Food Chemistry 237 (2017) 488–498

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Does oolong tea (*Camellia sinensis*) made from a combination of leaf and stem smell more aromatic than leaf-only tea? Contribution of the stem to oolong tea aroma

Lanting Zeng [a,b,1], Ying Zhou [a,1], Xiumin Fu [a], Xin Mei [a], Sihua Cheng [a,b], Jiadong Gui [a,b], Fang Dong [c], Jinchi Tang [d], Shengzhou Ma [e], Ziyin Yang [a,b,*]

[a] Key Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement & Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China
[b] University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing 100049, China
[c] Guangdong Food and Drug Vocational College, Longdongbei Road 321, Tianhe District, Guangzhou 510520, China
[d] Tea Research Institute, Guangdong Academy of Agricultural Sciences & Guangdong Provincial Key Laboratory of Tea Plant Resources Innovation and Utilization, Dafeng Road 6, Tianhe District, Guangzhou 510640, China
[e] Zhenjiang Institute of Agricultural Sciences in Hill Area of Jiangsu Province, Ninghang Road 112, Jurong 212400, China

ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 16 May 2017
Accepted 28 May 2017
Available online 29 May 2017

Keywords:
Amino acid
Aroma
Camellia sinensis
Stem
Tea
Volatile

ABSTRACT

The raw materials used to make oolong tea (*Camellia sinensis*) are a combination of leaf and stem. Oolong tea made from leaf and stem is thought to have a more aromatic smell than leaf-only tea. However, there is no available evidence to support the viewpoint. In this study, sensory evaluation and detailed characterization of emitted and internal volatiles (not readily emitted, but stored in samples) of dry oolong teas and infusions indicated that the presence of stem did not significantly improve the total aroma characteristics. During the enzyme-active processes, volatile monoterpenes and theanine were accumulated more abundantly in stem than in leaf, while jasmine lactone, indole, and *trans*-nerolidol were lower in stem than in leaf. Tissue-specific aroma-related gene expression and availability of precursors of aroma compounds resulted in different aroma distributions in leaf and stem. This study presents the first determination of the contribution of stem to oolong tea aroma.

1. Introduction

Tea (*Camellia sinensis*) is the second most consumed beverage worldwide after water. The popularity of tea depends on its pleasant flavor that is divided into taste (non-volatile compounds) and aroma (volatile compounds) (Hara, Luo, Wikremasinghe, & Yamanishi, 1995). Aroma is an essential component in the evaluation of sensory scores (Yang, Baldermann, & Watanabe, 2013). More than 600 volatile compounds have been identified as constituents of black tea aroma (Schuh & Schieberle, 2006). Although volatile compounds are only present in low quantities in tea, comprising around 0.01% of the total dry weight, they have a large impact on flavor, owing to their low human odor perception thresholds (Rawat et al., 2007). The amounts of volatile compounds depend on cultivars and pre-harvest treatments (cultivation), and post-harvest treatments (processing methods) (Yang et al., 2012; Fu et al., 2015). Many studies have shown that different manufacturing processes influence the formation of tea aroma. For exam-

Abbreviations: ANOVA, analysis of variance; C, catechin; CL, combined leaf; CS, combined stem; EC, epi-catechin; ECG, epi-catechin-3-gallate; EGC, epi-gallocatechin; EGCG, epi-gallocatechin-3-gallate; EF 1, encoding elongation factor 1; GBVs, glycosidically bound volatiles; GC, gallocatechin; GCG, gallocatechin-3-gallate; GC–MS, gas chromatography–mass spectrometer; GDP, geranyl diphosphate; GT, glycosyltransferase; IW, indoor-withering; LOX, 9/13-lipoxygenase; MEP, methylerythritol phosphate; OSE, organic solvent extraction; P, freshly plucked tea leaves; PD, β-primeverosidase; qRT-PCR, quantitative real time PCR; SBSE, stir bar sorptive extraction; SL, separated leaf; SPME, solid-phase microextraction; SS, separated stem; SW, solar withering; T1–T5, turned over 5 times; TPS, terpene synthase; TSA, tryptophan synthase α-subunit; TSB, tryptophan synthase β-subunit; VCDs, volatile carotenoid derivatives; VFADs, volatile fatty acid derivatives; VPBs, volatile phenylpropanoids/benzenoids; VTs, volatile terpenes.

* Corresponding author at: South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China.
E-mail address: zyyang@scbg.ac.cn (Z. Yang).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.foodchem.2017.05.137
0308-8146/

ple, the unique manufacturing process of oolong tea contributes to the formation of its sweet, fruity, and floral aroma (Gui et al., 2015; Wang, Kubota, Kobayashi, & Juan, 2001). During the oolong tea manufacturing process, tea leaves are exposed to various stresses, including plucking (wounding), solar withering (drought, heat, and UV radiation), indoor withering (drought), and turn over (wounding) (Cho et al., 2007; Gui et al., 2015). Aroma formation in tea leaves during the oolong tea manufacturing process may result from the defense responses of tea leaves against these various stresses (Baldermann et al., 2014). For example, levels of three aroma compounds (indole, jasmine lactone, and *trans*-nerolidol) significantly increased at the turn over stage of oolong tea manufacture, which was due to continuous mechanical damage stress from the turn over stage (Gui et al., 2015). A detailed investigation of volatile profiles from more than 38 tea products, including green teas, oolong teas, and black teas from different production areas and tea cultivars, indicated that these three compounds were characteristic aroma compounds, and were accumulated abundantly in oolong teas (Baldermann et al., 2014). Important odorants in oolong tea have also been identified by gas chromatography–olfactometry (GC–O), showing that the three characteristic aroma compounds, and other aromas (including furaneol, δ-decalactone, linalool, vanillin, β-ionone, 3-methylnonane-2,4-dione, and β-damascenone), contributed to the sweet, fruity, and floral qualities of oolong teas (Gui et al., 2015; Sheibani, Duncan, Kuhn, Dietrich, & O'Keefe, 2016).

Tea manufacturers usually remove stems during the tea manufacturing process, as their size and color can lead to a non-uniform and unpleasant appearance. However, the raw materials used to make oolong tea are a combination of leaf and stem. Oolong tea made from leaf and stem is generally considered to contain more aromas than leaf-only tea. To date, there is no available evidence to support the viewpoint, and the contribution of the stem to oolong tea aroma is unknown. Two key questions remain to be answered: (1) Does the presence of stem really improve oolong tea aroma? (2) What is the contribution of stem to the quality of oolong tea? To answer these questions, sensory evaluations and analyses of flavor-related compounds, including aroma compounds, amino acids, and polyphenols in oolong teas made from a combination of leaf and stem, or leaf only, were performed. Changes in levels of aroma compounds and aroma-related gene expression were monitored during the enzyme-active manufacture processes of tea made from a combination of leaf and stem, leaf only, and stem only. Furthermore, we investigated whether the leaf and stem interacted during oolong tea manufacture. The results will help to elucidate the actual contribution of the stem to oolong tea aroma, and highlight differences in aromas between leaf and stem.

2. Materials and methods

2.1. Chemicals and regents

Benzaldehyde, benzyl alcohol, β-damascenone, δ-decalactone, ethyl decanoate, furaneol, 2-hexen-1-ol, 3-hexenyl acetate, indole, β-ionone, jasmine lactone, 3-methylnonane-2,4-dione, methyl salicylate, and 2-phenylethanol, and vanillin, were purchased from Wako Pure Chemical Industries Ltd, Japan. α-Farnesene, (*Z*)-3-hexen-1-ol, linalool, linalool oxides, methyl jasmonate, *trans*-nerolidol, geraniol, polyvinylpolypyrrolidone (PVPP), XAD-2, catechin (C), gallocatechin (GC), gallocatechin-3-gallate (GCG), epicatechin (EC), epi-catechin-3-gallate (ECG), epi-gallocatechin (EGC), epi-gallocatechin-3-gallate (EGCG), and ninhydrin were purchased from Sigma-Aldrich Company Ltd., USA. The QuickRNA isolation kit was purchased from Huayueyang Biotechnology Co., Ltd., Beijing, China. Sodium dodecyl sulfate (SDS), and iTaq™ Universal SYBR® Green Supermix were purchased from Bio-Rad Laboratories, CA, USA.

2.2. Plant materials and manufacturing processes of oolong teas

All materials were collected from the *C. sinensis* var. Jinxuan plant, which is widely cultivated and often processed into oolong tea in South China. They were picked by hand (a conventional picking method) from the Tea Experiment Station at the South China Agricultural University (Guangzhou, China) at around 9:00 am in October 2015. Three types of materials were processed according to the oolong tea manufacturing process, following previously reported methods (Gui et al., 2015; Zeng et al., 2016) as shown in Fig. 1A. The first type of material comprised three parts, namely one bud, three leaves (bud and leaf are collectively referred to as leaf in this study), and a stem. Stem–leaf separation was carried out from the material after each manufacturing process, producing combined stem (CS) and combined leaf (CL) (Fig. 1A). The second type of material, separated leaf (SL), was produced by manually removing the stems from the first type of material once picked. The third type of material, separated stem (SS), was produced by manually removing the leaves from the first type of material once picked. The oolong tea manufacturing process was as follows: freshly plucked samples (P) were exposed to sunlight for 70 min to achieve solar withering (SW). Afterwards, the samples were indoor-withered (IW) at 30 °C to achieve a relative humidity of 70%, and subsequently turned over five times (T1–T5) at 1.5 h intervals. The samples were then parched in a tea-firing roller machine at 250 °C for 2–3 min to inactivate enzymatic activity and fix the sample (F). Finally, the samples were rolled (R) at room temperature for 15 min and dried (D) at 105 °C for 1.5 h. Samples at every stage were frozen immediately with liquid nitrogen and stored at −80 °C for further study. Each replicate used more than 250 g of fresh tea. Three replicates were processed according to the manufacturing procedure.

2.3. Sensory evaluation

Thirty participants, including 7 trained panelists and 23 normal consumers, were recruited to take part in the sensory evaluation of dry tea and tea infusion. For dry tea, tea product (10 g) was placed into a sealed container. For tea infusion, tea product (2 g) was infused with boiling distilled water (100 mL, 98 °C), and the tea slurry was poured out after brewing for 5 min. Participants were instructed to provide a degree of aroma liking for each sample using a five-point category scale with anchors from "dislike very much" (1) to "like very much" (5). Participants were carefully instructed about the test protocol before the test began.

2.4. Analysis of contents of total polyphenols and phenolic compounds in tea samples

Finely powdered sample (200 mg) was extracted twice with 70% MeOH (4 mL) at 75 °C for 10 min. The extracts were combined and diluted to 10 mL with the extraction solvent. The extract was used to analyze the contents of total polyphenols and phenolic compounds.

The total polyphenol contents of the tea products were determined using the Folin–Ciocalteu method with some modifications (Singleton, Joseph, & Rossi, 1965). Before reaction, the extract was diluted 100-fold with distilled deionized H_2O. Diluted extract or standard gallic acid solution (1 mL) was decanted into a 15 mL centrifuge tube. Then, 10% Folin–Ciocalteu reagent (5 mL) was added to the tube and the mixture was shaken. After 4 min, 7.5% Na_2CO_3 solution (4 mL) was added to the mixture. After incubating for