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Improving the Stability and Antioxidant Properties of Sesame Oil: Water-Soluble Spray-Dried Emulsions from New Transesterified Phenolic Derivatives

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Hydrosoluble sesame oil fatty acid transesters having enhanced antioxidant activities were synthesized in a two-step process. The key step involved the biocatalyzed (lipase from *Candida antarctica*) acylation of sesame oil methanolic ester with either vanillyl (VNA) or piperonyl benzylic alcohols, or 5-hydroxymethyl resorcinol (5-HMR). These substrates were selected to introduce phenolic or sesamol structurally related frameworks. The VNA and 5-HMR-derived transesters were obtained with 20−40% yields and retained the starting proportions of sesame oil linoleic, oleic, and saturated acids, these fatty acids also being the only constituents of the nonesterified fraction. The VNA-derived transester showed the best antioxidant capacity in standard assays and was processed as the unique lipid phase of spray-dried emulsions containing a high level of linoleic acid phenolic ester. These emulsions provided a high degree of protection to UV-irradiated fibroblasts, through the potential synergy between VNA antioxidant action and replenishment of damaged membranes by unsaturated fatty acids.

KEYWORDS: Sesame oil; enzymatic transesterification; vanillyl alcohol; spray-dried emulsions; antioxidant capacity; ESR spin trapping; DEPMPO; 3T3 fibroblasts; UV irradiation

INTRODUCTION

Sesame seed (*Sesamum indicum L.*) is the source of one of the oldest edible oils in the world, being widely cultivated in tropical and subtropical areas and used worldwide for human nutrition. Depending upon the origin and the variety of the seeds, sesame oil contains high concentrations of unsaturated fatty acids, including oleic (38−43%) and linoleic (35−48%) acids, and minor proportions of saturated fatty acids (SFA) such as palmitic and stearic acids (*1*−*4*). The unsaponifiable fraction of sesame oil contains biologically active molecules of great interest such as sterols, phenolic antioxidants (including γ-tocopherol), lignans (the most abundant being sesamin, sesamolin, and sesamol) and lignan glucosides which together confer to the oil a relatively good thermal stability, despite the high level of unsaturated compounds (*5*). The antioxidant constituents of sesame oil could play a key role in a variety of pharmacological properties, eg., antiaging and antimutagenic effects, lowering of high fat-induced cholesterol absorption and synthesis in rats, inhibition of key enzymes activated upon oxidative stress such as p38 MAPK or caspase-3, or enhancement of hepatic detoxication (*3*, *6*−*9*).

Because exploitation of natural or processed sesame oil represents a great economic potential in several sectors such as the food, cosmetics, chemical, and pharmaceutical industry, many studies have focused on the conditions influencing plant cultivation and production (*4*, *10*). In the food industry, sesame oil is widely used as a bioresource to obtain edible oil cakes rich in proteins and antioxidants or utilized as a substrate for bioprocesses to produce enzymes, antibiotics, or biopesticides (*11*, *12*). More recently, the use of sesame oil methylesters obtained by transesterification as an energy source to produce biodiesels or fuels has been proposed (*12*, *13*).

To achieve a better action in hydrophilic systems, dry emulsions have been recently developed, which contain sesame oil either as active biodisinfectant in microcapsules or as a constituent of the lipidic phase to improve the bioavailability of active substances in oil-in-water (o/w) emulsions (*14*, *15*). However, there are two main drawbacks for the use of sesame oil as antioxidant in microcapsules or emulsions, i.e., its very high lipophilicity, which destabilizes emulsions at high dosages, and the fact that its intrinsic antioxidant properties are far lower than that of compounds such as γ-tocopherol, resveratrol, or even

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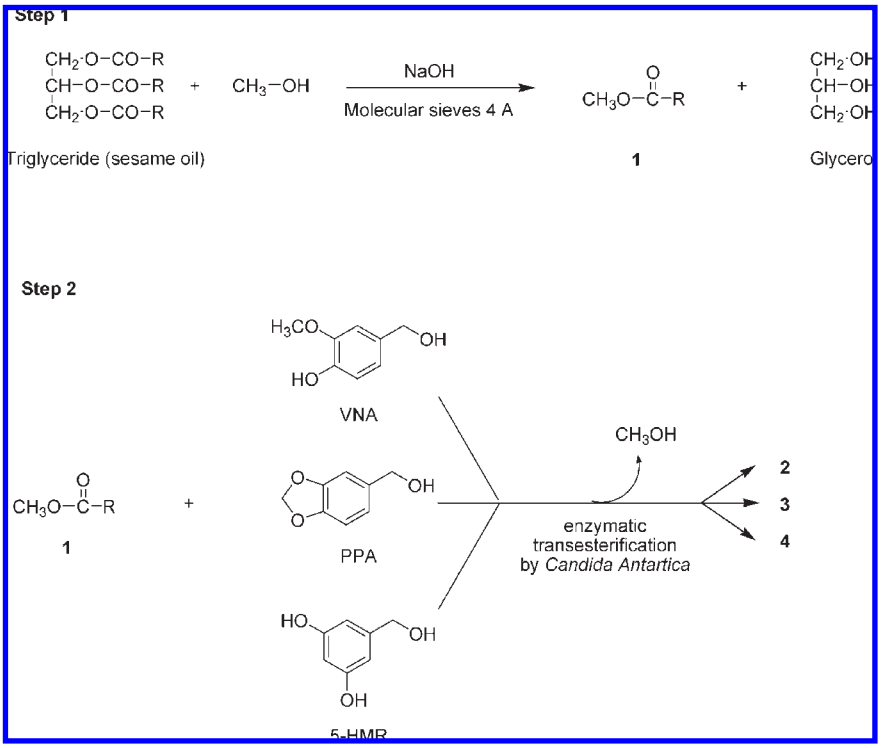

Figure 1. Two-step transesterification of sesame oil with benzylic alcohol derivatives VNA, PPA, or 5-HMR via intermediate formation of **1**.

sesamol, which can be moreover included into emulsions in higher, well-controlled concentrations (*16*−*18*).

The present study was undertaken to test whether synthetic modifications of the main fatty acids in sesame oil would enhance its global antioxidant activity and hydrosolubility. For this purpose, we carried out a recently described enzymatic transesterification method (*19*, *20*) to obtain fatty acid esters from three alcohols (**Figure 1**) which are either recognized (i.e., vanillyl alcohol (VNA), see refs *21* and *22*, or potential (5-(hydroxymethyl)-resorcinol (5-HMR)) phenolic antioxidants or nonphenolics structurally related to sesamol, such as piperonyl alcohol (PPA). These new sesame oil derivatives were first screened in vitro for their free radical scavenging and antioxidant activities using electron spin resonance (ESR) and spectrophotometric methods. The best compound was then processed as the lipid constituent and active agent of new spray-dried emulsions (i) in which fatty acids essential for repairing cell damage (such as linoleic acid) would be preserved (*23*) and (ii) that will advantageously compete with standard antioxidants in a situation of UV-induced cellular oxidative stress.

MATERIALS AND METHODS

General Procedure. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 300 spectrometer (Germany) operating at 300 and 75 MHz, respectively, in $CDCl_3$ or DMSO-d_6 as indicated. Chemical shifts are given on a parts per million scale with tetramethylsilane as internal standard. Coupling constants are expressed in hertz. The fatty acid composition of sesame oil was determined by ^{1}H NMR according to ref *2*. For transesterified sesame oil derivatives, the percentage of esterified phenolic fatty acid was calculated relative to the ^{1}H NMR integration of methyl-end protons of the fatty acid chain in the reference compounds (see below), taken at 100%. Elemental analyses were performed using a ThermoFinnigan (model FlashEA 1112) elemental analyzer (USA). ESR spectra were acquired on a Bruker ESP 300 spectrometer operating at X band (9.8 GHz) with a TM-4103 cylindrical cavity and a 100-kHz modulation frequency.

Chemicals. All chemicals used in the study were of analytical grade (∼98−99%) from the following commercial suppliers: sesame oil and γ-tocopheryl acetate (vitamin E acetate) were from Cooper (Melun, France); VNA (4-(hydroxymethyl)-3-methoxyphenol), *cis*,*cis*-9,12-octadecadienoic acid (linoleic acid), (9*Z*)-octadec-9-enoic acid (oleic acid), NH_4SCN, and $BaCl_2$ were from Fluka Chemika (France); catalase and xanthine oxidase (both from bovine liver), superoxide dismutase (SOD) from bovine erythrocytes, lipase acrylic resin from *Candida antarctica* (Novozym 435), sesamol (3,4-methylenedioxyphenol, SEOH), D-(+)-saccharose, sodium caseinate (SC, from bovine milk), sodium ascorbate, KH_2PO_4, H_2O_2, $FeSO_4$, $FeCl_3$, hypoxanthine, 2-deoxy-D-ribose (deoxyribose), 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), sodium ascorbate, cumene hydroperoxide, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma-Aldrich (France); PPA (1,3-benzodioxol-5-ylmethanol), 5-HMR (5-(hydroxymethyl)-benzene-1,3-diol), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and molecular sieves (4 Å, 8−12 mesh) were from Acros Organics (France); methanol, *n*-hexane, and NaOH pellets were from Carlo Erba Reactifs (Val de Reuil, France); hydroxypropylmethyl cellulose (HPMC; Pharmacoats 603) was from Shin-Etsu Chemical Co. (Japan); and Labrafil M1944CS was from Gattefossé SAS (St Priest, France). Deuterated solvents were purchased from Eurisotop (Gif-Sur-Yvette, France), and when required, doubly distilled, deionized water was used. The spin trap 5-(diethoxyphosphoryl)-5 methyl-1-pyrroline *N*-oxide (DEPMPO) was prepared and purified as previously described (*24*). For cell culture media, Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), trypsin-EDTA, and fetal calf serum were purchased from Life Technology Inc. (USA).

Syntheses. *Synthesis of Sesame Oil Methylester* (*1*). Sesame oil was transesterified in 100% excess methanol using NaOH as a catalyst according to a modification of ref *25*. Ten grams of sesame oil and 0.7 mL of 1 N NaOH in methanol (160 mL, 3.95 mols) were stirred at 60 °C for 1 h in the presence of molecular sieves. The pale yellow residue was cooled to room temperature, filtered through a fritted funnel, and processed by rotary evaporation under vacuum to afford a brown-yellow oil, which was dissolved in 100 mL of methylene chloride and washed three times with brine (40 mL) to remove traces of moisture, glycerol phase, and unreacted methanol. The organic layer was dried overnight over anhydrous $MgSO_4$ and filtered, and the solvent was removed under reduced pressure to give 7.5 g of **1**, which was used without purification in further steps.

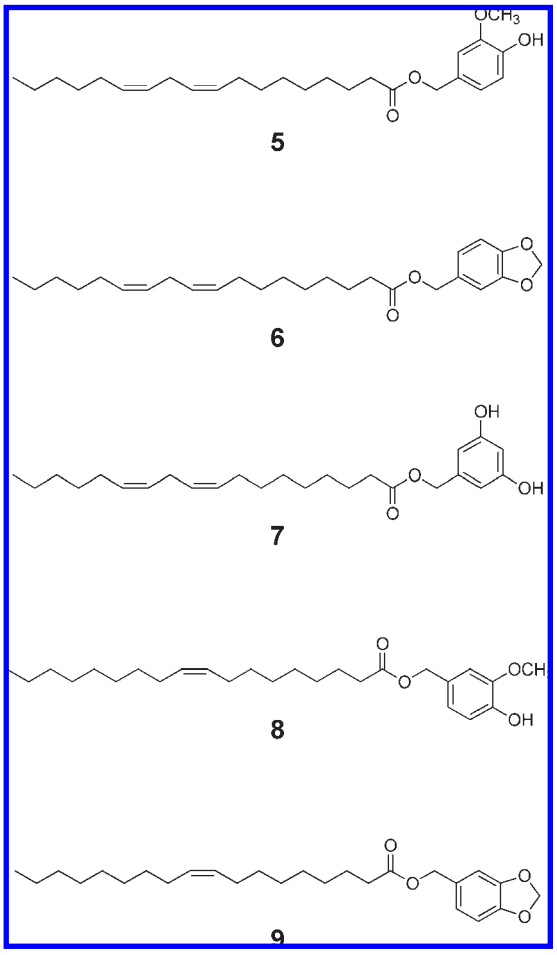

Figure 2. Structure of phenolic fatty acid esters.

*NMR Data for **1***. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.29 (m, 2H, −HC=CH−), 3.61 (s, 3H, CH_3O−), 2.72 (t, J = 5.7 Hz, 2H, −CH=CH−CH_2−CH=CH−), 2.25 (t, J = 7.4 Hz, 2H, −OOC−CH_2−), 1.98 (m, 4H, −CH_2−CH=CH−CH_2−), 1.58 (m, 2H, −OOC−CH_2−CH_2−), 1.26−1.21 (m, ∼20H, −CH_2−), 0.84 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.21 (−COO−), 130.12, 129.96, 129.93, 129.68, 127.99, 127.85 (−HC=CH−), 51.34 (CH_3O−), 34.03, 31.86, 29.63, 29.11, 29.08, 27.15, 24.89, 22.63 (−CH_2−CH=CH−CH_2−, −CH_2−), 14.04 (−CH_3).

*General Procedure for Transesterification of **1** with Alcohols*. The reaction of **1** with VNA, PPA, or 5-HMR was catalyzed by the immobilized lipase from *Candida antarctica* (*19*, *20*). Ten grams of **1** and 2 g of the alcohol (i.e., 13.0, 13.1, and 11.9 mmol for VNA, PPA, and 5-HMR, respectively) were mixed with 0.2 g of Novozym 435 (2%, w/w) in a 100 mL round-bottom flask. The mixture was vigorously stirred and heated at 60 °C for 72 h in the absence of any reflux condenser to favor evaporation of formed methanol in order to prevent any back reaction. The reaction mixture was then diluted with 100 mL of *n*-hexane and filtered to remove insoluble products and any degradation products. The organic layer was washed three times with brine and rotary evaporated to yield the corresponding sesame oil phenolic fatty esters **2**, **3**, or **4**, for VNA, PPA, and 5-HMR, respectively.

The following NMR assignements for **2**, **3**, and **4** have been facilitated by the synthesis of esters of linoleic acid with VNA, PPA, and 5-HMR, i.e., compounds **5**, **6**, and **7**, respectively (see below and **Figure 2**).

*Data for **2***. Brown-orange oil (7.5 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.85 (m, 3H, Ar), 5.32 (m, 2H, −HC=CH−), 5.01 (s, 2H, VNA-CH_2OOC−), 3.87 (s, 3H, CH_3O−), 2.75 (t, J = 5.9 Hz, 2H, −CH=CH−CH_2−CH=CH−), 2.30 (m, 2H, −OOC−CH_2−), 2.00 (m, 4H, −CH_2−CH=CH−CH_2−), 1.61 (m, 2H, −OOC−CH_2−CH_2−), 1.28−1.24 (m, ∼20H, −CH_2−), 0.86 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.75, 173.22, 172.80 (−COO−), 130.12, 129.92, 129.64, 127.99, 127.88, 127.82 (−HC=CH−), 146.43, 145.70, 121.91, 114.33, 111.21 (aromatic VNA), 68.82, 66.23, 64.94, 62.04 (VNA-CH_2OCO−), 55.81 (CH_3O−), 34.29, 34.00, 33.94, 31.83, 31.45, 29.76, 29.69, 29.62, 29.45, 29.24, 29.08, 29.02, 27.12, 25.55, 24.87, 24.79, 22.61, 22.49 (−CH_2−CH=CH−CH_2−, −CH_2−), 14.04, 13.99 (−CH_3).

*Data for **3***. Brown-orange oil (8 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.81−6.73 (m, 3H, aromatic PPA), 5.92 (s, 2H, −OCH_2O−), 5.32 (m, 2H, −HC=CH−), 4.98 (s, 2H, PPA-CH_2OOC−), 2.75 (t, J = 5.9 Hz, 2H, −CH=CH−CH_2−CH=CH−), 2.30 (q, 2H, J = 7.6, 7.0, 14.3 Hz, −OOC−CH_2−), 1.99 (m, 4H, −CH_2−CH=CH−CH_2−), 1.60 (m, 2H, −OOC−CH_2−CH_2−), 1.28−1.24 (m, ∼20H, −CH_2−), 0.86 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.28, 173.10, 172.69 (−COO−), 129.60, 129.41, 129.33, 129.16, 129.13, 127.51, 127.48, 127.35 (HC=CH), 147.23, 147.02, 121.61, 108.41, 107.63, 100.57 (aromatic PPA), 67.60, 65.48, 64.45 (PPA-CH_2OCO−), 33.76, 33.51, 33.46, 31.36, 30.98, 29.29, 29.21, 29.15, 29.12, 28.98, 28.91, 28.77, 28.54, 26.65, 25.08, 24.36, 24.31, 22.13, 22.02 (−CH_2−CH=CH−CH_2−, −CH_2−), 13.56, 13.51 (−CH_3).

*Data for **4***. Red oil (8 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.15 (s, 3H, aromatic 5-HMR), 5.30 (m, 2H, −HC=CH−), 4.86 (s, 2H, 5-HMR-CH_2OOC−), 2.72 (t, J = 5.8 Hz, 2H, −CH=CH−CH_2−CH=CH−), 2.30 (t, J = 7.3 Hz, 2H, −OOC−CH_2−), 2.13 (t, J = 7.3 Hz, 2H, −OOC−CH_2−), 1.98 (m, 4H, −CH_2−CH=CH−CH_2−), 1.49 (m, 2H, −OOC−CH_2−CH_2−), 1.22 (m, ∼20H, −CH_2−), 0.83 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.00, 173.36, 172.94 (−COO−), 130.13, 129.93, 129.63, 128.00, 127.83 (−HC=CH−), 157.38, 107.09 (aromatic 5-HMR), 65.46 (5-HMR-CH_2OCO−), 34.19, 33.97, 31.85, 31.46, 29.71, 29.64, 29.61, 29.54, 29.47, 29.27, 29.11, 29.05, 27.13, 25.56, 24.79, 24.77, 22.63, 22.52 (−CH_2−CH=CH−CH_2−, −CH_2−), 14.04, 14.00 (−CH_3).

*(9Z,12Z)-4-Hydroxy-3-methoxybenzyl Octadeca-9,12-dienoate (Vanillyl Linoleate, **5**)*. This ester was obtained by stirring 2 g of VNA (13.0 mmol) with 4 g of linoleic acid (14.3 mmol), and 120 mg of Novozym 435 (2%, w/w) at 60 °C for 24 h in a nonrefluxing device as described for **1**. The oil obtained was solubilized in 60 mL of dichloromethane and washed with brine to remove excess linoleic acid, and the organic layer was concentrated in vacuo and resolubilized in 60 mL of ethylacetoacetate/*n*-hexane (8:2, v/v). The solution was then filtered on a silica bed (20 g), and the solvent was removed yielding **5** as an orange oil (2.75 g, yield = 51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.87 (m, 3H, aromatic VNA), 5.33 (m, 4H, −HC=CH−), 5.02 (s, 2H, VNA-CH_2OOC−), 3.88 (s, 3H, CH_3O−), 2.76 (t, J = 6.3 Hz, 2H, −CH=CH−CH_2−CH=CH−), 2.32 (t, J = 6.9, 7.7 Hz, 2H, −OOC−CH_2−), 2.03 (m, 4H, −CH_2−CH=CH−CH_2−), 1.62 (m, 2H, −OOC−CH_2−CH_2−), 1.29 (m, 14H, −CH_2−), 0.88 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.31 (−COO−), 129.69, 129.50, 129.21, 127.54, 127.46, 127.40 (−HC=CH−), 145.99, 145.27, 121.48, 113.89, 110.79 (aromatic VNA), 65.80 (VNA-CH_2OCO−), 55.38 (CH_3O−), 33.86, 31.01, 29.31, 29.25, 29.16, 29.07, 29.01, 28.83, 28.64, 28.58, 26.68, 25.11, 24.44, 22.06 (−CH_2−CH=CH−CH_2−, −CH_2−), 13.56 (−CH_3). Anal. Calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 75.02; H, 9.85. ^{1}H NMR data were comparable with those reported in ref *26*.

*Esters of PPA and 5-HMR with Linoleic Acid (**6** and **7**, respectively)*. The procedure was the same as that described for **5**, starting with 4 g of linoleic acid and 2 g of PPA or 5-HMR (13.1 and 11.9 mmol, respectively). The esters **6** and **7** were obtained as a yellow-orange (2.65 g, yield = 49%) or yellow (0.43 g, yield = 9%) oil, respectively.

*Data for (9Z,12Z)-Benzo[d][1,3]dioxol-5-ylmethyl Octadeca-9,12-dienoate (Piperonyl Linoleate, **6**)*. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.83−6.75 (m, 3H, aromatic PPA), 5.94 (s, 2H, −OCH_2O−), 5.33 (m, 4H, −HC=CH−), 4.99 (s, 2H, PPA-CH_2OOC−), 2.76 (t, J = 5.9 Hz, 2H, −CH=CH−CH_2−CH=CH−), 2.31 (t, 2H, J = 7.6 Hz, −OOC−CH_2−), 2.02 (m, 4H, −CH_2−CH=CH−CH_2−), 1.61 (m, 2H, −OOC−CH_2−CH_2−), 1.29−1.25 (m, 14H, −CH_2−), 0.88 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.59 (−COO−), 129.96, 129.67, 127.98, 127.85 (−HC=CH−), 147.72, 147.50, 130.13, 122.12, 108.91, 108.14 (aromatic benzodioxol), 101.7 (−O−CH_2−O−), 65.97 (PPA-CH_2OCO−), 34.26, 31.84, 31.46, 29.70, 29.61, 29.51, 29.46, 29.28, 29.25, 29.08, 29.03, 27.13, 25.56, 24.86, 22.62, 22.51 (−CH_2−CH=CH−CH_2−, −CH_2−), 14.04, 14.00 (−CH_3). Anal. Calcd. for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 74.87; H, 9.41.

Data for (9Z,12Z)-3,5-Dihydroxybenzyl Octadeca-9,12-dienoate (5-HMR Linoleate, 7). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.16 (m, 3H, aromatic 5-HMR), 5.30 (m, 4H, −HC=CH−), 4.87 (s, 2H, 5-HMR-CH_2OOC−), 2.72 (t, J = 5.8 Hz, 2H, −CH=CH−CH_2−CH=CH−), 2.29 (t, J = 7.5 Hz, 2H, −OOC−CH_2−), 1.99 (m, 4H, −CH_2−CH=CH−CH_2−), 1.47 (m, 2H, −OOC−CH_2−CH_2−), 1.23 (m, 14H, −CH_2−), 0.85 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, DMSO): δ 174.61 (−COO−), 129.84, 127.89 (−HC=CH−), 129.73, 158.65, 105.78, 102.17 (aromatic 5-HMR), 65.46 (5-HMR-CH_2OCO−), 33.91, 31.53, 31.14, 29.56, 29.32, 29.26, 29.11, 28.97, 28.88, 28.81, 26.83, 25.39, 24.72, 22.32, 22.21 (−CH_2−CH=CH−CH_2−, −CH_2−), 14.05 (−CH_3). Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51. Found: C, 73.94; H, 9.58.

*Esters of VNA and PPA with Oleic Acid (**8** and **9**, respectively; See Structures in **Figure 2**).* The procedure was the same as that described for **5**, starting with 4 g of oleic acid (14 mmol) and 2 g of VNA or PPA (13.0 and 13.1 mmol, respectively). The esters **8** and **9** were obtained as a pale yellow (0.45 g, yield = 8%) or yellow (1.82 g, yield = 33%) oil, respectively.

*Data for 4-Hydroxy-3-methoxybenzyl Oleate (Vanillyl Oleate, **8**).* ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.86−6.87 (m, 3H, aromatic VNA), 5.33 (m, 2H, −HC=CH−), 5.02 (s, 2H, VNA-CH_2OOC−), 3.87 (s, 3H, CH_3O−), 2.32 (t, J = 6.9, 7.7 Hz, 2H, −OOC−CH_2−), 2.00 (m, 4H, −CH_2−CH=CH−CH_2−), 1.62 (m, 2H, −OOC−CH_2−CH_2−), 1.26 (m, 14H, −CH_2−), 0.87 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.77 (−COO−), 129.96, 129.69, (−HC=CH−), 146.41, 145.70, 127.96, 121.96, 114.30, 111.20 (aromatic VNA), 66.26 (VNA-CH_2OCO−), 55.87 (CH_3O−), 34.34, 31.87, 29.61(2), 29.25(3), 29.02(3), 27.14(2), 24.88, 22.61 (−CH_2−CH=CH−CH_2−, −CH_2−), 14.04 (−CH_3). Anal. Calcd. for $C_{26}H_{42}O_4$: C, 74.60; H, 10.11. Found: C, 74.14; H, 10.54.

*Data for Benzo[d][1,3]dioxol-5-ylmethyl Oleate (Piperonyl Oleate, **9**).* ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.83−6.73 (m, 3H, aromatic PPA), 5.93 (s, 2H, −OCH_2O−), 5.33 (m, 2H, −HC=CH−), 4.99 (s, 2H, PPA-CH_2OOC−), 2.31 (t, J = 6.9, 7.7 Hz, 2H, −OOC−CH_2−), 2.00 (m, 4H, −CH_2−CH=CH−CH_2−), 1.61 (m, 2H, −OOC−CH_2−CH_2−), 1.27 (m, 14H, −CH_2−), 0.87 (m, 3H, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.50 (−COO−), 129.85, 129.61, (−HC=CH−), 147.68, 147.46, 127.81, 122.05, 108.86, 108.06 (aromatic PPA), 101.01 (-O-CH_2−O-), 65.90 (PPA-CH_2OCO−), 34.20, 31.87, 29.67(2), 29.23(3), 28.99(3), 27.11(2), 24.82, 22.58 (−CH_2−CH=CH−CH_2−, −CH_2−), 14.00 (−CH_3). Anal. Calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 74.71; H, 10.01.

Thermal Stability of Vanillyl Fatty Acid Esters. In order to check for possible acellular degradation processes, 0.1 mM solutions of **5** and **8** in ethanol were added to 10 mL of PBS and heated in sealed vials at either 40 or 60 °C for 18 or 2 h, respectively, under stirring. Aliquots (2 mL) were sampled in a timely manner, cooled to room temperature, and extracted with 5 mL of dichloromethane, and the organic phase was evaporated to dryness to give a syrup which was analyzed by ^{1}H NMR in $CDCl_3$. Degradation was measured by comparing integrations of VNA-CH_2-O signals to the methyl-end protons of the fatty acid chain as described above.

Preparation of Spray-Dried Emulsions from 2. Dry emulsions were prepared by spray-drying (SD) according to a modification of the procedure described in ref *18*. To solutions, prepared by adding HPMC (2 g) or SC (4 g) at 40 °C to saccharose (230 or 228 g for HPMC and SC, respectively) in ∼70 mL water, was added 36 g of **2**, and the final volume was adjusted to 315 mL with water (giving a total weight of 400 g). Homogeneization of the mixtures for 2 min at 24,000 rpm in a Ultra-Turrax T25 basic high-speed colloid mill (IKA Labortechnik, Germany) yielded the HPMC- or SC-containing starting liquid o/w emulsions (termed as **2**-HPMC-LE and **2**-SC-LE, respectively) which were then pulverized by SD. This process was carried out using a Büchi mini spray dryer B-290 (Switzerland) with 100% aspiration and a feeding rate of emulsion of 9 mL/min. The final dry emulsions **2**-HPMC-$SD_{180\ °C}$ and **2**-SC-$SD_{150\ °C}$ were obtained using inlet (outlet) air temperatures (±2.0 °C) of 180 °C (95 °C) or 150 °C (85 °C), respectively. Control HPMC- or SC-containing liquid emulsions in which **2** was omitted and replaced by 36 g of Labrafil were prepared using the procedure described above (termed as CTR-HPMC-LE and CTR-SC-LE, respectively). The yield of the SD process was calculated from the percentages of recovered and added emulsion masses as reported in ref *27*.

Assay of Hydroperoxide Content of Sesame Oil and Hemisynthetic Derivatives. To check if esterification or SD procedures could affect the oxidative status of hemisynthetic derivatives and emulsions, lipid hydroperoxides were assayed as described in ref *28* using extraction with isooctane/2-propanol (3:1, v/v). After centrifugation (3,400*g*), the resulting organic phase (0.2 mL) was added to 2.8 mL of methanol/butanol (2:1, v/v), and the mixture was added to aliquots of aqueous (final concentrations given) NH_4SCN (15 mM), $FeSO_4$ (7 mM), and $BaCl_2$ (6 mM). The absorbance was then determined at 510 nm, and the mean hydroperoxide concentration (n = 3) was calculated relative to a standard cumene hydroperoxide solution.

Free Radical Scavenging and Antioxidant Activities of Starting and Reconstituted Liquid Emulsions. Reconstitution of 2-HPMC-$SD_{180\ °C}$ and 2-SC-$SD_{150\ °C}$ was performed by dissolving the dry emulsions in water (2 mg/mL) and rotating the solutions for 2 min at 9,500 rpm.

DPPH Radical Scavenging Assay. The antioxidant effect of untreated or Fenton-reacted (see below) test compounds was evaluated using the DPPH assay and compared to SEOH and standard antioxidants (vitamin E acetate and Trolox). Stock solutions of compounds (0.6 mM) were prepared either in water/methanol (1:2, v/v) or in dichloromethane, 10−750 μL-aliquots were added to 1.500 mL of a 75 mg/mL DPPH solution (in either methanol or dichloromethane), and the appropriate solvent was then added to adjust the final sample volume to 2.25 mL. The absorbance at 517 nm was read 5 and 30 min after the addition of each test compound. All analyses were run in triplicate and averaged.

Residual Antioxidant Capacity Following Exposure to a Hydroxyl Radical (HO•)-Generating Fenton System. Aliquots of 0.6 mM aqueous solutions of VNA, **5**, starting liquid emulsions **2**-HPMC-LE and **2**-SC-LE, and reconstituted dry emulsions **2**-HPMC-$SD_{180\ °C}$ and **2**-SC-$SD_{150\ °C}$ were incubated for 1 h at 37 °C with a Fenton reagent consisting of H_2O_2 and $FeCl_3$ (both at 50 μM), and sodium ascorbate and EDTA (both at 0.1 mM). The reaction was stopped by adding 5 U/mL catalase, and aliquots were sampled for the analysis of their capacity to reduce DPPH.

Deoxyribose Assay. This method for determining the capacity of a potential antioxidant to protect deoxyribose against HO•-induced degradation was performed according to a modification (*18*) of a previously reported procedure (*29*). Aqueous solutions of test compounds (final concentration 10−100 μM) were incubated in 20 mM phosphate buffer (pH 7.4) for 1 h at 37 °C with a mixture of a Fenton reagent (consisting of H_2O_2 (1 mM), $FeCl_3$ (0.1 mM), and sodium ascorbate (0.1 mM)), deoxyribose (2.8 mM), and EDTA (1.1 mM). A mixture of 0.5 mL of TBA (10 g/L) and 0.5 mL of acetic acid was then added to the solution, which was heated at 95 °C for 15 min and cooled down to room temperature, and the absorbance of the malondialdehyde (MDA)-TBA chromogen was read at 532 nm (UVmc^2 spectrophotometer, Saphas, Monaco). All analyses were run in triplicate and averaged.

Evaluation of Superoxide Radical (O_2•$^-$) Scavenging Activity by ESR Spin Trapping. The O_2•$^-$ scavenging activity of VNA, **2**, **5**, and the two reconstituted liquid emulsions was assessed by their ability to dose-dependently inhibit the formation of the O_2•$^-$/DEPMPO spin adduct (DEPMPO-OOH). Solutions of test compounds (final concentration 0.3−50 mM) were incubated at room temperature in DTPA (2 mM)- and DEPMPO (15 mM)-supplemented phosphate buffer (40 mM; pH 7.0) with 0.4 mM hypoxanthine and 0.04 U/mL xanthine oxidase. Two-scan DEPMPO-OOH ESR spectra were recorded 1 min after the addition of the enzyme in 50-μL calibrated glass capillary tubes (Hirschmann Laborgeräte, Germany) using the following parameters: microwave power, 10 mW; receiver gain, 5×10^5; time constant, 20.48 ms; modulation amplitude, 0.63 G; and scan rate, 1.4 G/s for a sweep width of 120 G. ESR signals were quantitated by double integration of the simulated spectra (using the program reported in ref *30*, assuming for the major *trans*-DEPMPO-OOH diastereoisomer the existence of two rotamers (65:35%) having the hyperfine coupling constants (in G) a_N = 13.3 (12.9); a_P = 53.9 (43.0); and $a_{H\beta}$ = 12.2 (8.6). Data were the means of 3−6 independent experiments for each concentration.

Experiments on 3T3 Cells. *Cell Culture and Exposure to UV Irradiation.* Murine 3T3 fibroblasts (ATCC-LGC Promochem, Molsheim, France) were maintained in culture at 37 °C in a 5% CO_2-humidified atmosphere in DMEM containing 1% glucose and supplemented with

10% calf serum, 100 U/mL penicillin, and 100 mg/mL streptomycin essentially as described earlier (*31*). Cells were plated in 24-well dishes, and the medium was replenished every 2 days until confluency, which was checked by microscopic observation. Stock solutions of test compounds and reconstituted liquid emulsions were prepared in phenol-red free DMEM medium containing 1% glucose, and aliquots (0.01−0.20 mL) were transferred into the culture wells previously filled with the appropriate volume of medium to reach a final volume of 0.5 mL/well. After 1 h of incubation, cells were irradiated for 1 h with a UV solar simulator (150 W, UVA/UVB, 280−400 nm, Lot-Oriel, France), and incubation was further prolonged for 4 h at 37 °C for cell recovery. Lactate dehydrogenase (LDH) extracellular release and cellular MDA−TBA contents were then assayed in culture medium and scrapped cells, respectively (see below). Results were compared to cells treated with DMEM medium alone (DMEM group). In additional experiments, 20-μL aliquots of DEPMPO (final concentration, 15 mM) were added to cell culture wells at the time the UV irradiation was switched off, and cells were incubated for 10 supplementary minutes before the supernatant samples were transferred into cryotubes and immediately stored in liquid N_2 for delayed ESR analysis (see below).

LDH Assay. The protective effect of test compounds on UV-induced cytotoxicity was evaluated as a function of the amount of cytosolic LDH released from damaged cells. LDH activity was assessed spectrophotometricelly at 490 nm in supernatant medium samples using a commercial detection kit (Roche Diagnostic, Mannheim, Germany). Data are representative of 6 independent experiments for each test compound concentration and are expressed in arbitrary units/mg of protein. Protein measurement was performed in 4 over 24 randomly selected wells by the method of Lowry et al. (*32*).

MDA Assay. At the end of the irradiation and recovery periods, cells were scrapped and homogenized in serine-borate buffer, and the MDA content of the suspension was measured by HPLC using UV−vis detection according to a modification (*33*) of the method of Draper et al. (*34*). Briefly, 0.5 mL of cell suspension was added to 1.4 mL of 5% trichloroacetic acid and 100 μL of 0.2% butylated hydroxytoluene. The mixture was incubated for 40 min at 95 °C, rapidly cooled down to room temperature, and centrifuged (1,000*g* at 4 °C), and the supernatant was mixed with an equal volume of saturated aqueous TBA solution and incubated again for 60 min at 95 °C. After rapid cooling, each sample was extracted with 2 mL of butanol/pyridine (15:1, v/v), and the absorbance of the MDA−TBA complex was determined at 532 nm. A calibration curve was obtained from standard MDA−TBA samples prepared by using 0.25 mL of 0.1−15 mM 1,1,3,3-tetramethoxypropane solutions instead of the cell suspension in the procedure described above. Data are representative of 3−6 independent experiments for each test compound and are expressed as μmol/mg protein.

ESR Experiments on Irradiated 3T3 Cells. DEPMPO-supplemented supernatant samples from irradiated cells obtained as described above were sequentially thawed, placed into calibrated 50-μL glass capillary tubes, and single-scanned for their free radical content using ESR spectrometry 1 min after thawing of the sample. Quantitation of the signals and acquisition parameters were as described above, except for receiver gain, 1×10^5; modulation amplitude, 0.5 G; and scan rate, 1.7 G/s for a sweep width of 140 G. Data represent the means of 3 independent experiments for each test compound.

Statistical Analyses. All values are expressed as mean ± the standard deviation. Differences were analyzed by one or two-way analysis of variance (ANOVA) followed by a posteriori tests. Intergroup differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Synthesis of Sesame Oil Derivatives. In a first step, NaOH-catalyzed methylation of sesame oil yielded the mixture of methylesters **1** with a ca. 75% yield relative to the starting weight of the oil. There are many examples in the literature of transesterifications of plant oils in the presence of methanol (which is here both the solvent and the reactant) using a strong alkaline catalyst (*25*, *35*, *36*).

Linoleic acid, contained in high proportions in sesame oil triglycerides, plays a key-role in membrane fluidity as compared to saturated fatty acids (*21*). It was therefore considered important to assess to what extent it was recovered among the constituents of **1**. First, in the NMR spectra of **1**, the lack of signals at 5.2−5.3 and 4.1−4.4 ppm (^{1}H), and 68 and 61 ppm (^{13}C) showed that **1** was obtained free of the glycerol backbone NMR signals reported in sesame oil (*2*). Computational ^{1}H NMR analysis of sesame oil and **1** according to ref *2* also revealed that the relative percentages of linoleic and oleic acids, and SFA in the starting material was fully preserved in **1** (**Table 1**). In our conditions, no proton NMR signal at 0.98 ppm, which was assigned to the methyls of linolenic acid contained at less than 1% in sesame oil (*2*), was seen in the starting material and in **1** (see Materials and Methods).

Table 1. Transesterification of Sesame Oil, its Methylester (**1**), or Fatty Acids in the Presence of Benzyl Alcohols

				composition of fatty acid residues (%)[b]		
source	alcohol	formed ester	yield (%)[a]	oleic	linoleic	SFA
sesame oil[c]	MeOH	**1**	75	52	36	12
	VNA	**2**	33−40	52	36	12
1[d]	PPA	**3**	19−24	38	20	42
	5-HMR	**4**	20−24	53	35	12
linoleic acid[d]	VNA	**5**	21	0	100	0
	PPA	**6**	49	0	100	0
	5-HMR	**7**	9	0	100	0
oleic acid[d]	VNA	**8**	8	100	0	0
	PPA	**9**	33	100	0	0

[a] Calculated from the integration of the 1-H NMR aromatic peak for derivatives **2**, **3** and **4** of vanillyl (VNA), piperonyl (PPA) and 5-(hydroxymethyl)resorcinyl (5-HMR) alcohols, respectively (from 4 independent experiments). [b] Determined by 1-H NMR according to ref *2*. SFA, saturated fatty acids. [c] NaOH as catalyst. [d] *Candida antartica* as catalyst.

Previous studies have reported on the use of immobilized lipases as catalysts for the direct transesterification of plant oils (*37*), including sesame oil (*20*). Although attempts to produce **1** enzymatically from sesame oil, including with *Candida antartica*, resulted in incomplete or no esterification of the di- and triglycerides (data not shown), this lipase was found to be a very efficient catalyst for the bioconversion of **1** to the transesters of a series of alcohols, the structure of which is shown in **Figure 1**. These reactions were carried out using **1** as reactant and solvent and afforded the phenolic transester-enriched **2**−**4** in good yields when the following optimized conditions were applied: ester/alcohol ratio of 5:1 w/w, proportion of lipase of 2%, w/w, and heating at 60 °C for 24 h (see Materials and Methods). When the temperature increased above 70 °C, the small ^{1}H NMR signals appearing at 4.0−4.3 ppm and not present in the spectrum of **1** (see Materials and Methods) are due to glyceric acid, a degradation product of acrylic acid contained in the immobilized enzyme system (data not shown).

Proton NMR analysis demonstrated that the sole nonesterified byproducts in **2**−**4** were the corresponding pure fatty acids of sesame oil (**Figure 1** and **Table 1**) so that our reported bioconversion of **1** could also be used as a convenient source of linoleic acid. Of the general bioprocesses used for transesterification, acylation, or acyl transfer of natural products, many are based on proteases or lipases (*38*, *39*) because of their high specific selectivities. Here, we selected *Candida antarctica* because of its known efficiency toward long-chain fatty acids (C_{12}−C_{18}) and phenolic compounds in solvent-free conditions (*39*−*42*). To determine the specificity of *Candida antartica* in the transesterification of **1**, we have compared the ^{1}H NMR signals of **2**, **3**, and **4** with that of the reference linoleate esters **5**, **6**, and **7**, respectively. The yield in fatty acid phenolic esters was first calculated from the couples of typical ^{1}H NMR spectra (such as that selected in **Figure 3** for [**2**, **5**])

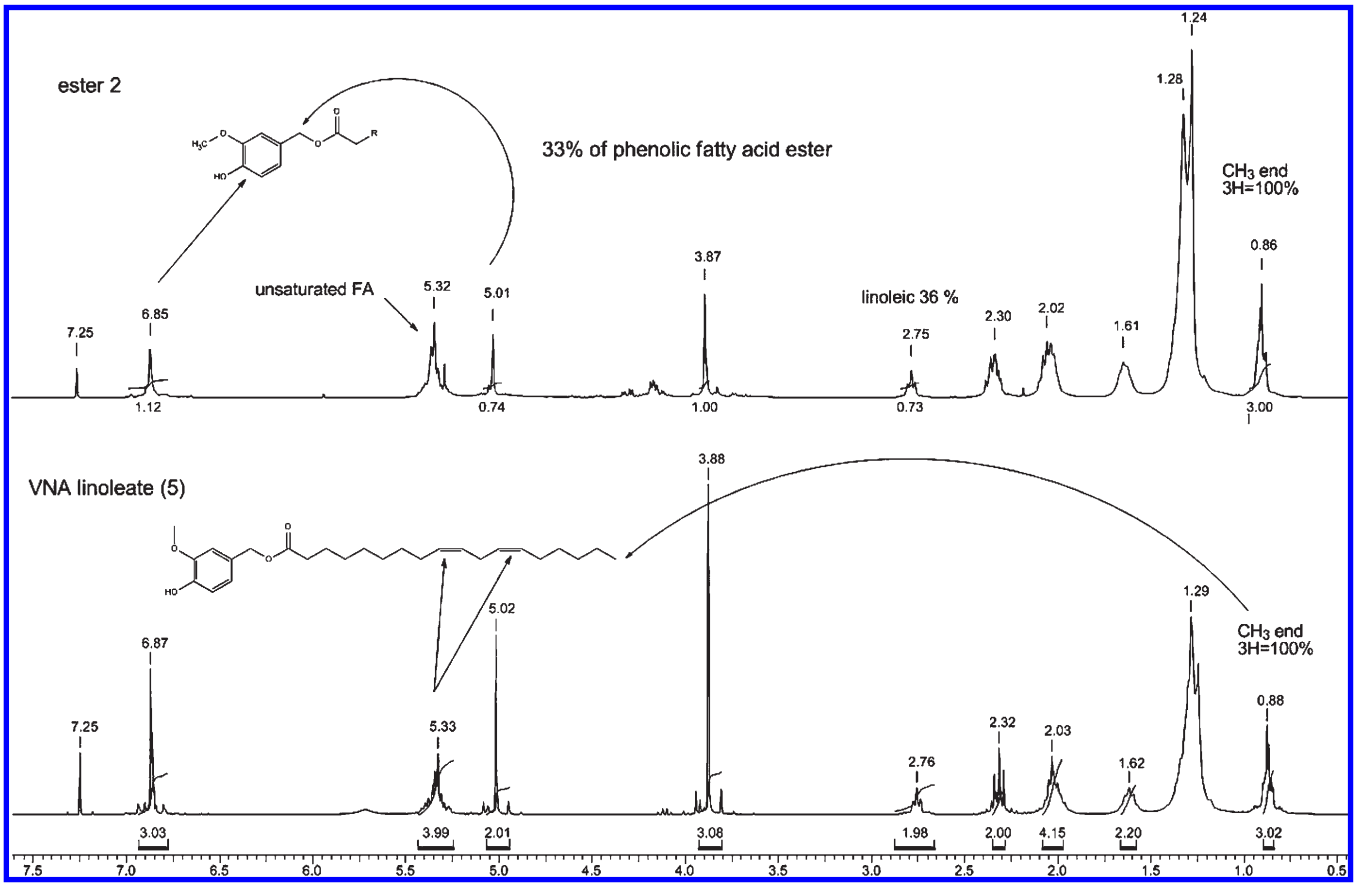

Figure 3. ^{1}H NMR spectra of **2** and its reference VNA ester **5** allowing both the assignements of lines and determination of the relative content of residue **5** in **2** (based on the integration of the fatty acid chain CH_3 end groups at 0.86−0.88 ppm taken at 100%).

by integration of the specific peaks of the aromatic region. In a second step, we investigated if the method of Vigli et al. (*2*), which afforded the global content of linoleyl and oleyl residues in compounds **2**−**4** (**Table 1**), could also discriminate between their free fatty acid versus transester forms.

For this purpose, we compared the ^{1}H NMR spectral features of **2** (**Figure 3**) with that of mixtures of the main fatty acids of sesame oil with different proportions of VNA esters **5** and/or **8**. We first checked that the signals from polyunsaturated acids (eg, in the 2.76 ppm region) of either sesame oil or **1** were comparable to that of a freshly prepared mixture containing (%, w/w) 52% oleic, 36% linoleic acid, and 12% stearic acids (data not shown) showing the accuracy of the ^{1}H NMR method described in ref *2* to determine the global fatty acid proportions. **Figure 4A** shows that the spectrum recorded when an excess of **5** (65%) was added to this mixture exhibited a multiplet at 2.32−2.33 ppm (instead of a triplet in **2**; **Figure 3**) because of the overlapping of the strong α methylene signal of the ester (t, 2.32 ppm) with the weaker acidic one (t, 2.33 ppm). Consistently, if the ester is added to the fatty acid mixture as a minor component (eg, ∼30%), then the expected concomitant decreases of the intensities of the ester-related aromatic methoxy (at 3.88 ppm) and α methylene signals were observed, but still the integration method in ref *2* allowed both the determination of the linoleyl residue proportion (at 2.76 ppm) and the nature of the added ester (see **Figures 4B**,**C** in the case of **5** and **8**, respectively). This was also true if a mixture of esters was added to the fatty acids, as illustrated by **Figure 4D** showing the ^{1}H NMR spectrum recorded in the case of the proportions calculated for **2** (**Table 1** and **Figure 3**), i.e., when the total added ester fraction, which contained **8:5** (55:45%), was 40% versus the fatty acid mixture.

From these above calibrations, we found that transesterification using *Candida antartica* (**Table 1** and **Figure 3**) showed that the relative percentage of unsaturated fatty acids was preserved for VNA and 5-HMR in **2** and **4**, respectively, while the reactivity of linoleic acid with PPA was much lower, yielding a very important fraction of SFA derivatives in **3** (**Table 1**).

Stability tests in acellular medium for vanillyl fatty acid esters **5** and **8** showed that heating at 40 °C resulted in a 30% and 50% degradation after 4 and 18 h, respectively. Heating for 2 h at 60 °C resulted in a 65% and 60% degradation for **5** and **8**, respectively.

The oxidative stability of sesame oil and hemisynthetic derivatives was determined on the total lipid fraction at each stage of the chemical, mechanical, and SD processes. Esterifications led to a strong elevation of hydroperoxide levels in **3** and **4** but not in **2** as compared to the antioxidant-rich (*5*) sesame oil, suggesting that VNA was the best protective residue (**Table 2**). Emulsification and SD strongly accelerated lipid oxidation in CTR-derived emulsions, while the presence of VNA in **2** significanty lowered hydroperoxide elevation. Emulsification-induced increase in lipid oxidation, attributed to the mechanical stress and enhanced oxygen distribution during homogenization, and heating upon pulverization, can be limited by incorporation of antioxidants (*28*, *43*). According to our previous study on sesamol-containing emulsions (*18*), we speculated that **2**-containing HPMC or SC-based SD emulsions would retain both oxidative stability within the multilayer interfacial membrane of the droplets and VNA-derived antioxidant effect.

Screening of Antioxidant Capacity Using the DPPH Test. We have first compared the DPPH scavenging activity of sesame oil, its different hemisynthetic derivatives, and fatty acid esters **5**−**9** with those of the antioxidants vitamin E acetate, Trolox,

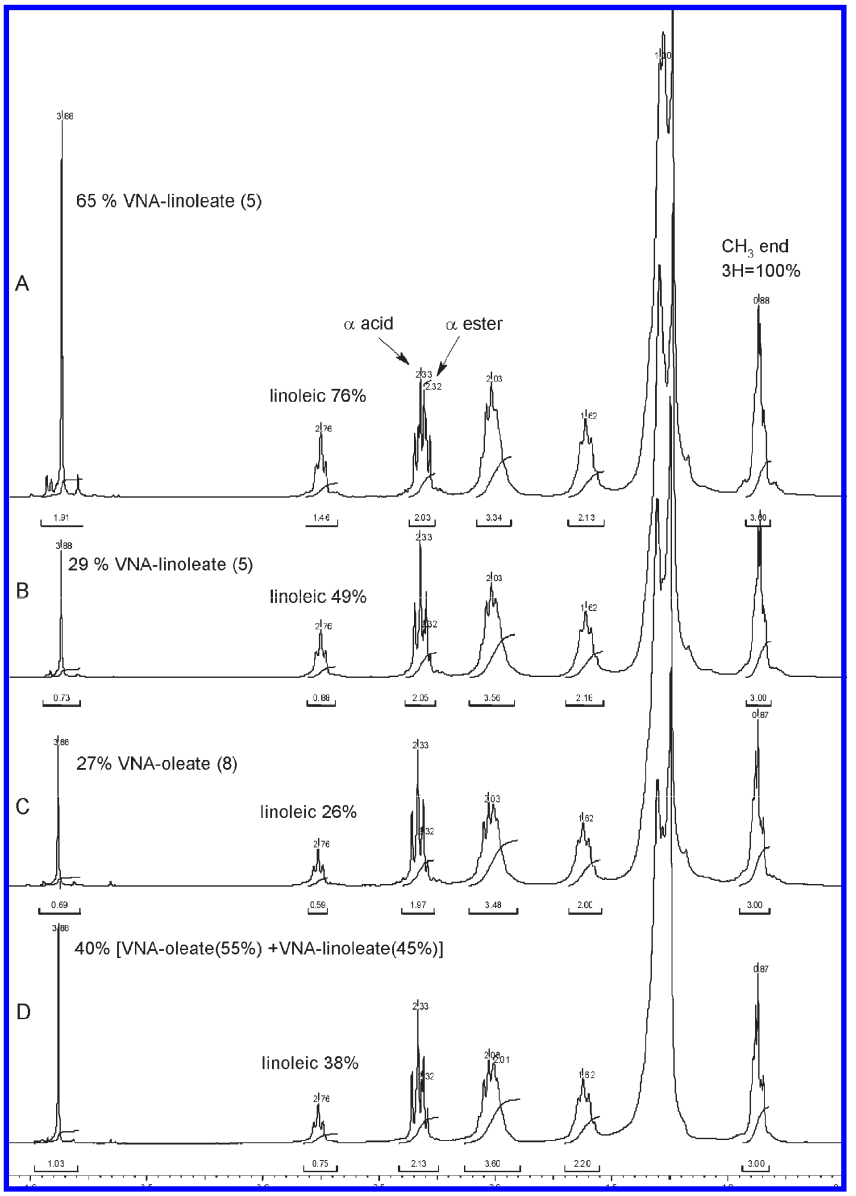

Figure 4. ^{1}H NMR spectra of a mixture of oleic (52%), linoleic (36%), and stearic (12%) acids supplemented with (relative %) (**A**) **5** (65); (**B**) **5** (29); (**C**) **8** (27); (**D**) **5**:**8** (22:18). Relative contents were calculated as described in the caption of **Figure 3**.

Table 2. Effect of Hemisynthetic and Emulsification Processes on Lipid Hydroperoxide Content

compound	hydroperoxides (mmol/kg oil)
sesame oil	2.9 ± 2.1
1	5.7 ± 1.7
2	4.9 ± 3.1
3	25.9 ± 3.1[a]
4	15.9 ± 2.2[a]
2-HPMC-LE	5.5 ± 0.9
2-SC-LE	7.8 ± 1.9
CTR-HPMC-$SD_{180\ °C}$	26.1 ± 1.2[a]
CTR-SC- $SD_{180\ °C}$	32.2 ± 2.5[a]
2-HPMC-$SD_{180\ °C}$	6.1 ± 1.2
2-SC- $SD_{180\ °C}$	7.2 ± 2.5

[a] Data are means ± standard deviation (n = 3). Statistics (one-way ANOVA followed by Newman-Keuls test): $P < 0.05$ versus sesame oil.

and SEOH, using a 5-min incubation protocol. A good solubility of sesame oil was achieved in dichloromethane, and **Figure 5A** shows that the oil dose-dependently reduced DPPH (50 mg/mL) absorbance, with a EC_{50} (the concentration decreasing the initial DPPH concentration by 50%) value of ~33 mg/mL. In the same conditions, the estimated EC_{50} values of **1**, **3**, and **4** were above 50 mg/mL, while that of **2** was significantly lower (i.e., 2 mg/mL; **Figure 5A**). This better activity of **2** in dichloromethane is in agreement with previous reports on an increased DPPH scavenging activity for methoxy-substituted phenolic compounds (*44*). The complex mechanism of the interaction of phenolics with DPPH has been found to strongly depend upon the solvent (*45*−*47*). We have therefore repeated the experiment in the more polar methanol in which all sesame oil phenolic derivatives and their precursor alcohols could be compared to standard antioxidants. For mixtures of esters, the molar concentrations used in the antioxidant and cytotoxicity tests described below were calculated from the average molecular weights derived from the yields reported in **Table 1** (i.e., 296, 327, 314, and 311 g for **1**−**4**, respectively).

From the concentration-dependent inhibition curves of **Figure 5B**, the estimated EC_{50} value for **2** was 0.39 mM, ranging between that of its parents VNA and **5** (i.e., 0.06 and 0.25 mM,

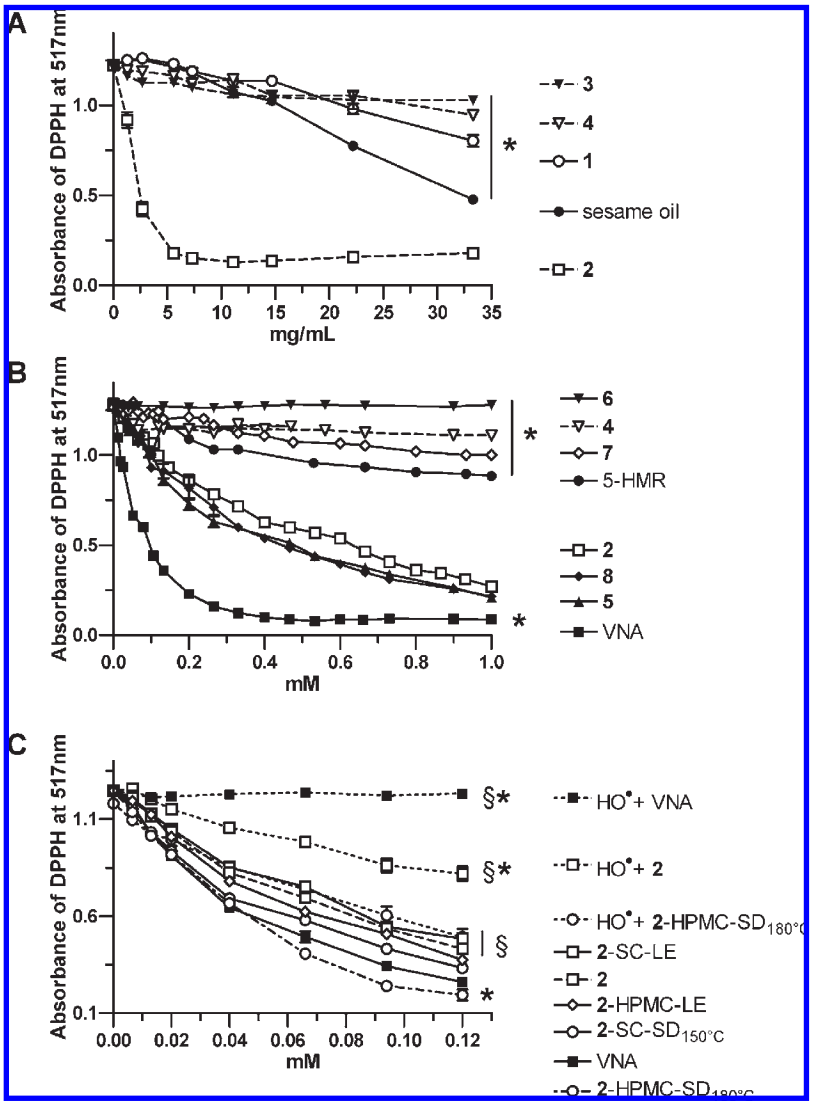

Figure 5. Concentration-dependent DPPH scavenging properties of (**A**) sesame oil and its hemisynthetic derivatives in methylene chloride after 5 min of DPPH incubation; (**B**) hemisynthetic esters and their alcohol and ester precursors in methanol after 5 min of DPPH incubation; (**C**) VNA and **2**-containing starting and reconstituted dry emulsions before or after pre-exposure to a Fenton reagent (the latter termed as HO• + compound abbreviation) in methanol after 30 min of DPPH incubation. Bars represent the standard deviation ($n = 3$). Statistics (two-way ANOVA and Newman-Keuls test): $^{*}P < 0.05$ versus **2**; $^{\S}P < 0.05$ versus 2-HPMC-$SD_{180\ °C}$ and VNA.

respectively) showing a partial inactivation of the antioxidant properties of the vanillyl phenolic moiety in the corresponding transesters. The complexity of the effect of substituents on the antioxidant activity of phenols has been intensively investigated (*43*, *48*) and a correlation between rate constants for the reaction with DPPH and calculated bond dissociation enthalpy has been established for the weakest, reactive O−H bond (*48*). The slower kinetics of DPPH scavenging by **5** could be due to increased molecular size and/or lipophilicity (*42*−*46*). The inactivation of the benzylic alcohol function of 5-HMR (EC_{50} ~2.1 mM) by the transesterification reaction was less pronounced than in VNA derivatives since the behavior of linoleate ester **7** was comparable (EC_{50} ~3.2 mM; **Figure 5B**). We suggest that the lack of DPPH scavenging activity of **4** in **Figure 5B** may specifically result from the relatively low yields of the transesterification reaction (**Table 1**). Consistently, no improvement in the poor DPPH scavenging activity of PPA seen in dichloromethane (**Figure 5A**) was obtained for **3** in methanol (data not shown). In this regard, the choice of fatty acids as substrates in our study was decisive since earlier work has demonstrated an increase in anti- $O_2•^-$ and DPPH scavenging activities in PPA-substituted coumarins (*49*).

Replacing the linoleate by an oleate residue in **9** (data not shown) did not improve the poor DPPH scavenging activity of ester **6** having the PPA alcohol function (**Figure 5B**) but maintained the efficiency of the vanillyl-oleate derivative **8** (EC_{50} ~0.31 mM). Altogether, these data prompted us to select **2** as the active antioxidant principle of sesame oil dry emulsions, keeping in mind that its EC_{50} value in the DPPH test (better than that of inactive vitamin E acetate) was an order of magnitude lower than that of SEOH and Trolox (i.e., 0.042 and 0.030 mM, respectively).

Preparation and in Vitro Antioxidant Properties of Vanillyl Fatty Acid Ester-Containing Spray-Dried Emulsions. Liquid o/w emulsions are mostly obtained using lipids and a carrier such as dextrin, lactose, or saccharose in the water-soluble phase (*50*, *51*). Generally, ingredients such as HPMC or SC are added to improve the physical stability of the emulsion when further submitted to SD (*18*, *27*, *50*, *51*). In the present work, we have synthesized two dry emulsions with ester **2** as the unique lipid constituent

and a hydrophilic phase containing combinations of saccharose with either HPMC or SC, using a SD procedure conducted at the optimal inlet air temperatures of 180 and 150 °C, respectively (*18*). The resulting powders **2**-HPMC-SD$_{180\ °C}$ and **2**-SC-SD$_{150\ °C}$, which were obtained with a satisfactory yield (15−20%, w/w), could be reconstituted in DMEM at concentrations up to 2,000 μg/mL, corresponding to ~6 mM in free **2**.

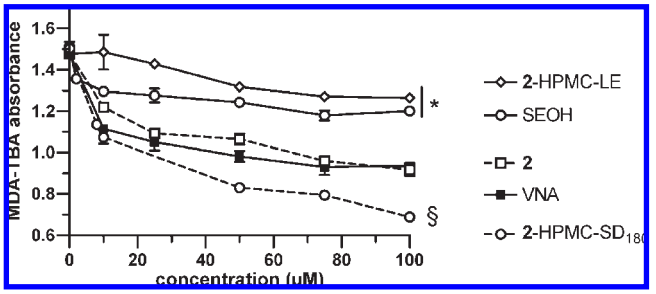

Figure 6. Concentration-dependent inhibition of deoxyribose degradation by **2**, its derived liquid and reconstituted dry emulsions, and SEOH. Bars represent the standard deviation ($n = 3$). Statistics (two-way and Newman-Keuls test): $^{*}P < 0.05$ versus **2** and VNA; $^{§}P < 0.05$ versus all other groups.

Investigation of the antioxidant properties of the new emulsions using the DPPH assay was performed in methanol using an extended 30-min incubation period. Under these conditions, the decrease of EC_{50} values was very significant for **2** (0.076 mM; **Figure 5C**) and relatively low for VNA and SEOH (0.045 and 0.033 mM, respectively) so that the two latter compounds can be considered as rapid antioxidants according to recently published ratings (*45*, *52*).

In methanol, reconstituted emulsions (0.06 to 0.3 mM in **2**) from **2**-HPMC-SD$_{180\ °C}$ and **2**-SC-SD$_{150\ °C}$ showed significantly increased DPPH scavenging properties as compared to the corresponding starting liquid emulsions **2**-HPMC-LE and **2**-SC-LE, or even **2** itself (**Figure 5C**), with EC_{50} values of 0.044, 0.052, 0.065, and 0.080 mM, respectively. To evaluate to what extent the antioxidant properties of these emulsions or their precursors VNA and **2** would be degraded by oxidative stress, they were prereacted with HO• before mixing with DPPH. **Figure 5C** shows that the DPPH scavenging activity is better protected in 2-HPMC-SD$_{180\ °C}$ than in HO•-challenged

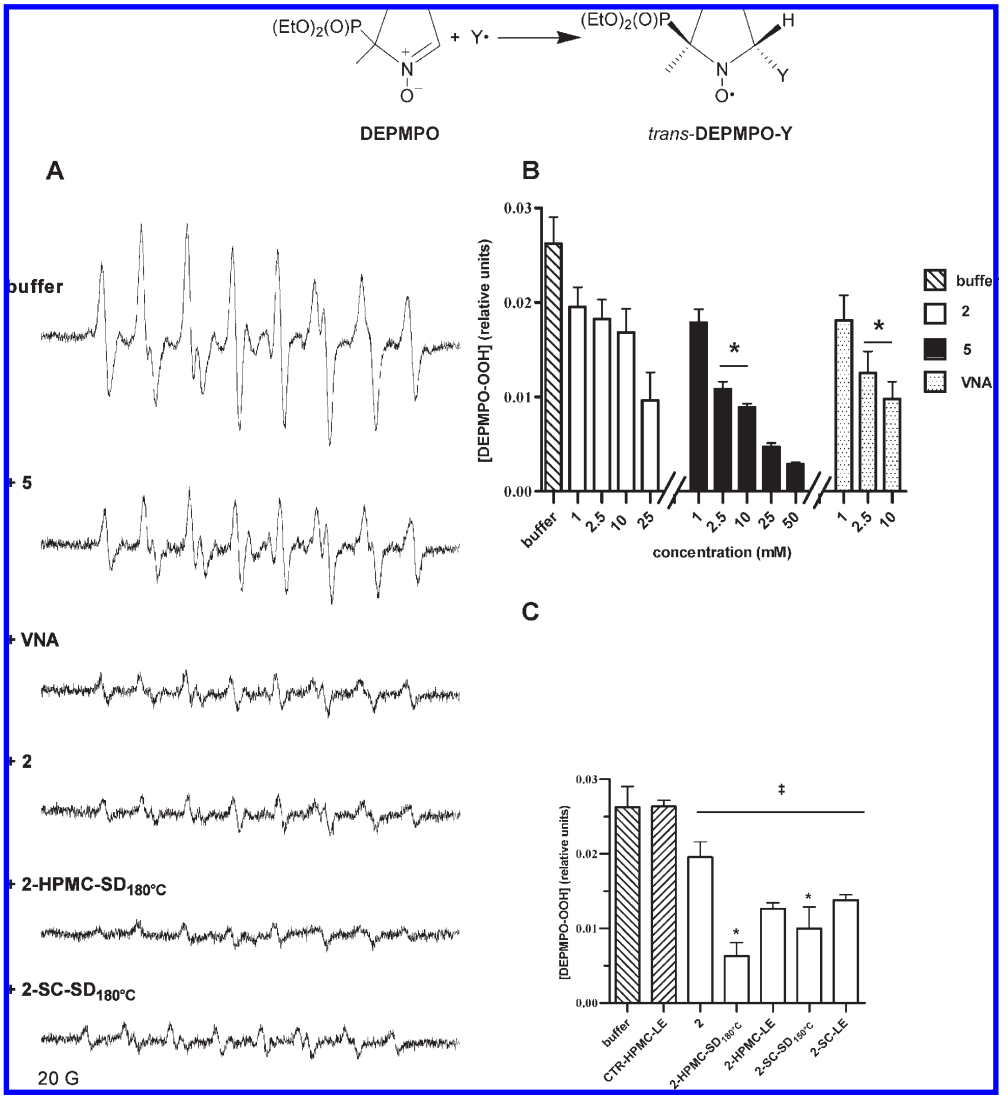

Figure 7. Scavenging of superoxide radicals determined by ESR spin trapping on DEPMPO in phosphate buffer. (**A**) Representative signals from mixtures containing 15 mM DEPMPO and 10 mM of an exact (VNA and **5**) or equivalent (**2** and its reconstituted emulsions) antioxidant solution. Dose-dependency of the superoxide scavenging on (**B**) active compounds; (**C**) reconstituted or starting liquid emulsions containing **2** (1 mM equivalent concentration). Bars represent the standard deviation ($n = 3$). Statistics (one-way ANOVA and Newman-Keuls test): $^{*}P < 0.05$ versus **2** at the same concentration; $^{‡}P < 0.05$ versus buffer and CTR-HPMC-LE.

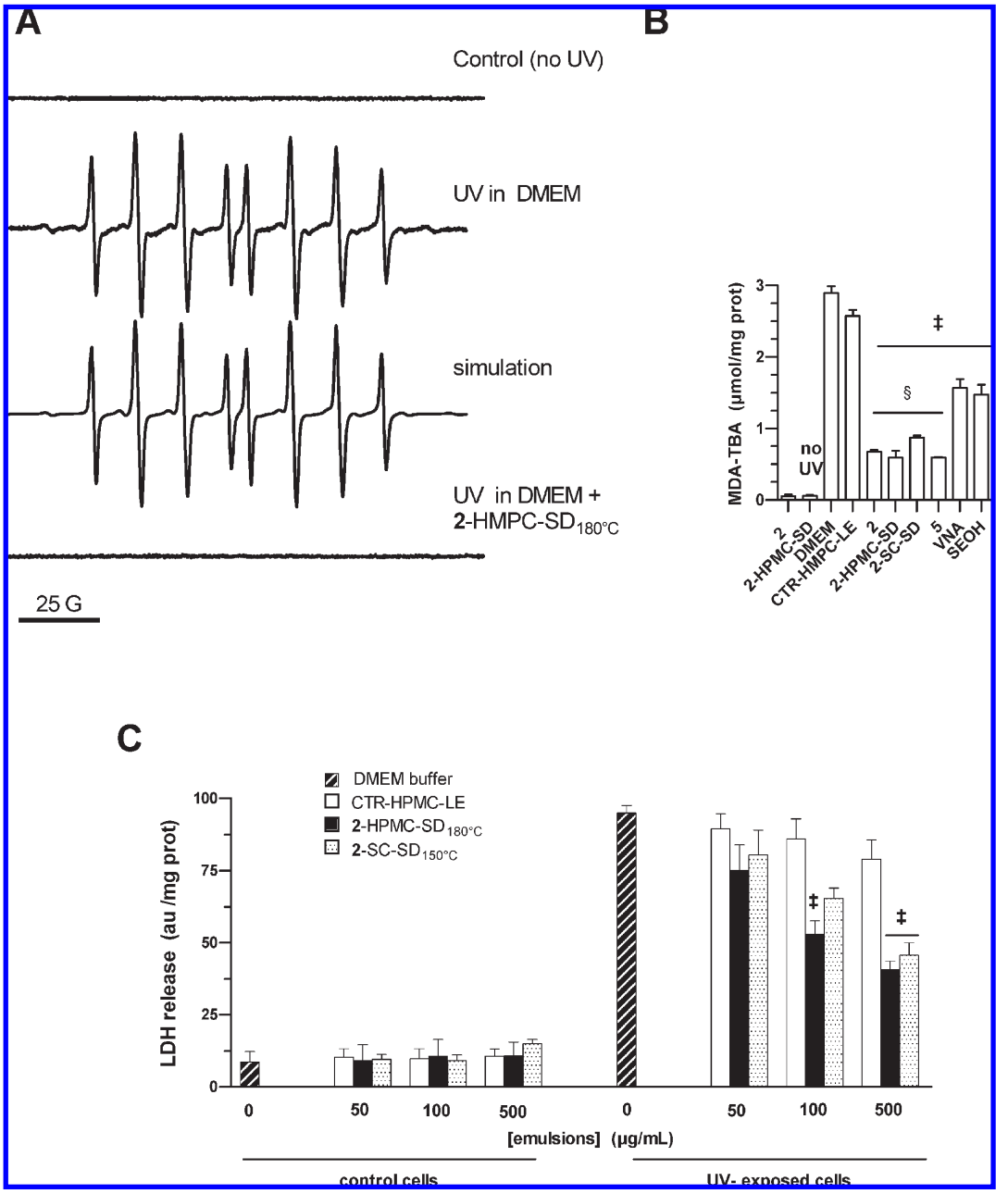

Figure 8. Antioxidant and protective effect of **2**-containing dry emulsions on UV-induced free radical formation in 3T3 cells. (**A**) Effect of adding **2**-HPMC-SD180 °C (1.5 mM in **2**) on the ESR signal from non- or UV-exposed cells upon postexposure addition of DEPMPO (15 mM). (**B**) Effect of **2**-based emulsions (100 μg/mL, i.e., 0.3 mM of **2**), one of its constituents **5**, and VNA and SEOH (both at 0.3 mM) on pre- and postirradiation MDA−TBA cellular content. (**C**) Dose-dependent effect of 2-based emulsions on pre- and postirradiation LDH extracellular release. Bars represent the standard deviation ($n = 3$). Statistics (one-way ANOVA and Newman-Keuls test): $^{\S}P < 0.05$ versus VNA; $^{\ddagger}P < 0.05$ versus DMEM and CTR-HPMC-LE.

2 (EC_{50} = 0.09 and 0.22, respectively) and that the effect of VNA was completely lost. Such a protective effect of encapsulation techniques toward free radical attack has been reported earlier for labile antioxidants (*16*−*18*, *23*).

In the deoxyribose assay, a dose-dependent inhibitory effect was observed within a pharmacological concentration range, which was comparable for VNA and **2**, and significantly better for **2**-HPMC-$SD_{180\,°C}$. The starting liquid emulsion **2**-HPMC-LE was significantly less effective and close to the effect of pure SEOH (**Figure 6**). Because formation of MDA−TBA is considered a consequence of nonspecific HO•-induced deoxyribose degradation when the Fenton reaction is performed in the presence of a strong chelator such as EDTA (*29*), there is evidence from the present and previous (*18*) data of a protective effect exerted by the shell surrounding the encapsulated antioxidant.

Superoxide being a primary species formed at the onset of oxidative stress in biological systems, we considered it pertinent to investigate its interaction with VNA and its derivatives using ESR spin trapping in phosphate buffer. Incubation of the xanthine−xanthine oxidase $O_2{\bullet}^-$ source with 15 mM DEPMPO yielded the long-lived nitroxide adduct DEPMPO-OOH, the ESR signal of which contains 12 main asymmetric lines characteristic of an equilibrium between two rotamers of the major *trans*-diastereoisomer (see Materials and Methods (*53*)). In these control spectra, 2−5% of the detected ESR signal was assigned to DEPMPO−OH (couplings in G: a_N = 14.0; a_P = 47.2, and $a_{H\beta}$ = 13.3; see ref *54*), the HO•/DEPMPO spin adduct (**Figure 7A**). The control

ESR signal was completely inhibited when the spin trapping reaction was performed in the presence of 10 units/mL SOD (10 units/mL) showing that HO• was not directly formed in the system (data not shown). The control ESR signal was inhibited by adding VNA, **2**, **5**, or the reconstituted dry emulsions **2**-HPMC-$SD_{180\ °C}$ or **2**-SC-$SD_{150\ °C}$ (**Figure 7A**). The mechanism of the inactivation of $O_2•^-$ by phenolic compounds is complex (*55*), and there has been evidence that VNA significantly decreased $O_2•^-$-induced luminol and lucigenin chemiluminescence signals in rats undergoing oxidative stress (*22*). In vitro inhibition of DEPMPO-OOH by VNA and its derivatives was dose-dependent (**Figure 7B**), and the observation that VNA and **5** were more efficient than **2** ($P < 0.05$) was consistent with the fact that the latter contains only 33% of active phenolics (**Table 1**). Since this decrease in anti-$O_2•^-$ efficacy for **2** versus VNA and **5** was only 20% at 1 mM, we considered it pertinent to investigate the spin trapping reaction in **2**-based reconstituted emulsions containing this concentration. **Figure 7C** shows that encapsulation of **2** resulted in a significant increase of its anti-$O_2•^-$ power in the starting liquid emulsions, likely because of the additional interaction of the free radical with HPMC or SC. Spray-drying significantly emphasized this trend only when HPMC was the excipient (**Figure 7C**), consistent with previous reports showing the benefits of this technology on antioxidant activities and stability of natural compounds including SEOH and linoleic acid (*18*, *23*).

Protective Effect of Dry Emulsions on UV-Irradiated Cells. Because **2**-based dry emulsions developed in this study could be used in active formulations for topical applications, we investigated their antioxidant and cytoprotective activities on UV-irradiated 3T3 fibroblasts. Thus, an exposure of these cells to UVA + UVB for 1 h in DMEM followed by incubation with 15 mM DEPMPO for 10 min resulted in a strong ESR signal (**Figure 8A**) which was satisfactorily simulated ($r = 0.996$) assuming a mixture of 90% DEPMPO−OH (which could be decomposed as a mixture of diastereoisomers (couplings in G): *cis*, $a_N = 14.05$, $a_P = 47.26$, $a_{H\beta} = 14.14$, $a_{H\gamma} = 0.48$ (1), 0.34 (3), 0.14 (1), and 0.11 (2); and *trans*, $a_N = 14.04$, $a_P = 47.26$, $a_{H\beta} = 12.73$, $a_{H\gamma} = 0.50$ (1), 0.34 (3), 0.19 (2), and 0.09 (1)) and 10% DEPMPO-H ($a_N = 14.94$, $a_P = 48.62$, $a_{H\beta} = 22.60$ (2), $a_{H\gamma} = 0.44$ (2), 0.31 (3), 0.28 (1), and 0.14 (1)). Cell culture medium was diamagnetic either when no UV was applied or when it contained 500 μg/mL **2**-HPMC-$SD_{180\ °C}$, correponding to 1.5 mM **2** (**Figure 8A**). DEPMPO-H, which was detected in spin trapping experiments on plant cell membranes, was believed to be a side-product of the Fenton reaction (*56*). However, a revisited mechanism of free radical formation in UVA-irradiated fibroblasts more likely implicates singlet oxygen or hydroperoxides rather than hydrogen peroxide or $O_2•^-$ as the primary species responsible for the observed oxidative stress within cells (*57*). In support, *cis*-DEPMPO−OH accounted for ∼30% in the spectrum of **Figure 8A**, a relatively low value as compared to that of Fenton-generated DEPMPO−OH (*54*).

A similar dose-dependent protection as seen for **2**-HPMC-$SD_{180\ °C}$ in **Figure 8A** was observed for all **2**-containing emulsions on irradiation-induced lipid peroxidation and LDH extracellular release. In these experiments, none of these damage indices arose in the absence of UV light, and a small protection on UV-exposed cells was provided only if **2**-free, CTR-SC-LE, or CTR-HPMC-LE emulsions were present above 500 μg/mL (**Figures 8B**,**C**). The protective effect of VNA or SEOH alone on cellular MDA−TBA formation was significantly lower than that of **2** or **5**, despite their better antioxidant performance in the DPPH assay (see above and also **Figure 5B**). Thus, the presence of fatty acid residues in **2**, particularly linoleate, likely plays the protective role we expected when we selected linoleic acid-rich sesame oil as a preferred substrate for transesterification. In our cell experiments, **5**, which resisted thermal degradation for hours in aqueous medium (see above), could therefore undergo cell uptake during preincubation to be hydrolyzed intracellularly by lipases or esterases, thus acting as a source of both VNA antioxidant and linoleic acid membrane constituents. Indeed, linoleic acid is among the first cell constituents affected during lipid peroxidation (*21*) so that an external source for its replenishment would be of great benefit for the damaged cell.

In conclusion, the present study clearly demonstrates that enzymatically obtained hemisynthetic derivatives of sesame oil with alcohols having either a known biological action (VNA) or used in cosmetic industry (5-HMR; (*58*)) show better antioxidant properties than the starting fatty acids and can be easily processed as the unique lipid and active phase in stable and hydrosoluble SD emulsions. For instance, the protection against UV-induced free radical formation in fibroblasts found for very low concentrations of **2**-HPMC-$SD_{180\ °C}$ and **2**-SC-$SD_{180\ °C}$ (i.e., 50−500 μg/mL, equivalent to 0.15−1.5 mM of **2**) was similar or even better than that reported for SEOH or VNA in other stressed cells (*18*, *21*, *59*). Because of the higher susceptibility of these natural phenolics to oxidative degradation, we believe the general principle behind the phenolic fatty acid esters and their corresponding emulsions described in this study may be a good alternative for the valorization of sesame or other natural plant oils.

ABBREVIATIONS USED

SFA, saturated fatty acids; o/w, oil-in-water; VNA, vanillyl alcohol; 5-HMR, 5-(hydroxymethyl)-resorcinol; PPA, piperonyl alcohol; ESR, electron spin resonance; SOD, superoxide dismutase; SEOH, sesamol; SC, sodium caseinate; TBA, 2-thiobarbituric acid; DTPA, diethylenetriaminepentaacetic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; HPMC, hydroxypropylmethyl cellulose; DEPMPO, 5-(diethoxyphosphoryl)-5 methyl-1-pyrroline *N*-oxide; DMEM, Dulbecco's modified Eagle's medium; SD, spray-drying.

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