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Analytical Methods

Profiling volatile compounds from Mucuna beans by solid phase microextraction and gas chromatography-high resolution time of flight mass spectrometry

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ABSTRACT

Four solid phase microextraction (SPME) fibres, polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA), and carboxen/polydimethylsiloxane (CAR/PDMS), were evaluated for profiling of volatile compounds during cooking of *Mucuna pruriens* beans. A gas chromatograph coupled to a high resolution time of flight mass spectrometer system was employed for separation, detection and identification of the volatile compounds. For the first time we report a total of 26 compounds, mostly alkyl benzenes and polycyclic compounds, identified in black, white, black–white, and yellow green Mucuna beans during head space sampling employing a CAR/PDMS fibre with subsequent detection with high resolution mass spectrometry. The number of volatile compounds sampled, most notably alkylbenzenes, decreased with each hour of boiling and discarding of water extracts. As the beans approached being fully cooked, benzoic acid 2-hydroxy methyl ester was the most dominant compound in all the four types of beans. These results are a first step towards addressing some of the occupational exposure associated with cooking Mucuna beans by rural communities.

1. Introduction

The legume, *Mucuna pruriens* (L.) DC. var. *utilis* (Wall ex Wight), (velvet bean) is of the family *Fabaceae*. The beans are native to Southern Asia and Malaysia and also grow widely in the tropics including South and North Africa (Pugalenthi, Vadivel, & Siddhuraju, 2005). There are over a 100 species of the genus *Mucuna*, five of which are the most grown with *M. pruriens* var. *utilis* being the most common (Lorenzetti, MacIsaac, Arnason, Awanga, & Buckles, 1998; Pugalenthi et al., 2005). The beans constitute an important source of food in the regions where they are grown and particularly during drought spells.

Research has shown that *M. pruriens* beans are very toxic due to the presence of high concentrations of *l*-dopa in the seeds which have been reported in quantities ranging from 3% to 7% (Lorenzetti et al., 1998). Consumption of inadequately cooked Mucuna beans led to death of people in Malawi (Gilbert, 2002) and severe psychosis in Mozambique (Infante et al., 1990). Cooking the legume for food remains a major challenge facing communities who grow or have access to Mucuna beans. Apart from rendering the beans edible, the cooking process which may take up to 14 h of continuous boiling must also detoxify them of *l*-dopa. This tedious cooking method is to date the most common and effective process of cooking Mucuna beans for food (Gilbert, 2002). The long cooking hours of the beans are accompanied by a variation of strong smell indicating the release of volatile compounds. In our field experience, women who are involved in cooking the beans have often complained of obnoxious vapours that lead to dizziness and headaches during the cooking processes. The vapours are said to be intense during the first 5–6 h of boiling. Sampling, identification and monitoring of the volatiles compounds during the cooking process is therefore very important as a critical step towards human health risk assessment.

Extraction and trapping of volatile compounds that are free of interfering matrices prior to identification have their own challenges. Chromatographic systems coupled to mass spectrometers though versatile in separation and unequivocal in identification of analytes are severely limited by not being able to directly handle complicated sample matrices (Kataoka, Lord, & Pawliszyn, 2000). Simultaneous distillation extraction (SDE), solid phase extraction (SPE), and liquid–liquid extraction (LLE) are some of the major techniques that have been employed in sampling of volatile compounds from various sample matrices (Chaintreau, 2001; Kataoka et al., 2000). These techniques use organic solvents to extract analytes from a sample. The drawbacks of these methods are that they are tedious and time consuming, and may also use solvents which

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are not only costly but also present health and environmental hazards (Kataoka et al., 2000).

Supercritical fluid extraction (SFE), on the other hand employs a pressurised gas, usually carbon dioxide. The equipment for SFE is bulky and requires electricity. It is not easy to adapt SDE, LLE and SFE to field analysis due to the elaborate equipment or glassware involved. Solid phase microextraction (SPME), a solventless extraction technique does not suffer from these disadvantages. It is easy to use, acts as a preconcentration step and is adaptable for field use (Arthur & Pawliszyn, 1990; Ouyang & Pawliszyn, 2006; Pawliszyn, Pawliszyn, & Pawliszyn, 1997). The fibre coating can be chosen and optimised to suit a targeted group of analytes. The technique of SPME has been used to analyse flavoured compounds in French beans (Barra et al., 2007). This paper presents the application of head space SPME as a sample clean up tool for analysis of volatile and semi volatile compounds during cooking of Mucuna beans. To the best of our knowledge, this is the first time SPME has been employed to study volatile compounds released during cooking of Mucuna beans.

2. Materials and methods

2.1. Materials

Dry Mucuna beans were collected in intact shells from Zomba and Balaka districts south of Malawi and were stored in air tight closed glass containers prior to boiling and sampling. Ultrapure water of 18.2 mΩ cm quality was obtained from a Millipore Milli Q system (Bedford, MA, USA) and was used throughout for washing and boiling the beans. A SPME fibre assembly, 30 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 85 μm polyacrylate (PA), and 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) fibre holders were purchased from Supelco (Bellefonte, PA, USA).

2.2. Screening of fibres and profiling of volatile compounds

In order to screen the volatile compounds released in the course of boiling, the four fibres were evaluated for the most suitable coating material and extraction mode. The separation of volatile compounds on GC with high resolution MS detection was also optimised for subsequent identification. Each fibre was conditioned at the temperature recommended by the manufacturers before extraction. Further, conditioning and sampling of water blanks (without seeds) between samples were carried out to detect any cross-contamination. Volatile compounds from about 11 g (8–9 bean seeds) of black, white, white–black and yellow–green Mucuna bean seeds were sampled during boiling, with and without seed coats. In addition the seed coats of each type of beans were similarly sampled for the volatiles. Each fibre was suspended in a Liebig condenser with water cooling over a flask of boiling whole beans headspace or directly exposed to the boiling flask contents (direct sampling) for 1 h. The Liebig condenser was left so as to mimic the traditional open cooking set up practised at the local villages and also to enable the beans to boil for long hours without charring. The boiling procedures were replicated five times for each sample and employed SPME and high resolution MS for identification.

2.3. SPME GC and GC–MS analysis

A 6820 Agilent (Agilent Technologies) gas chromatograph equipped with a flame ionisation detector (FID) maintained at 300 °C was employed for preliminary evaluation of the ability of the fibres to sample the volatiles and subsequent separation on a 30 m, 0.25 μm internal diameter and 0.25 μm film thickness HP–Innowax (Agilent) cross-linked polyethylene glycol column. Nitrogen was employed as a carrier gas. The same column was used for separation on a 6890 Waters gas chromatograph coupled to a Waters GCT Premier Micromass Technologies (Manchester, UK) time of flight mass spectrometer. The injection port at the GC–MS was maintained at 250 °C in a splitless mode. Helium was used as a carrier gas at a flow rate of 1 ml s^{-1}. Electron ionisation (EI) was employed to generate ions at 70 eV. The mass of the volatile compounds was scanned from 50 to 500 amu. The optimised temperature programming for the GC is given in Table 1.

Table 1
Temperature programme for GC–MS.

Ramp	°C/min	Temperature (°C)	Hold (min)
Initial		30	1
1	3	168	5
2	2.5	200	2.5

2.4. Identification of volatile compounds

The identification of the volatile compounds was carried out based on the National Institute of Standards and Technology (NIST) NIST05 MS Library and MS Search Program V.2.0d (NIST 2005). The identity of the compounds from the acquired spectra was arrived at based on three values; match factor, relative match, and probability as generated by the NIST, 2005 software. Additional information from MS/MS processes (data not shown) for the target analytes was also used to support the NIST library data.

3. Results and discussion

3.1. Selection of fibre for SPME for Mucuna volatile compounds

Out of the four types of fibres, CAR/PDMS coating yielded the highest number of peaks for the total ion chromatogram with a time of flight mass spectrometer (TOF-MS) employing electron ionisation (EI) as shown in Fig. 1. The CAR/PDMS, coated as a porous solid dispersed in liquid polymer matrix, employs partition and adsorption for sampling of volatile and low molar mass analytes (Augusto & Valente, 2002). The CAR/PDMS fibre was chosen for further sampling to profile the volatile compounds during boiling based on its wide sampling capacity and sensitivity.

Head space (HS) sampling gave the highest number of compounds signified by the number of chromatographic peaks detected compared to direct sampling for all fibre coating materials. All the peaks obtained with the PDMS, PDMS/DVB and PA fibres were also detected and identified after employing the CAR/PDMS fibre. Subsequent sampling of the volatile compounds was therefore carried out employing CAR/PDMS fibre in HS mode in order to capture as many volatile compounds as possible. Total ion chromatograms were obtained at various intervals during cooking of the beans and the volatiles were identified with GC–MS. A typical total ion chromatogram obtained with a GC–MS for whole Mucuna beans sample during boiling with a CAR/PDMS in the HS mode is shown in Fig. 2.

3.2. Identification of volatile compounds

The highest number of peaks was detected with sampling by the CAR/PDMS fibre but only those that scored high values on the NIST05 were recorded as identified. A total of 26 compounds sampled from headspace at various cooking stages were identified in accordance to the NIST05 software protocol as listed in Table 2. None of the compounds that were identified employing the high

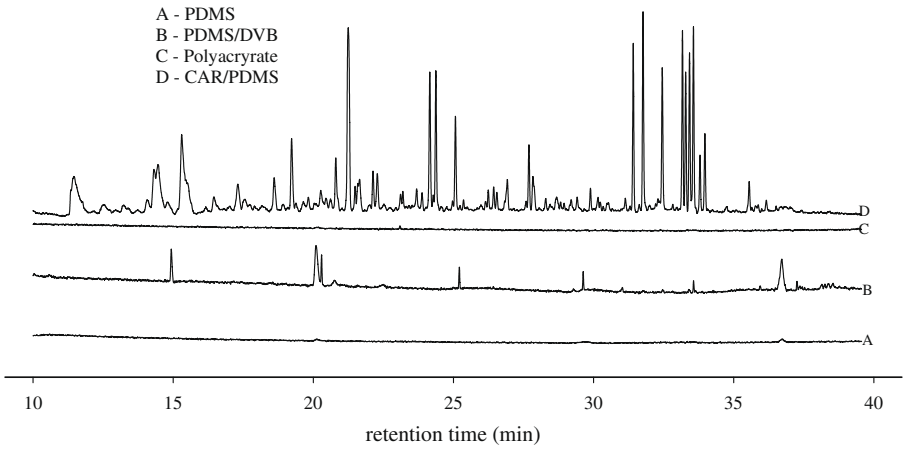

Fig. 1. Total ion chromatograms of volatile compounds from boiling Mucuna beans after sampling with four types of fibres: (A) PDMS, (B) PDMS/DVB, (C) PA, and (D) CAR/PDMS. GC-TOEFMS parameters: column; HP-Innowax 30 m by 0.25 μm; carrier gas; helium at 1 ml s^{-1}; injection; splitless at 250 °C; ionisation; EI at 70 eV.

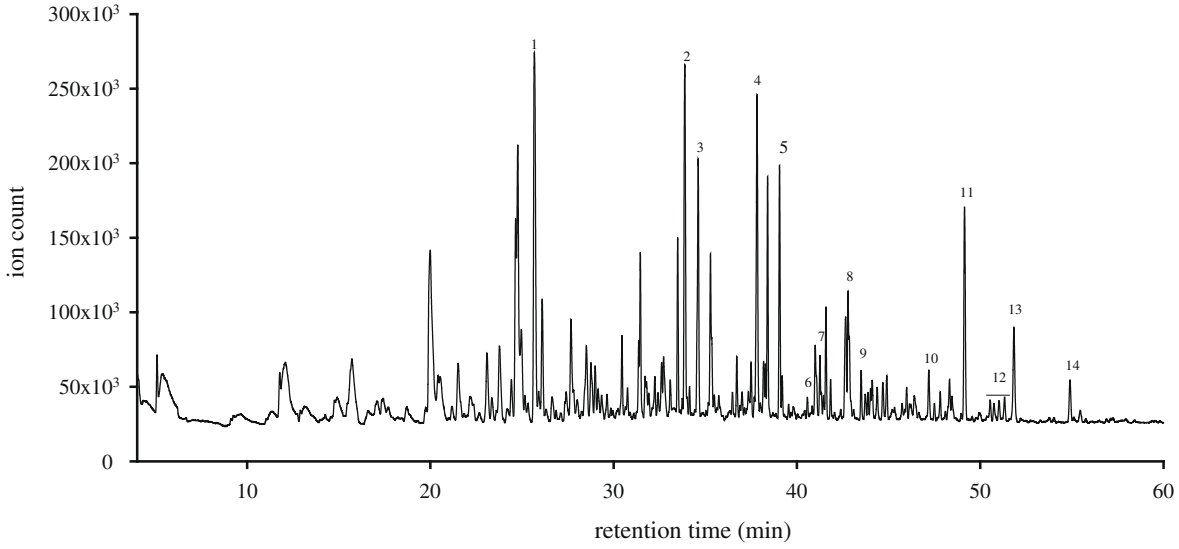

Fig. 2. Total ion chromatogram of compounds sampled for 1 h during boiling whole black beans sample. 1 = benzaldehyde, 2 = naphthalene, 3 = benzene-1,3-dimethoxy, 4 = naphthalene-1-methoxy, 5 = naphthalene-2-methoxy, 6 = naphthalene-2,6-dimethyl, 7 = benzene(1-propylnonyl) 8 = benzene (1-ethyldecyl), 9 = diphenylether, 10 = acenaphthene, 11 = acenaphthylene, 12 = 2,6-diisopropylnaphthalene, 13 = dibenzofuran, 14 = fluorine. Sampling mode: HS with CAR/PDMS fibre. GC-TOFMS parameters: column; HP-Innowax 30 m by 0.25 μm, carrier gas; helium at 1 ml s^{-1}, injection; splitless at 250 °C, ionisation; EI at 70 eV.

resolution MS were present in the water blanks. The main proportion of compounds was a class of linear alkylbenzenes (LABs), (see Fig. 3) of varying alkyl chain lengths. A small fraction of small polycyclic hydrocarbon related compounds were also identified. The proportion of compounds decreased sharply after the first hour of boiling. The commonality among the compounds was their non-polar nature which renders them more volatile and suitable candidates for sampling with CAR/PDMS (Augusto & Valente, 2002).

The major variation in the structures of the identified LABs was on the chain lengths of the R and R′ groups. All the spectra of the LABs had a common intense peak at 91 m/z as shown in the mass spectrum of benzene (1-butylhexyl) in Fig. 4. This peak most likely corresponds to the tropylium ion $C_7H_7^+$. A second peak, at m/z = 77 was also common and is consistent with the phenyl group which can be assigned to $C_6H_5^+$.

3.3. Profile of Mucuna bean volatile compounds during boiling

It was observed that most of the compounds especially the LABs were detected during the first hour of boiling most notably for the black and white–black beans. The white Mucuna beans yielded LABs with a reduced intensity. The intensity of the peaks in the total ion chromatograms for the LABs decreased considerably with increase in boiling time and more also without the seed coats. It was further observed that 4 h of continuous boiling after removing seed coats, benzoic acid 2-hydroxy methyl ester was the most dominant compound in all the four types of beans and a more pleasant flavour started to emerge.

On the other hand, the most dominant compound during boiling of seed coats alone was 2,6-diisopropylnaphthalene (DNPH) as shown in Fig. 5. It was observed that DNPH, corresponded to between four and six peaks within the same total ion chromatogram and exhibited very similar mass spectra shown in Fig. 5. The base peak for all the DNPH spectra was at m/z 197 and in addition had common peaks at m/z 212, and m/z 155. The presence of multiple peaks of DNPH could possibly be due to the structural isomers of the compound as reported by Brzozowski, Skupiński, Jamróz, Skarżyński, and Otwinowska (2002). The difference arising from structural isomers, is likely to introduce differences in polarity which in turn affects the separation on the GC column. The presence of DNPH in the seed coats adds to the significance of Mucuna

Table 2
Volatile compounds identified in Mucuna beans during boiling after sampling with CAR/PDMS fibre as identified with NIST 05 (NIST, 2005) protocol.[a].

Compound	Match factor	Relative match	Probability
1 H-Indene, 1-ethyldene	891	909	22.5
2,6-Diisopropylnaphthalene	881	888	88.1
Acenaphthene	810	908	68.5
Acenaphthylene	902	946	51.5
Azulene	890	913	40.3
Benzaldehyde	918	935	73.7
Benzene (1-butylheptyl)-	731	824	81.7
Benzene (1-butylhexyl)-	922	923	94.9
Benzene (1-butyloctyl)-	867	888	89.7
Benzene (1-ethylnonyl)-	857	861	88.1
Benzene (1-ethyloctyl)-	850	854	80.9
Benzene (1-pentylheptyl)-	864	906	90
Benzene (1-pentylhexyl)-	876	880	92.1
Benzene (1-propylnonyl)-	917	917	94.6
Benzene (1-propyoctyl)-	893	895	890
Benzene (ethyldecyl)-	815	860	92.5
Benzene1,3-dimethoxy-	917	928	94.2
Benzoic acid 2-hydroxymethylester	840	907	74.5
Biphenylene	898	924	43.5
Dibenzofuran	913	932	89.7
Diphenylether	844	887	79.9
Fluorene	905	907	68.4
Naphthalene	890	914	40.3
Naphthalene, 2-methyl	901	910	37.1
Naphthalene,1,6,7-trimethyl	863	875	37.8
Naphthalene,2,6-dimethyl-	895	931	17.7

[a] Match factor: a perfect match scores a value of 999. A value of 900 is designated excellent, 800–900 a good match; 700–800 a fair match. Relative match has same values as match factor. Probability has values between 1 and 100.

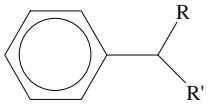

Fig. 3. General structure of linear alkyl benzene, where R ≠ R′ alkyl groups of variable chain lengths.

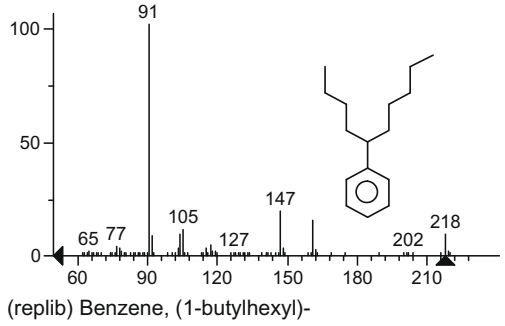

Fig. 4. Mass spectrum of a typical LAB (benzene, (1-butylhexyl)) from Mucuna beans. Characteristic fragmentation patterns of a linear alkylbenzene obtained with NIST05 mass spectra software after sampling from whole boiling beans.

beans. It is reported that DNPH is used as a plant growth regulator and as a solvent for manufacturing of printing materials (Brzozowski et al., 2002; Genter et al., 2006; Höke & Zellerhoff, 1998).

Sampling at different points showed variation in the range of volatile compounds released from Mucuna beans. The compounds could be naturally present or were by-products produced due to high temperatures during cooking. It is certain however that the detected compounds volatilise during the process of boiling Mucuna beans. None of the identified compounds is among those that have been reported in uncooked dry or fresh Mucuna beans before.

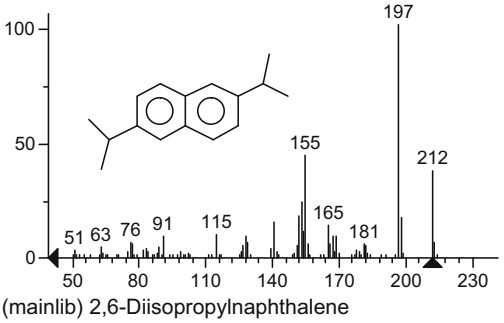

Fig. 5. Mass spectrum of 2,6-diisopropylnaphthalene from seed coats of Mucuna beans.

This is not surprising since previous studies have concentrated on liquid extracts and not volatile compounds.

There is certainly potential for adverse effects from exposure to some of the compounds based on their toxicity information. In particular fluorene, dibenzofuran and acenaphthene are known to be carcinogenic (Perugini et al., 2007; Shemer & Linden, 2007). This is of concern since during cooking of the beans there is exposure of these compounds for long periods of time. In addition, being unaware of the dangers, cooking may be carried out in poorly ventilated kitchens, which is not uncommon in villages.

Finally, the versatility of SPME and high resolution GC–MS for screening of volatiles compounds during cooking of Mucuna beans has been demonstrated. Out of the four fibres evaluated, a CAR/PDMS fibre was found to be the most suitable for sampling the volatile compounds. The major challenge was that the nature of the compounds was unknown prior to sampling and GC separation. This paper therefore sets an exciting agenda for further work in sampling, identification and detection of compounds released under various stages of cooking Mucuna beans.

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