Comparative study on volatile compounds in Turkish green tea powder: Impact of tea clone, shading level and shooting period

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ABSTRACT

The objective of this study was to determine volatile compounds in green tea powders produced from a clone of two different teas (*Camellia sinensis* (L.) O. Kuntze) grown under different shade levels and harvested in two consecutive shooting periods. Both hydrodistillation and solid phase microextraction (SPME) methods were comparatively performed to identify maximum number and amount of volatile compounds. SPME method enables the identification of the greatest number of volatile compounds which principally comprise limonene, α-terpineol and heptanal. A few specific volatile compounds were identified for differentiation of green tea samples depending on the treatments, such as, heptanal in 1st shooting period, ethyl benzene, xylene and benzenacetal for 2nd shooting period, and phytol and tridecane for shading treatments. The treatments were significantly clustered either as tea clones or shooting period by the volatile compounds i.e. linalool, α-terpineol, 3-methylbutanal, 2-methylbutanal and *p*-cresol, 2,6-di-*tert*-buthyl determined in hydrodistillation method and tridecane, heptanal, linalool, nonanal, hexanal, α-terpineol, 1-pentanol, pentanal, dimethylsulfide, 2,2,4-trimethylhexane, limonene and 1-hexanol in SPME method as shown by principal component analysis (PCA).

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1. Introduction

Green tea quality varies by tea clones, climate, soil properties, altitude, and horticultural practices. In addition, plucking season, sorting (grading) of the leaves, processing, and storage are the other key factors affecting green tea quality (Ku et al., 2009). Green tea contains high amounts of catechins especially epigallocatechin gallate. The highest quality green tea has high content of free amino acids such as theanine (Song, Kelman, Johns, & Wright, 2012) and terpens such as linalool (Kato & Shibamoto, 2001).

Hirai et al. (2008) reported that shading treatment of tea plants for a few weeks before the harvest enriches tea leaves with free amino acids such as theanine and increases the quality of green tea. According to Ku et al. (2009), shading causes tea leaves to become greener, sweeter, and have less astringent taste, all of which indicate higher quality. A special product produced from shade grown tea is green tea powder (*matcha*), consumed in Japanese tea ceremony after whisking with a special whisker (*chasen*) in a bowl (*chawan*). It is also used as an additive in production of beverages, chocolates,

candy, cakes and pastries, cookies, pudding, ice cream, etc. (Tokunaga, 2004).

Green tea powder is made of young shoots of tea bushes shaded for a few weeks before hand-harvest followed by processing into green tea powder through different steps, namely steaming, drying, removing of stems, midribs and veins, and fine grinding by stone milling (Tokunaga, 2004). There are many studies on this product especially related to milling methods, particle characteristics and foaming properties (Haraguchi, Imada, & Sawamura, 2003; Maeda, Hibi, & Hayakawa, 1999; Sawamura, Haraguchi, Yasuda, & Matsusaka, 2009; Sawamura, Ichitani, Ikeda, & Sonoda, 2012; Sawamurai, Haraguchi, Ikeda, & Sonoda, 2010). Green tea powder is also studied in catechins (Li, Taylor, & Mauer, 2011; Weiss & Anderton, 2003) however, there is no study on volatile constituents of green tea powder which was studied by our group and is being reported here.

Tea has been produced in Turkey for more than 80 years. Unlike many tea producing countries, the distribution of tea crops is atypical in Turkey. After a severe winter, the temperature suddenly rises in the spring. The tea season starts in May and the crop is ready for harvesting at nearly the same time at different locations in the eastern Black Sea region of the country. Tea is plucked three times until October, depending on the weather conditions. In other words, there are three shooting (flushing) periods in a season (Ozdemir, Gokalp, & Nas,

1992, 1993). In Turkey, all of the tea is being processed into black tea only. During the last decade, the production of green tea has been started and efforts are underway to process green tea powder these days.

To the best of our knowledge, no study has been reported on Turkish green tea powder. The main purpose of the current study was to identify volatile constituents of green tea powder produced from two different Turkish tea clones (Derepazari 7 and Fener) depending on shooting period and shading degree. In addition, analyses of volatile constituents of the green tea powder recovered by both hydrodistillation and SPME methods were comparatively investigated.

2. Material and methods

2.1. Material

Young shoots of tea (C. sinensis (L.) O. Kuntze) clones (Derepazari 7 and Fener) were plucked as two leaves and a bud in the two consecutive shooting periods (May and July 2010) from Hayrat tea plantation (latitude:41°01′57″ N, longitude: 40°29′86″ E, altitude: 140 m) which belongs to the Turkish Tea Board Experimental Station in Rize. The tea bushes are 35–40 years old. They [nearly 60 bushes in 75 m^2 for each shading degree] were randomly selected and shaded [control, light (50%) and dark (90%)]. The plants were grown under recommended agricultural practices without pesticide application. Young shoots of the tea clones were meticulously harvested by hand. On each occasion, about 40 kg of homogenous leaves were immediately processed into the green tea powder at the experimental tea station of the Turkish Tea Board (ÇAYKUR). The tea was processed through a series of steps, namely steaming (120 °C, 90 s), cooling, drying (95 ± 2 °C), removing of stems, midribs and veins, and milling (D_{90} < 107.1 μm).

2.2. Extraction of volatile constituents

2.2.1. Distillation of volatile constituents

30 g of green tea powder was distilled by a Clevenger type steam distillation assembly. The volatile constituents were collected in diethyl ether for 6 h (supernatant of the condensate collecting arm) and directly injected to the injection port of the GC–MS system.

2.2.2. HS-SPME procedure

HS-SPME analysis was also employed to determine the volatile constituents of the green tea powder. Preliminary tests were carried out to optimize analytical conditions to identify high number of volatile components. Sample amounts, extraction conditions (dry or dispersed in water), extraction parameters such as selection of fiber, extraction time and temperature, and absorption and desorption time of volatiles were comprehensively studied in the preliminary tests. The best conditions were determined as 0.2 g dry sample in 20 mL headspace vial, 65 μm PDMS/DVB fiber (Supelco, Bellefonte, Pennsylvania, USA), 40 min extraction at 60 °C without agitation followed by 20 min absorption to fiber, and finally 3 min desorption in the injection port. These conditions resulted in the highest amount of volatile components of green tea powder.

2.3. Evaluation of volatile constituents

2.3.1. Gas chromatography–mass spectrometry (GC–MS) analysis

The volatile constituents of green tea powder were analyzed by a GC–MS system (Shimadzu QP2010 Plus) equipped with a TRB-5MS (30 m × 0.25 mm × 0.25 μm) column.

Elution of the volatiles recovered by distillation method (in diethyl ether) was performed under the following conditions: column temperature was programmed from 50 °C (held 2.8 min) to 140 °C at the rate of 5.5 °C/min and followed by increasing from 140 °C (held

for 1 min) to 220 °C at the rate of 4.5 °C/min. Then from 225 °C (held for 2 min) to 265 °C at the rate of 3.4 °C/min and held at 265 °C for 5 min. The temperature of injection port and transfer line was 250 °C. Helium (%99.99) was used as carrier gas with a constant flow rate of 1.37 mL/min, and injection volume was 1 μL at split ratio of 1/15 (Zhu, Li, & He, 2008).

For SPME analysis, column temperature was programmed from 40 °C (held 3 min) to 200 °C with 4 °C/min and held at 200 °C at 5 min. The temperature of injection port and transfer line was 220 °C. Column flow rate was 1.37 mL/min and split ratio was 1/25.

MS conditions for both analyses were EI 70 eV, mass range 40–400 amu and scan rate 769 s/scan.

2.3.2. Identification of compounds

Alkane standard (C_7–C_{40}) was used to determine retention index (RI) of each compound. Identification of the volatile compounds was first attempted using mass spectral libraries of Wiley 7 and NIST 02. They were also confirmed by comparing RI and mass spectra with literature (Lee, 2009; Wang et al., 2008; Yamanishi, 1978) and online library (Anonymous, 2012).

2.4. Statistical analysis

The data set including all volatile components from the treatments were subjected to analysis (PCA) and agglomerative hierarchical cluster analysis (HCA) by using XLSTAT software (Addinsoft, New York, NY). Two dimensional principal components were created from the data matrices, each row corresponding to relative peak areas of the volatile compounds and each column was represented by the treatments. Ward's procedure and Euclidean distance methods were used to generate dendrograms for HCA.

3. Results and discussion

3.1. Volatile constituents determined by hydrodistillation

Total volatile compounds of the green tea powders produced from the leaves of Derepazari 7 and Fener clones, grown under different light intensity and harvested in two shooting periods were extracted by hydrodistillation method. Fig. 1 illustrates the extraction efficiency of the volatile compounds as total peak area. It shows that the green tea powders produced from the Derepazari 7 clone had a greater amount of volatile compounds in comparison with that produced from Fener except control samples of 1st shooting period. In case of shooting period comparison, the product of the 1st shooting period had more volatile compounds. The amount of volatile compounds was increased by lowering the light intensity in the growing conditions for both clones. No evidence was reported on effect of light intensity and tea clone on volatile constituents of either green tea
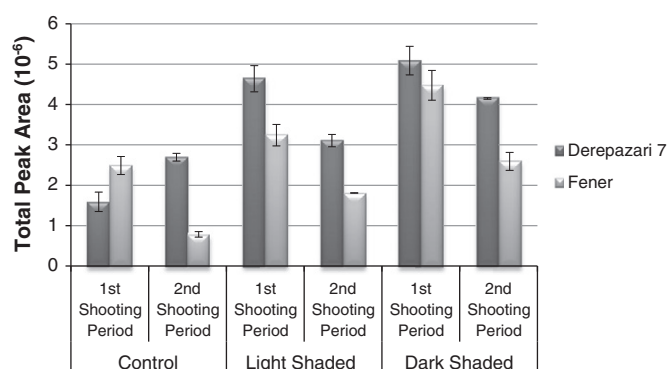

Fig. 1. Total peak area of volatiles extracted by hydrodistillation.