Comparative studies on the physicochemical and antioxidant properties of different tea extracts

Haixia Chen · Yu Zhang · Xueming Lu · Zhishuang Qu

Revised: 24 July 2010 / Accepted: 27 July 2010 / Published online: 7 February 2011
© Association of Food Scientists & Technologists (India) 2011

Abstract Tea is one of the most popular drinks next to water. Tea polyphenol is one of the main bioactive constituents of tea with health functions. In order to find the most bioactive tea polyphenols, polyphenol extracts from green tea, black tea and chemical oxidation products of green tea extracts were comparatively studied on the physicochemical and antioxidant properties. Results showed physicochemical and antioxidant properties of polyphenol extracts changed greatly after the chemical oxidation. Hydrogen peroxide induced oxidation products (HOP) possessed the highest antioxidant ability among the four tea polyphenol extracts. Thirteen phenolic compounds and one alkaloid in HOP were identified by reversed phase high-performance liquid chromatography coupled to diode array detection and electrospray ionization mass spectrometry (RP-HPLC-DAD-ESI-MS). Hydrogen peroxide induced oxidation of tea polyphenol extracts could improve the antioxidant activity and could be used to produce antioxidants for food industry.

Keywords Tea polyphenols · Comparative studies · Physicochemical properties · Antioxidant activity · HPLC-MS

Introduction

Tea (*Camellia sinensis* L.) was originally used as a beverage thousands of years ago. It was a complex mixture containing multiple compounds from simple phenolics, catechins to polysaccharides and complex thearubigins, many of which had been reported to have health functions such as antiallergic (Suzuki et al. 2000), antioxidative (Jhoo et al. 2005), antimutagenic and anticarcinogenic (Standley et al. 2001), antiatherosclerotic (Yokozawa et al. 2002), antibacterial (Yildirim et al. 2000), and hypoglycemic (Zhou et al. 2007) activities.

Tea has been consumed for centuries in the forms of unfermented (green tea), semi-fermented (oolong), and fermented (black and pu-erh or red) by ancient cultures for its medicinal properties (Lin et al. 2008). The content and constituents of the tea polyphenols are different in different forms. There are four major tea catechins existed in green tea because there was no enzymatic oxidation in the process procedure of green tea. While during the fermentation process of black tea, four major tea catechins originally contained in fresh leaves are enzymatically oxidized and converted to various oxidation products comprising black tea polyphenols. Because the enzymatic oxidation procedure produces a complex and small amount of oxidation products, only little oxidation products had been studied (Sava et al. 2001, Tanaka et al. 2005). An alternative approach, using a model oxidation system, had thus been developed for the study of polyphenolic compounds. For example, oxidation in model systems has been carried out both chemically by using ferrous sulphate (Oszmianski et al. 1996) or hydrogen peroxide (Zhu et al.

H. Chen (✉) · Y. Zhang · X. Lu · Z. Qu
Tianjin Key Laboratory for Modern
Drug Delivery & High-Efficiency,
School of Pharmaceutical Science and Technology,
Tianjin University,
Tianjin 300072, People's Republic of China
e-mail: chennhxx@yahoo.com.cn

2000) and enzymatically with polyphenoloxidase (Guyot et al. 1996). But until now there are no reports on the comparative studies on the polyphenol extracts from green tea, black tea and chemical oxidation products of green tea extracts.

This paper aimed to comparatively study the physicochemical and antioxidant properties of the polyphenol extracts from green tea, black tea and chemical oxidation products of green tea extracts with hydrogen peroxide and potassium hexacyanoferrate respectively. The constituents of the most bioactive tea polyphenol extract among the four extracts were determined by HPLC-DAD-ESI-MS analysis.

Materials and methods

Materials Green tea and black tea were produced in Huangshan Mountain, Anhui, China. They were purchased from the local market in Tianjin, P.R.China. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St, Louis, MO, USA). HPLC solvents were obtained from Concord Technology Co., Ltd (Tianjin, P.R. China). All other chemicals and reagents were purchased locally and were of analytical grade.

Extract isolation from green tea and black tea One hundred grams of dry green tea or black tea leaves was extracted for 1 h with 1500 ml of boiling water. After cooling to 45 °C, the aqueous infusion was concentrated using vacuum rotary evaporator (RE-52CS Rotary Evaporator, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) and then extracted with chloroform and ethyl acetate for three times (1:1, v/v), respectively. The ethyl acetate extract was vacuum dried and lyophilized to obtain the main polyphenol extract. The yields of tea polyphenol extracts were 10.2 g and 9.0 g for green tea (named as GTP) and black tea (named as BTP), respectively.

Chemical oxidation of green tea extracts Chemically oxidation of green tea extracts by hydrogen peroxide was done according to (Li and Xie 2000). Briefly, 10% of the green tea polyphenol extract with aquatic solution as substrate was mixed with 2.5% (v/v) of hydrogen peroxide, and reacted at 45 °C for 6 h with stirring at medium velocity. This is followed by ethyl acetate (1:1, v/v) extraction for three times. The hydrogen peroxide oxidized tea polyphenols (named as HOP) was obtained by drying the ethyl acetate extract in vacuum and freeze-dried. Chemical oxidation of green tea extracts by potassium hexacyanoferrate was done according to Wan et al. (1997)

and the potassium hexacyanoferrate oxidized tea polyphenols (named as POP) were obtained.

Physicochemical analysis of tea extracts Solubility of different tea extracts was measured by employing AOAC (1984) methods. The pH was recorded with a Schott pH-meter (Model CG840, Schott Instruments, Mainz, Germany) at the concentration of 1.0 mg/ml in aqueous solution of each tea polyphenol extracts. UV absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolics in tea extracts were determined according to the method reported by Chen (1997) using gallic acid as standard. Contents of total phenolics in GTP, BTP, HOP and POP were expressed as gallic acid equivalents in milligrams per g tea polyphenol extract weight (mg GAE/g TEW). The results were averages of triplicate analyses.

Scavenging effects on DPPH radicals Scavenging effects of the extracts on DPPH radicals were determined according to Duan et al. (2006). Hundred microliters of various concentrations of tea extracts was mixed with 2900 µl DPPH solution (120 µM) in ethanol and incubated at 37 °C for 30 min in the dark. The absorbance was recorded at 517 nm. Scavenging effects of extracts GTP, BTP, HOP and POP on DPPH free radicals in percentage (%) was calculated and ascorbic acid was used as positive controls. All tests were carried out in triplicate.

Measurement of ferric reducing power (FRP) FRP potential of the extracts was determined according to Gow-Chin Yen et al. (1995). The reaction solutions (0.1 ml) containing different concentrations of samples in 0.2 M PBS (pH 6.6) were mixed with 30 mM aqueous potassium hexacyanoferrate solution (0.7 ml). After incubating at 50 °C for 20 min, 10% trichloroacetic acid (2.0 ml) was added and centrifuged at 3000×g for 10 min. The supernatant (1.0 ml) was mixed with 1.7 mM aqueous FeCl₃ (3.0 ml) and absorbance at 700 nm was determined. The ascorbic acid was used as the control.

RP-HPLC analysis Sample of HOP (the most active extract) was analyzed by RP-HPLC. A rapid resolution, 1200 series HPLC system with a quaternary SL pump, well plate autosampler, thermostat and heated column compartment and diode array detector (Agilent Technologies, Palo Alto, CA, USA) was used. A Zorbax 80SB-C18 column (150×2.1 mm, 3.5 µm) (Agilent Technologies, Palo Alto, CA, USA) was used for the separation of phenolic compounds. Sample HOP was prepared at a concentration of 1.0 mg/ml in water, and filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter (Whatman, Florham

Park, NJ). Sample was injected into a 20 µl injection loop and the column was maintained at 40 °C. Gradient elution was performed with water/0.1% formic acid (solvent A) and MeOH (solvent B) at a constant flow rate of 200 µl/min. An increasing linear gradient (v/v) of solvent B was applied: (t (min),%B): (0, 5), (50, 30), (70, 100). Chromatograms were recorded at 273 nm, with peak scanning between 200 and 600 nm.

Qualitative Analysis by HPLC-DAD/ESI-MSⁿ Sample of HOP was analyzed by HPLC-DAD/ESI-MSⁿ. An angilent 6310 ion trap mass spectrometer fitted with an electrospray interface coupled online to HPLC system as described above was used. Experiments were performed in negative and positive ion modes. Scan range and scan rate were 150–2000 and 13000 m/z/sec, respectively. The drying gas temperature was 300 °C. High spray voltage was set at 3500 V. Nitrogen was used as the dry gas at a flow rate of 12 l/min. MS/MS and MS³ were carried out using helium as the target gas. Identifications were achieved on the basis of the ion molecular mass, MSⁿ, and UV-visible spectra.

Statistical analysis Values were expressed as means±standard deviation (SD) of three replicates, and Student's test was used for the statistical analysis. The values were considered to be significantly different when the *P* value was less than 0.05.

Results

Physicochemical analysis Oxidation change of the physicochemical properties of tea extracts is shown in Table 1. There were similar solubility of GTP and BTP. Amongst all tea extracts, the solubility for HOP was the highest and that for POP was the lowest. The pH values of the four polyphenol extracts were similar. All extracts had an acidic

pH, ranging from 4.8 to 5.3. The UV spectra (190–340 nm) of tea extracts showed two absorption maxima, namely λ_{max1} and λ_{max2}, which were similar for each extracts. Overall, the range of λ_{max1} was 232–233 nm, whereas λ_{max2} ranged from 269–273 nm. All tea extracts were compared for their total polyphenol contents, in terms of gallic acid equivalents. HOP showed relatively greater reactivity to the Folin-Ciocalteu reagent, while POP was the least reactive. The total polyphenol content in tea extracts was in the following order: HOP>GTP>BTP>POP.

DPPH radical scavenging effects DPPH radical is a stable free radical and is commonly used as a substrate to evaluate antioxidant activity (Kaur et al. 2008). The scavenging effects of four tea extracts and vitamin C on DPPH free radicals were shown in Fig. 1. All of the four samples, GTP, BTP, HOP and POP, exhibited a strong ability to quench DPPH radicals. Stronger scavenging effects were observed with increasing concentrations used in the test (from 1.7 µg/ml to 13.3 µg/ml). The IC₅₀ value of GTP, BTP, HOP, POP and Vitamin C were 1.7, 2.3, 1.4, 2.5 and 2.1 µg/ml, respectively. Although no significant difference (*P*>0.05) was observed between the scavenging rate of HOP and vitamin C at a relatively high concentration, the DPPH radical-scavenging activity of HOP at low concentrations (1.7, 3.3, 6.7 and 13.3 µg/ml) was significantly higher (*P*<0.05) than that of vitamin C at the same concentration. This indicated that HOP was a good antioxidant with strong DPPH radical-scavenging activity.

Ferric reducing power of tea extracts Ferric reducing power of tea extracts is shown in Fig. 2. All the tea polyphenol extracts showed good FRP potentials. GTP, BTP, HOP and POP had dose-dependent reducing power with the coefficient *R*²=0.9615, 0.9301, 0.9336 and 0.9595, respectively. Although weaker than reference antioxidant vitamin C, HOP had the strongest activity in FRP activities

Table 1 Physicochemical Characteristics of four different tea polyphenol extracts^a

Characteristic	GTP ^b	BTP ^b	HOP ^b	POP ^b
Solubility (mg/ml)	13.8	13.5	15.6	5.3
UV _{λ_{max}} (nm)	232, 271	232, 272	233, 269	232, 269
pH (1.0 mg/ml aqueous solutions)	5.3	4.9	5.1	4.9
Total polyphenols (mg of gallic acid/g of extract)	381.1	300.1	501.1	226.3

^a Values are the mean of three analyses;

^b GTP: Tea polyphenol extracts from green tea; BTP: Tea polyphenol extracts from black tea; HOP: Hydrogen peroxide oxidized tea polyphenols; POP: Potassium hexacyanoferrate oxidized tea polyphenols

Fig. 1 DPPH scavenging activities of four different tea polyphenol extracts. Each value is the mean $\pm$ SD of triplicate measurements. GTP: Tea polyphenol extracts from green tea; BTP: Tea polyphenol extracts from black tea; HOP: Hydrogen peroxide oxidized tea polyphenols; POP: Potassium hexacyanoferrate oxidized tea polyphenols. ^b $P < 0.05$ compared to the group of Vc

among the four tea polyphenol extracts. The activity order of the four tea polyphenol extracts for FRP potential were HOP>GTP>BTP>POP.

Identification of chromatographic peaks Figure 3 showed the LC-DAD chromatograms of HOP, which was the most bioactive extract in the four tea polyphenol extracts. UV-visible characteristics and LC/MS/MSⁿ data were given in Table 2. Based on the LC-MS data and the identity of tea polyphenols previously established (Del Rio et al. 2004, Lee et al. 2005, Krishnan and Maru 2006), peaks 2, 3, 5, 6, 7, 8, 10, 11 and 13 were identified as gallic acid, 5-galloylquinic acid, (–)-galocatechin, (+)-catechin, (–)-epigallocatechin, HHDP-galloylglucose, (–)-epigallocatechin-3-gallate, (–)-epicatechin, (–)-galocatechin-3-gallate, (–)-epicatechin-3-gallate, respectively. Peaks 12 and 15 were the polymeric polyphenols which was identified as (–)-gallocatechin-3-gallate dimer and EGCG quinone dimer (Tanaka et al. 2005). Other major peaks in the chromatograms, 8, 16 and 17 of HOP, were the glycoside conjugate that were identified as HHDP-galloylglucose, quercetin-3-galactoside and kaempferol-3-rutinoside. The positive ion mass spectra recorded molecular ions at m/z 195 (peak 9) was identified as caffeine. Peaks 14 were partially identified as HHDP derivatives.

Unidentified Peaks in HOP There are still some constituents corresponding to the peaks that we are unable to identify according to the LC-DAD and LC-MS data (Table 2). Peaks 1 and 4 displayed a UV spectrum similar

to that of gallic acid, and there was a molecular ion at m/z 169 in the MSⁿ analysis. So they were gallic acid conjugates but no reference data were available for further confirmation. Isolation and NMR identification are needed for an unequivocal structural elucidation of all unidentified peaks.

Discussion

Two chemical oxidation methods (H_2O_2 induced polyphenol chemical oxidation and potassium hexacyanoferrate induced chemically oxidation) were adopted to obtain two types of tea polyphenol extracts, HOP and POP. Compared to extracts from original green tea and black tea, there were great changes on the physicochemical properties. Antioxidant assays of scavenging abilities on DPPH free radicals and ferric reducing power showed that H_2O_2 -induced chemical oxidation of tea polyphenol extracts had the highest antioxidant ability which was in accordance with the total polyphenol content.

In this study, hydrogen peroxide induced oxidation of tea polyphenol extracts could improve the antioxidant activity. Thirteen phenolic compounds and one alkaloid in HOP were identified by reversed phase high-performance liquid chromatography coupled to diode array detection and electrospray ionization mass spectrometry (RP-HPLC-DAD-ESI-MS). Among the constituents identified in HOP, (–)-Epigallocatechin-3-gallate (EGCG), was still the major compound, which is typical in *Camellia* species. Two

Fig. 2 Ferric Reducing Power of four tea polyphenol extracts. Each value is the mean $\pm$ SD of triplicate measurements. GTP: Tea polyphenol extracts from green tea; BTP: Tea polyphenol extracts from black tea; HOP: Hydrogen peroxide oxidized tea polyphenols; POP: Potassium hexacyanoferrate oxidized tea polyphenols

Fig. 3 Profile of HPLC-DAD chromatograms at 273 nm of hydrogen peroxide oxidized tea polyphenols (HOP). 1, unidentified; 2, gallic acid; 3, 5-galloylquinic acid; 4, unidentified; 5, (–)- gallo catechin; 6, (+)- catechin; 7, (–)-epigallocatechin; 8, HHDP-galloylglucose; 9,

caffeine; 10, (–)- epigallocatechin-3-gallate; 11, (–) – epicatechin; 12, (–)-gallo catechin-3-gallate Dimer; 13, (–)-epicatechin-3-gallate; 14, HHDP derivatives; 15, EGCG quinone Dimer; 16, quercetin-3-galactoside; 17, kaempferol-3-rutinoside

dimers of catechin identified in HOP suggested that the extract was only partially oxidized. The higher antioxidant activity of HOP compared to that of GTP indicated that the polymerization of tea polyphenols could improve the antioxidant activities. These results agree with the previous report (Li and Xie 2000), in which the antioxidant activity of tea catechin oxypolymers was equal to or even more notable than that of tea catechin. The mechanism might due to the increase of solubility and the conformation change of the partially oxidized tea polyphenol, which might make it

more easily to bind free radicals (Ding et al. 2005). The detailed structural analysis and evaluation of the bioactivities mechanism of the partially oxidized tea polyphenols will need further study.

In conclusion, it can be stated that the polyphenol extracts from green tea, black tea and chemical oxidation products of green tea extracts with hydrogen peroxide and potassium hexacyanoferrate respectively were found to have different physicochemical and antioxidant properties. Our finding indicated that H_2O_2 induced oxidation of tea

Table 2 Identification of compounds in HOP^a by using their spectral characteristics in LC-DAD, negative ions and positive ions in LC-MS and MSⁿ

peak	t_R (min)	UVdata (nm)	$[M-H]^-$ (m/z)	MS^2/MS^3 (m/z)	tentative identification
1	2.7	210, 270	961	791,747,639	unidentified
2	4.1	210, 272	169	–	gallic acid
3	5.1	210, 275	343	191,169	5-galloylquinic acid
4	5.5	210, 270	946	775,731,623,301	unidentified
5	8.2	210, 270	305	221,179	(–)- gallo catechin
6	19.8	210, 275	289	245,205	(+)- catechin
7	20.3	210, 270	305	261,219,179	(–)-epigallocatechin
8	22.0	210, 265	633	463,301,275	HHDP-galloylglucose
9	29.4	210, 270	195 ^b	–	caffeine
10	31.5	225, 275	457	331,193,169	(–)- epigallocatechin-3-gallate
11	33.9	210, 275	289	245,203,123	(–) - epicatechin
12	40.1	225, 275	883	457, 331,193,169	(–)-gallo catechin-3-gallate Dimer
13	46.8	210, 275	441	331, 289, 169	(–)-epicatechin-3-gallate
14	48.9	220, 275	635	465, 447,313,169	HHDP derivatives
15	59.6	275, 360	929	–	EGCG quinone Dimer
16	60.1	270, 370	463	301	quercetin-3-galactoside
17	62.2	270, 350	593	447,285	kaempferol-3-rutinoside

^a HOP: Hydrogen peroxide oxidized tea polyphenols.

extracts could improve the antioxidant activities and this method could be used to produce antioxidants for food industry.

Acknowledgements This research is funded by the National Natural Science Foundation of China to H. Chen (Grant No. 30600470).

References

- AOAC (1984) Official methods of analysis, 14th edn. Association of Official Analytical Chemists, Arlington, VA
- Chen FK (1997) Measurement of the effective components of common Chinese Herb Medicine. People's Medical Publishing House, Beijing, p 51
- Del Rio D, Stewart AJ, Mullen W, Burns J, Lean MEJ, Brighenti F, Crozier A (2004) HPLC-MSⁿ analysis of phenolic compounds and purine alkaloids in green and black tea. *J Agric Food Chem* 52:2807–2815
- Ding L, Tu YY, Chen XM (2005) Progresses on the bioactivities of modified tea catechin. *Tea Sci Technology* 15:1–3
- Duan XJ, Zhang WW, Li XM, Wang BG (2006) Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem* 95:37–43
- Guyot S, Vercauteren J, Cheynier V (1996) Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. *Phytochem* 42:1279–1288
- Jhoo JW, Lo CY, Li S, Sang S, Ang CYW, Heinze TM, Ho CT (2005) Stability of black tea polyphenol, theaflavin, and identification of theanaphthoquinone as its major radical reaction product. *J Agric Food Chem* 53:6146–6150
- Kaur R, Arora S, Singh B (2008) Antioxidant activity of the phenol rich fractions of leaves of *Chukrasia tabularis* A. Juss *Bioresour Technol* 99:7692–7698
- Krishnan R, Maru GB (2006) Isolation and analyses of polymeric polyphenol fractions from black tea. *Food Chem* 94:331–340
- Lee JH, Johnson JV, Talcott ST (2005) Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. *J Agric Food Chem* 53:6003–6010
- Li CM, Xie BJ (2000) Evaluation of the antioxidant and pro-oxidant effects of tea catechin oxypolymers. *J Agric Food Chem* 48:6362–6366
- Lin SD, Liu EH, Mau JL (2008) Effect of different brewing methods on antioxidant properties of steaming green tea. *LWT Food Sci Technol* 41:1616–1623
- Oszmianski J, Cheynier V, Moutounet M (1996) Iron-Catalyzed oxidation of (+)-catechin in model systems. *J Agric Food Chem* 44:1712–1715
- Sava VM, Galkin BN, Hong MY, Yang PC, Huang GS (2001) A novel melanin-like pigment derived from black tea leaves with immuno-stimulating activity. *Food Res Int* 34:337–343
- Standley L, Winterton P, Marnewick JL, Gelderblom WCA, Joubert E, Britz TJ (2001) Influence of processing stages on antimutagenic and antioxidant potentials of rooibos tea. *J Agric Food Chem* 49:114–117
- Suzuki M, Yoshino K, Maeda-Yamamoto M, Miyase T, Sano M (2000) Inhibitory Effects of Tea Catechins and O-Methylated Derivatives of (–)-Epigallocatechin-3-O-gallate on Mouse Type IV Allergy. *J Agric Food Chem* 48:5649–5653
- Tanaka T, Matsuo Y, Kouno I (2005) A novel black tea pigment and two new oxidation products of epigallocatechin-3-O-gallate. *J Agric Food Chem* 53:7571–7578
- Wan XC, Nursten HE, Ya C (1997) A new type of tea pigment from the chemical oxidation of epicatechin gallate and isolated from tea. *J Sci Food Agric* 74:401–408
- Yen GC, Chen HY (1995) Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem* 43:27–32
- Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V (2000) Comparison of antioxidant and antimicrobial activities of *Tilia* (*Tilia Argentea* Desf Ex DC), *Sage* (*Salvia Triloba* L.), and *Black Tea* (*Camellia Sinensis*) Extracts. *J Agric Food Chem* 48:5030–5034
- Yokozawa T, Nakagawa T, Kitani K (2002) Antioxidative activity of green tea polyphenol in cholesterol-fed rats. *J Agric Food Chem* 44:3549–3552
- Zhou XL, Wang DF, Sun PN, Bucheli P, Li L, Hou YF, Wang JF (2007) Effects of Soluble Tea Polysaccharides on Hyperglycemia in Alloxan-Diabetic Mice. *J Agric Food Chem* 55:5523–5528
- Zhu N, Huang TC, Yu Y, LaVoie EJ, Yang CS, Ho CT (2000) Identification of oxidation products of (–)-Epigallocatechin Gallate and (–)-Epigallocatechin with H₂O₂. *J Agric Food Chem* 48:979–981