechins (flavan-3-ols).[4,5] Epigallocatechin gallate (EGCG) is the predominant catechin in green tea, but is less abundant in black tea due to the formation of polymerized catechins.[6] EGCG has a four-ring structure with eight hydroxyl groups and is therefore highly hydrophilic. It renders its bioactivities or health effects mainly in aqueous environments or water compartments in body tissues. In humans, EGCG may have limited bioavailability due to its physical and chemical characteristics, which dictate its rate of absorption through the gastrointestinal tract, metabolism, and elimination from the body.

EGCG is a powerful antioxidant, possessing the highest antioxidant potency among all tea catechins,[7] and is known to inhibit oxidation in various food systems, including pork, fish, and highly unsaturated marine oils.[8−10] It also plays a protective role against oxidative stress in biological environments. A number of researchers have reported that EGCG effectively suppresses lipid oxidation in biological systems and subcellular fractions such as microsome and low-density lipoprotein (LDL) cholesterol.[11] EGCG retards lipid oxidation and depletion of endogenous lipid-soluble antioxidants such as α-tocopherol and β-carotene in the human blood plasma.[12] The antioxidant potential of EGCG arises from its polyphenolic structure and may vary depending on the reaction environment (e.g., temperature, pH, lipophilicity, type of oxidants, presence of synergist/antagonist, etc.) involved.

EGCG, like many other natural antioxidants, exhibits good solubility in aqueous media. The hydrophilic nature of EGCG may negatively affect its effectiveness in protecting lipophilic systems, such as fats, oils, and lipid-based foods or cosmetic formulas and emulsions as well as biological environments. In addition, the poor solubility of EGCG poses a problem for the homogeneity and appearance of the lipid product and hence its ease of application. Therefore, EGCG can be structurally modified to improve its lipophilicity and hence expand its application in the food, medicinal, and cosmetic industries. Structure modification of EGCG via esterification with aliphatic molecules such as long-chain fatty acids can serve as a useful tool in altering its physical properties such as solubility and miscibility and hence its antioxidant activity and bioavailability. Additional advantages or synergism can be expected when long-chain omega-3 polyunsaturated fatty acids (PUFA) are selected as acyl donors; long-chain PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to render a broad range of health benefits, including cardioprotective, immuno-enhancing, and anticancer effects, among others.[13,14] This study aimed to investigate lipophilization of EGCG via its esterification with different fatty acids and to examine its effect on the antioxidant activity of products.

## MATERIALS AND METHODS

**Materials.** EGCG was kindly supplied by GlaxoSmithKline Consumer Healthcare (Parsippany, NJ). Stearoyl chloride was purchased from Nu-chek Prep Inc. (Elysian, MN). Eicosapentaenoic acid (EPA) was obtained from Fuso Pharmaceutical Industries, Ltd. (Osaka, Japan)

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and DHA single cell oil (DHASCO) from Martek Bioscience Corp. (Columbia, MD). Trolox was purchased from Acros Organics (Fair Lawn, NJ). Other chemicals were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). All solvents used were obtained from Fisher Scientific Ltd. (Ottawa, ON, Canada). The solvents employed were of HPLC or reagent grade.

**Preparation of Crude EGCG Derivatives.** Long-chain fatty acids, namely, EPA and DHA, used for acylation were prepared from EPA ethyl ester and DHASCO (containing 40% DHA), respectively. EPA was obtained following saponification, and DHA was prepared by a urea complexation process following saponification as described by Wanasundara and Shahidi.[15] Briefly, KOH-catalyzed saponification was carried out in 95% ethanol at 62 ± 2 °C under reflux condition. The unsaponifiable matters were removed with hexane, and the pH of the aqueous phase was adjusted to 1.0 with HCl. Free fatty acids were then extracted into hexane, and the solvent was removed using a rotary evaporator. For urea complexation, the free fatty acids were mixed with a urea solution (20% in 95% ethanol) and allowed to stand for 24 h at 4 °C for urea−fatty acid adduct crystallization. The mixture was then filtered to obtain the non-urea complex fraction, the pH of which was subsequently adjusted to 4−5 with HCl. DHA was extracted into hexane, and the solvent was removed by evaporation. The identity of the fatty acids (EPA and DHA) so obtained was confirmed by GC-MS. EPA and DHA were then converted to their corresponding acyl chlorides by reaction with thionyl chloride. Stearoyl chloride was a commercial product and used as such. Esterification of EGCG was carried out with acyl chlorides (stearoyl, eicosapentaenoyl, and docosahexaenoyl chloride) at a mole ratio of 1:1. Acyl chloride was added dropwise to EGCG dissolved in ethyl acetate. The reaction was carried out in the presence of pyridine, which removed the released HCl from the medium. The mixture was then heated in an oil bath at 50 °C under a nitrogen blanket with constant stirring. The reaction mixture upon completion of the esterification was cooled to ambient temperature and filtered. The filtrate was then washed three times with distilled water (60 °C), and the ethyl acetate layer was collected and passed through a cone of anhydrous sodium sulfate. The dry powder of crude products containing a mixture of EGCG esters (at different degrees of substitution) was obtained by evaporating the solvent.

**Purification and Identification of EGCG Derivatives.** The composition of the reaction mixture was determined by reversed phase HPLC-MS, using an Agilent 1100 HPLC unit (Agilent Technologies, Palo Alto, CA) with a UV−diode array detector (UV-DAD). Separation was achieved on a C-18 column (4.6 mm × 250 mm coupled with a guard column, Agilent) by gradient elution with a methanol/water mobile phase (95:5−100:0 from 0 to 30 min) at a flow rate of 1 mL/min, and fractions were detected at 280 nm. LC flow was further analyzed online by the MS detector system (LC-MSD-Trap-SL, Agilent) with atmospheric pressure chemical ionization (APCI) at positive mode for identification of each fraction. The MS conditions were as follows: drying gas flow rate, 5 L/min; nebulizer pressure, 60 psi; drying gas temperature, 350 °C; APCI temperature, 400 °C; and capillary voltage, 110 V.

To obtain individual EGCG derivatives for subsequent structure elucidation and bioactivity evaluation, the crude products of EGCG esters were purified by flash column chromatography. EGCG esters were eluted on a silica column with a gradient of hexane/ethyl acetate/formic acid (90:10:2−50:50:2, v/v/v). Fractions corresponding to each band were collected and solvents removed using a rotary evaporator. The identity of each fraction was confirmed by HPLC-MS as described above. The predominant fractions of EGCG esters with stearic acid (SA), EPA, and DHA (referred to as compounds **1**, **2**, and **3**, respectively) were analyzed for their specific structures and evaluated for their antioxidant activity in vitro.

$^{1}$H and $^{13}$C NMR analyses were carried out for purified EGCG esters (compounds **1**, **2**, and **3**) to identify their molecular structures, that is, the location of fatty acid incorporation in the EGCG molecule. The $^{1}$H and $^{13}$C spectra were recorded on a Bruker Avance 500 MHz NMR spectrometer (Bruker Biospin Co., Billerica, MA) operating at 500.13 and 125.77 MHz, respectively. The samples were dissolved in dimethyl sulfoxide (DMSO)-$d_6$ containing TMS as an internal standard. Signal processing and interpretation were performed with the Topspin 1.3 (Bruker Biospin Co.) and MestRe Nova (Mestrelab Research SL, Santiago de Compostela, Spain) software. Structure elucidation was accomplished by comparing the chemical shifts of EGCG derivatives with those of the parent EGCG molecule.

**Determination of Lipophilicity.** The lipophilicity of the identified EGCG derivatives was determined as octanol−water partition coefficient ($P$) by a shake flask method. Briefly, a flask containing a mixture of octanol (100 mL) and deionized water (100 mL) was shaken in a shaking water bath at room temperature (22 °C) for 24 h. The content was then allowed to stand for 24 h for separation into two phases. Test compounds (0.2 $\mu$mol) were dissolved in 5 mL of the upper phase (presaturated octanol), and the absorbance ($A_0$) was read at 280 nm. A blank with no sample was prepared. Five milliliters of the bottom phase (presaturated water) was added afterward, and the mixtures were vortexed for 1 min and allowed to stand for 24 h for separation. Absorbance ($A_x$) of the upper phase in the vials was measured and the octanol−water partition coefficient ($P$) calculated using the following equation: $P = \log A_x/(A_0 - A_x)$, where blank-corrected absorbance was used.

**Antioxidant Activity.** The antioxidant activity of the lipophilic EGCG derivatives was evaluated as DPPH radical scavenging capacity using EGCG as the reference. The DPPH scavenging capacity of test compounds was determined by electron paramagnetic resonance (EPR) according to the method of Madhujith and Shahidi[16] with slight modifications. Trolox standards (50−300 $\mu$M) and test compounds (25 $\mu$M) were dissolved in ethanol, to which 2 mL of ethanolic DPPH solution (0.18 mM) was added. Contents were mixed well and injected to the sample cavity of a Bruker e-scan EPR food analyzer (Bruker Biospin Co.) through capillary tubing. The spectrum was recorded after 1 min. The operating parameters for EPR were as follows: 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100.000 G sweep width, 3495 G center field, 5.12 ms time constant, 9.795 GHz microwave frequency, and 86 kHz modulation frequency. Reduction of the DPPH radical concentration in the presence of test compounds was monitored by change in the corresponding signal intensity. DPPH radical scavenging capacity (%) was calculated using the equation

$$\%\ \text{scavenging} = 100 \times (1 - \text{signal intensity}_{\text{sample}}/\text{signal intensity}_{\text{control}})$$

where control contained no test compounds or Trolox. A standard curve was constructed, and DPPH scavenging capacities for test compounds were expressed as Trolox equivalents (TE).

**Statistical Analysis and Data Interpretation.** One-way analysis of variance (ANOVA) with pairwise comparisons (Tukey's HSD) was performed at a $P < 0.05$ level using Sigmastat for Windows version 2.0 (Jandel Corp., San Rafael, CA) to determine the significant differences.

## ■ RESULTS AND DISCUSSION

**Synthesis of EGCG Derivatives.** Several strategies for improving the functional and biological properties of EGCG through structural modification have been investigated. For example, introduction of alkoxyl groups and peracetylation have been shown to increase the lipid membrane permeability and chemical/metabolic stability of EGCG, thereby improving bioactivities under physiological conditions.[17,18] In this study, we intended to enhance the lipophilicity of the water-soluble EGCG while maintaining its antioxidant activity. This was achieved by

Table 1. Composition EGCG−Fatty Acid Polyesters in Crude Products[a]

| | tetraester (%) | pentaester (%) | hexaester (%) |
|---|---|---|---|
| EGCG-SA | 63.3 ± 1.99 c | 36.7 ± 1.99 a | tr |
| EGCG-EPA | 81.1 ± 0.59 b | 18.9 ± 0.59 b | tr |
| EGCG-DHA | 94.7 ± 0.63 a | 5.33 ± 0.63 c | tr |

[a] Reaction mixture also contained unreacted EGCG, which was not calculated as a component of crude products. EGCG-SA, EGCG-EPA, and EGCG-DHA are ester products of EGCG with SA, EPA, and DHA, respectively. Values (mean ± SD of three replicates) in the same column with different letters were significantly different at $P < 0.05$. tr, trace.

partial esterification and incorporation of long-chain saturated or polyunsaturated fatty acids into the EGCG molecule. This is the first report of the incorporation of the health-beneficial omega-3 PUFA into the EGCG molecule; the products so produced may provide additional perspectives for application to food, as natural health products, or even as pharmaceuticals.

The more lipophilic derivatives of EGCG were prepared via reaction with various acylating agents, namely, the acyl chlorides of SA, EPA, and DHA. The yields (calculated as total amount of esters obtained/amount of esters expected if the fatty acid and EGCG are reacted fully) of the crude products containing a series of EGCG polyesters (tetraester and higher) were 56.9, 42.7, and 30.7% for SA, EPA, and DHA esters, respectively. Long-chain PUFA showed lower rates of incorporation, possibly because of their nonlinear (bent) structure, which renders steric hindrance in the acylation reaction. Saturated fatty acids such as SA are known to allow facile packing and close intermolecular interactions. Esterification by chemical means, with less selectivity and specificity, has been reported to result in higher degrees of substitution (DS) in the products than those catalyzed by enzymes. For example, polyesters of sucrose with fatty acids (DS 4−8) were prepared using acylating agents such as acyl chloride or aryl esters,[19] whereas lipases were effective for the regioselective synthesis of mono- and diesters of sucrose (DS 1−2).[20] The electrophilic acyl group may react with different numbers of hydroxyl groups located in different positions depending on the nature of the electrophile, the reaction conditions, and the catalysts employed.[21] In the current study, polyesters of EGCG with high DS (≥4) were formed during acylation under the conditions employed. It was noted that the composition of the reaction mixture varied depending on the ratio of the starting materials (EGCG/fatty acid); esters with higher DS (7−8) were produced at lower ratios of EGCG/fatty acid (<1) (data not shown). As the DS increases, the lipophilicity increases, whereas hydrolysis by lipolytic enzymes is limited due to steric hindrance,[22] hence the higher membrane permeability and metabolic stability of the esters. The antioxidant activity of the derivatives so prepared would presumably be preserved because hydroxyl groups were only partially acylated. According to the results from HPLC-MS analysis, esterification of EGCG with fatty acids (at 1:1 ratio) yielded predominantly tetraesters (DS 4) in all crude products of SA, EPA, and DHA esters, but at different proportions (Table 1). The chain length of the fatty acids appeared to play a role in their incorporation into the EGCG molecule. Pentaesters were produced at the highest level in the EGCG−SA crude product (36.7%) followed by EGCG−EPA (18.9%) and EGCG−DHA (5.33%), the fatty acids of which were less effective in approaching the acyl acceptor and packing in the synthesized molecules.

**Identification of EGCG Derivatives.** The crude products of EGCG esters were separated into different fractions by flash column chromatography, and the major products corresponding to tetraesters (named compounds **1**, **2**, and **3** for the stearate, eicosapentaenoate, and docosahexaenoate, respectively) were collected and subjected to lipophilicity and antioxidant activity assessment tests. The three compounds were identified by HPLC-MS; their mass spectra are shown in Figure 1.

For compound **1**, the molecular ion peak detected showed a $m/z$ at 1524.5, representing $[M]^{\bullet+}$ of the EGCG-tetrastearate ($C_{94}H_{154}O_{15}$), which might result from loss of a hydrogen atom from the $[M + H]^+$ or its migration to the fragments. The presence and abundance of $[M]^{\bullet+}$ in relation to $[M + H]^+$ in APCI-MS have been reported to be dependent on the mobile phase in the LC as well as the hydrogen atom affinity of the analyte.[23] The peaks at $m/z$ 1258.0, 991.6, 725.5, and 459.3 showed one or multiple mass loss of 266.5 from the molecular ion, representing the ions of $[M - 266.5]^+$, $[M - 2 \times 266.5]^+$, $[M - 3 \times 266.5]^+$, and $[M - 4 \times 266.5]^+$, respectively. These appeared to be the fragments with 1, 2, 3, or 4 acyl groups (mass 267.5 for stearoyl moiety and a hydrogen atom migrating to the fragment ions) dissociated from the molecular ion. The peaks located at $m/z$ 822.9, 555.9, and 289.7 corresponding to $[M - 2 \times 266.5 - 169]^+$, $[M - 3 \times 266.5 - 169]^+$, and $[M - 4 \times 266.5 - 169]^+$, respectively, originated from additional cleavage of the gallic acid moiety (mass 169). On the basis of the presence of the molecular ion and fragments in the mass spectrum, the identity of compound **1** was confirmed to be EGCG tetrastearate.

Similarly, compound **2** showed a molecular ion peak at $m/z$ 1596.5 (mass of EGCG tetraeicosapentaenoate, $C_{102}H_{130}O_{15}$). The peaks at $m/z$ 1596.5, 1312.4, 1028.0, and 743.3 had a difference in mass of 284.5, indicating the cleavage of an eicosapentaenoyl group (mass 285.5 minus a migrating H). The peak at $m/z$ 455.8 ($[M - 4 \times 285.5]^+$) might be derived from loss of all four eicosapentaenoyl moieties in the molecular ion ($m/z$ 1596.5) without the addition of four hydrogen atoms. Peaks at $m/z$ 859.2 and 573.8 indicated additional cleavage of the gallic acid group (mass 169) from the fragments $m/z$ 1028.0 and 743.3, respectively. All of the above provides support for the identification of compound **2** as EGCG tetraeicosapentaenoate.

Similar ionization and fragmentation patterns were found for MS of compound **3**. The molecular ion at $m/z$ 1700.9 implied the presence of EGCG tetradocosahexaenoate ($C_{110}H_{138}O_{15}$, MW 1700.3), which was further confirmed by its fragments. Peaks at $m/z$ 1390.7 and 1080.1 correspond to the fragments with one and two docosahexaenoyl moieties (mass 311.6 minus a migrating H) cleaved off, respectively. The fragments from dissociation of three and four docosahexaenoyl groups were detected as sodium adducts at $m/z$ 792.2 (769 + 23) and 481.6 (458 + 23), respectively. Further cleavage of the gallic acid moiety (mass 169) from the fragments $m/z$ 769, 1080, and 1390 gave rise to peaks at $m/z$ 600.4, 911.2, and 1221.5, respectively. Thus, compound **3** was positively identified as EGCG tetradocosahexaenoate.

**Structure Elucidation of EGCG Derivatives.** The purified EGCG derivatives (compounds **1**, **2**, and **3**) were identified by MS as EGCG tetraesters (DS 4). However, because EGCG has eight hydroxyl groups, the location of groups undergoing esterification needed to be established. This was determined using $^1$H and $^{13}$C NMR and by comparing the chemical shifts of the derivatives with the parent EGCG molecule. The chemical shifts detected for EGCG in both $^1$H and $^{13}$C NMR were very close to those reported in the literature,[24] whereas the spectra for the three derivatives displayed additional signals for corresponding

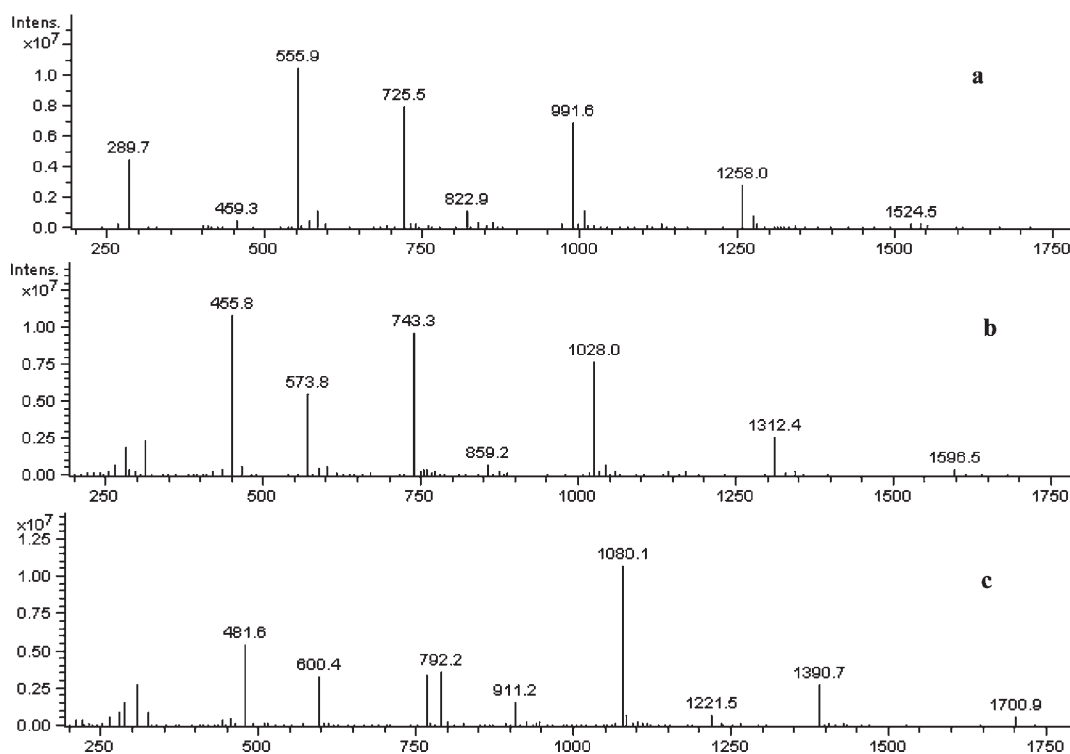


**Figure 1.** Mass spectra of EGCG derivatives (a, compound **1**; b, compound **2**; c, compound **3**).

alkyl groups in the expected range, that is, $\delta$ 0.79−2.31 for $^1$H and $\delta$ 15.01−34.25 for $^{13}$C NMR (spectra not shown). The presence of alkyl protons and carbon provided evidence for incorporation of fatty acids in the EGCG molecule.

When the chemical shifts of protons in the derivatives were compared with those in EGCG, a downfield shift was observed for all proton signals of EGCG (Table 2), which is in agreement with the findings of Islambekov et al.,[25] who demonstrated that acetylation of catechin leads to a downfield displacement of all of the proton signals. Because there is no proton attached to the carbon bearing the hydroxyl substituent in the aromatic rings of EGCG, large downfield shifts ($\Delta\delta$ 1.0−1.2) normally observed for geminal protons were not detected.

Chemical shifts of protons in all three derivatives followed the same trend. The H-2′ and H-6′ showed a downfield shift of $\Delta\delta$ 0.09 in comparison with the parent EGCG molecule, indicating the occurrence of acylation in the B-ring. Downfield shifts of H-2, H-3, and H-4 in the C-ring decreased in the order H-2 > H-3 > H-4, due to the increasing distance from the acylation site in the B-ring. Acylation sites were tentatively assigned to the D-ring as well on the basis of the downfield shifts of H-2″ and H-6″ ($\Delta\delta$ 0.04−0.05), which appeared to be smaller than that of H-2′ and H-6′ in the B-ring, possibly because of the ester bond in the gallic acid moiety. Only minor shifts were found for H-6 and H-8 in the A-ring, suggesting that the A-ring was not the acylation site. On the basis of the $^1$H NMR results, a tentative conclusion was reached that the fatty acids were incorporated in the B- and D-rings of the EGCG molecule.

The specific positions of hydroxyl groups being acylated were further confirmed by $^{13}$C NMR. The general strategy established by Yoshimoto et al.[26] was employed for structure elucidation of the EGCG derivatives. As described by Yoshimoto et al.,[26] acylation of a hydroxyl group of the substrate resulted in a downfield shift of the *O*-acylated carbon and an upfield shift of the neighboring carbon. As presented in Table 2, a large downfield shift ($\Delta\delta$ 4.25−4.58) was found for C-3′ and C-5′, indicating these might be the positions of acylation. The remarkable upfield shift ($\Delta\delta$ 0.72−3.20) observed for C-4′, the carbon adjacent to both C-3′ and C-5′, suggested the presence of a free hydroxyl group at C-4′ and also further confirmed acylation of C-3′ and C-5′. The other neighboring carbons (C-2′ and C-6′) of the acylation site did not show any upfield shift, possibly due to the steric conformation of the fatty acids incorporated, which is believed to affect the $^{13}$C chemical shifts. It has been demonstrated that $^{13}$C chemical shifts may be determined by more complex stereochemical factors, whereas electronic influences dominate chemical shifts of $^1$H.[27] Similarly, the positions of acylation in the D-ring were assigned to C-3″ and C-5″, on the basis of the downfield shifts of C-3″ and C-5″ and upfield shift of C-4″. The absence of downfield or upfield shifts for carbons in the A-ring implied that hydroxyl groups in the A-ring were not acylated, which is in agreement with the $^1$H NMR results. Thus, structure characterization of the EGCG derivatives was conclusive based on the combined $^1$H and $^{13}$C NMR, and compounds **1**, **2**, and **3** were identified as EGCG-3′,5′,3″,5″-*O*-tetrastearate, EGCG-3′,5′,3″,5″-*O*-tetraeicosapentaenoate, and EGCG-3′,5′,3″,5″-*O*-tetradocosahexaenoate, respectively, as shown in Figure 2.

**Lipophilicity of EGCG Derivatives.** EGCG is highly hydrophilic with a large polar molecular surface area and is among those considered to be poorly absorbed in the body.[28] The hydrophilic nature of EGCG also poses a serious disadvantage/challenge for its use as a food preservative in stabilizing fats, oils, and lipid-based food systems. Therefore, structure modification of EGCG may serve as a means to alter its physicochemical properties and hence improve its effectiveness as an antioxidant in more diverse systems. Many water-soluble bioactives have structurally been modified for improved lipophilicity, hence expanding their applications in more lipophilic

**Table 2. $^1$H and $^{13}$C Chemical Shifts ($\delta$) of EGCG and Its Derivatives[a]**

| C/H position | EGCG $^{13}$C | EGCG $^1$H | compd 1 $^{13}$C | compd 1 $^1$H | compd 2 $^{13}$C | compd 2 $^1$H | compd 3 $^{13}$C | compd 3 $^1$H |
|---|---|---|---|---|---|---|---|---|
| 2 | 77.56 | 4.91 | 77.47 | 4.99 | 77.47 | 4.99 | 77.28 | 5.00 |
| 3 | 69.09 | 5.32 | 69.29 | 5.38 | 69.06 | 5.36 | 69.24 | 5.37 |
| 4 | 26.82 | 2.61 | 26.77 | 2.63 | 26.21 | 2.65 | 26.26 | 2.61 |
| | | 2.88 | | 2.91 | | 2.91 | | 2.89 |
| 5 | 156.71 | | 156.68 | | 156.77 | | 156.58 | |
| 6 | 95.41 | 5.78 | 95.40 | 5.79 | 95.45 | 5.79 | 95.46 | 5.79 |
| 7 | 157.58 | | 157.60 | | 157.58 | | 157.70 | |
| 8 | 96.60 | 5.88 | 96.67 | 5.89 | 96.67 | 5.90 | 96.76 | 5.90 |
| 9 | 157.58 | | 157.60 | | 157.58 | | 157.70 | |
| 10 | 98.47 | | 98.44 | | 98.44 | | 98.35 | |
| 1′ | 129.71 | | 129.61 | | 129.52 | | 129.65 | |
| 2′ | 106.57 | 6.36 | 106.53 | 6.45 | 106.53 | 6.45 | 106.60 | 6.45 |
| 3′ | 146.47 | | 150.72 | | 150.72 | | 151.05 | |
| 4′ | 133.45 | | 131.97 | | 130.25 | | 132.73 | |
| 5′ | 146.47 | | 150.72 | | 150.72 | | 151.05 | |
| 6′ | 106.57 | 6.36 | 106.53 | 6.45 | 106.53 | 6.45 | 106.60 | 6.45 |
| 1″ | 120.39 | | 120.40 | | 120.40 | | 120.32 | |
| 2″ | 109.76 | 6.77 | 109.52 | 6.81 | 109.76 | 6.82 | 109.84 | 6.82 |
| 3″ | 146.72 | | 151.26 | | 151.25 | | 151.05 | |
| 4″ | 139.64 | | 137.04 | | 137.05 | | 137.23 | |
| 5″ | 146.72 | | 151.26 | | 151.25 | | 151.05 | |
| 6″ | 109.76 | 6.77 | 106.52 | 6.81 | 109.76 | 6.82 | 109.84 | 6.82 |
| COO | 166.29 | | 166.28 | | 166.26 | | 166.36 | |

[a] Proton and carbon in alkyl chain of incorporated fatty acids are not listed.

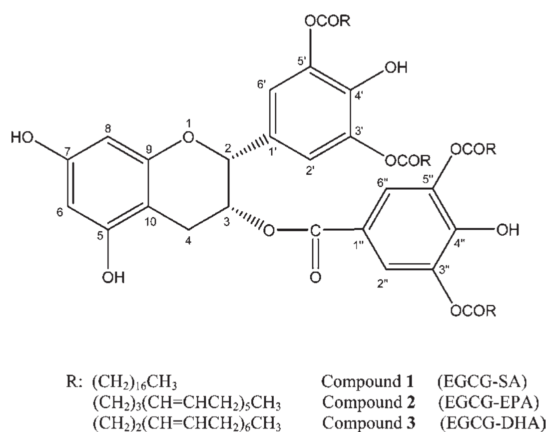


**Figure 2.** Structures of EGCG derivatives.

**Table 3. Lipophilicity of EGCG Derivatives as Octanol−Water Partition Coefficient (*P*)[a]**

| | EGCG | compd 1 | compd 2 | compd 3 |
|---|---|---|---|---|
| *P* | 0.48 ± 0.01 c | 1.42 ± 0.02 a | 1.10 ± 0.06 b | 1.03 ± 0.10 b |

[a] Values (mean ± SD of three replicates) with different letters were significantly different at $P < 0.05$.

environments. Ascorbyl palmitate, frequently used in the food industry as the lipophilic alternative to ascorbic acid, is one of the most successful examples. A number of phenolic antioxidants have also been lipophilized in a similar manner, including ferulic, chlorogenic, cinnamic, sinapic, *p*-coumaric, and caffeic acids,[29−33] which were esterified with various aliphatic alcohols, as well as ascorbic acid, tyrosol, protocatechuyl and vanillyl alcohol, esculin, rutin, naringin, genistein, and daidzein,[34−39] which were converted into their corresponding fatty acid esters. In addition, sterols including cholesterol, cholestanol, and sitosterol were esterified with long-chain fatty acids for better water-holding properties of the resultant esters.[40] Many of these studies used saturated fatty alcohols or acids as alkyl chain donors to enhance the lipophilicity of the molecules of interest. In this work, we investigated the incorporation of a saturated fatty acid as well as two long-chain omega-3 PUFA into the EGCG molecule. Long-chain omega-3 PUFA such as EPA and DHA are the major bioactive components in fish oils that are believed to render a myriad of health benefits and are used as supplements, pharmaceutical substances, functional food ingredients, and natural health products as well as in cosmetics applications. Acylation of EGCG with these fatty acids not only will increase the lipophilicity of EGCG but also takes advantage of the health-promoting effects of long-chain omega-3 PUFA. Additional perspectives may also be expected such as synergism between the two moieties, namely, EGCG and the omega-3 fatty acids.

The EGCG derivatives prepared were evaluated for their lipophilicity in terms of octanol−water partition coefficient (*P*). Higher *P* values indicate higher lipophilicity of the compound. As expected, acylation with fatty acids resulted in increased lipophilicity, as all three derivatives showed higher *P* values than their parent EGCG molecule (Table 3). Compound **1** had the highest *P* value, due to the presence of the highly lipophilic stearic acid side chain. Compounds **2** and **3** did not differ significantly in their lipophilicities. The lipophilicity of the test compounds was in agreement with their retention times observed in the reversed phase HPLC, which was in the order compound **1** (13.4 min) > compound **3** (7.1 min) ≥ compound **2** (6.2 min) > EGCG (2.4 min). The enhanced lipophilicity of EGCG derivatives may lead to their improved incorporation into the lipid bilayers of cell membrane and hence better bioavailability in the body as well as greater potential in liposome-based drug delivery. Hashimoto et al.,[41] in a study on the interaction of tea catechins with lipid bilayers, reported that the incorporation rate was positively associated with the partition coefficient in octanol−water.

**Antioxidant Activity.** EGCG and other catechins are powerful antioxidants acting as free radical scavengers, reducing agents, and metal chelators. In vitro assays have shown the antioxidant effectiveness of EGCG in scavenging DPPH radical, reducing ferric ion to ferrous ion and chelating metal ions.[42−45] Studies on the antioxidant mechanism of EGCG have suggested that EGCG may have multiple reaction pathways for exerting oxidation/antioxidation effects depending on the reaction environment. The hydroxyl groups at positions 5 and 7 on the A-ring were thought to be not so important in radical scavenging of EGCG.[46] However, a study on oxidation products of EGCG and $H_2O_2$ demonstrated that the A-ring of EGCG might be a site for rendering antioxidant activity.[47] The trihydroxyphenyl B-ring was found to be the most active site of antioxidant action in DPPH-induced oxidation of EGCG.[42,47] Nevertheless, the gallate moiety, which accounts for increased phospholipids/water partition coefficient, may lead to a higher antioxidant potential in vivo.[48]

The EGCG derivatives in this study, as identified by MS and NMR, had four hydroxyl groups acylated with fatty acids, and the

**Figure 3.** EPR spectra of DPPH scavenging of EGCG and its derivatives (compounds **1**, **2**, and **3** are EGCG tetraesters of SA, EPA, and DHA, respectively).

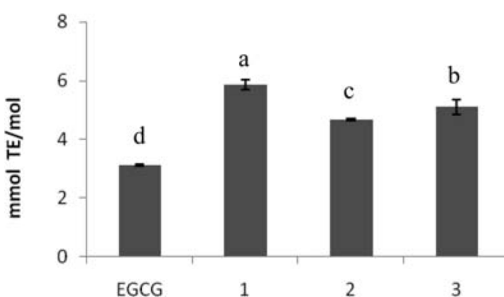


**Figure 4.** DPPH scavenging capacity in millimoles of Trolox equivalents (TE) per mole (compounds **1**, **2**, and **3** are EGCG tetraesters of SA, EPA, and DHA, respectively; bars with different letters are significantly different at $P < 0.05$).

remaining hydroxyl groups on the aromatic rings of EGCG may contribute to the antioxidant property of the derivatives. However, the antioxidant efficacy of the derivatives may differ from that of EGCG, due to possible electronic and steric effects rendered by the incorporated fatty acid chains. The antioxidant activity of the modified EGCG was evaluated as scavenging capacity against DPPH radical, a stable hydrophobic radical frequently used in antioxidant assessment. Figure 3 shows the EPR spectra of DPPH radical as affected by the test antioxidants. The presence of test compounds significantly decreased the resonance signal intensity, indicating the scavenging effect of EGCG and its derivatives against DPPH radical. When their radical scavenging capacities were compared, compound **1** showed the highest capacity as Trolox equivalents, followed by compounds **3** and **2** (Figure 4). All derivatives displayed a higher scavenging activity against DPPH than EGCG itself. The trend for antioxidant activity among test compounds was in accordance with that of their octanol−water partition coefficient.

Lipophilicity has been found to play a role in the antioxidant activity of flavonoids. The lipophilic derivatives of EGCG may have greater accessibility/affinity to the lipophilic DPPH radical than the hydrophilic EGCG. Moreover, acylation may have an effect on the hydrogen atom donation capability of EGCG by altering its electron density and distribution on the aromatic

rings. Maintenance and even enhancement of the antioxidant activity of EGCG derivatives suggest that these derivatives may be used as antioxidants or EGCG alternatives in more lipophilic environments. Further investigation on the antioxidant effectiveness of EGCG derivatives in different model systems is needed for exploring their full potential for application in food and as natural health products.

**Conclusions.** Esterification of EGCG with long-chain saturated (SA) or polyunsaturated (EPA and DHA) fatty acids yielded mainly EGCG tetraesters. The specific structures of the synthesized EGCG derivatives were confirmed by $^1$H and $^{13}$C NMR as EGCG-3′,5′,3′′,5′′-*O*-tetraester of the fatty acids involved. These derivatives had higher lipophilicity than EGCG itself. The antioxidant activity of all EGCG derivatives was superior to that of EGCG in terms of DPPH radical scavenging capacity, suggesting their potential use as antioxidants and EGCG alternatives in more lipophilic environments. Moreover, incorporation of long-chain omega-3 PUFA into the EGCG molecule may render additional health benefits or possible synergistic effects in vivo, which will be further investigated.

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