Industrial Crops & Products xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Use of simultaneous distillation-extraction, supercritical fluid extraction and solid-phase microextraction for characterisation of the volatile profile of *Dipteryx odorata* (Aubl.) Willd.

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ARTICLE INFO

Keywords:
Head-space solid phase microextraction
Simultaneous distillation-extraction
Supercritical fluid extraction
Tonka beans
Volatile

ABSTRACT

Head-space solid phase microextraction (HS-SPME), simultaneous distillation-extraction (SDE) and supercritical fluid extraction (SFE) were employed to prepare volatile extracts of tonka beans (*Dipteryx odorata*). A total of 190 compounds were assigned by gas chromatography/mass spectrometry (GC/MS) together in comparison with retention indices. These included 156 (HS-SPME), 77 (SDE) and 36 (SFE) compounds (alcohols, carbonyl compounds, acids, esters, terpenes, terpenoids, lactones, aliphatic and aromatic hydrocarbons, and other non-categorised compounds). Semiquantitative evaluation of individual compounds was performed using gas chromatography coupled with a flame ionisation detector. The main constituent detected in all extracts was coumarin (51–85%, despite the extraction method) belonging to the group of lactones. A comparison was made among the three methods in terms of volatile profiles, categorisation of compounds and representation of individual groups of compounds. Extracts prepared by HS-SPME were rich in alcohols, carbonyls and acids than other extracts. Results can provide essential information for the application of different treatment of tonka beans in flavor industries.

1. Introduction

Dipteryx odorata (Aubl.) Willd. (*Fabaceae*) is a large tree native to the tropical rainforests of Central and Northern South America. In total, the genus *Dipteryx* involves 14 species. Ten of them are typical to the Amazon region; the other two are found in the Northeast and Central Brazil and the last two might be found in Central America. (Allen and Allen, 1981)

D. odorata, which is also commonly known as the “tonka bean tree”, is the most studied species of the genus. It produces seeds commonly called “tonka beans”, which have been already examined for the content of coumarin (Ehlers et al., 1995; Ehlers et al., 1996; Oliveros-Bastidas et al., 2013). This compound is liberated from the glycoside melilotoside (an ether of glucose bonded with an ester bond to coumarin) (Dolan et al., 2010) by fermentation during the drying of the seeds which have been soaked in alcohol for 24 h. Its content is variable and usually moves in the range of 1–3% in fermented tonka beans (Ehlers et al., 1995). As reported by Givel (Givel, 2003), “numerous studies, beginning in 1855, have indicated that coumarin has toxic effects on the nervous system, heart, blood vessels, and liver of animals as well as inducing cancerous tumours and toxic conditions in humans. In 1954, the US Food and Drug Administration (FDA) banned coumarin in food, but not tobacco products in the USA based on the results of animal research. Also, since 1954, many European countries have either banned or greatly restricted coumarin because of its toxic properties”. In 2004, the European Food Safety Authority (EFSA) established present Tolerable Daily Intake (TDI) of 0–0.1 mg coumarin/kg body weight (EFSA, 2004), which was confirmed by EFSA in 2008 (EFSA, 2008) on the base of toxicity and clinical studies that have become available since 2004.

Coumarin naturally occurs in other plants, for example flavouring herbs deers tongue (Haskins et al., 1972), cinnamon and cassia (Rychlik, 2008; Wang et al., 2013) or herbs used for making tea like woodruff (Laub and Olszowski, 1982; Rychlik, 2008) and melilot (Nair et al., 2010). There are several publications about on the usage of coumarin-occuring plants in traditional or seasonal food products or beverages (Ballin and Sørensen, 2014; Sproll et al., 2008) when TDI values could possibly be reached simply by consuming these products. These results led to the question of whether a re-evaluation of the maximum levels is necessary. It was also mentioned in the Opinion of EFSA (EFSA, 2008) in which it was concluded that exposure to coumarin resulting in an intake 3 times higher than the TDI for one to two

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https://doi.org/10.1016/j.indcrop.2018.01.055
Received 20 February 2017; Received in revised form 15 January 2018; Accepted 20 January 2018

weeks is not of safety concern. In 2008, new maximum limits for coumarin in traditional or seasonal bakery products, breakfast cereals and desserts containing cinnamon were established by the European Regulation No 1334/2008 (Council, 2008).

Due to the various scents, which are vanilla, cinnamon, saffron, almond or cloves, the extracts of tonka beans have a widespread use, particularly as additives in flavouring snuff, cigarettes, cigars, and also in perfumes or liquors (Sullivan, 1982). Moreover, *D. odorata*, mainly its seeds, flowers and bark, have been studied for its phytochemical properties. There are works dealing with isolation of isoflavones (Januario et al., 2005), fatty acids (Oliveros-Bastidas et al., 2013) and diterpenoids (Godoy et al., 1989). Seeds of *D. odorata* have been furthermore examined for contents of ethyl acetate-soluble extract which acts as a naturally occurring cancer chemopreventive agent (Jang et al., 2003).

Despite the fact that extracts of tonka beans are substantially aromatic, there aren't enough reports dealing with volatile profile of the tonka beans. The only work dealing with volatile profile was published by Wörner et al. (Worner and Schreier, 1991). Wörner isolated volatile compounds by using solid-liquid extraction with subsequent high-vacuum distillation/extraction of dry tonka beans. Separation and identification of the constituents were carried out by high resolution capillary gas chromatography in connection to mass spectrometry. In total, 138 compounds were identified. Andrade et al. (Andrade et al., 2003) analysed the volatile profile of the fresh flowers of *Dipteryx odorata* by using simultaneous distillation-extraction (SDE) and GC/MS. They identified 32 compounds, mainly terpene hydrocarbons.

The aim of the present work is the determination of the volatile compounds from tonka beans by different methods of extraction (HS-SPME, SDE and SFE).

2. Materials and methods

2.1. Chemicals and materials

Tonka beans (origin South America) were purchased in a local shop in Mallorca (Balearic Islands, Spain). Prior to the analysis, beans were grated and sieved (Mesh size 16 (Standard Mesh, US)).

n-Alkane mixture standard solution (C8-C40) was purchased from Restek (Bellefonte, PA, USA) in concentrations of 500 μg mL^{-1} dissolved in carbon disulphide/dichloromethane (3/1, V/V). Carbon dioxide (purity 4.5 and 2.8), and nitrogen (purity 4.0) were purchased from Linde Gas a.s. (Prague, Czech Republic). *n*-Hexane and *n*-heptane were purchased from Sigma-Aldrich (Prague, Czech Republic).

Fragrance Material Test Mix (ethyl butyrate, limonene, eucalyptol, geraniol, benzoic acid, (E)-cinnamaldehyde, hydroxycitronellal, thymol, cinnamyl alcohol, cinnamyl acetate, vanillin, benzyl salicylate) was purchased from Restek (Bellefonte, PA, USA). Menthol, α-terpineol, carvone, *p*-anisaldehyde were purchased from Sigma-Aldrich (Prague, Czech Republic).

SPME fibers 100 μm PDMS (polydimethylsiloxane), 85 μm Carboxen/PDMS and 50/30 μm StableFlex DVB/CAR/PDMS (divinylbenzene/carboxene/polydimethylsiloxane) were purchased from Sigma-Aldrich (Prague, Czech Republic).

2.2. Head-space solid phase microextraction procedure

2.2.1. HS-SPME at constant temperature

100 mg of the sample was placed into a 20 mL headspace vial and closed by a cap with a Teflon septum, and conditioned at an initial extraction temperature for 20 min. HS-SPME was carried-out according to the following conditions: 85 μm Carboxen/PDMS fibre, extraction temperature 62 °C, and extraction time 39 min. After that, volatile compounds were desorbed from the fibre in the GC injector port, set up at 200 °C.

2.2.2. Optimisation of HS-SPME at constant temperature

The selection of a suitable fiber coating is one of the most crucial steps in the developing of an SPME method. In the present work, three different SPME fibres (100 μm PDMS; 50/30 μm DVB/CAR/PDMS and 85 μm Carboxen/PDMS) were simply tested for the suitability of isolation of volatile compounds from tonka beans. The selection procedure was carried out based on a sum of peaks detected after incubation and extraction steps (20 + 30 min) at three different temperatures (40, 60, 80 °C).

The optimisation procedure was contented from 12 experiments for the chosen fibre (85 μm Carboxen/PDMS). The extraction factors observed were extraction temperature in the range from 35 to 95 °C and extraction time that ranged from 10 to 60 min. The incubation time was 20 min for each analysis, while the temperature of the incubation was kept at the same level as the extraction. The evaluation of the whole experiment was done according to the number of peaks (NoP) in individual chromatograms using the method of response surface modelling in STATISTICA data analysis software, version 12 (StatSoft, Inc., www.statsoft.com). Critical values of independent variables were in the maximum of the response surface of the model, and the optimum extraction conditions were found to be 62 °C for 39 min.

2.2.3. HS-SPME at decreasing temperature

100 mg of the sample was placed into a 20 mL headspace vial and closed by a cap with a Teflon septum, and conditioned at an initial extraction temperature for 20 min. Extraction of volatile compounds was carried-out according to the following conditions: 85 μm Carboxen/PDMS fibre, at temperatures decreasing spontaneously from 100 °C to 30 °C. After that, volatile compounds were desorbed from the fibre in the GC injector port set at 200 °C.

2.3. Supercritical fluid extraction

Supercritical fluid extractions were performed on an SE-1 instrument from SEKO-K (Brno, Czech Republic). All extractions were performed with supercritical CO_2 in dynamic mode. The stainless steel extraction vessel (4.5 mL) was packed with a mixture of 100 mg of sample and glass sand. A silica tube restrictor (15 cm, i.d. 50 mm) was used to collect the extracted analytes. The restrictor outlet was immersed into a liquid *n*-hexane trap. Components of the sample were extracted using the dynamic extraction mode by 5.2 L of carbon dioxide at 31 MPa and 103 °C. The obtained extract was transferred into a 2 mL volumetric flask and filled up to the mark with *n*-hexane. The sample was extracted in triplicate.

2.4. Simultaneous distillation-extraction

SDE was performed using apparatus of Clevenger type (Kavalierglass a.s., Prague, Czech Republic). 10 g of grated and sieved tonka beans (see 2.1) were distillated with 500 mL of water for 5 h. Volatile compounds were extracted using 1 mL of *n*-hexane in a separator. The SDE of sample was performed three times.

2.5. GC instrumentation

For the analyses, a gas chromatograph, model GC-2010 Plus coupled to mass spectrometry detector TQ-8030 and auto-sampler AOC-5000 Plus (all from Shimadzu, Kyoto, Japan) was used. A capillary column SLB–5 ms (30 m × 0.25 mm; 0.25 μm) from Supelco (Bellefonte, PA, USA) was employed for separation. As a carrier gas, helium 5.0 (Linde Gas a.s., Prague, Czech Republic) was used at a constant linear velocity of 30 cm/s (column flow rate was 0.69 mL/min at initial conditions of separation). The temperature of the injector was maintained at 200 °C. The temperature gradient was programmed as follows: the initial temperature at 40 °C was held for 3 min and then increased by a rate of 2 °C/min up to 250 °C (10 min). The mass spectrometer was operated in