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Enantiomeric and quantitative analysis of volatile terpenoids in different teas (*Camellia sinensis*)

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ABSTRACT

Volatile terpenoids play important roles in the formation of tea aroma quality due to their pleasant scents and low odor thresholds. Most volatile terpenoids contain stereogenic centers, which results in various stereo distributions of their enantiomers and diastereoisomers in different types of tea. However, the distribution characteristics of terpenoid enantiomers in teas were still unclear, which poses an obstacle to the scientific understanding of tea aroma. In this work, a new and efficient analysis approach based on headspace solid phase microextraction (HS-SPME)-chiral gas chromatography-mass spectrometry (GC–MS) was established to analyze 12 pairs of familiar terpenoid enantiomers in different teas. The extraction efficiency of the HS-SPME method to extract volatile terpenoids in teas was the greatest when using CAR-DVB-PDMS (50/30 μm) fibers and 1:10 proportions between tea and boiling water at a 50 °C extraction temperature for 40 min, and the stability observation of enantiomeric ratios of the terpenoids well proved the feasibility of the extraction method. The favorable limits of detection, limits of quantitation, repeatability, linearity, and concentration ranges of each terpenoid enantiomer demonstrated the repeatability and reliability of the analytical approach. The enantiomeric and quantitative analyses indicated that *S*-limonene, *S*-linalool, (2*S*, 5*S*)-linalool oxide A, (2*S*, 5*R*)-linalool oxide B, *R*-4-terpineol, (2*S*, 5*R*)-linalool oxide C, (2*S*, 5*S*)-linalool oxide D, *S*-α-terpineol, *R*-α-ionone, peak 1 of theaspirane A and peak 2 of theaspirane B were the major terpenoid components in most Chinese teas; instead, higher proportions of the opposite enantiomers of the above terpenoids were frequently detected in black teas with large leaf origin and Indonesia white teas. Besides, great diversities of enantiomeric ratios and concentrations among different teas were observed. Furthermore, partial least-squares discriminant analyses were performed to distinguish the concentration differences of the terpenoid enantiomers among different teas; the analysis results indicated that highly significant concentration differences existed between large and small leaf origins of black teas, and significant differences of the concentrations of linalool oxides A–C were observed between green, white and dark teas. The successful application of this chiral analysis technique of tea aroma will lay a scientific foundation for further quality assessment, botanical origin determination and authenticity assessment of teas.

1. Introduction

Essential oils of tea (*Camellia Sinensis*) are composed of diverse volatile compounds at various concentrations, and the volatiles can be classified into aliphatics, aromatics and volatile terpenoids based on their basic carbon chains and parents of compounds [1]. Terpenoids have a significant influence on the formation of tea aroma quality due to their low odor thresholds and pleasant fragrances, and hundreds of them have already been separated and identified by gas chromatography-mass spectrometry (GC–MS) [2]. Most identified terpenoids contain one or more stereogenic

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Abbreviations: CAR-DVB-PDMS, carboxen/divinylbenzene/polydimethylsiloxane; DVB-PDMS, divinylbenzene/polydimethylsiloxane; PA, polyacrylate; ER, enantiomeric ratio; GC, gas chromatography; HCA, hierarchical clustering analysis; HS-SPME, headspace solid-phase microextraction; LOD, limits of detection; LOQ, limits of quantitation; PLS-DA, partial least-squares discriminant analysis; R^2, correlation coefficients; RSD, relative standard deviation; TIC, total ion chromatogram; VIP, variable importance in the projection.

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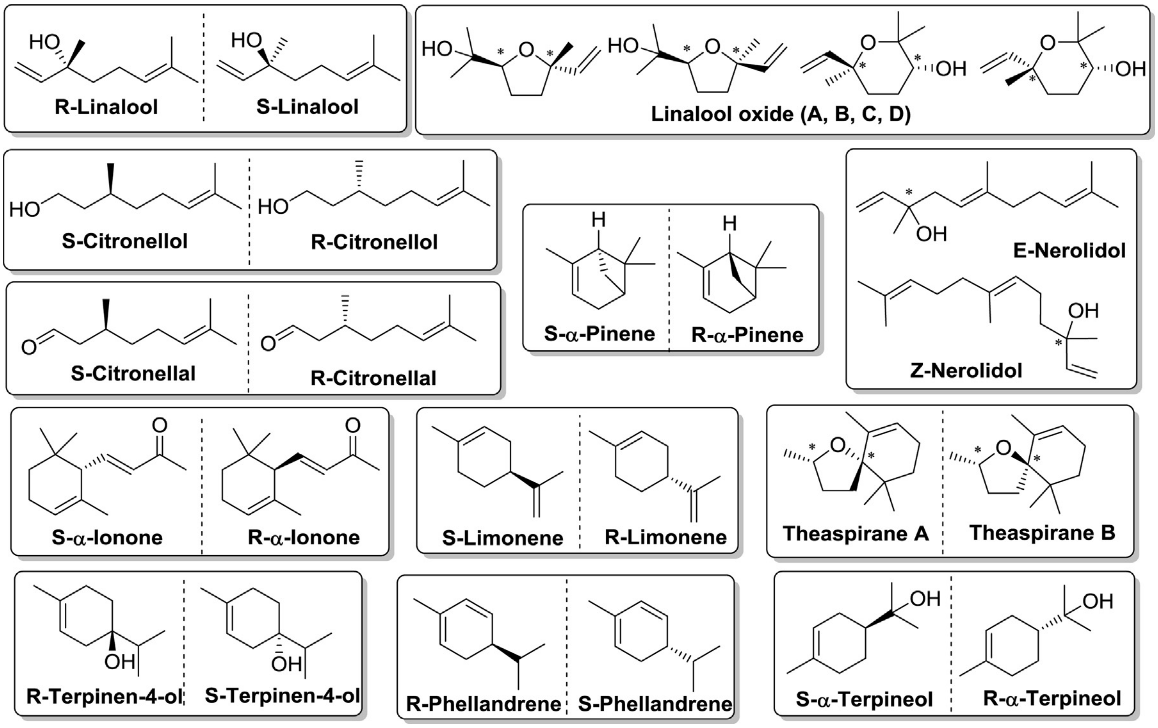

Fig. 1. The familiar volatile terpenoids with high contents in teas.

center, which results in various stereo distributions of their enantiomers and diastereoisomers in different teas. Among them, linalool, linalool oxides (A–D), citronellol, nerolidol (*Z*, *E*), citronellal, α-ionone, theaspirane (A, B), α-terpineol, α-pinene, limonene, phellandrene and 4-terpineol are considered to be the familiar aroma components with high contents in most teas [3] (Fig. 1).

It has been verified that different enantiomers of the same molecule may possess different properties including odor characteristics, odor thresholds and even biological activities [4]. For instance, *R*-linalool (odor threshold of 0.8 μg/L) has a woody and lavender-like smell [5] and shows sedative effects on autonomic nerve activity and mood states at a very low intensity [6], whereas *S*-linalool (odor threshold of 7.4 μg/L) has a sweet, flowery and orange-like aroma [7] and shows no sedative effects [6]. *S*-Citronellol presents a gentle rose-like smell, but *R*-citronellol presents a sweet and rose-like smell [8]. *S*-α-Ionone (odor threshold of 20–40 μg/L) has a woody odor, whereas *R*-α-ionone (odor threshold of 0.5–5 μg/L) has a violet-like aroma, and racemic α-ionone has a strong sweet-floral odor, reminiscent of violets [9]. *S*-Limonene exhibits a lemon-like aroma, whereas *R*-limonene exhibits a turpentine smell [10]. In view of the different aroma characteristics of enantiomers, it is speculated that different enantiomeric distributions of terpenoids could trigger various fragrance characteristics, and would significantly influence the aroma quality of tea due to permutations and combinations of terpenoids with different enantiomeric excess values [4] and concentrations. Therefore, determining the enantiomeric distributions of volatile terpenoids in different types of tea is a significant and valuable research topic.

Enantiomeric analysis of terpenoids is currently widely studied in the field of food chemistry. It has been found that *R*-linalool exists in basil oil, hops and Japanese pepper, whereas *S*-linalool exists in strawberries, orange oil, and cocoa products [11,12]. Racemic linalool oxides have four chemical constructions named linalool oxide A–D; the chiral separation of the four pairs of enantiomers in linalool oxides has been studied in grape [13], honey [14] and food essential oil [15]. *R*-α-Ionone has been detected in raspberry [16], violet [17] and carrot [18]. Additionally, enantiomers of α-terpineol, α-pinene, limonene, phellandrene and 4-terpineol have been successfully separated in essential oils of lamiaceae [19], cardamom [20], citrus [21], lemon [22] and passion fruit [4]. In light of the enantiomeric distribution characteristics of terpenoids in certain foods, chiral analysis technology has been successfully applied in the quality assessment, determination of botanical origin and authenticity assessment of honey, *Melaleuca alternifolia* oils and fruit beverages [14,23,24].

Although great progress has been made, research on the enantiomeric analysis of volatile terpenoids in teas is still at an initial stage. The enantiomers of linalool and linalool oxides in black and oolong teas were originally separated in 1994 [25], and it has proven that the enantiomeric ratios and even the major configurations of linalool and linalool oxides presented different distributions in the different teas; very recently, the enantiomeric distribution of linalool in various types of teas, tea cultivars and tea samples during processing has been reported by our group [26], and a similar distinction was observed. Beyond those, however, few chiral separations of other terpenoids that likely play important roles in the formation of tea aroma quality have been reported.

Enantiomeric analysis of terpenoids has generally been performed using chiral gas chromatography. However, multifarious structures, similar boiling points and different adhesive abilities in chiral stationary phases of terpenoids cause great difficulty separating them in a single chiral chromatographic system. The multi-peak overlap phenomenon among different terpenoids and the unsuccessful separation of terpenoid enantiomers frequently occurred by using unsuitable chiral GC columns. Therefore, the optimization of chiral separation efficiency of terpenoids is the most critical step of the chiral analysis and presents many challenges. On the other hand, in prior work, identification of terpenoid enantiomers was frequently performed through maintaining consistency between the retention time of peaks of both standard and samples to be tested under the same experimental conditions by using a GC-flame ionization detector. Nevertheless, erroneous judgment of peaks might occur from similar retention times of other compounds with