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Metabolomic profiling delineate taste qualities of tea leaf pubescence

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ABSTRACT

The amount of pubescence on leaf epidermis is an important morphological marker for the quality of green tea, and the tea with plenty of pubescence is generally recognized as having a better taste. However, there is no systematic study on chemical compositions of tea leaf pubescence. The contributions of pubescence to taste properties are far from clear. In this research, 114 components were identified from the tea leaf pubescence of yunkang 10, a broad-leaf tea cultivar with plenty leaf pubescence, for the first time with a non-targeted metabolomics approach using ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UHPLC-Q-TOF-MS). Compared to the tea leaf with the pubescence removed (non-pubescent leaf), the pubescence obtained from the same shoots had relatively higher contents of amino acids and lower contents of polyphenols. It was also found that the umami of pubescence was elevated, while the bitterness and astringency were significantly declined. Partial least-squares (PLS) analysis suggested that the polyphenols and amino acids accounted for the taste quality. To the best of our knowledge, this is the first time that the metabolites in tea leaf pubescence were profiled. The results offer the direct concrete evidence on the contributions of pubescence to the tea taste properties.

1. Introduction

Tea (*Camellia sinensis*) is the second most widely consumed beverage after water (Fraser et al., 2014). The quality of tea is mainly assessed through sensorial properties (aroma, flavor and appearance) that are influenced by various factors, including genetic background, growth environment (climate, soil, and altitude), horticultural practices, harvest season, processing and storage (Daglia, Antiochia, Sobolev, & Mannina, 2014). Nowadays, tea consumption has moved beyond sensual pleasure and cultural significance since multiple health-promoting effects have been ascribed to this widespread beverage, e.g. antioxidative, anticarcinogenic, antihypertensive, neuroprotective and cholesterol-lowering properties (Hayat, Iqbal, Malik, Bilal, & Mushtaq, 2015). It was reported that these health-promoting effects are related to major compounds in tea, polyphenols, amino acids, and caffeine (Fraser et al., 2014). In addition, the sensory qualities are also influenced by the content and composition of the compounds in tea, which impose different tastes of bitterness, astringency, and umami (Liu et al., 2016). The bitter and astringent tastes are believed to be mainly contributed from the polyphenols, whereas the free amino acids account for the umami taste (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006; Narukawa, Kimata, Noga, & Watanabe, 2010). The tender tea plucked in earliest spring is generally recognized as having a better taste and found to have higher levels of free amino acids and lower levels of polyphenols (Dai et al., 2015). Besides, the tender tea was also found to have more pubescence than old one, thus the amount of pubescence on the leaf epidermis is considered as an important morphological marker for the quality of the green tea (Jin, Wang, Xia, Xu, & Pei, 2005). However, the actual contributions of pubescence to the tea taste and the constituents in pubescence account for the taste have never been reported.

The pubescence on the leaf epidermis is constituted by a combination of non-glandular trichomes and glandular trichomes (Ye et al., 2010). Usually the pubescence has the capacity to produce, store and secrete large amounts of different kinds of secondary metabolites. Some of secondary metabolites provide protection against herbivores and pathogens for the plant, and can be used as food additives or pharmaceuticals (Glas et al., 2012; Tissier, 2012). Furthermore, the contents and compositions of secondary metabolites may be different in the pubescence and the leaf (Ye et al., 2010), which may lead the difference in taste properties and health-promoting effects of tea. Although previous investigations have been conducted to delineate the relationship between the metabolites and the tastes of the tea leaves with different ages (Jiang et al., 2013; Lee et al., 2011), the results actually reflected the total effects of the pubescence and the ages of the leaves, and the contribution of pubescence alone to the taste properties and health-promoting effects was not still clear.

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Yunkang 10 (*C. sinensis* var. *assamica* cv. yunkang 10) is a broad-leaf tea cultivar bred from Menghai county of China. It has passed national examination in China, and been listed on the State New Varieties. It has the plenty of leaf pubescence and excellent capabilities of drought, cold and disease resistance. Nowadays, the cultivated area of yunkang 10 is >60 thousand hectares, which accounts for around 25% of total tea garden area in Yunnan province, China. In the current study, we aimed to establish a non-targeted metabolomics approach using ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) to identify the constituents in the leaf pubescence of yunkang 10, and determine the difference of the metabolites in the leaf pubescence and the non-pubescent leaf which were harvested from the same shoots. Then we evaluated the influence of leaf pubescence on the tea taste properties, and surveyed some novel potential compounds responsible for the taste of the pubescence. To the best of our knowledge, the research profiles the metabolites in tea leaf pubescence for the first time, and the results offer valuable information for elucidating the contributions of pubescence to the tea taste properties.

2. Materials and methods

2.1. Chemicals and materials

MS grade acetonitrile, methanol and water were purchased from J.T. Baker (Danville, PA). MS grade formic acid was provided by Sigma-Aldrich (St. Louis, MO). The standards of catechin (C, PubChem CID-9064), epicatechin (EC, PubChem CID-72276), gallocatechin (GC, PubChem CID-9882981), epigallocatechin (EGC, PubChem CID-72277), epicatechin-3-O-gallate (ECG, PubChem CID-107905), epigallocatechin-3-O-gallate (EGCG, PubChem CID-65064), gallocatechin-3-O-gallate (GCG, PubChem CID-199472), apigenin 6-C-glucoside-8-C-arabinoside (isoschaftoside, PubChem CID-3084995), apigenin 6-C-arabinoside-8-C-glucoside (schaftoside, PubChem CID-442658), apigenin 6-C-glucoside (isovitexin, PubChem CID-162350), apigenin 8-C-glucoside (vitexin, PubChem CID-5280441), rutin (PubChem CID-5280805), quercetin 3-O-galactoside (PubChem CID-5281643) quercetin 3-O-glucoside (PubChem CID-5280804), myricetin (PubChem CID-5281672), kaempferol (PubChem CID-5280863), gallic acid (PubChem CID-370), methyl gallate (PubChem CID-7428), and ellagic acid (PubChem CID-5281855) were obtained from Shanghai Tauto Biotech (Shanghai, China), and their purities were >95%.

Fifteen samples of the first new shoots (one bud) in the cultivar of yunkang 10 were collected in March 2015 from Menghai County in China. The voucher specimens (Voucher No. tea-201501-201515) were deposited at State Key Laboratory of Quality Research in Chinese Medicine, Macau.

2.2. Sample preparation

The leaf pubescence was isolated using the method described by Yerger et al. (1992) with slight modifications. The first new shoots were freeze-dried, and transferred to the 1.5 mL Eppendorf tube. The liquid nitrogen was added to maintain the tube at cold temperature. Then, approximately 1 cm^3 of finely powdered dry ice was added immediately followed by vortex. The contents in the tube were lightly filtered through the mesh, and the free pubescence and the particles of dry ice passed through the screen, while the tea leaf with the pubescence removed (non-pubescent leaf) retained on the screen. Finally, the pubescence and the non-pubescent leaf were fully grinded with liquid nitrogen to small powders, and stored at 4 °C until use.

2.3. Extraction of tea samples

The sample (0.1 g) was extracted with 1 mL of 70% methanol for 30 min in ultrasonic bath at room temperature. The extraction was repeated for three times, and the supernatants were combined and filtered through a 0.22 μm membrane. Three replicates were used for each sample.

2.4. UHPLC-Q-TOF-MS analysis

The UHPLC system (1290 Infinity LC System, Agilent Technologies, Santa Clara, CA) coupled with Q-TOF MS (6550 iFunnel, Agilent Technologies) was used for the analysis of the samples. The UHPLC system consisted of a thermostated autosampler (maintained at 6 °C), a thermostated column compartment, a degasser, and a binary pump. The Q-TOF-MS system was equipped with a dual Agilent Jet Stream electrospray ion source (dual AJS ESI) which was set as negative ion mode. One microliter of sample was injected onto a reverse-phase Eclipse XDB-C_{18} column (2.1 × 100 mm, 1.8 μm, Agilent Technologies). The column temperature was maintained at 35 °C, and the flow rate was 0.3 mL/min. Solvents A and B were 0.1% formic acid and 0.1% formic acid-containing acetonitrile, respectively, and the solvent gradient was set as follows: 0–5 min, 98% A; 5–35 min, 98% to 70% A; 35–40 min, 70% to 5% A. Five minutes of post-run re-equilibration was conducted before the next injection. The MS parameters were set as follows: dry gas temperature, 250 °C; drying gas flow, 15 L/min; nebulizer, 25 psig; sheath gas temperature, 300 °C; sheath gas flow, 11 L/min; capillary voltage, −3500 V; nozzle voltage, 2000 V. The system was operated across the range of 100–1700 *m/z* in full scan mode for relative qualitative analyses. The tandem mass spectrometry (MS/MS) spectra were obtained using the Data-Dependent scan mode and the collision energy was set as 30 eV. Accurate mass measurements were obtained by means of an automated calibrant delivery system for real time mass correction using purine at *m/z* 119.0363 and hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine at *m/z* 966.0007 as the references.

2.5. Data analysis

Raw data were analyzed by the Molecular Feature Extraction in MassHunter Qualitative Analysis Software (Agilent Technologies) to perform meaningful data mining. Compound lists, including the information of retention time, *m/z*, and MS signal intensity of all metabolites, were created in a batch model by using an offline DA Reprocessor software (Agilent Technologies). The background noise and unrelated ions (e.g. plasticizers) in the blank were excluded in the samples. The alignment and filtering of data were then performed with Mass Profiler Professional software (Agilent Technologies), and the tolerance windows for the retention times and *m/z* values were set at 0.5 min and 20 ppm, respectively. The metabolites were identified based on the standards, MS/MS spectra, literatures, and/or the metabolome database of HMDB (http://www.hmdb.ca/). Then the identified metabolites were selected as variables, and principal component analysis (PCA) and orthogonal projection to partial least-squares discriminant analysis (PLS-DA) were performed using Simca-P 14.0 software (Umetrics AB, Umeå, Sweden) to assess the relationships between phytochemical metabolites (profiles) of the two kinds of tea samples. After the multivariate approaches, the significance of each metabolite in group discrimination was further measured by Student's *t*-test. Tea metabolites for grouping were selected to meet VIP (variable importance in the projection) > 1.0, and significance at $p < 0.05$.

2.6. Taste analysis

The tea taste quality was assessed according to the method described by Dai et al. (2015) and Ferrer-Gallego, Hernandez-Hierro, Rivas-Gonzalo, and Escribano-Bailon (2014) with slight modifications. Briefly, tea sample was infused with freshly boiling water for 5 min prior to filtration with a stainless steel strainer. Then the tea infusion was evaluated by the sensory panel consisting of 10 assessors (5 females/5 males, 20–40 years in age), who had no history of known