ELSEVIER

Food Chemistry 80 (2003) 115–118

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Chemiluminescence determination of free radical scavenging abilities of 'tea pigments' and comparison with 'tea polyphenols'

Zhao Yaping[a,*], Yu Wenli[a], Wang Dapu[a], Liang Xiaofeng[b], Hu Tianxi[b]

[a]*School of Chemistry and Chemical Technology, Shanghai Jiao Tong University, Shanghai, 200240, PR China*
[b]*Institute of Biological Medicine, Shanghai Jiaoda Only Co. Ltd., Shanghai, 200030, PR China*

Received 8 January 2002; received in revised form 14 May 2002; accepted 14 May 2002

Abstract

The objectives of this paper were to determine the free radical scavenging abilities of 'tea pigments' towards superoxide radical anion ($O_2^{-\bullet}$) and hydroxyl radical ($OH^{\bullet}$), and to make a comparison with 'tea polyphenols' using a chemiluminescence technique. $O_2^{-\bullet}$ and $OH^{\bullet}$ were generated from pyrogallol autoxidation and Fenton-type reaction, respectively. 'Tea pigments' could scavenge $O_2^{-\bullet}$ and $OH^{\bullet}$ at EC_{50} values of 0.08 and 0.003 mg/ml, respectively, and the kinetics of the scavenging reactions varied with the concentration, suggesting the operation of different mechanisms. In addition, the free radical scavenging abilities and kinetics of 'tea pigments' were similar to those of 'tea polyphenols'.

Keywords: Antioxidant; 'Tea pigments'; Superoxide radical anion; Hydroxyl radical; Chemiluminescence

1. Introduction

Tea, the most ancient but popular beverage worldwide, has been suggested to be beneficial in the prevention of many human chronic diseases, such as cancer and cardiovascular diseases, and its benefits are partly ascribed to its antioxidant components, such as 'tea polyphenols' (Hara, 2000). 'Tea polyphenols', which are colourless, traditionally have been known to be mainly composed of four catechins including (−)-epicatechin (EC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), and epigallocatechin (EGC). Recently another group of polyphenolic compounds, which are found in black tea and are formed at the fermentation stage of black tea manufacture (Xiao, Zhong, Xiao, & Li, 1998) have aroused interest. These are: catechin dimer, trimer or multipolymer (Nursten, 1997). Since they can give a yellow or dark brown colour, they have the general name, 'tea pigments' in order to distinguish them from the traditional 'tea polyphenols'. In some recent animal and clinical studies, 'tea pigments' exhibited a significant role in treating hypertension, decreasing blood sugar, preventing cancer and atherosclerosis (Morse, Kresty, Steele, Kellof, Boone, Balentine et al., 1997; Ye, 1997). Also, 'tea pigments' can increase superoxide dismutase (SOD) activity and decrease lipid peroxidation levels in patients with coronary heart disease, and reduce oxidative damage by free radicals in mice and in guinea pigs (Li, Han, & Wang, 1998; Ren, Zheng, & Xu, 1998). However, so far, no evidence has been produced to show the direct free radical scavenging abilities of 'tea pigments'.

In previous work, we established free radical chemiluminescence models to evaluate the free radical scavenging abilities of antioxidants (Yu, Zhao, Xue, Jin, & Wang, 2001). In the present paper, we evaluate the free radical scavenging abilities of 'tea pigments' towards $O_2^{-\bullet}$ and $OH^{\bullet}$ using these chemiluminescence models.

2. Materials and methods

2.1. Chemicals

'Tea polyphenols' and 'tea pigments', black tea extracts, were purchased from Hai Nang Qun Li Medical

* Corresponding author. Tel.: +86-21-547-42814; fax: +86-21-547-41297.
E-mail address: ypzhao@mail1.sjtu.edu.cn (Z. Yaping).

0308-8146/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0308-8146(02)00241-8

Co. Ltd. (Hai Nan, China). They were standardised commodities. Their HPLC spectra are shown in Fig. 1. Four catechin standard samples of EGCG, EC, EGC and ECG were purchased from Hang Zhou Tea Factory (Zhejiang, China). Pyrogallol was from Beijing Chemical Co. Ltd (Beijing, China). Luminol and 1,10-phenanthroline were from Sigma Chemical Co. HCI, H_2O_2 and CuCl were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Carbonic acid buffered saline solution (CBSS, pH = 10.2) and triple distilled water were prepared in our laboratory.

2.2. Chemiluminescence technique

The free radical scavenging abilities of tea pigments were assessed on an SH-G biochemistry chemiluminescence meter (BCM) (Shanghai Measurement Equipment Factory, Shanghai, China). The BCM is composed of three parts: an automatically rotating sample support, in which 12 sample cells (glass tubes, diameter = 10 mm, height = 20 mm) can be placed, a chemiluminescence monitor and a data processor. Each sample cell can rotate and cross the monitor at a set time interval according to a self-set programme. When testing, the chemiluminescence intensity (CL) of a reaction system can be recorded in the data processor at the set time interval.

For one $O_2^{-\bullet}$ assay (Yu et al., 2001), $O_2^{-\bullet}$ was generated by pyrogallol autoxidation. The reaction mixture contained 50 μl pyrogallol (1×10^{-3} M), 700 μl CBSS (pH = 10.2), 20 μl luminol (1×10^{-3} M). A sample cell loaded with the mixture was first placed in the BCM. When the cell crossed the monitor, a known concentration of the sample was injected into the cell in situ. The CL was simultaneously recorded in the processor and simultaneously was recorded once every 6 s (CBSS replaced the sample in the control). The scavenging rate was obtained according to the formula: Scavenging rate (%) = (CL(control)−CL(sample)) × 100/CL(control).

For another $OH^{\bullet}$ assay (Yu et al., 2001), $OH^{\bullet}$ was generated by a Fenton-type reaction. The reaction mixture included 20 μl $FeCI_2$ (1×10^3 M), 3 μl 1,10- phenanthroline (1×10^3 M), 800 μl CBSS and 50 μl H_2O_2 (0.6%). The testing procedure and scavenging rate formula were similar to those for the first $O_2^{-\bullet}$ assay.

2.3. HPLC analysis of 'tea polyphenols 'and 'tea pigments'

'Tea polyphenols' and 'tea pigments' were assessed on a Waters 2690 HPLC using a 5 μm particle size Hypersil C18 column (4.6 × 200 mm) (Chrompack, USA). The flow phase was methanol and the flow rate was 1 ml/min. The elution peaks were detected at 273 nm.

2.4. Statistical analysis

Chemiluminescence data were processed using origin 6.0 software (Microcal Software, Inc., Northampton, MA, USA). The experiments were repeated three times.

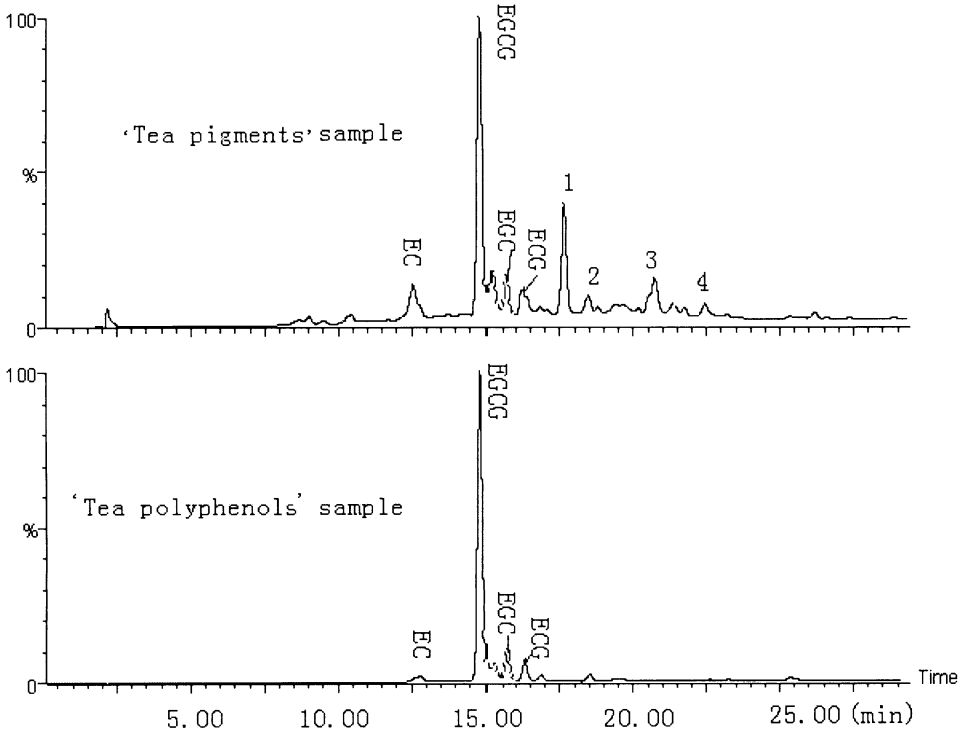

Fig. 1. HPLC of 'tea pigments' sample and 'tea polyphenols' samples 1, 2, 3, 4 are catechin dimer or multipolymer.

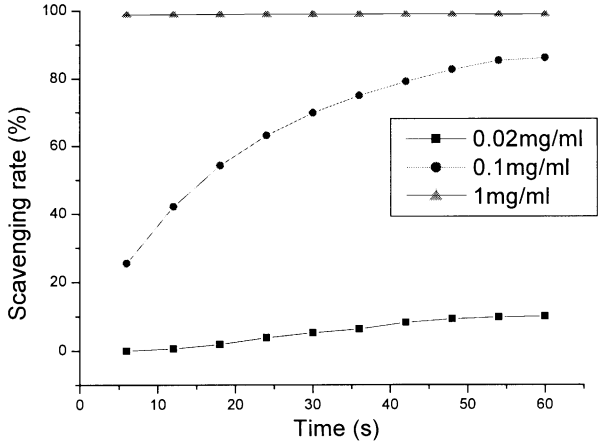

Fig. 2. Time-dependent scavenging effect of various concentrations of 'tea pigments' on superoxide radical anions.

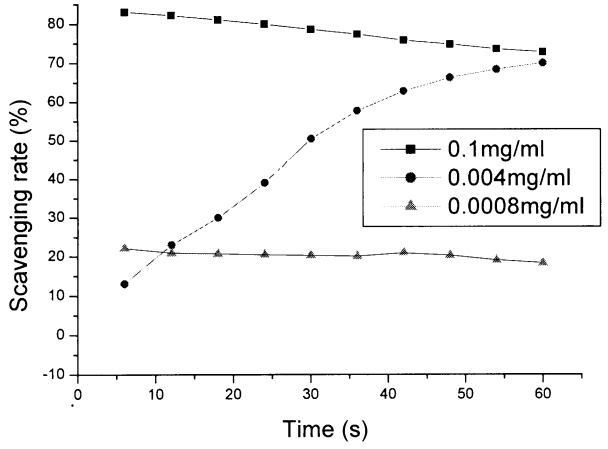

Fig. 3. Time-dependent scavenging effect of various concentrations of 'tea pigments' on hydroxyl radicals.

3. Results and discussions

3.1. Identification of 'tea polyphenols 'and 'tea pigments'

Fig. 1 shows HPLC spectra of 'tea polyphenols' and 'tea pigments'. In the HPLC spectrum of 'tea polyphenols', EGCG, EC, EGC, and ECG have been identified according to their own standard spectrum. EGCG was the main component while EC, EGC and ECG accounted for only a small proportion. In the HPLC spectrum of 'tea pigments', four peaks (peak 1, 2, 3, 4) appeared, except for the peaks of EGCG, EC, EGC, and ECG, which were ascribed to the oxidative and/or polymeric products of catechins occurring as catechin dimer, trimer, or multipolymer.

3.2. Interaction of 'tea pigments' with $O_2^{-\bullet}$

'Tea pigments' scavenged $O_2^{-\bullet}$ in a dose-dependent fashion (scavenging rate was 99.0% at 1 mg/ml, but not higher than 10% at 0.02 mg/ml) (Fig. 2). In addition, the time course of the scavenging rate varied: the rate reached 99% from the beginning of reaction and maintained this level until 60 s (the endpoint of the test) at 1 mg/mI, but rose at the start and then tended to a plateau (85% or so) at 0.1 mg/ml, and maintained a low level (not higher than 10%) at 0.02 mg/ml. The different kinetics suggested the presence of different scavenging mechanisms at different concentrations.

3.3. Interaction of 'tea pigments' with $OH^{\bullet}$

'Tea pigments' scavenged $OH^{\bullet}$ also in a dose-dependent fashion (scavenging rate was 80.0% at 0.1 mg/ml, but only 20% or so at 0.0008 mg/ml) (Fig. 3). The time course of scavenging rate also varied: the rate reached 80% from the beginning of reaction and then dropped

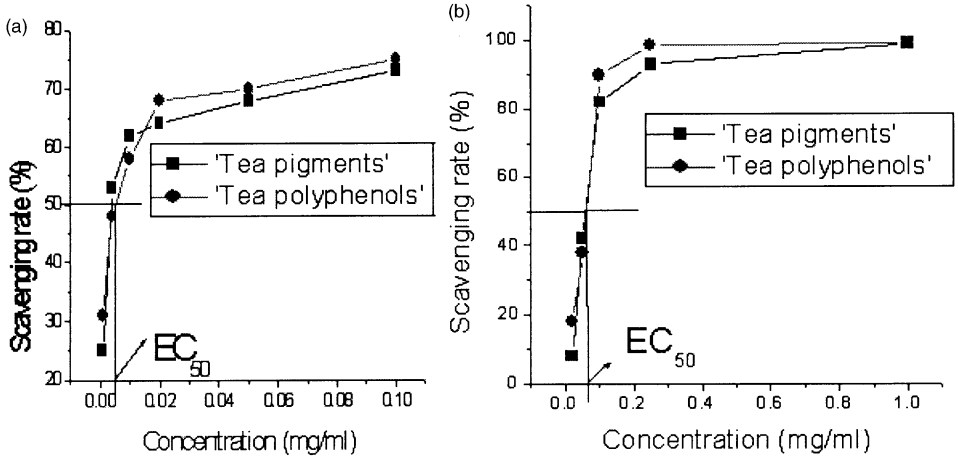

Fig 4. Variation of free radical scavenging rate with the concentration of 'tea pigments' and 'tea polyphenols': (A) $OH^{\bullet}$ (B) $O_2^{-\bullet}$.

Table 1
EC_{50} values of 'tea pigments' and 'tea polyphenols' towards $O_2^{-\bullet}$ and $OH^{\bullet}$

	'Tea pigments'	'Tea polyphenols'
$OH^{\bullet}$ (mg/ml)	0.003	0.004
$O_2^{-\bullet}$ (mg/ml)	0.08	0.08

slightly with time at 0.1 mg/ml, but rose at the start and then tended to a plateau (70% or so) at 0.004 mg/ml, and maintained at a low level (20% or so) at 0.0008 mg/ml.

3.4. Comparison between 'tea pigments' and 'tea polyphenols'

Comparing 'tea pigments' with 'tea polyphenols' showed that they scavenged $O_2^{-\bullet}$ and $OH^{\bullet}$ in a similar fashion, related to dose (Fig. 4), their EC_{50} values being similar (Table 1). In addition, the kinetics of 'tea polyphenols' (not reported) scavenging free radicals were similar to these of 'tea pigments' (Figs. 2 and 3). The similarities between them are no doubt due to 'tea pigments' being the polymeric and/or oxidative products of catechins and the two bear the same active polyphenolic groups; in consequence exhibit very similar free radical scavenging abilities.

Reactive oxygen species, such as $O_2^{-\bullet}$ and $OH^{\bullet}$, are found to do harm to the body by damaging lipids, DNA, amino acid and other biomolecules, thus leading to various diseases, such as cancer, heart disease and ageing (Freeman & Crapo, 1982). Plant antioxidant substances, which can scavenge free radicals, have been hypothesized to be useful to prevent diseases and delay ageing and much evidence supports this hypothesis (Halliwell, Murcia, Chirico, & Aruoma, 1995). 'Tea pigments' are a category of antioxidant polyphenolic components found in black tea manufacture, and up to now only some of their constituents have been defined (Nursten, 1997; Wan, Nursten, & Cai, 1997). Our demonstration that 'tea pigments' are effective scavengers of $O_2^{-\bullet}$ and $OH^{\bullet}$ and that their scavenging abilities are similar to those of 'tea polyphenols' supports the antioxidant mechanism for the role of 'tea pigments' in disease prevention (Ye, 1997).

References

Freeman, B. A., & Crapo, J. D. (1982). Biology of disease: free radicals and tissue injury. *Labratory Investigation*, *47*, 412–428.

Halliwell, B., Murcia, M. A., Chirico, S., & Aruoma, O. I. (1995). Free radicals and antioxidants in food and in vivo: what they do and how they work. *Critical Reviews in Food Science & Nutrition*, *35*, 7–20.

Hara, Y. (2000). *Green tea: health benefits and applications*. New York: Marcel Dekker.

Li, N., Han, H., & Wang, Z. (1998). Protective effect of tea pigments on oxidative damage by free radical in guinea pig. *Zhong Guo Zhong Yi Yao Technology (in Chinese)*, *29*, 23–24.

Morse, M. A., Kresty, L. A., Steele, V. E., Kelloff, G. J., Boone, C. W., Balentine, D. A., Harbowy, M. E., & Stoner, G. D. (1997). Effects of theaflavins on *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis. *Nutrition and Cancer—an International Journal*, *29*, 7–12.

Nursten, H. E. (1997). Chemistry of tea infusions. In R. Schubert, & M. Spiro (Eds.), *Chemical and biological properties of tea infusions* (pp. 10–83). Frankfurt: German Medical Information Services.

Ren, M., Zheng, Y., & Xu, S. (1998). The inhibitory effect of tea pigments on lipid peroxidation in mice. *Jiang Xi Medical Acta (in Chinese)*, *38*, 49–51.

Wan, X. C., Nursten, H. E., & Cai, Y. (1997). A new type of tea pigment—from the chemical oxidation of epicatechin gallate and isolated from tea. *Journal of the Science of Food & Agricuture*, *74*, 401–408.

Xiao, W., Zhong, J., Xiao, H., & Li, D. (1998). The mechanism of formation of tea pigments during the industrial processing of tea known as "fermentation". *Fujian Tea*, *3*, 8–12.

Ye, Y. (1997). The study and application of tea pigment. *China Food Additives (in Chinese)*, *4*, 23–24.

Yu, W., Zhao, Y., Xue, Z., Jin, H., & Wang, D. (2001). The antioxidant properties of lycopene concentrate extracted from tomato paste. *Journal of the American Oil Chemists Society*, *78*, 697–701.