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Green tea flavour determinants and their changes over manufacturing processes

Zhuo-Xiao Han [a,1], Mohammad M. Rana [a,b,1], Guo-Feng Liu [a], Ming-Jun Gao [c], Da-Xiang Li [a], Fu-Guang Wu [d], Xin-Bao Li [d], Xiao-Chun Wan [a], Shu Wei [a,*]

[a] State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, 130 Changjiang Ave W., Hefei, Anhui 230036, China
[b] Bangladesh Tea Research Institute, Srimangal-3210, Moulvibazar, Bangladesh
[c] Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, Saskatoon, Saskatchewan S7N 0X2, Canada
[d] Tea Company of September 16, Shucheng, Anhui 231334, China

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ABSTRACT

Flavour determinants in tea infusions and their changes during manufacturing processes were studied using *Camellia sinensis* cultivars 'Bai-Sang Cha' ('BAS') possessing significant floral scents and 'Fuding-Dabai Cha' ('FUD') with common green tea odour. Metabolite profiling based on odour activity threshold revealed that 'BAS' contained higher levels of the active odorants β-ionone, linalool and its two oxides, geraniol, epoxylinalool, decanal and taste determinant catechins than 'FUD' ($p < 0.05$). Enhanced transcription of some terpenoid and catechin biosynthetic genes in 'BAS' suggested genetically enhanced production of those flavour compounds. Due to manufacturing processes, the levels of linalool and geraniol decreased whereas those of β-ionone, linalool oxides, indole and *cis*-jasmone increased. Compared with pan-fire treatment, steam treatment reduced the levels of catechins and proportion of geraniol, linalool and its derivatives, consequently, reducing catechin-related astringency and monoterpenol-related floral scent. Our study suggests that flavour determinant targeted modulation could be made through genotype and manufacturing improvements.

1. Introduction

Consumption of tea has been correlated with reduced incidence of chronic pathologies, such as cancer (Masood & Muhammad, 2009) and cardiovascular diseases (Stangl, Dreger, Stangl, & Lorenz, 2007). Green tea may have even greater benefits owing to higher levels of bioactive compounds (such as polyphenols) than in black tea (Lin, Tsai, Tsay, & Lin, 2003; Wang et al., 2011). However, compared to fermented black and semi-fermented Oolong teas, green tea generally tastes more bitter and astringent and is less fragrant.

Tea flavour (aroma and taste) is basically determined by the chemical constituents in the tea leaves (Chaturvedula & Prakash, 2011), which are largely dependent on tea genotype and the manufacturing process. The myriad of flavour qualities resulting from diverse tea genotypes suggests there is great potential to improve green tea flavour by using suitable tea genotypes (Lv et al., 2014). A recent advance in green tea flavour improvement can be exemplified with the high consumption preference of albino green tea in China, which tastes less astringent and more umami due to its lower levels of catechins and higher levels of theanine compared to 'Fuding-Dabai Cha' ('FUD') (Feng et al., 2014), which is a widely used standard control cultivar in China.

Great efforts have been made to understand the differences in the volatile constituents of green tea (Hara, Luo, Wickremasinghe, & Yamanishi, 1995). Studies on profiling the volatiles of brewed green tea infusions improves the understanding of tea flavours directly perceived by consumers (Baba & Kumazawa, 2014; Schuh & Schieberle, 2006). Volatiles detected from green tea infusions do not contribute equally to the tea aroma (Kumazawa & Masuda, 2002). Investigations on potent odorants of green tea infusions have identified some volatiles, such as isoeugenol and linalool, which are crucial to the characteristic aroma of the tea (Baba & Kumazawa, 2014; Kumazawa & Masuda, 1999). However, variations in levels of the potent odorants crucial to the characteristic aroma of green tea infusions are rarely reported in terms of their biosynthesis in raw fresh tea leaves and subsequent alteration during manufacture.

Tea leaf metabolites including carotenoids, lipids, phenylpropanoids, terpenoids and their glycoside derivatives are aroma

* Corresponding author.
E-mail address: weishu@ahau.edu.cn (S. Wei).
[1] ZXH and MMR are equal contributors.

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precursors, from which volatile esters, alcohols, aldehydes, hydrocarbons, benzenoids and terpenoids can be generated (Ho, Zheng, & Li, 2015). Among these volatiles, small terpenoids including carotenoid-derived norisoprenoids were reported as key aroma compounds in teas since they are usually abundant and have a relatively low detection threshold (Schuh & Schieberle, 2006). These compounds are basically synthesised through condensation of isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) produced from both cytosolic mevalonate (MVA) and plastidial methylerythritol phosphate (MEP) pathways (Hemmerlin, Harwood, & Bach, 2012). *In silico* analysis of many genes in the terpenoid biosynthetic pathways (Fig. 1) for tea plants has been published (Xiang et al., 2013). Catechins including (−)-epigallocatechin gallate flavan-3-ols (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC) account for up to 30% of the dry weight of fresh tea leaves (Ho, Cheng, Weng, & Leu, 2009), and are another category of key flavour determinants contributing to bitterness and astringency (Narukawa, Kimata, Noga, & Watanabe, 2010) as well as the colour of tea infusions. These compounds are produced through several specific steps downstream of the specialised flavonoid pathways in tea (Punyasiri et al., 2004) (Fig. 1B). Transcriptomic analysis revealed that enhanced flavour (aroma) due to altered expression levels of the genes in the corresponding pathways occurs in some tea cultivars or results from insect infestation of fresh leaves (Gohain et al., 2012).

Manufacturing techniques play key roles in tea flavour by affecting the conversion of chemicals during the processing procedure. Production of aroma compounds from the Maillard reaction and precursors of carotenoids, lipids, and glycosides is affected by the manufacturing process (Ho et al., 2015). Green tea is typically made from the plucked fresh tea leaves, which may undergo withering procedure, are then subjected to heat deactivation of enzymes, rolling and final drying for dehydration; this results in minimal oxidation compared with manufacturing of other teas such as black tea (Wang et al., 2011). For enzyme deactivation, pan-fire and hot steam treatments are conventionally applied (Baba & Kumazawa, 2014). Pan-fired green teas usually have a typical roasted taste (Kumazawa & Masuda, 1999) while steamed teas often have a green note (Jumtee, Komura, Bamba, & Fukusaki, 2011).

In this study, efforts were made to investigate the key flavour determinants released from green tea infusions and their changes over the manufacturing processes, using a widely used reference cultivar 'FUD', and a new tea cultivar, 'Bai-Sang Cha' (BAS) with a unique taste and strong floral aroma.

2. Materials and methods

2.1. Plant materials

Eight-year old tea plants of the two cultivars 'BAS' and 'FUD' were grown on two neighbouring plots at the university experimental tea farm in Shucheng, Anhui, China (31°31′ 87″ N, 117°02′ 84″ E), under the same cultivation practices. Shoot tips with two unfolded leaves typically used for commercial processing of top quality green tea products were plucked on April 4th 2014 and either deep frozen in liquid nitrogen and maintained at −80 °C for later assays or subject to different processing treatments immediately. For fresh leaf metabolite profiling, the frozen samples were further freeze dried for 48 h under a vacuum of 14 Pa and −80 °C using Labconco Freezone 4.5L Freeze Dry System (BioSurplus, USA). Steamed tea was processed by steaming at 102 °C for 1.5 min immediately after plucking, followed by rolling and drying at 120 °C for 1 h. Pan-fire processing procedure typically applied in local tea industries involved an initial indoor spreading of harvested tea crops at about 0.5–1.0 kg m^{-2} for 2–3 h at room temperature immediately after plucking; tea leaves were then heated in a pan at 220 °C for 5 min, followed by rolling and drying (120 °C for 1 h). Dried tea samples were deep frozen in liquid nitrogen and stored at −80 °C for future analysis.

2.2. Chemicals

Authentic standards linalool, linalool oxides, geraniol, citral, β-myrcene, limonene, β-ocimene, nerol, *trans*-nerolidol, farnesene,

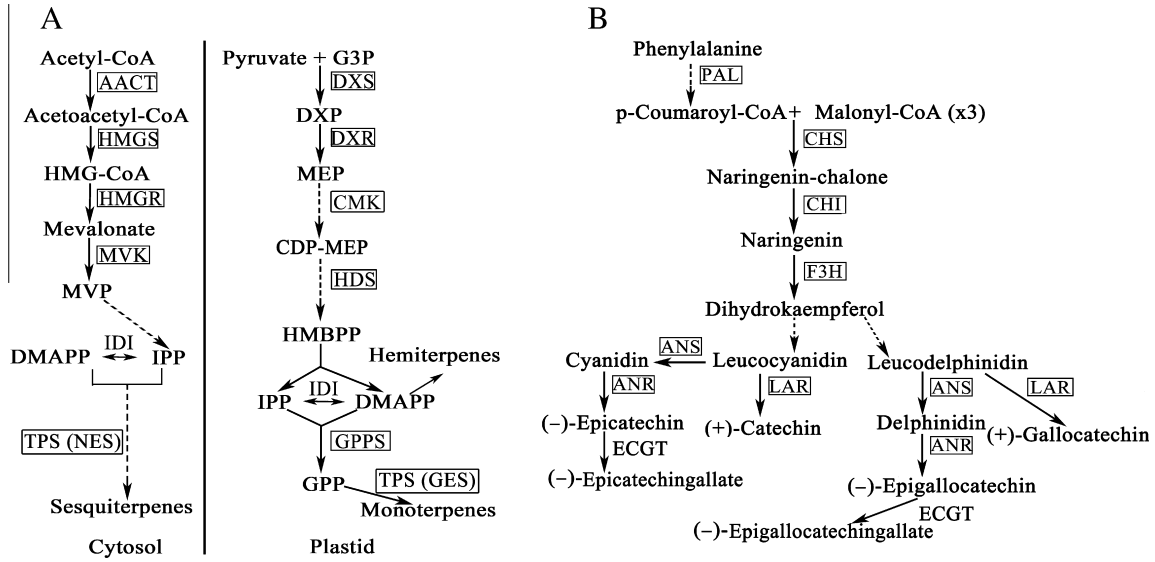

Fig. 1. Biosynthetic pathways of volatile terpenoids and catechins in tea plants. (A) Terpenoids; (B) Catechins. The genes coding the enzymes in boxes were quantified in this study. AACT, acetoacetyl-CoA thiolase; HMGS, 3-hydroxy-3-methyl-glutaryl coenzyme A synthase; HMGR, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase; MVK, mevalonate kinase; NES, neralidol synthase; CMK, 4-cytidine 5′-diphospho-2-C-methyl-D-erythritol kinase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-xylulose-5-phosphate reductoisomerase; HDS, hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase; GPPS, geranyl pyrophosphate synthase; GES, geraniol synthase; PAL, phenylalanine ammonia lyase; CHS, chalcone synthase; CHI chalcone isomerase; F3H, flavonoid-3-hydroxylase; LAR, leucoanthocyanidin reductase; ANS, anthocyanidin synthase; ANR, anthocyanidin reductase. Solid arrows indicate a one-step reaction; dashed arrows indicate multi-step reactions. The transcript levels of the genes encoding the enzymes in empty boxes were quantified in this study.