Food Chemistry 112 (2009) 1016–1021

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Effects of genetic variability, parts and seasons on the sterol content and composition in bamboo shoots

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ARTICLE INFO

Article history:
Received 29 March 2008
Received in revised form 25 May 2008
Accepted 22 June 2008

Keywords:
Bamboo shoot
Sterol
Genetic variability
Parts
Harvest seasons
UPLC-APCI-MS

ABSTRACT

Bamboo shoots are regarded as potential sources of sterols. The effects of genetic variability, parts and harvest seasons on the sterol content and composition in the bamboo shoots have been determined using a novel ultra-performance liquid chromatographic atmospheric pressure chemical ionisation mass spectrometer method. The results showed that the representative sterols in bamboo shoots were β-sitosterol, campesterol, stigmasterol, ergosterol, cholesterol and stigmastanol; exception stigmastanol, the significant differences were observed in the sterol content of different species (112.4–279.6 mg/100 g dry wt), different harvest seasons (195.3–279.6 mg/100 g dry wt) and different parts (253.6–321.8 mg/100 g dry wt); the sterol composition was similar in different species and different harvest seasons, however, it was significantly different between shoot bodies and shoot shell. The genetic variability, parts and harvest seasons could significantly affect the sterol composition in the bamboo shoots. The spring shoot shell of *Phyllostachys pubescens* contained the highest sterol content (321.8 mg/100 g dry wt).

1. Introduction

Sterols are members of the 'triterpene' family and more than 200 types of sterols are known to be found in nature (Lagarda, Garcıa-Llatas, & Farre, 2006). Sterols have received particular attention due to their capability to lower serum cholesterol levels in humans, resulting in significant reduction of the risk of cardiovascular diseases (Plat & Mensink, 2001). Furthermore, they were also regarded as a kind of natural product with anti-inflammatory (Bouic, 2002; Navarro, De las Heras, & Villar, 2001), anti-bacterial (Ovesna, Vachalkova, & Horvathova, 2004), and anti-carcinogenic properties (Awad, Downie, & Fink, 2000; Raicht, Cohen, Fazzini, Sarwal, & Takahashi, 1980). Sterols are the bioactive components occurring in all vegetable foods and representing the major part of the non-saponifiable fraction of lipids (Lagarda et al., 2006; Phillips, Ruggio, & Ashraf-Khorassani, 2005), hence it is crucial to get accurate quantitative data on the distribution of these nutritionally important lipids.

Bamboos are giant, woody grasses growing in tropical and subtropical (cosmopolitan) climate, with distribution ranges covering wide areas of Asia, Africa, the Caribbean and Latin America (Lu, Wu, Tie, Zhang, & Zhang, 2005). Bamboo shoots are the tender, young offspring of bamboo, which are generally harvested after a growth period of two weeks. The young shoots are crisp and tender, and are widely used as a vegetable in Asian cooking. Furthermore, bamboo shoots are commonly available as canned food, even though fresh bamboo shoots are far superior in taste and texture (Zhu, Ma, & Fu, 1994).

Bamboo shoots have a long history of being used as a source of both food and medicine in China and Southeast Asia (Bao, 2006). They were regarded as a traditional Chinese medicinal material for more than 2000 years and, according to archaic Chinese medicinal books such as "Ben Chao Qiu Zheng", "Ben Jing Feng Yuan", "Yao Pin Hua Yi" and "Jing Yue", were said to be beneficial to the human health, notably by promoting the peristalsis of the stomach and the intestine, helping digestion and preventing and curing cardiovascular diseases and cancers. However, little scientific evidence has supported such claims until now. Previous studies on nutritional benefits of bamboo shoots mainly focused on non-soluble and/or water-soluble components, such as dietary fiber, protein, amino acids and vitamins (B1, B2 and C) (Xu, Cao, Song, & Fang, 2005), but quantitative data on lipid-soluble components, especially sterol, are limited. The report in the reference (He & Lachance, 1998; Lachance & He, 1998) is the identification of the three predominant sterols (β-sitosterol, campesterol and stigmasterol), but the minor sterols have not been analysed. Furthermore, comprehensive and accurate data on the sterol content of various bamboo shoots (different genetic variants, different parts and different harvest seasons) is not available.

In this paper, the sterol content and composition in bamboo shoots of four species (*Pleioblastus amarus*, *Phyllostachys pubescens*,

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doi:10.1016/j.foodchem.2008.06.059

Dendrocalamus latiflorus and *Phyllostachys praecox*), six parts (shoot bodies and shoot shell) and three harvest seasons (winter, spring and summer) of *P. pubescens*, were evaluated widely, using a UPLC-APCI-MS method (Lu, Zhang, Wu, & Shi, 2007), in order to facilitate dietary recommendations and comprehensive utilisation of bamboo shoot resources.

2. Materials and methods

2.1. Bamboo shoot samples

Bamboo shoots of four species (*P. amarus*, *P. pubescens*, *D. latiflorus* and *P. praecox*) and three harvest seasons (winter, spring and summer) of *P. pubescens* were purchased from a market in Hangzhou, Zhejiang Province, People's Republic of China in 2006, and identified by the Research Institute of Subtropical Forestry of the Chinese Academy of Forestry (Hangzhou, China).

2.2. Sterol analysis

Based on the UPLC-APCI-MS method (Lu et al., 2007), the sterols in the tested samples were evaluated. The powder of bamboo shoots was obtained by comminution and filtration (20–40 mesh). 1.2 g of bamboo shoot powder were spiked with 100 μL of a 200 μg mL^{-1} solution of 6-ketocholestanol in methanol as internal standard. Sterols were extracted by supercritical carbondioxide in a 10 mL-extraction vessel, supplying supercritical CO_2 at a flow rate of 8 L min^{-1}, discharging under a pressure of 450 bar and a temperature of 55 °C for 2 h using a SFE-ed SFE-2 supercritical fluid extractor (Applied Separation, Allentown, PA, USA). All extracts were stored for UPLC-MS analysis in amber bottles, purged with nitrogen, at 18 °C. About 0.1 g extract was mixed with 5 mL of ethanolic KOH (2 M) for 2 h at 80 °C. The saponified material was transferred to a separatory funnel and the flask was rinsed with 5 mL of water. The unsaponifiable matter was extracted twice with 5 mL of diethyl ether. The combined organic phases were washed with 5 mL water twice and dried with anhydrous sodium sulfate, filtered and then evaporated to dryness by rotatory evaporation at 30 °C and redissolved in 15 mL of a hexane–ethyl acetate (95:5, v/v) mixture. A Waters Sep-Pak Vac (500 mg, 6 cc) silica cartridge was conditioned with 15 mL of hexane. The solution of the sample dissolved in hexane–ethyl acetate (95:5, v/v) was administered to the cartridge and the sterol fraction was eluted with 5 mL of a hexane–ethyl acetate (60:40, v/v) mixture. The eluate was dried under a nitrogen stream, redissolved in 2 mL of absolute methanol and filtered with 0.45 μm membrane filters before LC injection.

Separation, identification and quantification of sterols were performed using a coupled liquid chromatography tandem mass spectrometry system consisting of an ACQUITY Ultra-Performance Liquid Chromatography (Waters, USA) and a Quattro Ultima Pt (Micromass, UK) tandem mass spectrometer. An ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm id, 1.7 μm) was used for LC separation. The column oven was at 35 °C, the flow rate was 0.1 mL min^{-1}, and the injection volume was 10 μL. Methanol and water with 1% acetonitrile were used as mobile phases. The methanol was linearly increased from 90% to 95% in 21 min, then increased to 100% in 1 min and held for 1.0 min, finally brought back to 90% in 0.2 min and held for 6.8 min until the next injection.

The mass spectrometer was operated with APCI interface in selective ion monitoring (SIM) mode. Interface parameters were set as follows: probe temperature, 500 °C; source temperature, 120 °C; corona discharge, 2.5 μA; cone voltage, 20 V; cone gas flow (N_2, 99.999%), 45 L h^{-1}; desolvation gas flow (N_2, 99.999%), 450–500 L h^{-1}. UPLC-MS determinations were performed by operating the mass spectrometer in positive ion mode.

6-Ketocholestanol, desmosterol, ergosterol, cholesterol, lanosterol, cholestanol, stigmasterol, campesterol, β-sitosterol and stigmastanol, were identified based on both retention time and characteristic ion peaks in mass spectra. Quantitative analyses were carried out by internal standard calibration. All analytical experiments were performed in duplicate. Results were averaged from the analytical data of three samples.

3. Result and discussion

3.1. Sterols in bamboo shoot

For further studies, mass spectra under APCI mode were acquired using direct sample infusion by FIA (flow injection analysis) at 10 μL min^{-1}. In the mass spectra, the molecular ion of the sterols could not be seen, although several attempts were undertaken to obtain it: addition of ammonium acetate and acidification of the sample with acetic or formic acid. Table 1 shows that the main fragments observed for APCI in positive mode. Throughout, two major ions were seen, with the most intense ions being the fragment ions $[M + H - H_2O]^+$, while $[M + H - 2H]^+$ were minor ions corresponding to the dehydrogenation of the molecules. A better separation of the sterols was obtained by reversed phase UPLC, retention times were 4.31, 8.81, 9.39, 11.41, 11.59, 12.77, 12.82, 12.86, 14.14 and 15.63 for 6-ketocholestanol, desmosterol, ergosterol, cholesterol, lanosterol, campesterol, cholestanol, stigmasterol, β-sitosterol and stigmastanol, respectively. According to retention time and MS data, β-sitosterol, campesterol, stigmasterol, ergosterol, cholesterol and stigmastanol were confirmed in bamboo shoot of *P. amarus*, *P. pubescens*, *D. latiflorus* and *P. praecox*, as shown in Fig. 1. According to the reported data (He & Lachance, 1998; Lachance & He, 1998), 17 phytosterols in bamboo shoot have been observed by the State University of New Jersey, β-sitosterol, stigmasta-3,5-dien-7-one, stigmast-4-en-3-one, stigmasterol, campestrol and two isomers of sitostanol (stigmastanol) have been identified. However, ergosterol and cholesterol were found in bamboo shoots for the first time.

3.2. Effects of different species on sterol content and composition

The effect of different species on the sterol content and composition in bamboo shoots was investigated using the UPLC-APCI-MS/MS method. The bamboo shoots of four species (*P. amarus*, *P. pubescens*, *D. latiflorus* and *P. praecox*) were collected and analysed. Fig. 2 shows that the total sterol content as well as the contents of β-sitosterol, campesterol, stigmasterol, ergosterol, cholesterol and stigmastanol in different species, respectively. The total sterol content in bamboo shoots ranged from 112.4 to 279.6 mg/100 g dry wt in four species, and the order was *P. pubescens* > *P. amarus* > *P. praecox* > *D. latiflorus*. Significant differences in the total sterol content of bamboo shoots of different species were found, but with an average of 194.8 mg/100 g dry wt. No species containing extremely high levels of sterols could be found. Furthermore, no correlation between the total sterol content and the size (weight) of the bamboo shoots could be observed (data not shown). From the viewpoint of the diet and the effect of these plants on human body, peoples could get the more abundant phytosterol from shoot of *P. pubescens* than from other bamboo shoots and shoot of *P. pubescens* could reduce cholesterol levels more effective.

As shown in Fig. 2, the β-sitosterol contents were 177.8, 233.0, 89.9 and 132.8 mg/100 g dry wt for *P. amarus*, *P. pubescens*, *D. latiflorus* and *P. praecox*, respectively. Thus, the β-sitosterol contents in the four examined species differed significantly. The corresponding contents of campesterol, stigmasterol, ergosterol, cholesterol and stigmastanol in four species were 13.6–28.3, 6.4–13.5, 0.7–1.5,