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Determination of catechins and flavonol glycosides in Chinese tea varieties

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ABSTRACT

A standardised profiling method based on high performance liquid chromatography combined with ultraviolet (UV) and mass spectrometric detection (MS) was established to analyse the phenolic compounds of selected tea varieties used for manufacturing of green, black and oolong teas. The composition and content of 24 tea constituents were analysed, including catechins, flavonol and flavones glycosides, phenolic acids and purine alkaloids. Each tea variety had a unique chemical profile. The compositions of catechins were lower in the tea varieties for green tea manufacturing, while the content of myricetin glycosides was the lowest in the tea variety for oolong tea manufacturing. The content of individual phenolic compounds in the selected tea varieties is highly variable. However, the content of total catechins is proposed to be helpful to classify tea according to the future application as non fermented green and fermented oolong or black tea.

1. Introduction

Tea plants are widely cultivated in Southeast Asia (Lin, Juan, Chen, Liang, & Lin, 1996). There is a body of evidence, that the main active ingredients of tea, such as catechins, flavonols and theaflavins, have antioxidant and other health promoting activities (Lambert & Yang, 2003; Wu & Butler, 2011; Yang et al., 2011). Different tea varieties are suitable for different tea manufacturing. Depending on the type of manufacturing, tea is generally divided into green, white, oolong, black, yellow and dark tea. Due to the different processing, each tea sample has a unique character, taste and chemical profile (Rio et al., 2004).

Many compounds of tea have been identified, such as flavan-3-ols, flavonols, gallic acid, theaflavins, purine alkaloids and amino acids (Friedman, Levin, Choi, Lee, & Kozukue, 2009; Friedman et al., 2005; Lin, Lin, Liang, Lin-Shiau, & Juan, 1998; Price, Rhodes, & Barnes, 1998; Wang et al., 2010). The main flavan-3-ols in tea are (−)-epicatechin and its gallate derivatives, which will be partly replaced by the theaflavins and thearubigins after enzymatic oxidation (called tea fermentation) (Balentine, Wiseman, & Bouwens, 1997; Finger, Kuhr, & Engelhardt, 1992). Major flavonols are quercetin, kaempferol and myricetin, which affect not only the astringent taste of tea but also its colour (Hollman & Arts, 2000; Lee et al., 2008). Flavonols are usually found in tea bound to sugars as *O*-glycosides, such as quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside (Aherne & O'Brien, 2002). Compared to the flavan-3-ols and theaflavins, the flavonol glycosides were found to induce a silky, mouth-drying, and mouth-coating sensation at very low threshold concentrations spanning from 0.001 to 19.8 mM (Scharbert, Holzmann, & Hofmann, 2004).

Analysis of these compounds is typically done by high-performance liquid chromatography (HPLC) combined with ultraviolet (UV) absorption or a diode array detector (DAD). Nowadays, mass spectrometry (MS), or even a tandem mass spectrometry (MS^n) are frequently used to obtain structural information after chromatographic separation (Sherma, 2003). The polyphenols in various parts of the tea plant used for manufacturing of tea products were analysed by HPLC (Wang, Lu, Miao, Xie, & Yang, 2008). Dou, Lee, Tzen, and Lee (2007) and Lin, Chen, and Harnly (2008) identified the main phenolic compounds in green and fermented teas by LC-DAD–ESI/MS, unfortunately without giving any quantitative results. Wang et al. (2008) analysed the catechins and purine alkaloids in the leaves of 22 tea varieties for manufacturing of oolong tea, without any results about the flavonols. Suteerapataranon, Butsoongnern, Punturat, Jorpalit, and Thanomsilp (2009) showed that the tea variety and type did not affect the caffeine content in Chiang Rai tea infusions. Rostagno et al. (2011) studied the content of catechins and some flavonol glycosides of green, white and black tea, unfortunately without indicating the tea varieties.

There are few studies in which both the flavan-3-ols and flavonols have been identified and quantified in tea varieties. Therefore, the objective of the present study is to analyse the compounds, including flavan-3-ols, flavonol glycosides, phenolic acids and purine alkaloids in representative tea varieties by HPLC–UV and HPLC–

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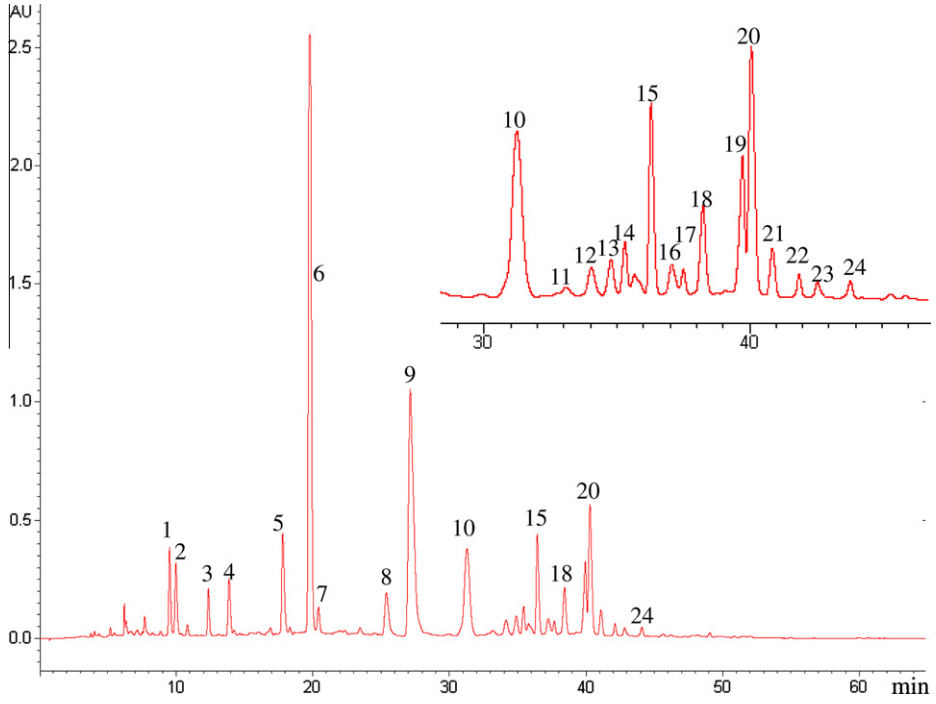

Fig. 1. RP-HPLC–UV chromatogram of a representative tea infusion (Fudingdabaicha). Peak numbers correspond to Table 1.

Table 1
Retention time and mass spectral data of phenolic compounds and purine alkaloids from a representative tea sample (Fudingdabaicha). Peak numbers and retention times correspond to Fig. 1.

Peak	Rt (min)	$[M-H]^-$ (m/z)	MS (m/z)	Compounds
1	9.62	343	191, 169	5-Galloylquinic acid
2	10.00	169	125	Gallic acid [b]
3	12.42	181[a]	–	Theobromine
4	13.93	305	261, 219, 139, 125	Gallocatechin
5	17.88	305	261, 219, 139, 125	Epigallocatechin
6	19.83	195[a]	138	Caffeine[b]
7	20.48	289	245, 205	Catechin[b]
8	25.50	289	245, 205	Epicatechin
9	27.18	457	305, 287, 169, 125	Epigallocatechin gallate
10	31.29	457	305, 287, 169, 125	Gallocatechin gallate
11	33.21	623	479, 317	Myricetin-3-*O*-rhamnosylglucoside
12	34.15	479	317	Myricetin-3-*O*-galactoside
13	34.95	479	317	Myricetin-3-*O*-glucoside
14	35.51	771	609, 463, 301	Quercetin-3-*O*-glucosyl-rhamnosyl-galactoside
15	36.51	771	609, 463, 301	Quercetin-3-*O*-glucosyl-rhamnosyl-glucoside
16	37.28	577	413, 293	Vitexin-2″-*O*-rhamnoside[b]
17	37.72	577	413, 293	Rhamnosylvitexin isomer
18	38.49	609	301	Quercetin-3-rutinoside[b]
19	39.99	463	301	Quercetin-3-D-galactoside[b]
20	40.35	441	289, 271, 169, 125	Epicatechin gallate
21	41.17	441	289, 271, 169, 125	Catechin gallate
22	42.15	593	285	Kaempferol-3-*O*-rutinoside
23	42.89	447	285	Kaempferol-3-*O*-galactoside
24	44.10	447	285	Kaempferol-3-*O*-glucoside

[a] $[M+H]^+$.
[b] Confirmed with standard compounds.

ESI-MS. The presented method is suitable to analyse green, black and oolong tea, respectively.

2. Materials and methods

2.1. Materials

Fresh tea leaves with a bud and three leaves were plucked from the tea garden of the Tea Science Institute, Zhejiang University of China in October 2010. Eight tea (*Camellia sinensis*) varieties were chosen for this study, including Fudingdabaicha, Huangyezao, Wuniuzao and Ziya for green tea manufacturing, Zhenghedabaicha and Zhenong 25 for black tea manufacturing, Shuixian and Dahongpao for oolong tea manufacturing. The fresh tea leaves were steamed at 100 °C for 3 min, dried at 100 °C for 5 min and then dried at 80 °C until the moisture in the dry matter was between 7% and 8%.

2.2. Chemicals

(+)-Catechin, gallic acid, caffeine, vitexin-2″-*O*-rhamnoside, rutin trihydrate, quercetin, myricetin quercetin-3-D-galactoside and