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Profiling of volatile compounds of *Phyllostachys pubescens* shoots in Taiwan

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ABSTRACT

This study examined the influence of heating temperature and duration on volatile aromatic components of spring and winter *Phyllostachys pubescens* shoots using SPME. Results from GC–MS analyses revealed that the main constituents in both bamboo shoots at ambient temperature include methoxy-phenyl oxime, followed by *n*-hexanol and 3*Z*-hexenal, which gives a fresh green aroma. Comparing the different compounds, between spring and winter shoots, revealed that spring bamboo shoots at ambient temperature comprise 12.30% methyl salicylate, which provides protection against insect attack, and 9.71% *epi*-cedrol; while winter bamboo shoots comprise 17.00% 1-octen-3-ol, which produces a distinct mushroom aroma. After heating at 100 °C for 60 min, a marked increase in relative content of benzyl salicylate (43.30%) and a significant decrease in methyl salicylate content in spring bamboo shoots were observed; while the major compound in winter bamboo shoots was *n*-heneicosane (78.09%) and the content of specific 1-octen-3-ol significantly decreased.

1. Introduction

Bamboo forests are widely distributed in Asia, Africa, the Caribbean region and Latin America. With its advantages of rapid and abundant growth, bamboo is an important forest resource (Liese, 1987). Belonging to the family of Gramineae, bamboo is a perennial evergreen plant and bamboo shoots are the main by-products of bamboo forests. Bamboo shoots are budding sprouts wrapped inside bamboo sheath (Lu, 2001) and are regularly consumed in the Orient because of their properties of high dietary fibre, low fat, and rich mineral content (Jiang, 1991; Satya, Bal, Singhal, & Naik, 2010). According to the survey conducted by the Forestry Bureau of the Council of Agriculture, Executive Yuan (2000), there exists a wide variety of bamboo cultivated over extensive areas, with the five main species being *Phyllostachys pubescens* Mazel, *Dendrocalamus latiflorus* Munro, *Phyllostachys makinoi* Hayata, *Eleba oldhami* Nakai, and *Leleba edulis* Odashimo (Huang, Lin, Lin, & Liu, 1999). These five types of bamboo all have distinctive aromas, flavours and textures; and many have been developed into local delicacies (Tsai, 2002). Among the aforementioned species, shoots of *P. pubescens* are the most popular and well-loved by people. Hence, the consumption of *P. pubescens* shoots as food and its economic value merit our attention (Luo, 2009). Satya et al. (2010) pointed out that the annual demand for *P. pubescens* shoots in Japan was high, amounting to 8000 tonnes, with 400 tonnes of processed *P. pubescens* shoots imported. Most of these imports are *P. pubescens* and *D. latiflorus* processed and canned in Taiwan.

Recent research on bamboo shoots tends to focus on its beneficial effects on health. Not only are bamboo shoots rich in Dendrocinino, a distinctive antifungal protein, they are also a natural source for the extraction of phenolic antioxidants (Park & Jhon, 2010). In addition, lipid content of bamboo shoots comprises mainly unsaturated fatty acids and phytosterol, which can lower serum cholesterol levels, inhibit inflammation and prevent prostate diseases (Lu et al., 2011). On the other hand, some studies have explored the aroma of bamboo shoots. Toshiyuki, Kohji, and Mitsuo (2010) examined the volatile compounds with odour in *P. pubescens* stems, while Fu, Yoon, and Bazemore (2002) studied the aroma-active components in fermented bamboo shoots.

Among the various approaches to extracting volatile organic compounds, such as phytoncide and essential oil, solid-phase microextraction (SPME) is the most widely employed (Yang & Peppard, 1994; Chung, Cheng, & Chang, 2008). SPME involves the use of a fibre coating, which extracts different kinds of components (including both volatile and non-volatile) from different kinds of media that can be in the liquid or gas phase. It is a simple and inexpensive technique with no need of solvents (Alexandra & Pawliszyn, 1996; Chen, Cheng, & Chang, 2010; Hsu, Chen, Wu, & Chang, 2006).

In view of its convenience in implementation and wide application in many areas, SPME was used in this study to extract volatile components of *P. pubescens* shoots. The volatile components were determined using gas chromatography–mass spectrometry (GC–MS). Comparison of the volatile components of bamboo shoots harvested in different seasons was also made at ambient temperature.

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In addition, the effects of different heating temperatures and durations on volatile compounds in bamboo shoots were investigated. Results thus obtained can shed light on the characteristics of winter and spring bamboo shoots and provide useful references for promoting its use in cuisine and nutritional value.

2. Materials and methods

2.1. Materials

Winter and spring shoots of moso bamboo (*Phyllostachys pubescens* Mazel) were obtained in November 2010 and March 2011, respectively, from the Xitou tract of the Experimental Forest of National Taiwan University. The winter bamboo shoots were buried in the soil and had to be dug up. They were of smaller size with base diameter ranging from 5 to 10 cm. The spring bamboo shoots had sprouted above the soil surface and were of larger size with base diameter ranging from 8 to 15 cm. When harvested, the fresh bamboo shoots were washed and then stored in a sealed bag at −80 °C without exposure to light prior to analysis.

2.2. Experimental conditions

Volatile compounds of spring and winter shoots of *P. pubescens* were extracted using SPME at ambient temperature (25 °C), 40 °C, 60 °C and 100 °C. In addition, the shoots were also steam-cooked at 100 °C for 5 min, 30 min and 60 min to examine the variations in volatile compounds of *P. pubescens* under different heating temperatures and durations.

2.3. Extraction of volatile compounds by SPME

The manual SPME device and fibre were purchased from Supelco Co. (Bellefonte, PA, USA). According to results obtained from the preliminary study, the 65 μm polydimethylsiloxane-divinylbenzene (PDMS/DVB) fibre was employed to extract the volatile compounds of spring and winter shoots of *P. pubescens*. PDMS/DVB fibre was conditioned as recommended by the manufacturer prior to the extraction. The SPME extraction procedure was slightly modified from the method used by Chen et al. (2010). Fresh bamboo shoots were clipped into particles with dimensions of 3 mm (longitudinal) × 2 mm (tangential) × 2 mm (radial) and 3 g of sample were placed in a 20-ml vial closed by a PTFE/silicone septum, and then heated for 30 min in 25 °C, 40 °C, 60 °C and 100 °C water baths, respectively. The adsorption time of each extraction was held for 30 min at different temperatures, and desorbed at a gas chromatography (GC) inlet for 5 min at 230 °C.

2.4. GC–mass spectroscopy (MS) and GC–FID analyses

Volatile compounds of spring and winter shoots were analyzed by a Trace GC PoLaris Q mass (ion source 200 °C, 70 eV) instrument, equipped with a DB-5 ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The oven temperature was held at 80 °C for 1 min, then programmed to increase from 80 to 200 °C at a rate of 8 °C/min and held for 5 min. Other parameters included injector temperature, 230 °C; split ratio, 1:10; and carrier gas, helium at a flow rate of 1 ml/min. Quantification was performed by percentage peak area calculations using the GC–FID. Identification of the major components of *P. pubescens* bamboo shoots was confirmed by comparison with standards, by spiking, and on the basis of their mass spectral fragmentation using the Wiley/NBS Registry of Mass Spectral library and NIST MS Search (Adams, 2007). The relative content of compounds was obtained by integrating the peak area of the spectrograms.

3. Results and discussion

3.1. Fragrant compounds of P. pubescens shoots at ambient temperature

Fig. 1 shows the GC–MS spectra of volatile compounds from spring and winter *P. pubescens* shoots at ambient temperature. Table 1 presents the volatile compounds and their relative contents. As can be seen, spring and winter bamboo shoots are releasing different proportions of volatile components at room temperature. The main components of spring bamboo shoots are 30.53% alcohol and 28.99% oxime compound, followed by 20.91% aldehyde. Moreover, spring bamboo shoots comprise both ester (12.30%) and ether compounds (3.12%), which are not found in winter bamboo shoots. Similarly, trends are also observed in winter bamboo shoots, with 34.39% alcohol and 32.79% oxime being the major compounds, followed by 26.94% aldehyde. Comparatively, winter bamboo shoots contain a greater proportion of these three compounds than spring bamboo shoots.

Table 1 shows the volatile compounds of *P. pubescens* shoots obtained by SPME at 25 °C. As can be seen, the spring and winter bamboo shoots contain nine and six volatile compounds, respectively, indicating that spring bamboo shoots have more volatile compounds than winter ones. The major volatile compound of spring bamboo shoots is methoxy-phenyl oxime (28.99%), followed by *n*-hexanol (20.82%), 3*Z*-hexenal (18.81%) and *epi*-cedrol (9.71%). Winter bamboo shoots comprise similar volatile compounds of different proportions. Methoxy-phenyl oxime (32.79%) constitutes the largest proportion, followed by 3*Z*-hexenal (22.84%), *n*-hexanol (17.39%) and 1-octen-3-ol (17.00%), which are not found in spring bamboo shoots. Among these volatile compounds, 3*Z*-hexenal generates the fresh green odour of newly picked vegetables and fruits, which has been reported in previous studies (Akikazu, 1993; Minke et al., 2002) and it is thus often used as aromatic food additive. Using gas chromatography–olfactometry (GC–O), Toshiyuki et al. (2010) studied the volatile compounds with characteristic odours in *P. pubescens* stems and found that 1-octen-3-ol produces a mushroom smell. At ambient temperature, spring bamboo shoots contain methyl salicylate (12.30%) and *epi*-cedrol (9.71%), which are not found in winter bamboo shoots. In the study of Jayasekara, Stevenson, Belmain, Farman, and Hall (2002), methyl salicylate is the principal volatile compound (90%) in the methanolic extract of *Securidaca longepedunculata* (Polygalaceae) root bark, which is often used by the Africans as a pesticide. Spring bamboo shoots have been found to contain a larger content of carbohydrates than winter bamboo shoots (Chen, Qiu, Huang, Fan, & Jiang, 1999); and they are exposed above the ground level, making them more vulnerable to attack from insects and other microorganisms. Fortunately, the hard shell of spring bamboo shoots with 12.30% of methyl salicylate contributes to the self-defense against insect attack during their growth.

3.2. Effect of heating temperature on volatile compounds of P. pubescens shoots

Firstly, 3 g of spring and winter bamboo shoots of *P. pubescens* were put into 20-ml test bottles, respectively, and then heated in water baths at ambient temperature (25 °C), 40 °C, 60 °C and 100 °C for 30 min, followed by SPME analysis. As shown in Table 2, winter bamboo shoots at ambient temperature did not contain any hydrocarbon compounds. However, when the temperature rose to 40 °C, 3.32% hydrocarbon compounds, such as *n*-heneicosane, were observed and their relative content increased further to 78.09% when heated at 100 °C (Table 3). These results revealed that when winter bamboo shoots were heated to 100 °C, *n*-heneicosane