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Electrochemical Characterization of Tea-derived Dissolved Organic Matter: Implications for Electron Shuttling

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Dissolved organic matter (DOM) is capable of taking part in biogeochemical electron transfer reactions due to its ability to facilitate redox reactions. Tea infusions are rich in DOM that show benefit impact on human health. In this study, electron transfer capacities (ETC) of tea-derived DOM (TDOM) were characterized with respect to the extent of fermentation. It appeared that the DOM originated from non-fermented and partially fermented tea had higher ETC values than those of fully fermentated tea. The electron acceptor capacity (EAC) values were ranged from 257~428 μmol_{e-} $(g{\cdot}C)^{-1}$ and the EDC values were in the range of 17~38 μmol_{e-} $(g{\cdot}C)^{-1}$ in the order: oolong tea > green tea > black tea > pu-erh. The electrochemical features of TDOM were proven to be correlated with its chemical structures. Element analysis, fourier-transform infrared (FTIR) and three-dimensional excitation/emission matrix fluorescence spectroscopy (3DEEM) revealed the abundance of aromatic and phenolic compounds in four tea samples, but their components differed in response to the manufacturing process. The fluorescence intensities of a peak at 370~390/460~470 nM (excitation (Ex)/emission (Em)) varied in the same trend as the ETC of all TDOM. Meanwhile, the same trend appeared in the TDOM -stimulated microbial reduction of iron(III) oxide, in which the TDOM acted as the electron shuttles to transfer electrons from the Fe(III)-reducing bacteria to the iron oxide. The results of the work provided potential biogeochemical implication of TDOM in natural environmental, which was the additional function of tea organic matters apart from benefiting human health.

Keywords: Electron transfer capacity, Tea leaf-derived dissolved organic matter, Fe(III) reduction, Electron shuttling

1. INTRODUCTION

Dissolved organic matter (DOM), a heterogeneous mixture of various organic compounds with different molecular sizes, structures, and functional properties, is widely distributed in the natural environment [1]. Numerous studies have proven that DOM plays a significant role in improving soil fertility, soil structure, and water-holding capacity and acts as a carbon sink in the global carbon cycle [2-4]. DOM also shows important functions in the transport and fate of heavy metals and organic pollutants due to its sorption and complexing properties [5,6]. In addition, DOM, rich in electrochemically active moieties, has biogeochemical redox properties by shuttling electrons between microorganisms and metal oxides (i.e. iron oxide, manganese oxide), organic pollutants, and so on [7,8]. In most cases, DOM has shown an electrochemical behavior similar to that of quinone model compounds (i.e. anthraquinone-2,6-disulfonate (AQDS), hydroquinone), suggesting that quinone-hydroquinone groups were the primary electrochemically active moieties in DOM [9]. Recently, the biogeochemical redox property of DOM was quantificationally evaluated by its electron transfer capacity (ETC) including electron accepting capacity (EAC) and electron donating capacity (EDC) [10,11]. The ETC of DOM were found to be source- and structure-dependent [12].

DOM in the natural environment is derived primarily from plant and microbial residues. Tea is one of the most widely consumed beverages in the world with a global production of about 3 million tons per year [13]. Once used, tea residues are usually dumped and go directly into the environment. Worldwide, the total area of tea plantations, usually located in tropical and subtropical regions, is about 2.4 million ha [14]. Therefore, tea leaves are important fresh organic matter in these regions, and defoliation can increase the organic carbon content in soil and water. Tea is classified as green, Oolong, white or black according to the processing conditions after harvested. Tea-derived solution contains nearly 500 components, mainly belong to eight categories: polyphenols, caffeine, amino acid, pigments, vitamins, esters polysaccharides, minerals and aromatic substances. The polyphenols include catechins and its complex oxidation products: theaflavins (TF), thearubigins (TR) and theabrownins (TB). The polyphenols are the most abundant and biologically active group of tea. Many biological functions of the polyphenols in teas have been previously studied, mainly including antioxidative activities, antimutagenic effects, anticarcinogenic effects, and nitrosation inhibition [15-17]. In the meanwhile, polyphenols are highly electrochemically active due to the abundance of electrochemically active catechol moieties [18]. Nurmi and Tratnyek [19] described that polyphenol compounds were important redox couples that could mediate the iron or manganese cycling. However, very little attention has been paid to the biogeochemical redox properties of tea-derived polyphenols or other tea-derived organic matter for stimulating the extracellular electron transfer in various environments.

The aim of this work was to explore the redox properties of tea-derived dissolved organic matter (TDOM), specifically its ETC for shuttling electrons between microorganisms and solid electron acceptors. As mentioned above, the ETC of DOM was related to its structure, whereas tea processing can change the structure and content of phenolic compounds and other organic matter. Therefore, TDOM derived from four types of teas with different manufacturing processes, including Oolong, green, black and Pu-erh tea, were tested in this study. The ETC of the four TDOM was measured and compared. The structure characterizations of all the TDOM were analyzed and correlated with their ETC. Iron reduction behavior of an iron-reducing microorganism was investigated in the presence of TDOM, and iron reduction rates were compared.

2. EXPERIMENTAL

2.1 Extraction of Dissolved Organic Matter from Teas (TDOM)

TDOM was extracted as previously reported [20]. All tea samples were purchased from the local market in Guangzhou city, China. The extraction process was as follows: TDOM was extracted with double-distilled water from the tea samples with a solid/boiling-water ratio of 1:10 (w/v, dry weight basis). Then the suspension was filtered through a 0.45-μm sterile membrane (GN-6 Metrice, Gelman Sciences, Ann Arbor, MI, USA). The above steps were repeated twice with the solid/boiling water ratio in the 3rd time being 1:5 (w/v, dry weight basis) instead of 1:10. The extracted TDOM in the filtrates was combined and stored in a sealed bottle at 4°C before use.

2.2 Electrochemical Measurements

Electrochemical measurements were performed using an electrochemistry workstation CHI660D (*Chenhua* Co. Ltd, Shanghai, *China*) with a conventional three-electrode cell at ambient temperature. The chronoamperometry (CA) measurements were performed to evaluate the EDC and EAC of TDOM in a N_2-saturated phosphate buffer solution (pH=7.0) at applied potentials under stirring as suggested by Yuan et al. [11]. A graphite plate with a projected surface area of 17.5 cm^2 was used as the working electrode, and Pt grid and Ag/AgCl electrode as the counter and reference electrode, respectively. The reversibility of electron transfer to and from the TDOM was measured by appling switchable potentials to the working electrode in the electrochemical system with the multiple-step potential technique.

2.3 Analytical Techniques

Fourier transform infrared spectroscopy (FTIR) and three-dimensional excitation/emission matrix (3DEEM) fluorescence spectroscopy were used to determine the functional groups in the extracted TDOM. Infrared absorption spectra were recorded on a Hitachi EPI Infrared Spectrophotometer using the KBr disk method with a solid/solid ratio of 1:50(w/w, dry weight basis). The 3DEEM spectra of DOM were recorded with a fluorescence spectrophotometer (Hitachi, Model F-4600) equipped with a 150 W Xenon arc lamp as the excitation source. A PMT voltage of 700V and an automatic response time were applied. Spectra were recorded using 5 nm excitation and 10 nm emission slits. Wavelengths were set from 280 to 510 nm for excitation, and from 320 to 540 nm for emission. Sigma Plot software was used to obtain the 3DEEM spectra. The total organic carbon (TOC) of the samples was measured with a TOC digital reactor block (DRB200, HACH, USA) equipped with a spectrophotometer (DR2700, HACH, USA). The relative ratios of C, H, and N atoms in the TDOM were determined using a CHNO/S analyzer (Perkin Elmer 2400, Shelton, Connecticut, USA).

2.4 Electron Shuttling Experiments

The capability of TDOM in shuttling electrons between iron-reducing microorganism and insoluble Fe(III) oxide was evaluated by monitoring changes in Fe(II) concentration as previously suggested by Li et al. [21]. In this study, iron-reducing bacterium *Shewanella putrefaciens* 200 (SP200) was used in the microbial Fe(III) reduction experiments. The strain was grown aerobically in glucose-containing Luria Broth (LB) at 30 °C on a shaker. After 48 h, cells were harvested by centrifugation at 8,000 r·min^{-1} for 10 min. The pellets were washed three times and resuspended in a sterile mineral salts medium (MSM) for future use. For the Fe(III) reduction tests, each bottle contained 1 × 10^{7} cells mL^{-1}, 20 mL MSM, 10 mM glucose (electron donor) and 25 mM α-FeOOH (electron acceptor) with or without TDOM (100 mg C /L, control).

3. RESULTS AND DISCUSSION

3.1 Electrochemical Characteristics of TDOM

It has already been known that tea-derived solutions contain large amounts of electrochemically active species, which provides an opportunity for the application of various electrochemical techniques to evaluate their characteristics. Here, ETC of TDOM was first evaluated with electrochemical methods.

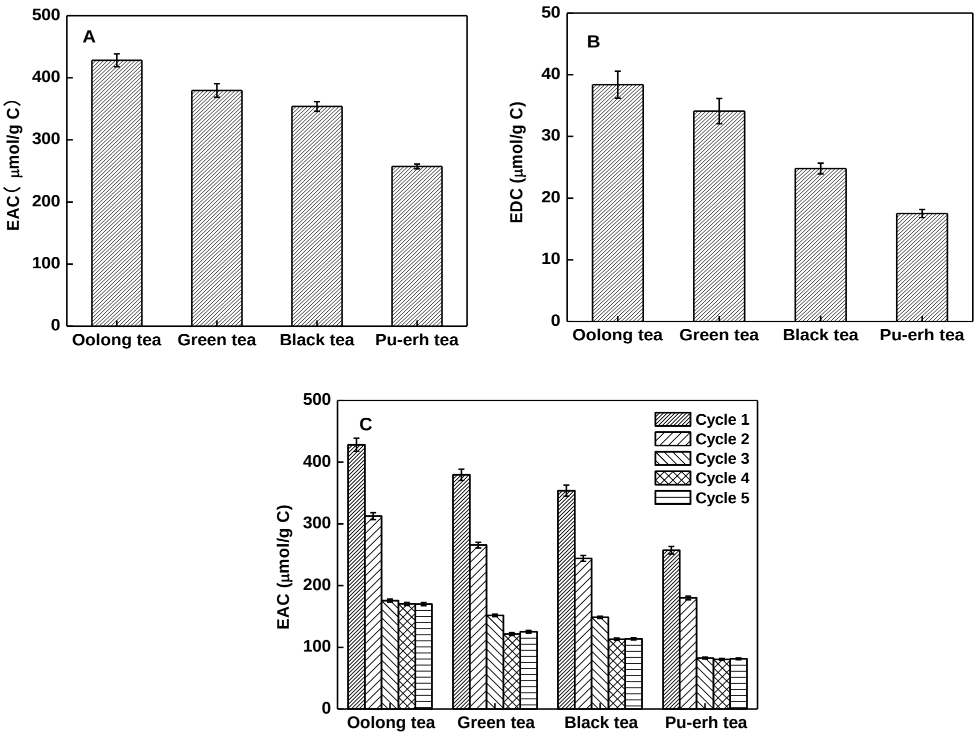

Figure 1. Electron-accepting capacities of the TDOM (A); Electron-donating capacities of the TDOM (B); Five successive measurements of the EAC of the TDOM at a potential of -0.6 V (The reduced TDOM was reoxidated at a potential of +0.5 V (vs. Ag/AgCl)) (C)

The measured EAC of TDOM was ranged from 257.2 to 428.2 μmol$_{e-}$ (gC)$^{-1}$ and the EDC was in the range of 17.5~38.4 μmol$_{e-}$ (gC)$^{-1}$ (Fig. 1A and B). Note that the EDC was far smaller than the EAC, which was probably due to the fact that most electrochemical species in tea solutions were fully oxidized when exposed to the air. The EAC was different among the four TDOM samples. The DOM derived from Oolong tea had the highest EAC while that from Pu-erh tea had the lowest. The results suggested that the four types of DOM contained different electrochemical components probably due to the different tea processing methods. It was revealed that green and Oolong teas were dominated by epigallocatechin gallate while black tea was dominated by theaflavin, leading to their different electrochemical activity [18]. To mediate electron transfer in natural environments, DOM needs to be capable of reversibly accepting and donating electrons, which is of particular interest in redox-dynamics studies. However, the reversibility of TDOM in successive reduction and oxidation cycles was rarely revealed. Herein, we investigated the reversible electron transfer of TDOM in terms of electron reversible rate (ERR) by applying two different potentials. The TDOM was first reduced at a potential of -0.6 V (vs. Ag/AgCl) and subsequently reoxidized at a potential of +0.5 V (vs. Ag/AgCl) in five full cycles. As shown in Fig. 1C, the measured EAC decreased greatly from the second cycle on, demonstrating that most functional moieties contained in TDOM could not reversibly accept and donate electrons. The ERRs were in the range of 41.3~48.3%, depending on the tea processing method.

Table 1. Main element contents and ratios of the TDOM

Sample	C%	N%	H%	C/N	C/H
Oolong tea	45.95	3.18	5.25	14.45	8.68
Green tea	40.73	3.09	5.83	13.18	6.98
Black tea	39.91	5.35	5.54	7.46	7.20
Pu-erh tea	39.61	6.53	5.15	6.07	7.68

3.2 Chemical Characteristics of TDOM

Differences in tea plant varieties, environmental effects and tea processing methods are the factors affecting the chemical composition of tea leaves, subsequently influences the ETC of TDOM. Element analyses, FTIR and fluorescent spectroscopes were employed to investigate TDOM’s chemical structures. Carbon, nitrogen, and hydrogen contents, along with ratios of C/N and C/H, were provided in Table 1. Differences in the element contents indicated different chemical compositions of the four TDOM. C/H ratio was believed to be associated with aromatic frameworks and portions of aliphatic functional groups [22]. The low ratio of C/H in the green tea suggested a high level of aliphatic carbon and a low level of unsaturated carbon [23]. It has been well known that the aerobic tea fermentation process is basically a series of enzymatic reactions where tea catechins are oxidized and polymerized to form polyphenols such as theasinesin, TF, TR and TB. Black and Pu-erh teas had higher levels of nitrogen content, suggesting that these two teas contain a higher proportion of functional nitrogen-containing moieties [24,25].

Further structure information of TDOM was obtained from the FTIR spectra. As shown in Fig. 2, all of the four TDOM had similar FTIR spectra. Absorption bands were identified according to published results [26,27]. All samples featured a broad band at 3,270 cm^{-1}, which was attributed to O-H stretching from the presence of phenols, and N-H stretching from amines and/or amides. The absorption band around 1,695 cm^{-1} indicated that C=C or C=O bond was present in all samples. The strong absorption band around 1,634 cm^{-1} was associated with the O-H bending in phenol functional groups and possible skeletal aromatic absorption. All the samples had peaks at around 1452 cm^{-1} and 873 cm^{-1}, which indicated C-H deformation of aliphatic groups and C-H bending of the substituted aromatic groups, respectively. Fluorescence spectra also revealed different compositions in the four TDOM. As shown in Fig. 3, two fluorescence peaks were identified from the 3DEEM fluorescence spectra, indicating two groups of fluorophores were present in the TDOM. Peak A at 370-390/460-470 nM (excitation (Ex)/emission (Em)) originated from flavonoids and catechins while peak B at 500/510-520 (Ex/Em) indicated the presence of phenolic compounds, such as theaflavins and thearubigins [28]. The different position of peaks on the spectra suggested different composition of the DOM. Catechins are the most abundant group of compounds in fresh tea and are oxidized by polyphenol oxidase during the fermentation process of black and Pu-erh teas. Thus, low fluorescence intensities of peak A were observed for black and Pu-erh teas. Although oolong tea is semi-fermented, a very high fluorescence intensity of peak A was still observed. Yen and Chen [29] suggested that the amount of caffeine in oolong tea extracts could be greater than that in the other three teas since caffeine content in tea extracts was found to be more remarkably affected by tea plant variety and cultivation conditions than tea processing methods. Therefore, the high fluorescence intensity of peak A for Oolong TDOM was possibly due to the high contents of caffeine or other fluorophores in it. The intensity of peak A (Table 2) was in the order of: Oolong tea (8.9) > green tea (4.3) > black tea (1.5) > Pu-erh (1.0). The ETC, including EAC and EDC, showed the same order. TDOM did not show a similar order in peak B intensity as in their ETC, indicating the complexity of phenonic compounds. Nevertheless, the higher fluorescence intensities of Oolong and green tea extracts than those of black and Pu-erh tea extracts suggested a higher content of phenolic compounds in non-fermented and semi-fermented teas, which was consistent with the findings by Yen and Chen [29].

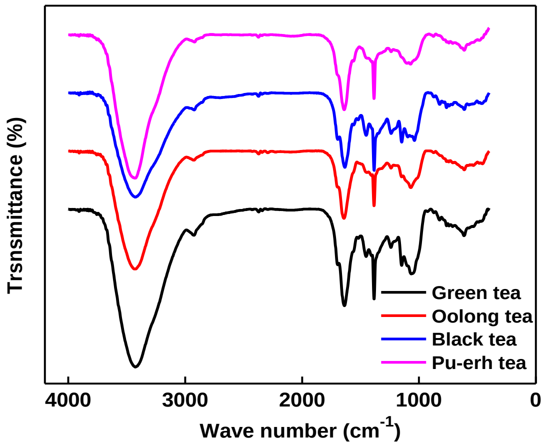

Figure 2. FTIR spectra of the TDOM

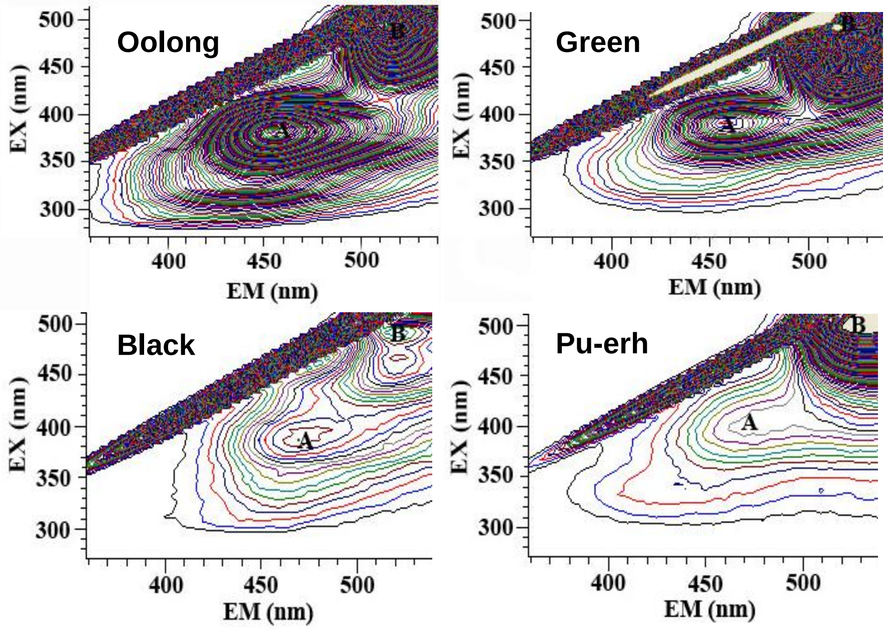

Figure 3. DEEM fluorescence spectra of the TDOM (Intensities are in Raman units)

Table 2. Chemical, spectra and electrochemical properties of the TDOM

Sample	pH	Fluorescence peak intensity		EAC μmol_{e-} $(gC)^{-1}$	EDC $\mu mol_{e-}(gC)^{-1}$	ERR (%)
		Peak A	Peak B			
Oolong tea	5.7	8.9	14.7	428.2	38.4	48.3
Green tea	5.6	4.3	54.0	379.5	34.1	43.7
Black tea	5.5	1.5	2.7	353.8	24.8	43.8
Pu-erh tea	4.8	1.0	11.7	257.2	17.5	41.3

3.3 Ferric Oxide Reduction Mediated by TDOM

In natural environments, DOM plays an important role in microbial activity, pollutant degradation, and metal mobility by shuttling electrons from donors to acceptors [30-32]. However, TDOM capability of stimulating electron transfer from bacteria to extracellular electron acceptors has not been probed. As shown in Fig. 4, Fe(III) reduction by *Shewanella putrefaciens* was accelerated in the presence of TDOM. After 25 days, no Fe(II) was detected in the abiotic control without glucose and only 0.09 mM Fe(II) was formed in the biotic control without any electron shuttles. In contrast, total Fe(II) concentration reached 0.71, 0.80, 0.87, 0.97 and 1.16 mM in the presence of such electron shuttles as AQDS and various TDOM. The results suggested that TDOM could function as electron transfer mediators to stimulate microbial Fe(III) reduction just as AQDS can. Fe(II) produced ranked in the same order as the ETC and ERR of TDOM: Oolong tea > green tea > black tea > Pu-erh tea (Table 2), which suggested that those redox active moieties might be responsible for shuttling electrons between bacteria and solid iron species.

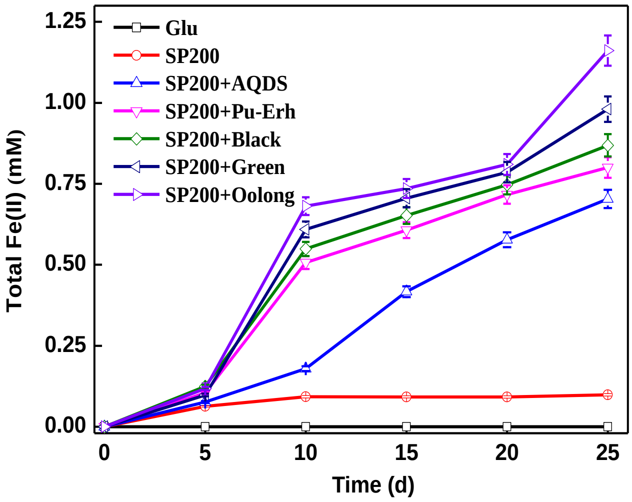

Figure 4. The production of total Fe(II) from the reduction of 25 mM α-FeOOH by *Shewanella putrefaciens* 200 using 10 mM glucose as the electron donor. Error bars represent SD of the mean (n = 3).

3.4 Environmental Implications

Tea is one of the most widely consumed beverages in the world and its health effects have been widely explored. Tea residues contain large amount of organic matter. After consumption, tea residues are usually discarded directly into the environment, which leads to possible accumulation of tea organic matter in the environments. The taste and health benefits of tea have been mainly attributed to the presence of polyphenols in it. These polyphenols's role as antioxidant and their biological activities have been widely studied. Recently, polyphenols as important components of humic-like substances in the environment are known to play an important role in the decontamination of heavy metals and organic pollutants by acting as electron shuttlers [6,30-33]. The results from this work imply that organic matters in tea have the same function as the polyphenols in natural DOM of stimulating extracellular electron transfer. Pesticide application is inevitable in tea production, which is introducing pesticide residues to tea extracts as well as to the soil. However, the presence of TDOM in the field possibly stimulates the microbial degradation of these pesticide residues by shuttling electrons between pollutants and microorganisms. Therefore, TDOM-based bioremediation techniques can be developed for pesticide-contaminated tea fields.

4. CONCLUSION

The results of electrochemical analysis showed that tea extracts were rich in organic matters capable of accepting or donating electrons. Fermentation after harvest had significant effects on the ETC of TDOM. The DOM extracted from semi-fermented Oolong tea tended to have the highest ETC while that from Pu-erh tea had the smallest ETC. Chemical analyses revealed that the main components of TDOM, which were possibly related to the ETC, varied with tea processing methods, Additionally, the DOM from Oolong tea had the strongest stimulating effects on microbial Fe(III) reduction compared to that from the other three teas, suggesting that the DOM from Oolong tea had the highest ETC or were the most efficient in transferring electrons between microorganism and Fe(III) oxides. The results provide an opportunity for probing the additional functions of tea organic matters in the environment.

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