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Characterisation of odorant compounds and their biochemical formation in green tea with a low temperature storage process

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ABSTRACT

We produced low temperature (15 °C) processed green tea (LTPGT) with higher aroma contents than normal green tea (Sencha). Normal temperature processed green tea (NTPGT), involved storing at 25 °C, and Sencha had no storing process. Sensory evaluation showed LTPGT had higher levels of floral and sweet odorants than NTPGT and Sencha. Aroma extract dilution analysis and gas chromatography–mass spectrometry–olfactometry indicated LTPGT had 12 aroma compounds with high factor dilution values (FD). Amongst LTPGT's 12 compounds, indole, jasmine lactone, *cis*-jasmone, coumarin, and methyl epijasmonate contributed to floral, fruity and sweet characters. In particular, indole increased initially, peaking at 16 h, then gradually decreased. Feeding experiments suggested [^{15}N]indole and [^{15}N]oxygenated indoles (OX-indoles) were produced from [^{15}N]anthranilic acid. We proposed the increase in indole was due to transformation of anthranilic acid during the 16 h storage and the subsequent decline in indole level was due to its conversion to OX-indoles.

1. Introduction

The tea plant is of great economic importance, and tea infusions are some of the most consumed beverages around the world. According to the Food and Agriculture organisation of the United Nations (2011), world tea production is 4.6 million tons per annum, and the main producers are China (35.1%), India (20.7%), Kenya (8.0%), and Sri Lanka (7.0%). Tea extracts are used as flavourings in sweets, bakery products, and bottled tea beverages.

Teas can be divided into three major categories: unfermented green teas, semi-fermented oolong teas, and fully-fermented black teas, which account for about 20%, some 2%, and nearly 80% of the total world tea production, respectively (Flaten, 2002). Around 300 types of teas have been described, of which only a very small number are known and consumed in non-tea-producing countries.

Tea quality is important for its market value and is defined by colour, freshness, strength, and aroma. To date, approximately 600 volatiles have been described in black tea, with fewer numbers in oolong and green tea, due to the lesser degree of fermentation when producing these teas, and thereby tea quality influences a certain market percentage. Fresh tea leaves of *Camellia sinensis* are steamed immediately after plucking to produce Japanese green tea (Sencha). Endogenous enzymes involved in aroma formation are inactivated by the steam treatment, producing low aroma contents. The commercial value of Sencha is mainly evaluated by umami (taste) and fresh green odour, whereas flowery and fruity

Abbreviations: LTPGT, low temperature processed green tea; NTPGT, normal temperature processed green tea; FD, factor of dilution value; GC–MS/O, gas chromatography–mass spectroscopy–olfactometry determination; AEDA, aroma extraction and dilution analysis; HR-MS, high resolution mass spectroscopy; ESI, electrospray ionisation; HIPV, herbivore-induced plant volatiles.

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odour is essential for black tea or semi-fermented tea (oolong tea). The volatile compounds in green tea, black tea and semi-fermented tea have been intensively analysed (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Takei, Ishiwata, & Yamanishi, 1976; Shimoda, Shigematsu, Shiratsuchi, & Osajima, 1995a; Shimoda, Shigematsu, Shiratsuchi, & Osajima, 1995b). The potent odorants of Japanese and Chinese green teas and black tea have been investigated based on aroma extract dilution analysis (AEDA) (Guth & Grosch, 1993; Kumazawa & Masuda, 1999; Kumazawa & Masuda, 2002; Kumazawa, Wada, & Masuda, 2006).

To enrich green tea with more aroma attributes, a selection of raw materials (tea cultivars) can be employed. Amongst several types of Sencha aroma-rich green teas, made from *C. sinensis* cultivars, Kohshun and Shizu 7132 have a sweet odour (Yang et al., 2009), and are becoming popular in Japan.

Modification of the manufacturing process is an alternative approach. It is known that floral and fruity aroma-rich green tea is produced from leaves harvested in cool mountainous areas, followed by storing at ambient temperature prior to processing. (Luo, Jin, Han, & Wen, 2009) However, neither sensory evaluation nor examination of the odorant compounds have been performed for green tea made from stored fresh tea leaves. The manufacturing process of oolong tea includes withering and bruising processes prior to a pan-frying step for inactivation of the endogenous enzymes. Black tea processing includes withering, rolling, cutting, and oxidation steps. During tea processing, many odorant compounds are produced by the action of glycohydrolases and oxidative enzymes. Thus, tea leaves are exposed to numerous mechanical and environmental stresses after plucking to yield higher amounts of volatile odorant compounds (Wang, Kubota, Kobayashi, & Juan, 2001).

The process of aroma production is similar to the defence response of plant leaves. As a defenceresponse to attacks by herbivores, such as the smaller tea tortrix (*Adoxophyeshonmai Yasuda*), tea leaves emit numerous volatiles such as (*Z*)-3-hexen-1-ol, linalool, α-farnesene, benzyl nitrile, indole, nerolidol, and ocimenes, in large amounts. Attack by Kanzawa spider mites (*Tetranychus kanzawai Kishida*), another major pest insect of tea crops, can induce the emission of α-farnesene and ocimenes (Yang, Baldermann, & Watanabe, 2013) from tea leaves. The exogenous application of jasmonic acid to tea leaves induced a volatile blend that was similar, though not identical, to that induced by the smaller tea tortrix. Most of the herbivore-induced plant volatiles (HIPV) were not stored in the tea leaves but emitted after herbivore attack.

As mentioned above, leaves have molecular responses to abiotic and biotic stresses. After plucking, the storing process may be similar to withering to affect a metabolic change in the green tea leaves, as clarified in oolong tea manufacturing (Wang et al., 2001). We examined the effects of temperature and the processing period on the aroma profiles of the final green tea products. Fresh green tea leaves were kept at 15 °C or 25 °C for 24 h, and were processed in the usual manner to yield aroma-endowed green tea. We investigated the characteristics of aroma by sensory evaluations and identified potent odorants using the AEDA technique. Furthermore, we examined the change in the amount of indole, a volatile compound contributing to the floral aroma, during the storage of green tea leaves, and analysed the metabolites of indole using metabolome analyses. The conversion of anthranilic acid to indole and its metabolites was also investigated using a stable isotope labelled precursor.

We describe the aroma characteristics, contributing volatile compounds, and the metabolic features which accumulate during the storage of green tea leaves.

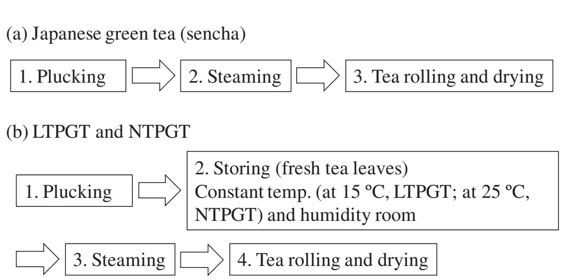

Fig. 1. Manufacturing process for Japanese green tea (Sencha), LTPGT, and NTPGT.

2. Materials and methods

2.1. Tea samples

Tea (*C. sinensis* cv Sayamakaori) leaves comprising a bud with three or four new leaves were harvested by a tea plucker in May 2012 and 2013 from the tea field of Tea Research centre, Shizuoka Prefectural Research Institute of Agriculture and Forestry, Shizuoka, Japan. The leaves (2 kg fresh weight)were spread with 10 cm thickness in a bamboo colander (90 W × 60 L × 20H cm) and stored at different temperatures (25 °C and 15 °C, relative humidity 70%) for 16 h under dark conditions, and they were processed according to the traditional green tea (Sencha) manufacturing process (Fig. 1). The green tea samples were then designated NTPGT (green tea stored at normal temperature, 25 °C, prior to processing) and LTPGT (green tea processed at low temperature, 15 °C, prior to processing), whereas the tea sample manufactured just after plucking was designated as Sencha.

2.2. Sensory evaluations by panellists

Sensory evaluation was performed in a sensory room by 15 panellists skilled in the evaluation of green tea or fragrance working at Takasago (Kanagawa, Japan) and the Local Tea Research Institute (Shizuoka, Japan). Tea samples (3 g) were extracted with hot water (90 °C, 150 mL). The tea infusion (25 mL) was presented to the panellists for orthonasal evaluation in a plastic cup (odour free, 90 mL, Asahi Kasei Pax Corporation). The panellists were first requested to freely describe the smell of the extracts. Seven types of aroma attributes were determined on the basis of the descriptions. The panellists scored the intensities of seven aroma attributes on a scale from 0 to 4 (0: no detection, 1: weak detection, 2: moderate, 3: intense, 4: very intense).

2.3. Preparation of aroma extracts from tea samples

The aroma compounds of each sample were concentrated using the adsorptive column method (Shimoda et al., 1995a; Shimoda et al., 1995b). Tea samples were powdered using a mill (Iwatani Corporation) and the powders (8 g) were suspended in hot distilled water (160 mL, 80–90 °C) for 5 min. The suspension was centrifuged for 10 min at 3000*g* at ambient temperature to separate supernatant from the residue. The supernatant (120 mL) was applied to a Porapak Q cartridge (2 mL of volume, 50–80 mesh; Waters Corporation, Milford, MA) conditioned with diethyl ether (5 × 4 mL), methanol (4 mL), and distilled water (4 mL). The cartridge was washed with 3 mL of water before the aroma compounds were eluted with 3 mL isopentane:diethyl ether (1:1, v/v). Ethyl decanoate (10 μg/10 μl) was added as an internal standard to the eluent. The eluate was dried over anhydrous sodium sulphate, and concentrated to 100 μL in a stream of nitrogen to give a sample for GC–MS and GC–MS/olfactory analyses.