



Short communication

Volatile constituents of fruit pulp of *Strychnos cocculoides* (Baker) growing in Malawi using solid phase microextractionT. Shoko^a, Z. Apostolides^b, M. Monjerezi^a, J.D.K. Saka^{a,*}^a Chemistry Department, Chancellor College, University of Malawi, P.O. Box 280, Zomba, Malawi^b Department of Biochemistry, University of Pretoria, Pretoria 0002, South Africa

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ABSTRACT

Volatile constituents of the edible pulp of *Strychnos cocculoides* (Monkey orange) were extracted using solid phase microextraction (SPME) and their identity established by GC–FID and GC–MS systems. Six compounds were extracted and identified: isobutyl acetate, 53.2%; 2-methylbutyl acetate, 12.8%; ethyl-2-methylbutyrate, 10.5%; 2, 6-ditetrabutyl-4-methyl-phenol, 8.4%; butyl-2-methyl butyrate, 6.7% and geranyl acetate, 3.1%. These accounted for 94.7% of the volatile constituents in the pulp. Thus, the acetate and butyrate esters were the most abundant volatiles in the edible pulp of the ripe fruit.

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1. Introduction

The Miombo or *Brachystegia* woodlands of Southern Africa including Malawi are rich in indigenous fruits that are consumed by the rural people at different times of the year and more importantly during the famine periods of the year (Chirwa and Akinnifesi, 2008). *Strychnos cocculoides* (monkey orange), which belongs to the family Loganiaceae (Mwamba, 2005) grows naturally in *Brachystegia* woodlands, mixed forests, deciduous woodlands and lowlands (Chirwa and Akinnifesi, 2008). Its fruits are known as *kabeza* in Malawi and are more important edible fruits than those from *Strychnos spinosa* which are somehow bitter (Chirwa and Akinnifesi, 2008). The pulp of *S. cocculoides* has a sweet taste (Mwamba, 2005), and provides good juices of comparable quality to those of exotic fruits (Saka et al., 2007). The main volatiles present in the peel of ripe fruits of *S. spinosa* include phenylpropanoids, with trans-iso Eugenol as the major compound using SPME (Sitrit et al., 2003). Despite the increasing importance of the monkey orange fruit; the composition and identity of its flavour compounds have not been investigated (Sitrit et al., 2003). This paper concerns the extraction of some of the flavour constituents of *S. cocculoides* fruits using SPME and

their identification by the gas chromatography–flame ionisation detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

Ripe fruits of *S. cocculoides* were collected from 10 trees, 50 m apart, from Sanga in Nkhata Bay, Malawi in September 2009. Samples were cleaned, packed in polythene bags and refrigerated at below –20 °C to minimize changes in flavour. An SPME (SUPELCO–Sigma Aldrich) device purchased from South Africa consisting of a fused silica fibre, coated with 100 µm polydimethylsiloxane (PDMS) polymeric adsorbent was used. Ten fruits from each tree were peeled and the fruit pulp mixed for analysis. A sample (30 g) of the composite pulp was placed in a 100 ml vial and tightly closed. The SPME fibre (5 mm) was inserted into the headspace of the vial for 20 min, and then directly inserted into the injection port for desorption (Viljoen et al., 2008). This process was replicated twice. The volatiles were analysed by the GC–MS Agilent 6890N GC system coupled directly to a 5973 MS (Viljoen et al., 2008). The splitless injection was carried out manually at 24.79 psi and an inlet temperature of 250 °C. The GC system was equipped with a HP–Innowax polyethylene glycol column (60 m × 250 µm i.d., 0.25 µm film thickness). The oven temperature programme was set at 60 °C for the first 10 min, rising to 220 °C at a rate of 4 °C/min and held for 10 min and then rising to 240 °C at a rate of 1 °C/min. The flame ionisation detection (FID) was kept at 250 °C. Helium was used as a carrier gas at a constant flow rate of 1.2 ml/min. The percentage

Abbreviations: SPME, solid phase microextraction; GC–FID, gas chromatography–flame ionisation detection; GC–MS, gas chromatography–mass spectrometry; FID, flame ionisation detection; RI, retention indices.

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Table 1

Identity and composition of volatile compounds in ripe *S. cocculoides* fruits using SPME (Mean \pm SD, n=2).

RRI	Compounds	Peak area (%)
<i>Esters</i>		
1037	Isobutyl acetate	53.2 \pm 2.4
1071	Ethyl-2-methyl butyrate	10.5 \pm 0.3
1141	2-Methylbutylacetate	12.8 \pm 0.4
1190	Butyl-2-methyl butyrate	6.7 \pm 0.4
1758	Geranyl acetate	3.1 \pm 0.3
Total esters		86.3
<i>Alcohols</i>		
1915	2,6-Di-tert butyl-4-methyl-phenol	8.4 \pm 0.5
Total alcohols		8.4
Total		94.7

compositions of the individual components were calculated from the total FID response. Spectra were obtained on electron impact at 70 eV, scanning from 35 to 550 *m/z*. The *n*-alkanes (C6–C24) were used as reference points in calculation of retention indices (RI). The volatiles were identified by comparison of the mass spectra and retention indices obtained in the literature using NIST®, MassFinder®, Flavour and the Baser library of essential oil constituents (Viljoen et al., 2008).

3. Results and discussion

The identity and chemical composition of the major volatile constituents of edible pulp of ripe *S. cocculoides* using SPME methods are provided in Table 1. Six volatiles accounting for 94.7% of the FID response were identified while the remaining 5.3% could not be identified under present conditions.

Acetate esters appeared the most dominant volatiles, accounting for 86.3% in the fruit pulp. Volatile acetate esters are very important contributors to the aroma of many plants, including the commercially important crops such as banana (*Musa acuminata*), apple (*Malus domestica*), melon (*Cucumis melo*), strawberry (*Fragaria ananassa*), and spice plants such as lavender (*Lavandula officinalis*) (Croteau and Karp, 1991). Geranyl acetate (3.1%) is one of main volatiles emitted by flowers of the scented rose variety (*Rosa hybrida*) (Shalit et al., 2003). Ethyl-2-methyl butyrate is considered to be one of the most important contributors to the characteristic sweet, apple-like odour of California skullcap (*Scutellaria californica* A. Gray) flowers (Takeoka et al., 2008). The butyrate has also been identified in *Parinari curatellifolia* collected from Venda in South Africa (Joulain et al., 2004). The compound, 2, 6-di-ter butyl-4-methyl-phenol is an important antioxidant and contributes to the

antioxidant capacity of various fruits (Alma et al., 2003) and is synthetically available. Therefore, the most important volatiles in the edible pulp of ripe fruits of *S. cocculoides* are acetate and butyrate esters; the former being four times higher. A volatile phenol with known antioxidant properties was also present.

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