Direct Analysis of Lipophilic Antioxidants of Olive Oils using Bicontinuous Microemulsions

Supporting Information

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Reference numbers correspond to reference numbers in the main text.

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SI-1 Scheme of quantitative analysis of antioxidant activity for oil samples

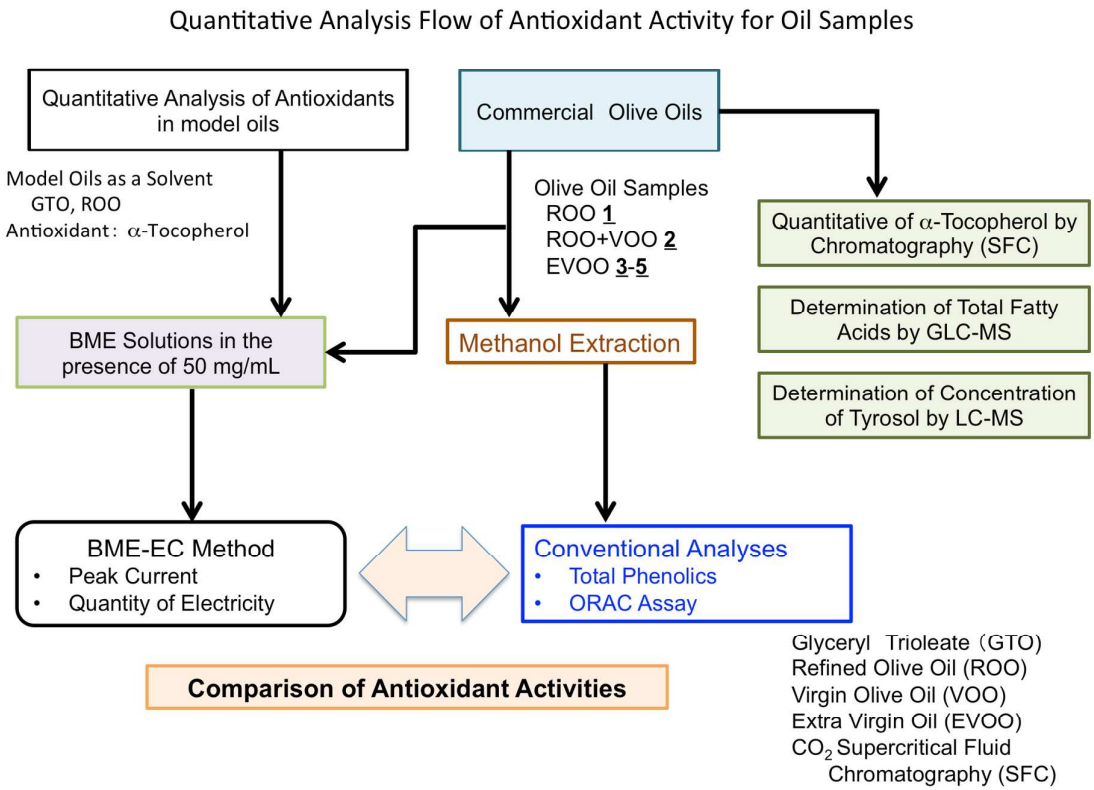

Figure S1. Quantitative analysis flow of antioxidant activities for olive oils.

SI-2 Electrochemical cell and preparation of electrodes

The CV measurements were conducted in a BME solution at 25°C without degassing using an ordinary three-electrode system. An electrochemical cell composed of polytetrafluoroethylene was used for a plate electrode (Parts No. 011951, BAS Inc., Tokyo, Japan).

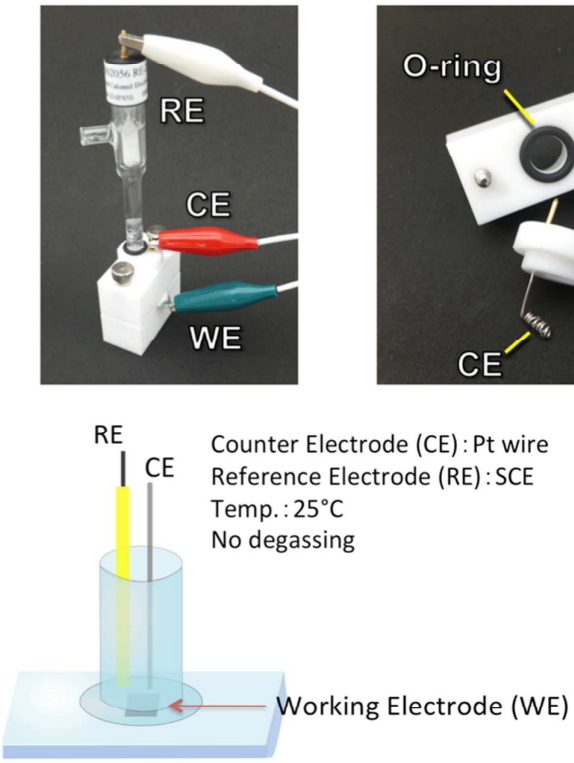

Figure S2. Typical setup of the electrochemical cell for antioxidant evaluation in olive oils by the BME-EC method.

The F-ECR electrode was prepared in accordance with previous reports [30-34]. First, a nanocarbon film electrode-material was deposited on highly doped silicon (100) substrates with electron cyclotron resonance (ECR) sputtering equipment (AFTEX-3200, MES-Afty, Tokyo, Japan) at room temperature. The microwave power and DC voltage applied to the carbon target were 500 W and 500 V, respectively. The argon gas pressure for sputtering was 5.0×10^{-2} Pa. During deposition, the irradiation ion current density was 5.8 mA cm^{-2}, and ion acceleration voltage at 75 V. The nanocarbon films were obtained in ~8 min and were typically 40 nm thick. Carbon electrodes were fluorinated using reactive ion etching (RIE) equipment (Model RIE-200L, SAMCO, Inc., Kyoto, Japan). (b) The radio frequency power was 40 W and CF_4 gas pressure and flow rate at 10 Pa and 10 sccm, respectively. Plasma treatment was performed for 30 s under the above conditions. Typical water

contact angle for the F-ECR electrode used was 86.8°. The F−ECR electrode is available from Universal Systems Co., Ltd. (Tokyo, Japan; http://www.universalsystems.jp/access.html)

Surprisingly, the F-ECR electrodes were able to be reused more than a hundred times because of their chemically inert surface and cleaning effects of BME solutions [29–34]. Prior to reuse, the electrode surface was flushed with methanol, 2-butanol, and toluene. When the electrode experienced at potential cycle beyond −1.5 or +2.0V vs. SCE, the electrode was damaged and gradually lost hydrophobicity as a result of introducing the oxygen-functional groups. Electrochemical operation at the potential region from −1.0 to +1.5V, used in this research, produced little damage in terms of CV reproducibility.

The ITO electrodes were cut to the required size (~1 cm^2) from the "flat" polished ITO-coated glass purchased from Kuramoto Co., Ltd. (Tokyo, Japan) and the "rough" indium tin oxide (ITO)-coated glass (Koshi Optical Industry Co. Ltd., Japan). The comparison of surface roughness on the ITOs has been described into our previous article[21]. There was basically no difference between the ITOs.

Prior to first use, the ITO electrodes were thoroughly cleaned in acetone, an alkali cleaning solution, and pure water with sonication. Typical water contact angle for the ITO was 16.5°. The ITO electrodes were also able to be reused. Prior to reuse, ITO electrodes were cleaned by flushing with methanol and pure water. Frequently, alkali cleaning solution or a commonly used plasma treatment was conducted for recovery of the hydrophilic surface.

SI-3 Determination of total fatty acids in olive oils

For determining fatty acid compositions, commercial kits were used for saponification, methylation, and purification of fatty acyl methyl esters from tested oils (Nacalai Tesque, Inc., Kyoto, Japan). Fatty acyl methyl esters were measured by gas-liquid chromatography-MS (GLC-MS; GCMS-QP2010 Plus, Shimadzu Corp., Kyoto, Japan). A DB-23 column of 30 m length, 0.25 mm i.d., and 0.25 μm thickness (Agilent Technologies, Inc., Santa Clara, CA, USA) was used with helium carrier gas at 0.83 mL/min. The GC's oven temperature program was 50 °C for 1 min, increased at 20 °C/min to 170 °C, increased again at 4 °C/min to 220 °C, then held for 4.5 min. The injector and detector temperatures were set at 250 °C and the mass range scanned from 30 to 600 daltons. GC/MS system control and data peak processing were managed using Shimadzu's GC/MS solution software, version 2.7.

Table 1. Compositions of fatty acids (%), concentrations of α-tocopherol and tyrosol, and antioxidant activities of tested olive oils.

Samples	Refined olive oil (ROO)	Refined olive oil and virgin olive oil (R-VOO)	Extra virgin olive oils samples (EVOO)		
	1	2	3	4	5
Source	Imagine, Inc. (Japan)	Spain	Spain	Italy	J-Oil Mills, Inc. (Japan)
Compositions of Fatty acids (%)					
Myristic, $C_{14:0}$	0.04	0.05	0.04	0.04	0.03
Palmitic, $C_{16:0}$	25.8	24.3	25.4	24.0	30.3
Palmitoleic, $C_{16:1}$	0.8	0.6	0.6	0.4	0.8
Stearic, $C_{18:0}$	6.6	6.2	6.6	5.6	5.7
Oleic, $C_{18:1}$	61.8	63.7	63.1	60.1	55.7
Linoleic, $C_{18:2}$	4.5	4.9	4.3	9.4	7.5
Linolenic, $C_{18:3}$	0.5	0.4	-	0.5	-
Concentrations of Antioxidant species					
α-tocopherol ± SD (mg/kg) [a]	203 ± 5	181 ± 3	193 ± 11	168 ± 18	105 ± 10
Tyrosol ± SD (mg/kg) [b]	Not detected (<1.0)	3.29 ± 0.11	11.4 ± 0.0	82.7 ± 0.6	11.0 ± 0.1
Antioxidant activities					
Total phenolics ± SD (GAE mg/kg) [c]	30.9 ± 9.1	52.9 ± 7.0	123 ± 8	146 ± 8	85.9 ± 5.3
ORAC value ± SD (mmol TE/kg) [d]	0.21 ± 0.02	0.97 ± 0.02	2.78 ± 0.04	3.04 ± 0.09	1.51 ± 0.04
BME-EC					
$Q_{0\text{-}1.2V}$ ± SD ($\mu C/cm^2$)[e]	7.31 ± 0.42	15.1 ± 0.22	22.3 ± 0.6	24.9 ± 0.1	15.9 ± 0.3

Compositions of fatty acids were measured by GLC/MS after saponification and methylation. Concentrations of α-tocopherol and tyrosol, total phenolics, ORAC values, and $Q_{0\text{-}1.2V}$ (charge passed from 0 to 1.2 V) measured by SFC[a], LC/MS[b], Folin-Ciocalteu method[c], and ORAC assay[d], and BME-EC[e], respectively; [a-e] n=3.