



# Study of the dynamic changes in the non-volatile chemical constituents of black tea during fermentation processing by a non-targeted metabolomics approach



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## ABSTRACT

Fermentation is a critical manufacturing process to produce black tea, in which the chemical compositions are greatly changed. However, the dynamic changes of this sophisticated process are far from clear, and were often characterized by determining a small number of compounds. In this study, we applied a non-targeted metabolomics approach based on ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry to comprehensively profile the variations of metabolites in tea samples with various fermentation durations of 0, 1, 2, 4, 6, 8, 10, 12 and 14 h. Principal component analysis indicated obvious stepwise alterations of tea metabolome during the fermentation. Relative quantitation of 61 identified metabolites including catechins, dimeric catechins, flavonol glycosides, amino acids, phenolic acids, alkaloids, and nucleosides revealed distinct changes of phenol pathway. Dynamic changes of a part of these compounds were mapped for the first time. To the best of our knowledge, this study offered the most comprehensive profiles of metabolite changes during the tea fermentation process.

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## 1. Introduction

Tea (*Camellia sinensis* L.) is one of the most traditional beverages consumed worldwide due to its health benefits, satisfactory taste and aroma (Hamilton-Miller, 2001). Unlike green tea (unfermented) and oolong tea (partially fermented), black tea is defined as one kind of fully fermented tea. The manufacturing process of orthodox black tea includes steps of withering, rolling, fermentation (oxidation) and drying. Among them, the fermentation process is a crucial step, which greatly changes the chemical compositions and subsequently the taste, aroma, color, and nutritional and biological properties of teas (Shinde, Das, & Datta, 2013).

A number of early studies have been carried out, focusing on the transformation of flavanols (catechins) during the complicated process of tea fermentation. The polyphenol oxidase (PPO) and peroxidase (POD) present in the leaves are considered responsible for most of the oxidation reactions. Flavanols (catechins) in fresh tea leaves are largely consumed and chemically transformed to various oxidation products including dimeric, oligomeric and polymeric compounds, such as

theaflavins, theacitrins, theasinensins, theanaphthoquinones, and thearubigins during the fermentation process (Kuhnert, Clifford, & Müller, 2010; U. Stodt & Engelhardt, 2013; Stodt et al., 2014). Besides the flavanols, other compounds, such as flavonol glycosides, amino acids, phenolic acids, and alkaloids are also crucial tastants and bioactive constituents, and were found changed during the tea fermentation (Kim, John, Choi, Lee, Kim, Kim et al., 2013; Lee, Lee, Chung, Shin, Lee, Lee, et al., 2011). For instance, Lee et al. monitored 14 main metabolites in green tea, partially fermented tea, and fully fermented tea using <sup>1</sup>H NMR spectroscopy approach, and found that theanine, caffeine, and sucrose decreased in fully fermented tea, while glucose and gallate increased (Lee, Lee, Chung, et al., 2011). However, the sophisticated fermentation process, in which numerous reactions take place simultaneously, was often characterized by determining only a small amount of compounds in these studies due to the limitation in analytical method. In addition, some of these studies were conducted using tea samples purchased from commercial markets, in which the results might be affected by other factors such as cultivar variety, plucking season, growing area, manufacturing process, and storage. Furthermore, the dynamic changes of chemical constituents except flavanols during the fermentation were of little concern. Therefore, a more comprehensive investigation to clarify the dynamic variations of the tea metabolome using well-controlled tea samples is in need.

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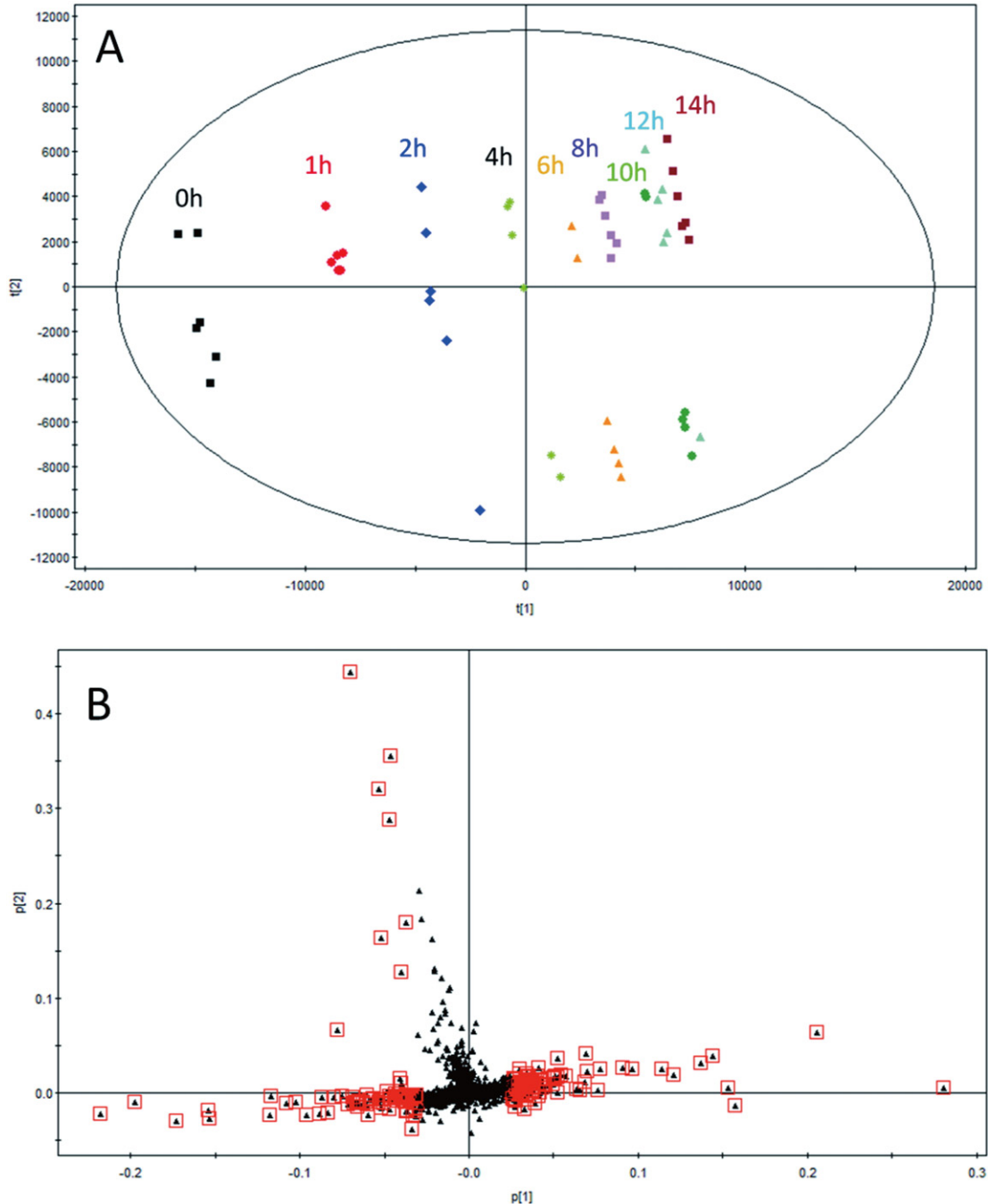
Metabolomics, emerging as an important part of system biology, has been widely applied in food and tea studies (Daglia, Antiochia, Sobolev, & Mannina, 2014; Jiang et al., 2013; Lee, Lee, Hwang, Ko, Chung, Kim, et al., 2011; Schmeda-Hirschmann, Quispe, & González, 2015; Zhao et al., 2013; Zhou et al., 2012). Non-targeted metabolomics detecting dozens of, even hundreds of, endogenous metabolites simultaneously provides an “unbiased” view of the global metabolome (Abu-Reidah, Ali-Shtayeh, Jamous, Arráez-Román, & Segura-Carretero, 2015; Daglia et al., 2014; Fraser et al., 2014). In this study, we applied a high-resolution mass spectrometry-based non-targeted metabolomics approach to comprehensively describe the dynamic changes of the tea metabolome during a long time fermentation process from 0 to 14 h. It offered a comprehensive and dynamic view for the tea fermentation

and is potentially helpful for the future studies on tea sensory quality, nutritional value, and biological properties of black tea.

## 2. Experimental

### 2.1. Chemicals

Deionized water was produced by a Milli-Q water purification system (Millipore, Billerica, Massachusetts). Methanol of LC–MS grade was purchased from Merck (Darmstadt, Germany). Ammonium acetate, formic acid, epigallocatechin (EGC), catechin (C), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epicatechin (EC), kaempferol-3-O-glucoside (astragalol), kaempferol-3-O-rutinoside, isovitexin,



**Fig. 1.** Metabolomics analysis of the tea metabolome changes during the fermentation process: (A) score plot of PCA demonstrating a stepwise metabolome change during the tea fermentation process from 0 h to 14 h. The principal components 1 and 2 explained 50.5% and 19.7% of total variance, respectively; (B) loading plot of PCA indicating primary differential metabolites.

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