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Original Article

Effect of shaking process on correlations between catechins and volatiles in oolong tea

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ABSTRACT

Shaking the tea leaves is the key manipulation to making oolong tea. It contributes to the formation of flavor and fragrance in oolong tea. The dynamic variations of catechins and volatile organic compounds (VOCs) during the shaking process were investigated. The results showed that the contents of epicatechin, epigallocatechin, epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) first decreased after the shaking and then increased to the initial value before the next shaking. Geraniol, linalool and its oxides, and phenylethyl alcohol showed similar variations. The contents of trans-β-ocimene, 1H-indole, and 3-hexenyl hexanoate increased after the second or third shaking (the late fermentation stage). However, the contents of aldehydes showed an opposite trend to other VOCs. The abundance of phenylethyl alcohol was positively related to the content of ECG and EGCG during fermentation, whereas the abundance of *cis*-3-hexenal was negatively related to the content of ECG. The correlations between catechin and VOCs indicated that shaking affected the chemical transformation of the compounds in oolong tea.

1. Introduction

Oolong tea is Taiwan's world renowned semifermented tea (partial oxidation) with an elegant floral aroma and mellow characteristics. The manufacturing process of this semifermented tea includes withering, several rounds of shaking and setting (fermentation or oxidation), firing (fixation), rolling, and drying. Tea fermentation is the most important stage for quality control. An oxidation reaction occurs in this stage; the catechins in tea leaves are oxidized with time [1,2]. In this process, catechin monomers are polymerized to form theaflavins, thearubigins, or other oxidation products such as theasinensins or oolongthenin [1,3,4]. These tea chemical components contribute to the color and taste of oolong tea.

The first step of tea making is withering the fresh tea leaves. As the moisture content of tea flushes decreases, withering reduces the semipermeability of the membranes, thus enabling the catechins stored in the leaf cell vacuoles to flow out of the cytoplasm and come into contact with the oxidase in the cell cytoplasm [5]. From this stage onward, the

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oxidation of catechins starts and tea fermentation begins. In the manufacturing of oolong tea, the withering process includes two stages: solar and indoor withering. In solar withering, other than the loss of moisture, the UV radiation of the sunlight also promotes gene expression of intracellular hydrolytic enzymes, β-primeverosidase and β-glucosidase, and facilitates the hydrolysis of the precursors of volatile organic compounds (VOCs), which are present in the glycosidic form [6,7]. After a short period of solar withering, the tea leaves are moved indoors to continue the withering process. During indoor withering, the tea leaves are shaken three to five times at intervals. Each shaking interval is about 2 hours and is dependent on the temperature, moisture, and the contents of leaves. The tea master decides when to shake the tea leaves, what the intensity should be, and the duration of the shaking by touching and smelling the leaves. The initial shaking is mild, mainly to redistribute the moisture from the stalk to the leaves, and the stomatal conductance of the tea leaf is decreased after plucking and solar withering [7,8]. After a heavy shaking, the catechins are released from the vacuoles to the cytoplasm [5]. The expression of oxidase is also significantly enhanced, indicating significant oxidation [9]. Moreover, the smell of the fermenting tea leaves changes during the entire shaking and setting process. The precursors of the aromatic VOCs undergo oxidation and hydrolysis after the shaking. The hydrolysis of terpene alcohol glycosides generates terpene alcohols as the VOCs, while the oxidation of carotenoids and lipids produces lactones, ketones/enols, and other VOCs [10–12]. Thus, the smell of the fresh tea leaves changes in the manufacturing process. Consequently, the resulting VOCs composition contributes to the unique and characteristic aroma of oolong tea.

The tea master monitors the entire process through his/her senses and decides the timing of each step, which is still an art depending on the traditional master–apprentice model (on-the-job training). This means that good tea can only be made by hand, not by machinery. If we want to produce tea of high quality, we need more information and parameters about tea fermentation. To establish a scientific tea manufacturing process, the changes in the chemical contents of tea leaves during the fermentation process should be elucidated. The fermentation duration of oolong tea can be up to 6–10 hours. We monitored the changes in the chemical contents of tea leaves during the fermentation stage, before and after each shaking step, to understand the changes in catechin monomers and VOCs in the manufacturing process of the oolong tea. We can determine the whole picture of the fermentation process through the change of catechins, the raw materials of tea fermentation, and the VOCs—what tea masters depend on. According to these data, we provide a theory of tea fermentation for procedures to monitor the manufacturing process.

2. Methods

2.1. Oolong tea manufacturing process

Tea flushes (3 leaves with 1 bud) were plucked from the tea plantations in the Taoyuan County, Taiwan. Two cultivars of tea (*Camellia sinensis* var. sinensis), *'Chin-Hsin-Dah-Pang'* and *'Chin-Hsin-Gan-Tzu'*, were used to make oolong tea in 2011 and 2013. We used the standard oolong tea manufacturing process which was followed by an experienced operator. Samples were collected from fresh tea leaves (F), the leaves after solar withering (SW), and indoor-withering stage which is separated into before each shaking (BSn, $n^{th} = 1, 2, 3,$ and 4) and 15 minutes after shaking during the setting period (ASn, $n^{th} = 1, 2, 3,$ and 4). The weight of each fresh leaf sample was ~100 g. Three replicates sampled simultaneously were obtained. Twenty grams of samples were dried at 105°C in the oven until a constant weight to measure the water content by the weight loss. Remaining samples were immediately frozen at −20°C and stored until analysis.

2.2. High performance liquid chromatography analysis of tea catechins

Freeze-dried tea leaves (20 g) were ground into a powder of < 40 mesh; 0.5 g of the ground tea leaves were extracted with 50-mL boiled deionized water for 20 minutes in a water bath at 90°C. The tea extract was filtered through a 0.45-μm Millipore filter before the analysis. A Jasco High Performance Liquid Chromatography System equipped with PU-2089, AS-2057, UV-2075, and LC-NetII/ADC (Shimadzu Co. Ltd., Kyoto, Japan) was used. A stainless steel Symmetry Waters column (4.6 mm internal diameter × 250 mm long; 5 μm particle size; WAT 054275, Milford, MA, USA) was used and maintained at a constant temperature of 40 ± 0.5°C. A flow rate of 1.0 mL/min was used during the separation; the injected volume was 8 μL. The mobile phase consists of a combination of solvent A [deionized water with 0.1% (volume/volume) formic acid] and solvent B (acetonitrile). The elution profile for catechin separation was as follows: 0–5 minutes, 100% A; 15 minutes, 90% A, 10% B; 29 minutes, 80% A, 20% B; 35 minutes, 78% A, 22% B; and 40 minutes, 75% A, 23% B. Absorbance at 280 nm was used for the real-time monitoring of peak intensities.

2.3. Extraction of volatiles and gas chromatography mass spectrometry analysis

Fresh frozen leaves (5 g) were mixed with liquid nitrogen to homogenize and release the volatiles, and then 0.03 g of the tea fragments were sealed in an airtight vial (30 mL). The samples were heated to 50°C for equilibration. Headspace gas sampling was conducted by solid-phase microextraction (SPME) using 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane (Supelco, St. Louis, MO, USA). A handheld SPME fiber was exposed to a conditioning sample headspace for 15 minutes. The SPME fiber was then injected into the gas chromatograph in the splitless injection mode at 250°C. Gas chromatography mass spectrometry was carried out using a HP 5890 gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with 5972 mass selective detector (Agilent, Santa Clara, CA, USA). A capillary column (DB-5 Hewlett-Packard – Agilent J&W GC Columns) 30 m long and 0.25 mm internal diameter with a 1-μm film thickness was used. The GC-operating condition is as follows: (1) hold at 35°C for 1 minute and then increase to 80°C at a rate of 25°C/min; (2) increase by 3°C/min from 80°C to 120°C, then further increase to 200°C