

Electronic Supplementary Information (ESI)

Reactive oxygen species induced by plant essential oil for effective degradation of *p*-phenylenediamine

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1. Experiment

1.1 Materials

Tween 20, Tween 80, Tween 85, polyethylene glycol 400 (PEG 400), ethanol (EtOH), isopropyl alcohol (IPA), methanol (MeOH), sodium hydroxide (NaOH) and ascorbic acid (ASC) were purchased from Kelong Chemicals Co. (Chengdu, China). Triton X-100 and L-glutathione reduced (GSH) were purchased from Shanghai Aladdin Biochemical Technology Co. (Shanghai, China). Limonene, *p*-phenylenediamine (PPD) and potassium bromide (KBr) were purchased from Shanghai Macklin Biochemical Technology Co. (Shanghai, China). Ammonia solution (NH₃·H₂O) and hydrochloric acid (HCl) were purchased from Chuandong Chemical Co. (Chongqing, China). Orange peel essential oil (OPEO) was purchased from Flower Code (Jinan, China). Hair dye was purchased from Foshan Modern Health Products Co. (Foshan, China). Human hair and orange peel were obtained from the local supermarket. The experimental water was deionized water.

1.2 Apparatus

Particle size analyzer (NanoBrook Omni, Brookhaven Instruments, UK). Scanning electron microscope (SEM, Gemini 300, Zeiss, Germany). Transmission electron microscope (TEM, Tecnai G2 F20, FEI, USA). Gas chromatography-mass spectrometer (GC-MS, GCMS-TQ8040, Shimadzu, Japan). Fourier transform infrared spectrometer (FT-IR, Nicolet iS50, Thermo Fisher Technology Co., Ltd, USA). X-ray diffractometer

(XRD, PANalytical X' Pert Powder, Spectris Pte. Ltd, Netherlands). Thermal gravimetric analyzer (TGA, TGA2, Mettler-Toledo, Switzerland). High performance liquid chromatograph (HPLC, 1220 Infinity II, Agilent, USA). High performance liquid chromatograph-mass spectrometer (HPLC-MS, QE Plus, Thermo Fisher Technology Co., Ltd, USA).

1.3 Preparation of self-emulsifying orange peel essential oil (SE-OPEO)

1.3.1 Selection of surfactant and co-surfactant

Surfactant was selected from Tween 20, Tween 80, Tween 85 and Triton X-100, while co-surfactant was selected from EtOH, IPA and PEG 400. It is worth noting that all these compounds and OPEO can be soluble with each other. OPEO, surfactant and co-surfactant were mixed in fixed ratios 15/42.5/42.5 (w/w/w) to obtain SE-OPEO, and the total mass of SE-OPEO is 1 g. Afterwards SE-OPEO (1 g) was added to distilled water (100 mL) at room temperature under magnetic stirring, and observed the phenomenon of self-emulsifying. The self-emulsifying efficiency of SE-OPEO was visually assessed according to the grading standards.¹ Grade A: the appearance of emulsion is clear or slightly bluish, and it can form rapidly within 1 min; grade B: the appearance of emulsion is slightly less clear and bluish white, and the self-emulsifying time is the same as grade A; grade C: emulsion is opaque bright white liquid (similar in appearance to milk), and it can form within 2 min; grade D: the appearance of emulsion is greyish white and slightly oily, and the self-emulsifying time is longer than 2 min; grade E: the surface of emulsion has large oil droplets, it is

difficult to emulsify.

1.3.2 Construction of pseudo-ternary phase diagram

The surfactant and co-surfactant selected were Triton X-100 and IPA respectively, and both of them were used with OPEO as three phases to construct the pseudo-ternary phase diagram. The mass fraction of OPEO was set as 10%, 15%, 20%, 25% and 30% respectively, following which Triton X-100 and IPA were mixed in fixed ratios (1/9, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2 and 9/1, w/w) under the above mass fraction of OPEO to prepare SE-OPEO at all 45 combinations. The total mass of each SE-OPEO is 1 g. Afterwards SE-OPEO (1 g) was added to distilled water (100 mL) at room temperature under magnetic stirring. If it is possible to form a clear or slightly bluish emulsion eventually, the corresponding component proportion was recorded. Pseudo-ternary phase diagram was mapped by the software Origin 2018. According to the self-emulsifying region, the mass fraction range of each component was determined.

1.3.3 Optimization of SE-OPEO formulation

On the basis of pseudo-ternary phase diagram, the simplex lattice design method was used to optimize the formulation of SE-OPEO. The total proportion of OPEO, Triton X-100 and IPA was fixed as 100%. According to the results of pseudo-ternary phase diagram and the proportional constraint of simplex lattice design method, the proportion of each component was limited as OPEO: 10-25%, Triton X-100: 37.5-

52.5%, IPA: 37.5-52.5%. With average particle size and polydispersity (PDI) were selected as the indicators, SE-OPEO formulation was designed by Design Expert 8.0.6 software. The experimental design is shown in **Table S1**. OPEO, Triton X-100 and IPA were mixed according to the 14 kinds of SE-OPEO formulations in **Table S1**, and the total mass of SE-OPEO is 1 g. Afterwards SE-OPEO (1 g) was added to distilled water (100 mL) at room temperature under magnetic stirring, and then average particle size and PDI of emulsion were determined by particle size analyzer. After that, the experimental data were analyzed by Design Expert 8.0.6 software. The response equation was obtained by fitting the mathematical model, and then the response surface diagram and contour plot diagram were drawn according to the response equation. Subsequently, the optimal SE-OPEO formulation was predicted by Design Expert 8.0.6 software. With the minimum average particle size and minimum PDI were used as the limitations, the component proportion of optimal formulation and the corresponding predicted values of average particle size and PDI were forecasted. The SE-OPEO was prepared according to the optimal formulation, and then the experimental values of average particle size and PDI were determined. Finally, the experimental values were compared with the predicted values to reflect the predictive performance of the mathematical model.

1.4 Synthesis of biochar (BC) and BC loaded with SE/OPEO (BC/SE-OPEO)

The fresh orange peel was washed with distilled water and dried in an air-dry oven at 60 °C overnight. After that, the dried orange peel was smashed into powder by

the crusher and passed through a 100-mesh screen. BC was prepared by oxygen-limited pyrolysis method.² The process of pyrolysis is performed as shown below: the crucible with orange peel powder was placed in the muffle furnace, the pyrolysis temperature was increased to the final temperature (900, 1000 and 1100 °C) at a rate of 10 °C/min, afterwards the pyrolysis temperature was decreased to room temperature at a rate of 5 °C/min after maintaining for a while (2, 3 and 4 h). The yield of the gained BC was calculated by the following formula:

$$\text{yield (\%)} = m_2/m_1 \times 100 \quad (1)$$

where m_1 is quality of orange peel before pyrolysis, m_2 is quality of orange peel after pyrolysis. Subsequently, BC was washed with distilled water until the pH of distilled water was close to 7, and then BC was dried in an air-dry oven at 80 °C overnight. Eventually, the dried BC was passed through a 100-mesh screen to collect for later use.

BC/SE-OPEO was prepared by absorbing optimized SE-OPEO into BC, and the proportion of SE-OPEO and BC was fixed as 0.4/1 (w/w). In brief, 0.4 g SE-OPEO was gradually added into 1 g BC in a mortar with constant stirring by a pestle, yielding the powders with suitably greasy appearance and good flowability.

1.5 Characterization

1.5.1 Morphological analysis

SEM and TEM were used to explore the morphology of BC and BC/SE-OPEO.

1.5.2 GC-MS

GC-MS was used to qualitatively detect OPEO on the column of Rtx-WAX (30 m × 0.25 mm × 0.25 μm) with nitrogen flow rate of 1 mL/min. The temperature-rising program was performed as follows: 40-200°C, 30°C/min; 200°C, 5 min; 200-210°C, 2°C/min, 210-230°C, 30°C/min; 230°C, 10 min. The mass spectrum (EI) acquisition mode was set to Q3 Scan and the scanning range was set from 22 to 600 m/z.

1.5.3 FT-IR

FT-IR was utilized to detect the FT-IR spectra of OPEO, Triton X-100, IPA, blank SE-OPEO and SE-OPEO with the resolution of 4 cm⁻¹ and the wavelength range of 500–4000 cm⁻¹. KBr pellet method was used to prepare samples.

1.5.4 HPLC

HPLC was used to qualitatively detect limonene, OPEO, SE-OPEO, BC/SE-OPEO, blank SE-OPEO and blank BC/SE-OPEO samples on the chromatographic column of Agilent TC-C18 (250 mm × 4.6 mm × 5 μm) with a flow rate of 1 mL/min at 30 °C. The mobile phase was consisted of distilled water and MeOH (v/v, 15/85). The injection volume was acquired at 10 μL and the UV detector wavelength was set at 205 nm.

1.5.5 XRD and TGA

XRD was utilized to detect the crystals of the BC and BC/SE-OPEO over the range of angle from 5° to 90° (2θ) for 10 min. Under the atmosphere of nitrogen, TGA measurement of the materials was gained from 25 °C to 900 °C with a heating rate of 10 °C/min.

1.5.6 Flow property

The flow property of BC/SE-OPEO was investigated by detecting angle of repose (θ), Hausner's ratio (HR) and Carr's index (CI). The injection method was used to detect angle of repose.³ Specifically, BC/SE-OPEO was slowly poured through a fixed funnel to form a conical pile until the cone height did not change. Afterwards, the height (h) and radius (r) of the conical pile were measured, and the angle of repose was calculated according to the formula:

$$\tan \theta = h/r \quad (2)$$

Whereafter, the measurements of HR and CI were performed according to the former reported ways.⁴ BC/SE-OPEO was gently added into a 10 mL measuring cylinder until it reached the maximum calibration, and then the cylinder was tapped until the volume was constant. Afterwards the powder volume before and after tapping and powder weight were recorded. The ratio of the powder weight and powder volume before tapping is the bulk density (BD) and the ratio of the powder weight and powder volume after tapping is the tapped density (TD). HR and CI were

calculated by the following formulas:

$$HR = TD/BD \quad (3)$$

$$CI (\%) = (TD - BD)/TD \times 100 \quad (4)$$

1.6 Adsorption and degradation experiments

The effects of pyrolysis temperature, pyrolysis time, dosage, contact time, contact temperature and solution pH on PPD removal were investigated. The adsorption and degradation experiments were performed by contacting BC/SE-OPEO with 5 mL of 20 mg/L standard PPD aqueous solution. The above experiment was set at corresponding BC pyrolysis temperature (900, 1000 and 1100 °C), BC pyrolysis time (2, 3 and 4 h), BC/SE-OPEO dosage (10, 30, 50, 70, 90, 110 and 130 mg), contact time (30, 70, 110, 150, 190 and 230 min), contact temperature (20, 30, 40, 50 and 60 °C) and solution pH (3, 5, 7, 9 and 11). 0.1 M HCl or 0.1 M NaOH were used to adjust the pH of PPD aqueous solution. Single factor optimization method was used to optimize the parameters. Afterwards, 1 g/L ASC aqueous solution was added to the above samples to stop the reaction and the concentration of PPD in the filtrate was detected by HPLC. The detection was carried out on the chromatographic column of Agilent TC-C18 (250 mm × 4.6 mm × 5 μm) with a flow rate of 0.6 mL/min at 30 °C. The mobile phase was consisted of 0.002% NH₃·H₂O and MeOH (v/v, 95/5). The injection volume was acquired at 20 μL and the UV detector wavelength was set at 290 nm. Removal efficiency (RE, %) was calculated by the following formula:

$$RE = (C_0 - C_t)/C_0 \times 100 \quad (5)$$

where C_0 (mg/L) is the initial concentration of PPD aqueous solution and C_t (mg/L) is the concentration of PPD aqueous solution after adsorption and degradation.

1.7 Analysis of actual sample

Human hair was washed with distilled water to remove exogenous PPD. 2 g hair dye was applied to 0.4 g human hair for 30 min. Distilled water was used to clean the hair, afterwards hair dye sewage and dyed hair sample were obtained respectively.

5 mg ASC was added into 5 mL hair dye sewage (sample 1). 50 mg BC/SE-OPEO was added to 5 mL hair dye sewage and reacted for 110 min at 40 °C, afterwards ASC was added to the above solution to stop reaction (sample 2). 5 mL distilled water was added to 0.2 g dyed hair sample, afterwards the sample was treated for 30 min by ultrasonic machine to extract PPD, and then 5 mg ASC was added into the solution after removing the hair (sample 3). 50 mg BC/SE-OPEO was placed into 0.2 g dyed hair sample contained 5 mL distilled water, and reacted at 40 °C for 110 min. Subsequently, the treatment of dyed hair sample was consistent with the preparation of sample 3 (sample 4). The above four samples were filtrated with 0.22 μ m membrane filters. Finally, the concentration of PPD in the above four samples were detected by HPLC and the program setting was performed the same as that in section 1.6.

1.8 Scavenging experiments of ROS

0.1 mol/L GSH and ASC were separately added into PPD aqueous solution. Subsequently, OPEO was put into the aforementioned PPD solution respectively. Afterwards, the samples were filtrated with 0.22 μm membrane filters after the reaction was sufficient. The filtrates were detected by HPLC and the program setting was performed the same as that in section 1.6.

1.9 Degradation intermediates and pathways

OPEO was added into PPD aqueous solution to react for a while, and then the sample was filtrated with 0.22 μm membrane filters and detected instantly with HPLC-MS. The determination of the sample was carried out on a Hypersil GOLD™ column (100 mm \times 2.1mm \times 3 μm) with a flow rate of 0.3 mL/min at 30 °C. The flowing phase composed of 0.002% $\text{NH}_3\cdot\text{H}_2\text{O}$ and MeOH (v/v) was as follows: 0-20 min, 5/95-5/95; 20-23 min, 5/95-10/90; 23-38 min, 10/90-70/30; 38-40 min, 70/30-5/95; 40-45 min, 5/95-5/95. The mass spectrometric ion mode was set to ESI⁺ and ESI⁻, scanning range was set from 50 to 750 m/z, and the spray voltage was set to 3.5 KV for positive ion and 3.0 KV for negative ion.

2. Supporting Figures

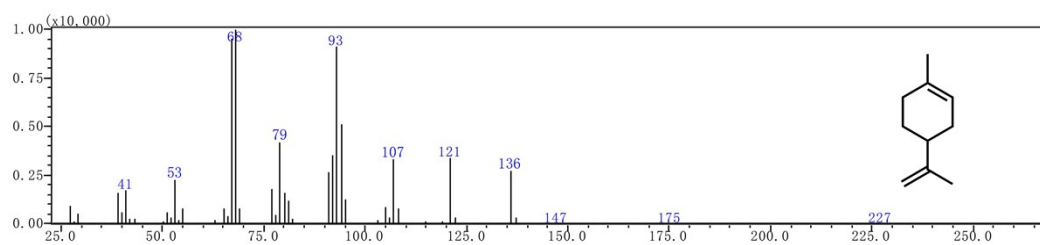


Fig. S1 Mass spectrum (EI) of limonene

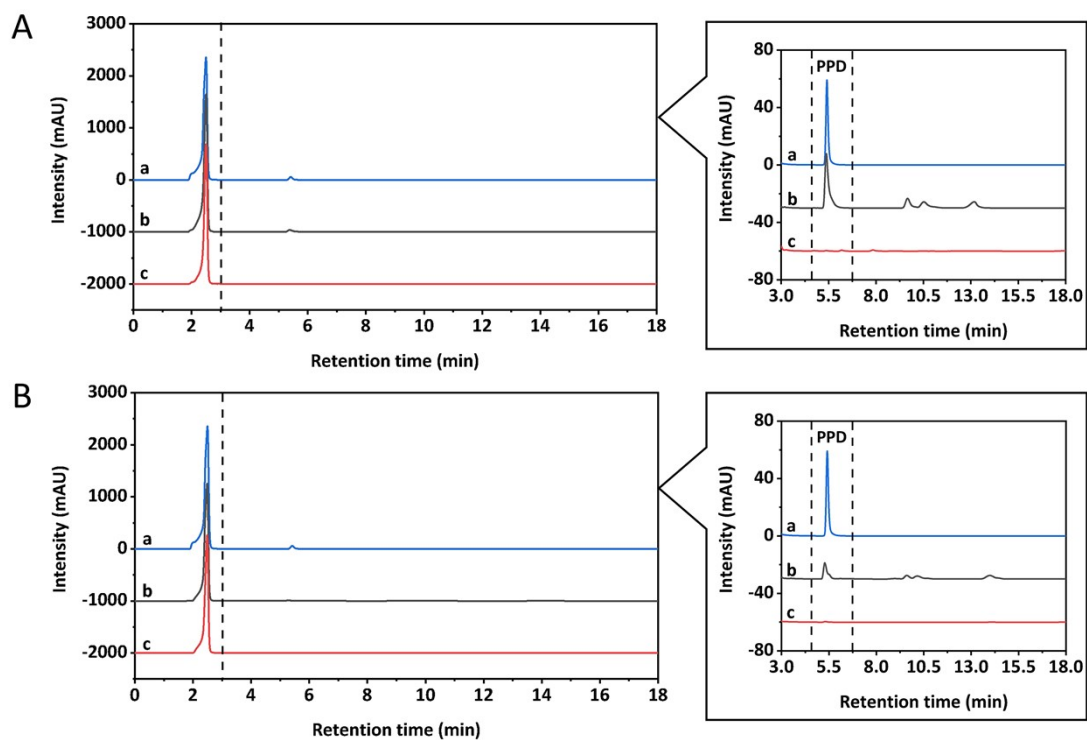


Fig. S2 (A) The overall HPLC chromatograms of PPD standard (a), hair dye sewage sample (b), hair dye sewage sample treated with BC/SE-OPEO (c); (B) The overall HPLC chromatograms of PPD standard (a), dyed hair extraction sample (b), dyed hair sample treated with BC/SE-OPEO (c).

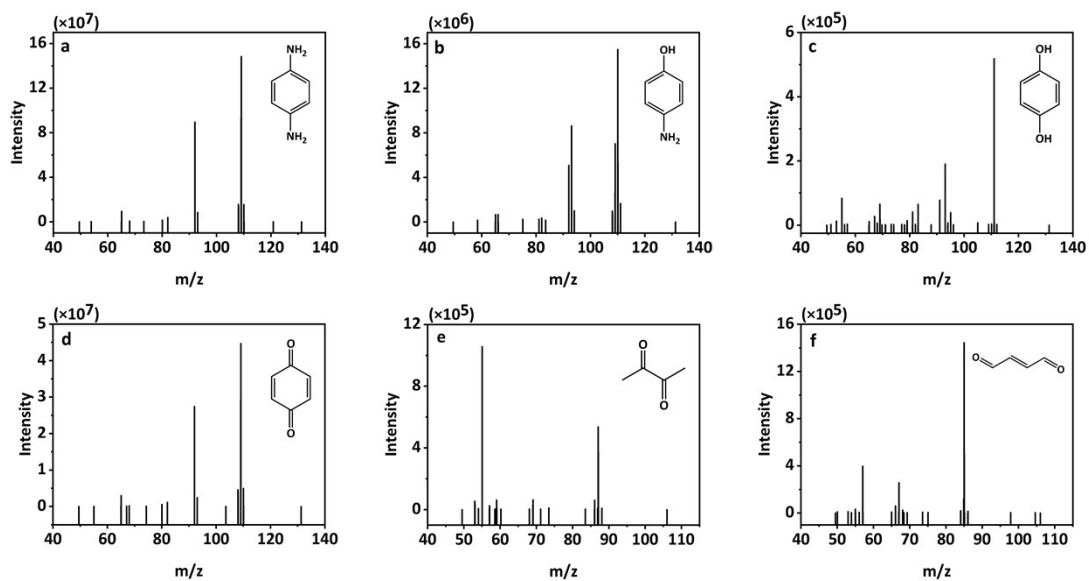


Fig. S3 Secondary mass spectrometry of *p*-phenylenediamine (a), *p*-aminophenol (b), hydroquinone (c), *p*-benzoquinone (d), 2,3-butanedione (e) and 1,4-butylene ketone (f).

3. Supporting Tables

Table S1 Experimental design and results of indicators

No.	A	B	C	Y ₁	Y ₂
	OPEO (%)	Triton X-100 (%)	IPA (%)	Average particle size (nm)	PDI
1	10.0	37.5	52.5	73.90	0.229
2	10.0	37.5	52.5	76.12	0.221
3	10.0	45.0	45.0	13.33	0.216
4	10.0	52.5	37.5	11.92	0.186
5	10.0	52.5	37.5	12.04	0.199
6	12.5	40.0	47.5	76.83	0.217
7	12.5	47.5	40.0	36.17	0.204
8	15.0	42.5	42.5	90.15	0.216
9	17.5	37.5	45.0	201.44	0.230
10	17.5	45.0	37.5	141.73	0.235
11	17.5	45.0	37.5	135.15	0.239
12	20.0	40.0	40.0	201.32	0.250
13	25.0	37.5	37.5	256.14	0.273
14	25.0	37.5	37.5	299.12	0.277

Table S2 GC-MS identification results of OPEO compositions

No.	T _R	Chemical formula	Molecular weight	Components
1	3.680	C ₁₀ H ₁₆	136	D-Limonene
2	3.988	C ₈ H ₁₆ O	128	Octanal
3	4.836	C ₁₀ H ₂₀ O	156	Decanal
4	4.960	C ₁₀ H ₁₈ O	154	Linalool
5	5.004	C ₈ H ₁₈ O	130	1-Octanol
6	5.044	C ₁₂ H ₂₀ O ₂	196	Linalyl acetate
7	5.272	C ₁₀ H ₂₀ O ₂	172	2-(4-Hydroxybutyl) cyclohexanol
8	5.332	C ₁₅ H ₂₄	204	(+)-epi-Bicyclosesquiphellandrene
9	5.368	C ₁₀ H ₁₆ O	152	(E)-p-2,8-Menthadien-1-ol
10	5.424	C ₉ H ₂₀ O	144	1-Nonanol
11	5.484	C ₁₅ H ₂₄	204	(E)-β-Farnesene
12	5.548	C ₁₀ H ₁₆ O	152	cis-p-Mentha-2,8-diene-1-ol
13	5.668	C ₁₀ H ₁₈ O	154	L-α-Terpineol
14	5.716	C ₁₂ H ₂₄ O	184	Dodecanal
15	5.868	C ₁₅ H ₂₄	204	Valencene
16	5.948	C ₁₀ H ₁₄ O	150	D-Carvone
17	6.004	C ₁₅ H ₂₄	204	Cadina-3,9-diene
18	6.076	C ₁₀ H ₁₈ O	154	Geraniol
19	6.216	C ₁₀ H ₁₄ O	150	Perilla aldehyde
20	6.292	C ₁₀ H ₁₆ O	152	cis-Carveol
21	6.748	C ₂₈ H ₅₆ O	408	Octacosanal
22	6.916	C ₁₅ H ₂₆ O	222	Cubebol
23	6.976	C ₁₀ H ₁₄ O	150	Piperitenone
24	7.224	C ₁₀ H ₁₆ O	152	2-(4-Methylenecyclohexyl)-2-propen-1-ol
25	7.308	C ₁₀ H ₁₆ O	152	Perilla alcohol
26	7.628	C ₈ H ₁₆ O ₂	144	Octanoic acid
27	7.700	C ₁₅ H ₂₆ O	222	Germacrene D-4-ol

28	7.844	$C_{15}H_{26}O$	222	Elemol
29	8.936	$C_{20}H_{32}$	272	m-Camphorene
30	9.316	$C_{15}H_{22}O$	218	2,6-Dimethyl-10-methylene-2,6,11-dodecatrienal
31	9.696	$C_{10}H_{18}O_2$	170	Limonene glycol
32	9.904	$C_{32}H_{66}$	450	Dotriacontane
33	10.648	$C_{15}H_{22}O$	218	α -Sinensal
34	14.108	$C_{15}H_{22}O$	218	Nootkatone
35	21.548	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid

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