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Enantiomeric analysis of linalool in teas using headspace solid-phase microextraction with chiral gas chromatography

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ABSTRACT

Linalool is a major volatile component of tea aroma. It occurs naturally as *R*-(−)- and *S*-(+)-linalool enantiomers, which exhibit entirely different sensorial properties. Using headspace solid-phase microextraction with chiral gas chromatography, we quantitated *R*-(−)- and *S*-(+)-linalool in teas. Optimal extraction conditions were as follows: CAR–DVB–PDMS fiber, 60 min at 60 °C, and tea/water ratio of 1:6 (w(g)/v(mL)). We measured linalool levels in five different teas, in fresh leaves of 14 different cultivars, and in samples collected during processing of green and black teas. Enantiomeric distributions of linalool were significantly different among the different teas and among the different tea cultivars. *R*-(−)-Linalool and *S*-(+)-linalool reached maximum levels during the rolling of black tea, with the levels declining drastically during green tea processing. Although tea germplasm was an important factor in determining the enantiomeric distribution of linalool in tea products, the processing steps also had a large impact on linalool levels.

1. Introduction

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is a terpene alcohol found in many plants (Cheng et al., 2012) with a floral aroma. It is a major volatile component of essential oils of several aromatic plant species and has marked effects on the central nervous system (Coelho et al., 2011). Linalool is an enantiomeric monoterpene compound that occurs naturally as two isomeric optical forms (Fig. 1), namely *R*-(−)-linalool and *S*-(+)-linalool, which exhibit entirely different sensorial properties (Siani et al., 2002), as well as different physiological and psychological effects (Kuroda et al., 2005). *R*-(−)-linalool (odor threshold of 0.8 μg/L) has a fresh, lavender aroma reminiscent of lily of the valley, whereas *S*-(+)-linalool (odor threshold of 7.4 μg/L) has an herbaceous, musty smell, which is often described as having a citric note (Bonnländer et al., 2006). *R*-(−)-Linalool has lavender-like sedative effects on autonomic nerve activity and mood states at a very low intensity, whereas *S*-(+)-linalool shows the opposite effects (Kuroda et al., 2005).

Enantiomer recognition is very important in food science because of its role in perception of flavors and fragrances. Enantiomeric excess can be used as an indicator for authenticity because biological interactions and biosynthetic processes are mostly stereospecific; thus, chiral components in natural products are often characterized by specific enantiomeric compositions (Cagliero et al., 2012). Enantiomeric analysis of linalool has shown that *R*-(−)-linalool predominates in basil oil, hops (Steinhaus et al., 2003), and Japanese pepper, whereas *S*-(+)-linalool is prominent in strawberries, orange oil, and cocoa products. Plants with a balanced distribution of both enantiomers, for example, pineapple, are also known (Bonnländer et al., 2006).

Linalool has been verified to be a very important contributor to the flavor and aroma of numerous foods (Bazemore et al., 2003; Bonnländer et al., 2006) such as strawberry (Schipilliti et al., 2011), mango (Sakho et al., 1997), and orange juice (Bazemore et al., 2003). In tea, linalool is one of the most important volatile constituents that contribute significantly to the aroma (Sharma et al., 2014; Wang et al., 1994). More specifically, linalool is one of the major odor-active components of black tea (Baldermann et al., 2014; Joshi and Gulati, 2015), dark tea (Lv et al., 2012), oolong tea (Lin et al., 2013; Wang et al., 2010), and green tea (Jumtee et al., 2011). However, little work has been carried out on the enantiomeric analysis

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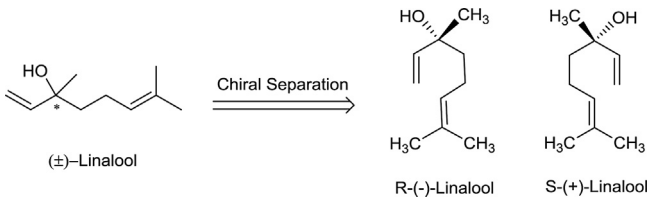

Fig. 1. Linalool and its enantiomers.

of linalool in teas, and the enantiomeric composition of linalool in tea has not been elucidated to date. To some extent, this has posed an obstacle to elucidation of the mechanism of tea aroma formation.

The accurate extraction of volatiles is critical in the determination of volatile compounds. At present, the most commonly used techniques for extracting essential oils from plant materials and foods are distillation-solvent extraction, direct solvent extraction, hydrodistillation, microwave-assisted extraction, supercritical-fluid extraction, and headspace solid-phase microextraction (HS-SPME) (Foudil-Cherif and Yassaa, 2012). Currently, HS-SPME is mostly used in the analysis of chiral volatile compounds in foods. In general, it has fulfilled analytical requirements (Flores et al., 2006), and many studies have successfully applied it (Cagliero et al., 2012; Fidalgo-Used et al., 2008; Schipilliti et al., 2011; Steinhaus et al., 2003; Verzera et al., 2014; Yassaa and Williams, 2007). It is a simple, fast, and solvent-free analytical technology and has been widely used in the analysis of volatile compounds in tea (Du et al., 2013; Lin et al., 2013; Lv et al., 2012; Ma et al., 2014; Wang et al., 2008). However, the combined use of HS-SPME and chiral gas chromatography (GC) has not been applied to the study of linalool in teas, and we believe that this approach warrants further investigation.

The present study was conducted to develop a simple but effective analytical method for the direct determination of the enantiomeric forms of linalool in teas using HS-SPME with enantioselective GC. In addition, this study examined the enantiomeric distributions of linalool in different teas and different varieties, including changes in the enantiomeric compositions during processing of green and black teas.

2. Materials and methods

2.1. Reagents and materials

(±)-Linalool, *R*-(−)-linalool, and ethyl decanoate (internal standard, IS) were purchased from Sigma–Aldrich (Shanghai, China). A stock solution of ethyl decanoate (0.2 mg/mL) in ethanol was prepared. Ethanol solutions (5 mg/mL) of (±)- and *R*-(−)-linalool were also prepared. The standard solutions were stored at 4 °C. Carboxen/divinylbenzene/polydimethylsiloxane (CAR–DVB–PDMS; 50/30 μm), divinylbenzene/polydimethylsiloxane (DVB–PDMS; 65 μm), and polyacrylate (PA; 85 μm) microextraction fibers were acquired from Supelco (Bellefonte, PA, USA).

2.2. Samples

All tea samples were ground to pass through a 40-mesh sieve and were stored in sealed containers for future use. The extraction of *R*-(−)- and *S*-(+)-linalool was optimized by using Yingde black tea. "Volatile-free" tea was prepared according to the method of Du et al. (2013) by repeatedly heating the tea infusion at 40 °C under reduced pressure in a rotary evaporator for 2.0 h to remove all *R*-(−)- and *S*-(+)-linalool, and then drying the tea powder at 100 °C for 1.0 h. Volatile-free tea was used as a blank matrix in the preparation of calibration curves.

Samples of green tea, black tea, white tea, oolong tea, and dark tea were purchased from a local market to allow comparison of the distribution of the studied chiral compounds among them. In addition, we prepared other tea samples to evaluate the influence of tea variety and processing methods on the distribution of *R*-(−)- and *S*-(+)-linalool. Freeze-dried samples of 14 different tea cultivars were prepared by using two fresh leaves and a bud of each that were plucked during spring of 2014 at the China National Germplasm Hangzhou Tea Repository, which was located at the Tea Research Institute of the Chinese Academy of Agricultural Sciences. After collection, the samples were preserved in liquid nitrogen and then freeze-dried for further analysis. In addition, green tea samples of the 14 different tea cultivars were prepared according to traditional processing methods for roasted green tea. To understand the changes in enantiomeric compositions during processing of green tea and black tea, samples were collected after each main step of processing. In this study, the main steps for green tea were fixing, rolling, and drying, and those for black tea were withering, rolling, fermentation, and drying. After collection, the samples were preserved in liquid nitrogen and then freeze-dried for further analysis.

2.3. Optimization of HS-SPME parameters

To establish the method used to extract *R*-(−)- and *S*-(+)-linalool by SPME, several experimental parameters that influence the HS-SPME procedure were evaluated in sequence by using the optimal condition obtained in the previous step. The examined parameters included the type of extraction fiber, the brewing proportions of tea and water, extraction temperature, and extraction time.

Three different microextraction fibers were tested: CAR–DVB–PDMS (50/30 μm), DVB–PDMS (65 μm), and PA (85 μm). Extraction using each fiber type was carried out in the same way. Each fiber was preconditioned for 5 min in the injection port of the GC, as recommended by the manufacturer. For each sample, 1 g of homogenized tea was accurately weighed into a 250 mL vial, and 6 mL of boiling water and 10 μL of ethyl decanoate (0.2 mg/mL, IS) were added. The vial was immediately placed in a water bath to equilibrate for 5 min at 60 °C before the SPME fiber was exposed to the headspace for 60 min while the sample temperature was maintained at 60 °C. The SPME fiber was then introduced into the GC injector and left inserted for 3 min to allow thermal desorption of the analytes. All samples were extracted and analyzed in triplicate. To evaluate the effects of extraction time, temperature, and brewing proportions of tea and water on the SPME of *R*-(−)- and *S*-(+)-linalool using each fiber, extractions were carried out at 30, 45, 60, 75, and 90 °C for 30, 45, 60, and 75 min by using different brewing proportions of tea and water (1:2, 1:6, 1:10, and 1:15; w(g)/v(mL)).

2.4. GC

GC of the enantiomeric distribution of linalool was carried out on an Agilent GC-7890A system (Agilent, USA) coupled to a flame ionization detector (FID). Separation was performed by using an Astec CHIRALDEX B-DM capillary column (30 m × 0.25 mm × 0.12 μm, Supelco, Bellefonte, PA, USA). Instrumental parameters for linalool analysis were as follows: the temperature of the injector was 250 °C; N_2 flow, 1.2 mL/min; column temperature program, initial temperature at 50 °C (held for 2 min), increased to 150 °C at 2 °C/min (held for 10 min), and increased at 4 °C/min to 180 °C (held for 5 min); the temperature of the detector was 250 °C.

R-(−)-Linalool and *S*-(+)-linalool were identified by comparison of the retention times with those of analytical standards analyzed under the same experimental conditions. The enantiomers were quantified from calibration curves prepared by using the IS ethyl decanoate and the respective standards that had been added to the sample.