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Quantification of Antioxidant Capacity in a Microemulsion System: Synergistic Effects of Chlorogenic Acid with α-Tocopherol

Wei Lin Sandra Sim,[†] Ming Yong Han,[‡] and Dejian Huang*,[†]

[†]Department of Chemistry, National, University of Singapore, 3 Science Dr. 3, Singapore 117543, Republic of Singapore and [‡]Institute of Materials Research and Engineering, Agency of Science Technology and Research, 3 Research Link, Singapore 117602, Republic of Singapore

We report herein a characterization of an oil-in-water (o/w) microemulsion consisting of 12% methyl linoleate (or styrene in weight %), 29% surfactant Tween-20, 15% *n*-butanol, and 44% 75 mM potassium phosphate buffer (pH 7.04). The oil phase droplet size, determined by dynamic light scattering, is 19.3 nm with polydispersity at 0.103. When methyl linoleate is replaced with styrene, the droplet size increases to 63 nm but with much narrower polydispersity at 0.047. The droplet size of styrene is confirmed by polymerization of the styrene in the microemulsion. The polystyrene particles isolated have diameters of ~70 nm determined by a scanning electronic microscope. Both microemulsions remain stable for two months as expected for a thermodynamically stable system. The methyl linoleate oxidation is induced by AAPH, and the effects of radical scavengers are evaluated in a high throughput fashion using an oxygen sensor coated 96-well microplate Oxygen Biosensor Systems. From the oxygen consumption kinetic curves, the antioxidant capacity can be calculated using Trolox as the standard. The synergistic effect of hydrophilic antioxidants and α-tocopherol was measured for representative flavonoids. Chlorogenic acid has the best synergistic effect of 44.8%. The assay, coined as $ORAC_E$ standing for oxygen radical absorbance capacity in microemulsion, provides a model system in evaluating antioxidant capacity of phenolic compounds in a heterogeneous system relevant to food and cosmetic applications.

KEYWORDS: Antioxidant; synergistic effect; chlorogenic acid; free radical; microemulsion

INTRODUCTION

Microemulsion is thermodynamically stable due to low interfacial tensions between oil and water domains. The droplet sizes of microemulsion are comparable to that of nanoemulsion (*1*), which, however, is a kinetically stable system and requires high energy input to be generated due to its thermodynamic instability. Typically, a microemulsion consists of water, oil, surfactant, and short chain alcohols as cosurfactant (*2*). The translucent liquid could be an important delivery vehicle to disperse lipid soluble ingredients into water phase for better availability of nutrients such as lipid soluble vitamins, antioxidants, and polyunsaturated fatty acids (*3*, *4*). It is foreseeable that the large interfacial areas between oil and water phases would make the lipids in microemulsion more vulnerable toward oxidation. Yet little was known about the antioxidant capacity in microemulsion. The long-term physical stability of the microemulsion is also ideal as a model system in evaluating antioxidant capacity as it provides a stable platform for comparative studies and provides insight for stabilization of the ingredients from oxidation in microemulsion. Microemulsion is also a suitable system for studying synergistic effects between lipid soluble antioxidants and water soluble antioxidants in vitro. A well studied synergism of antioxidants is α-tocopherol regeneration by vitamin C experimentally demonstrated in vitro (*5*) and suggested in vivo (*6*).

Antioxidant activity in heterogeneous systems can be very different from the bulk phase, due to the phase distribution of antioxidants according to their solubility in oil, interfacial, and water phases (*7*). Antioxidant paradox is often used to refer to the phenomenon that lipophilic antioxidants works better in hydrophilic environments and hydrophilic antioxidants in lipid systems (*8*−*11*). It is recommended that model systems be developed specifically for the quantification of antioxidant activity in an emulsion bearing some similarity to certain food mixtures (*12*). Conventionally, antioxidant capacity in emulsion was measured in thermodynamically unstable and optically translucent (macro)emulsions with an array of methods used in measuring oxidation progress. The headspace volatile approach measures the secondary oxidation products such as propanal by gas chromatography (*13*). Conjugated diene peroxide quantitation (UV 234 nm) is also applied, but it requires sample extraction and suffers interferences from a wide range of compounds that also absorb at 234 nm (*14*). Lipid peroxide quantification by iodometric

*Corresponding author. Tel: 65-6516-8821. Fax: 65-6775-7895. E-mail: chmhdj@nus.edu.sg.

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assay is sensitive to oxygen (*15*), while modified ferrous oxidation xylenol orange (FOX-2) suffers from carbohydrate interferences and requires sample purification (*16*). The transparent and thermodynamically stable microemulsion offers much advantage for monitoring the oxidation kinetic in real time using UV−vis or fluorescence methods. We report herein a fluorescence based, high throughput assay to quantify antioxidant capacity in microemulsions consisting of methyl linoleate, Tween-20, *n*-butanol, and phosphate buffer. The oxidation reaction is initiated by radical initiator 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), and the reaction progress is monitored by oxygen concentration change over time. In line with the $ORAC_{FL}$ assay (*17*), which measures antioxidant capacity using fluorescein as the probe in water, we refer to the assay reported here as the $ORAC_E$ (oxygen radical absorbance capacity in microemulsion) assay. The synergistic effect of typical flavonoids and α-tocopherol is examined using the $ORAC_E$ assay.

MATERIALS AND METHODS

Materials. α-α′-Azodiisobutyramidine dihydrochloride (AAPH) and styrene were bought from Fluka (Steinheim, Germany). Methyl linoleate (97%) was purchased from TCI Tokyo Kasei (Tokyo, Japan). Polyethylene glycol sorbitan monooleate (Tween-20) and *n*-butanol were obtained from Merck (Germany). (±)-α-Tocopherol, (+)-catechin, quercetin, ascorbic acid, and naringin were purchased from Sigma Chemical Co. (St Louis, MI). Trolox was purchased from Acros Organics (New Jersey). Chlorogenic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI). Baicalin was extracted from the plant, *Scutellaria baicalensis* Georgi. The oxygen biosensor system (OBS), a specially ruthenium dye coated 96-well microplate, was purchased from BD Biosciences Discovery Labware (Bedford, MA) and was received as a gift from Brunswick Laboratories, LLC (Norton, MA).

Instruments. Autoxidation of methyl linoleate was monitored by a Synergy HT microplate fluorescent reader fitted with an excitation filter at 485 nm and an emission filter of 590 nm (Biotek Instruments Inc., Winooski, VT, USA) with an oxygen sensor biosystem. To cross-validate the results of the plate reader, the kinetics of reaction was also studied with 5300A Biological Oxygen Monitor (Yellow Spring Instruments Co., Ohio). Light scattering experiments were performed using a BI-200SM Research Goniometer with an argon ionic laser (λ = 514.5 nm) (Brookhaven Instrument Corporation, NY). The scanning electron microscopy (SEM) image was obtained using Joel Fesem Jsm 6700F Scanning Electron Microscope (Tokyo, Japan).

Preparation of the o/w Microemulsion. Two different microemulsions were prepared, one with methyl linoleate and another with styrene. The compositions of both systems were modified from literature (*18*). In a 15 mL test tube, oil (ML or styrene, 1.2 g), Tween-20 (2.9 g), *n*-butanol (1.5 g), and potassium phosphate buffer (4.4 g) were mixed and vortexed to give a transparent solution. The droplet sizes of both microemulsions were determined using a photon-correlation spectroscope. All dynamic light scattering experiments were performed at 37 °C, and the digital correlator analyzed the intensity of the scattered light collected at an angle of 90°. The refractive index and viscosity value for continuous phase at 37 °C were 1.334 and 2.875 cP, respectively.

Polymerization of Styrene in Microemulsion. Styrene microemulsion (2.0 g) was bubbled with nitrogen for 20 min to remove dissolved oxygen. AAPH (100 μL, 0.20 g/mL in phosphate buffer) was then added to the solution, which was maintained in a 37 °C water-bath for 2 h, and the solution turned milk white. The precipitate was centrifuged for 15 min, washed three-times with deionized water, and dried in vacuo. The sample was dispersed in deionized water, and one droplet was placed on a 2 × 2 mm glass slide and dried at room temperature for SEM imaging.

$ORAC_E$ Assay. Trolox solutions (20 μL, with concentrations at 500, 250, 125, 62.5, and 31.25 μM) and samples (20 μL with a given concentration in phosphate buffer) were added to respective wells of an OBS microplate. The standards were pipetted in duplicate (D2 to D6, D7 to D11). Each sample was run in triplicate. The perimeter wells were not used due to the potential edge effect of the microplate for kinetic studies. The oxidation substrate, 200 μL of methyl linoleate microemulsion, was then pipetted into the wells. The mixture of samples and microemulsion were incubated for 10 min at 37 °C prior to the addition of AAPH (20 μL, 0.20 g/mL in phosphate buffer) to all of the wells. A control well that contains 200 μL of microemulsion and 40 μL of phosphate buffer was required for normalization of the fluorescence reading. The final volume of the reaction mixture in each well was 240 μL. The kinetics of the reaction were monitored for 2 h at 37 °C with one reading every 2 min, and the plate was shaken for 20 s at low intensity before each reading to ensure sufficient mixing. The collected fluorescence data were normalized and converted to the oxygen concentration at each point of the reaction. Hence, the graph of oxygen concentration versus time was plotted for each sample and standard. The area under the curve (AUC) and net AUC ($AUC_{sample} - AUC_{blank}$) of the standards and samples were then calculated. The AUC approach combined inhibition time and degree to one quantity and was very powerful in measuring the antioxidant capacity of complex food mixtures. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final $ORAC_E$ values were calculated using the regression equation between Trolox concentration and the net AUC was expressed as micromole Trolox equivalents per micromole of sample. The AUC was calculated as:

$$AUC = \{[O_2]_o + 2[O_2]_2 + ... + 2[O_2]_i + ... + [O_2]_{120}\}$$

where $[O_2]_o$ = initial oxygen concentration at 0 min; $[O_2]_i$ = oxygen concentration at time *i*; and $[O_2]_{120}$ = oxygen concentration at 120 min.

The relative Trolox equivalent $ORAC_E$ value was calculated as:

$$\text{relative } ORAC_E \text{ value} = [\text{Trolox equivalent from regression equation}]/[\text{sample}]_{well}$$

The unit of $ORAC_E$ was μmolTE/g for solid samples or μmolTE/L for liquid samples.

Antioxidant Capacity Measured Using Oxygen Electrode. To the microreactor of the Biological Oxygen Monitor, an oxygen electrode was connected. The reaction chamber was stirred by a magnetic rod-shaped bar and was maintained at 37 °C by circulating water. Freshly prepared methyl linoleate microemulsion (500 μL) and Trolox (50 μL, 62.5 μM) were syringed into the chamber. The reaction mixture was then incubated for 10 min before AAPH (50 μL, 0.20 g/mL in phosphate buffer) was added to initiate the reaction. The oxygen concentration reading was taken every 20 s until the reading reached zero. The measurement was also repeated with 0, 125 μM, 250 μM, 500 μM, and 1000 μM of Trolox. Each measurement was then repeated. The graph of oxygen concentration versus time was plotted, whereby net AUC ($AUC_{sample} - AUC_{blank}$) was obtained for each standard. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the three measurements for each concentration.

Measuring Synergistic Effects between Lipophilic and Hydrophilic Antioxidants. A stock solution of α-tocopherol

(1.17 g/L in *n*-butanol) was prepared before dilution to the appropriate concentration of the working solution. In this case, the *n*-butanol that was used to prepare the microemulsion was being replaced by the working solution. The microemulsion containing α-tocopherol and the blank were prepared. The $ORAC_E$ assay was then applied to investigate the possible synergistic effects between lipophilic and hydrophilic antioxidants. Two hundred microliter microemulsions were pipetted into wells in the 96-well OBS plate. The hydrophilic antioxidants (20 μL 500 μM in buffer) were added to microemulsions with or without α-tocopherol (41.7 μM final concentration) in triplicate. The mixtures were incubated at 37 °C for 10 min before the addition of AAPH (20 μL, 0.2 mg/mL in PBS) to initiate the reaction. The reactions were monitored for 2 h, and the net areas under the oxygen consumption curves were calculated and converted to the μmol TE equivalency.

RESULTS AND DISCUSSION

Preparation and Characterization of the o/w Microemulsion. Methyl linoleate was chosen as the oxidation substrate because it is chemically similar to polyunsaturated fatty acids and has been used to assess antioxidant activity extensively (*19*).Tween-20 has the suitable lipophilic and hydrophilic balance (LHB) of 16.7 for stabilizing the microemulsion with the help of *n*-butanol as cosurfactant, which further decreases the interfacial tensions required for the formation of microemulsions and hence their thermodynamic stability (*2*). The selected microemulsion contains 12% oil, 44% phosphate buffer, 29% Tween-20, and 15% *n*-butanol and is transparent for a few months at 4 °C. If the oil increases beyond 15%, the solution will turn cloudy. At room temperature, the prepared microemulsions stay transparent for a long time, whereas the macroemulsion system readily undergoes phase separation (**Figure 1**). Twelve percent oil is applied in the selected microemulsion formula to ensure the presence of a large excess of substrates compared to oxygen concentration. Methyl linoleate is susceptible to autoxidation, and to avoid possible accumulation of lipid peroxides upon storage, the microemulsion is prepared fresh and used within 24 h. Methyl linoleate may be replaced by styrene and presumably also other lipids of interests such as n-3 polyunsaturated fatty acids found in many fish oils. Dynamic light scattering data revealed that the average oil droplet size was 19.3 nm with a polydispersity of 0.103, whereas the average droplet size for the styrene system was much larger at 63.1 nm but has much narrower polydispersity of 0.047. It is likely due to the molecular shape and polarity difference between styrene and methyl linoleate. Nonetheless, two microemulsions showed similar autocorrelation function trends as smooth exponential decay (**Figure 2A** and **B**) indicating the similarity between the two. To confirm the droplet sizes measured by dynamic light scattering, the styrene containing the microemulsion was degassed (to remove oxygen) and then polymerized by the addition of AAPH as a radical initiator. The polystyrene nanoparticles were isolated, and scanning electronic microscopy showed the relatively uniform particle sizes of 70 nm (**Figure 2C**), larger than the oil droplet; this is likely due to swelling of the polymer as the packing can no longer be as compact as in the monomer form. Our result is consistent with the fact that microemulsion was a commonly used system in the preparation of nanoparticles including nanosized polystyrene (*20*).

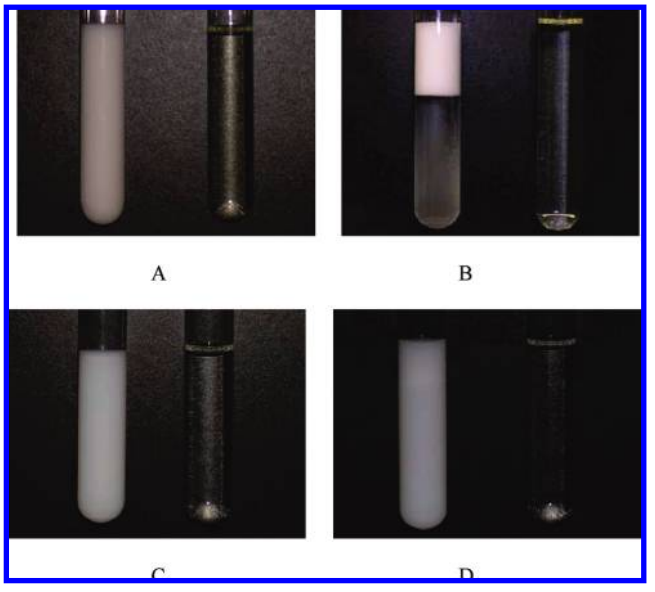

Figure 1. Images of oil-in-water microemulsion and macroemulsion of methyl linoleate and styrene. (**A**) Methyl linoleate, immediately after preparation; (**B**) 2 h after preparation; (**C**) styrene immediately after preparation; (**D**) styrene 2 h after preparation. Microemulsions are transparent, whereas the macroemulsions are milky. The phase separation is apparent in the macroemulsion system of methyl linoleate and Tween-20.

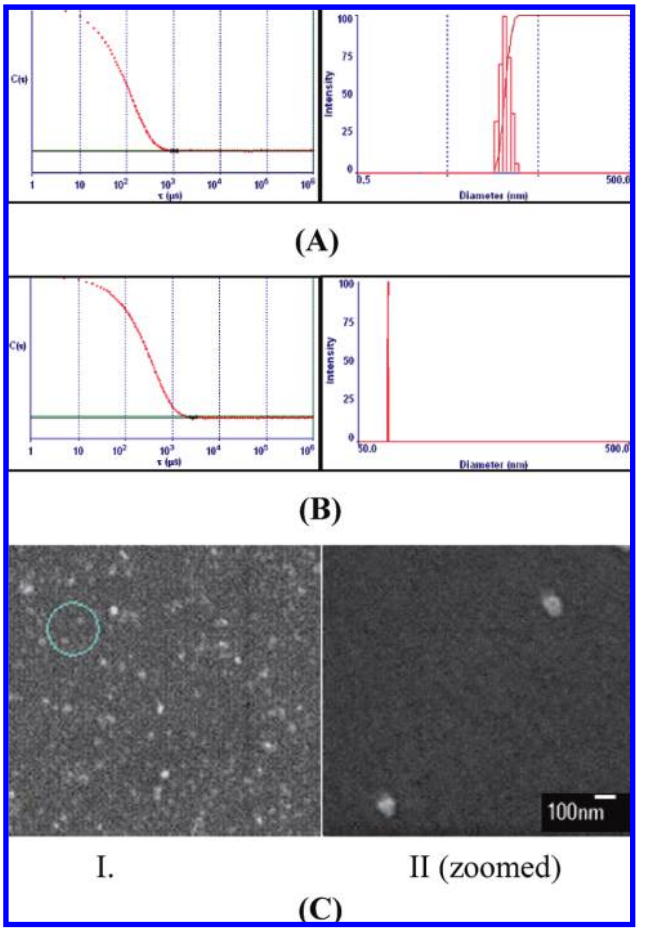

Figure 2. (**A**) Autocorrelation function and droplet size distribution for methyl linoleate and (**B**) that of styrene microemulsion. (**C**) Topographic images of polystyrene obtained after polymerization of the microemulsion in **B**. The average particle size is 70 nm.

$ORAC_E$ Assay. With the fully characterized microemulsion in hand, we applied it for the evaluation of antioxidant activity of common phenolic compounds. The radical chain oxidation reaction of ML in the microemulsion was initiated by AAPH, and the oxygen consumption rates during oxidation was monitored by the low throughput Clark electrode method and by the high throughput method using Oxygen Biosensor System (OBS) (*21*), a 96-well microplate coated with tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride in a silicone matrix at the bottom of the plate. The detailed description of the use of OBS was given in the literature (*22*). In an uninhibited reaction, the oxygen concentration decreases linearly until all of the oxygen is consumed. Oxygen (~200 μM) is the limiting reagent under the assay conditions as the methyl linoleate (10%) is in excess. The oxygen consumption rate was proportional to the square-root of AAPH concentration (**Figure 3A**), indicating that the uninhibited reaction kinetic followed the simple radical chain oxidation of fatty acid in the microemulsion system. Typical kinetic curves of noninhibited and inhibited oxidation were shown in **Figure 3**. In the presence of Trolox, the lag phase appeared as expected, and the net areas under the curve had a good linear response (R^2 = 0.99) to the concentration of Trolox in the range of 0−41.7 μM Trolox. With the ability to monitor 80 reactions in just 2 h, this assay can analyze hundreds of samples per day and thus greatly improves the efficiency. In addition, it provides a tool for rapid screening of both hydrophilic and lipophilic antioxidants in emulsions and is also matrix-interference free.

To verify the high throughput assay independently, we utilized the Clark oxygen electrode to monitor the inhibited oxidation reaction of the ML microemulsion. **Figure 3** shows the graphs of oxygen concentration versus time obtained for each concentration of Trolox. The net AUC and Trolox concentration has an excellent linear response, and the linear regression equation is very similar to that obtained from the microplate. This result suggests that the microplate approach is a sound method in monitoring oxygen concentration, and compared to the Clark electrode, the efficiency increases by nearly one hundred times. The %CV of 2.83 (n = 8) for the blank and accordingly the limit of detection (3σ) and limit of quantitation (10σ) are calculated to be 1.7 and 5.6 μMTE, respectively. The present assay approach provides a platform for the different emulsion systems needed for specific types of compositions and applications.

$ORAC_{FL}$ versus $ORAC_E$. Using the assay, we measured the $ORAC_E$ values of representative polyphenolic compounds listed in **Table 1**. Naringin is a flavanone found in grapefruit; catechin is the representative flavanol in tea and chocolate; chlorogenic acid is the major phenolic acid in coffee; quercetin is a flavone in apple; and baicalin is the major flavone found in *Scutellaria baicalenisis* Georgi, an important herbal medicine with anti-inflammatory properties. For a majority of the compounds, the antioxidant activity is lower than that of Trolox with quercetin as an exception. This is in sharp contrast to their $ORAC_{FL}$ values. Vitamin C is a fairly potent radical scavenger in aqueous systems, yet it becomes ineffective in microemulsions. Most noticeably, catechin and quercetin have very high antioxidant activity measured by the $ORAC_{FL}$ values, yet their performance is greatly compromised in the microemulsion systems. However, α-tocopherol exhibits higher antioxidant activity in microemulsions. The lipophilicity (log P) of these compounds were listed in **Table 1**. From the data, it is apparent that lipophilicity plays an important role in

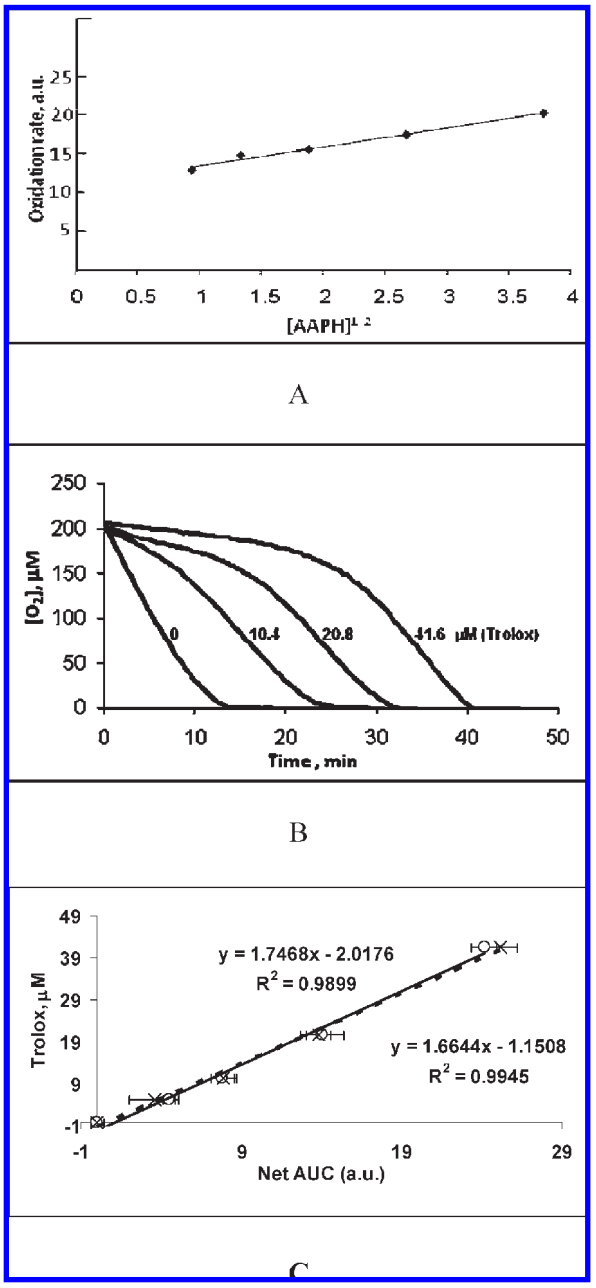

Figure 3. (**A**)The initial reaction rate of methyl linoleate and its dependence on AAPH concentration ($mM^{1/2}$). (**B**) Change of relative oxygen concentration in the absence and presence of Trolox (concentration labeled on the curve) at 37 °C in the presence of AAPH as the radical initiator for methyl linoleate oxidation in the microemulsion, monitored by an oxygen electrode. (**C**) The standard curves obtained by the 96-well microplate (y = 1.7468x − 2.0176, solid line, n = 3) and Clark oxygen electrode (y = 1.6644x − 1.1508, X, dotted line, n = 3).

antioxidant activity; although there are no linear correlations, the compounds (quercetin, Trolox, and α-tocopherol) with positive log P values have higher $ORAC_E$ values than those with negative log P. Taken together, we concluded that the high antioxidant activity in an emulsion system requires the optimal combination of lipophilicity and the chemical nature of the phenolic compounds. It is reasonable to assume that only the antioxidants in the methyl linoleate phase can act as radical chain breakers because the autoxidation of methyl linoleate takes place in the oil phase. Since the AAPH and the charged peroxyl radicals derived from it are highly hydrophilic, the antioxidants in the water phase act not as

Table 1. Antioxidant Capacity of Phenolic Compounds Measured by the $ORAC_E$ Assay

name	$ORAC_E$ value (μmolTE/μmol)	$ORAC_{FL}$ value (μmolTE/μmol)	log P^c
chlorogenic acid	0.42 ± 0.04	3.14 ± 0.19[a]	−0.97
baicalin	0.33 ± 0.02	1.71 ± 0.15[d]	−0.31
Trolox	1.00	1.00	3.01
catechin	0.20 ± 0.01	6.76 ± 0.22[a]	0.49
quercetin	1.54 ± 0.09	7.28 ± 0.22[a]	2.07
naringin	0.19 ± 0.02	1.30 ± 0.20[d]	−0.53
ascorbic acid	0.24 ± 0.01	0.95 ± 0.02[a]	−2.41
α-tocopherol	0.79 ± 0.05	0.50 ± 0.02[b]	11.4

[a] The values were taken from Ou et al. (*24*). [b] The values were taken from Huang et al. (*25*). [c] Calculated value. [d] Measured in-house.

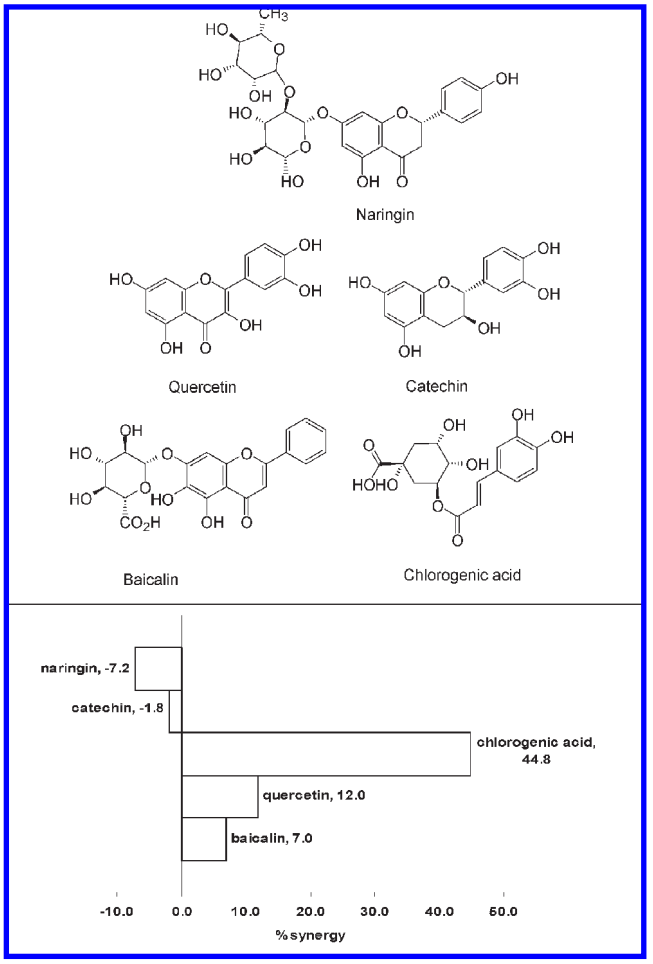

Figure 4. Structures of common water soluble antioxidants and their synergistic and antagonistic effects with α-tocopherol (CV < 12%) in microemulsions.

chain-breaking antioxidants, instead they scavenge the radical to prevent the radical chain initiation. It is foreseeable that the $ORAC_E$ values of compounds are also dependent on the radical initiators used. However, due to the limited availability of good lipophilic radical initiators, we were not able to confirm this at present.

Chemically, the $ORAC_{FL}$ assay and $ORAC_E$ assay have many parameters in common. Both assays use AAPH as free radical initiators and are run under the same pH, temperature, and standard (Trolox). In both assays, the linearity relationship between the net area under the curve and the antioxidant concentrations are excellent. The difference lies in the nature and concentration of the substrates. The $ORAC_{FL}$ assay utilizes fluorescein as the substrate and the probe, whereas in the $ORAC_E$ assay, the substrate is in large excess, and the probe is the oxygen sensor. $ORAC_{FL}$ requires the reaction media to be clear, whereas the $ORAC_E$ assay can tolerate cloudiness of the mixture because the probe is coated under the bottom of the plate and does not mix with the reaction substrate. For lipophilic samples, the $ORAC_{FL}$ assay requires the utilization of a solubility enhancer, randomly methylated cyclodextrin, whereas for $ORAC_E$ assay, there is no need to use a solubility enhancer because the microemulsion has two phases and can simultaneously measure the hydrophilic antioxidant and lipophilic antioxidant.

Understanding the synergistic effects between polyphenolic compounds with vitamin E is of great interest. The antioxidant capacity of a mixture of equal concentrations of α-tocopherol and the phenolic compounds was measured individually and also in an equal molar mixture in the microemulsion, and the percentage of synergy was calculated as follows (*23*):

$$100\%\{[ORAC_E \text{ value of the mixture} - (ORAC_E \text{ value of } \alpha\text{−tocopherol} + ORAC_E \text{ polyphenolic compound})]/ ORAC_E \text{ value of the mixture}\}$$

Surprisingly, there is no synergistic effect between vitamin C and vitamin E in our system. Naringin shows a significant antagonistic effect, whereas catechin has a negligible synergistic effect. Baicalin, chlorogenic acid, and quercetin exhibit synergistic effects with chlorogenic acid, which shows the strongest synergy at 44.8%. Since α-tocopherol is mainly in the oil phase, to regenerate the tocopheryl radical, the polyphenolic compound must be in oil phase or in the interface layer. This might explain the synergistic effect of quercetin as it is sufficiently lipophilic but is contradictory with the fact that the less lipophilic chlorogenic acid and baicalin have significant synergistic effects. One needs to take into account the reactivity between the tocopheryl radical and polyphenolic compounds. Nonetheless, the chlorogenic acid and its analogue are the major phenolic compounds found in coffee. Since coffee is one of the most consumed beverages, it would be worthwhile to investigate whether chlorogenic acid can regenerate α-tocopherol in vivo.

In summary, we report here the preparation and characterization of a microemulsion consisting of methyl linoleate as a model system for assaying antioxidant capacity in high throughput fashion using an oxygen biosensor system. The assay has a limit of detection and a limit of quantitation of 1.7 and 5.6 μMTE and a linearity range of 5.6 to 41.7 μMTE. The $ORAC_E$ assay allows convenient and rapid evaluation of antioxidant capacity in a heterogeneous system more relevant to food and cosmetic products where lipids are delivered in emulsion for enhanced availability of the active ingredients. This high throughput assay conveniently monitors the autoxidation process of lipid in microemulsion in the presence of a radical initiator and antioxidants. With the ability to perform 80 reactions in just 2 h, it greatly improves the efficiency as compared to the conventional methods. Our preliminary results show that lipophilic antioxidants exhibit potent antioxidant activity on AAPH induced methyl linoleate oxidation in the microemulsion system. The $ORAC_E$ assay provides a platform for studying the synergistic effect between α-tocopherol and water soluble antioxidants. Our results demonstrate that chlorogenic acid has a strong

synergist effect. This may have implications for the nutritional values of drinking coffee, which is rich in chlorogenic acid and its analogues. More detailed studies in terms of the kinetics of chlorogenic acid and α-tocopherol concentration changes are underway to provide insight into the synergism. A comparative study of antioxidant behavior in bulk oil and in emulsion is also underway and will be reported in due course.

ABBREVIATIONS USED

$ORAC_{FL}$, oxygen radical absorbance capacity using fluorescein as the probe; $ORAC_E$, oxygen radical absorbance capacity in a microemulsion system containing methyl linoleate as the substrate; AAPH, 2,2′-azobis-2-methyl-propanimidamide; AUC, area under the curve; ML, methyl linoleate; LHB; lipophilic and hydrophilic balance; TE, Trolox Equivalent.

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