Short communication

Headspace volatiles of the edible fruit pulp of *Parinari curatellifolia* growing in Malawi using solid phase microextraction

T. Shoko a,⁎, J.D.K. Saka a, Z. Apostolides b

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

**Abstract**

Headspace volatiles of the edible pulp of the mobola plum (*Parinari curatellifolia*) were extracted using solid phase microextraction (SPME), and their identities determined by GC–FID and GC–MS systems. The SPME method extracted eleven major compounds accounting for 99.0% of the volatile constituents. The volatiles were ethyl butyrate, 28.7%; ethyl isovalerate, 19.3%; ethyl valerate, 12.4%; ethyl hexanoate, 3.7%; ethyl benzoate, 2.5%; isomyl isovalerate, 0.3%; phenol, 10.5%; α-bergamotene, 1.1%; β-farnesene, 3.0%; 2,6-diterbutyl-4-methylphenol, 3.1% and phenylacetonitrile, 14.4%. Thus, the valerate and butyrate esters are the most abundant volatiles in the head-space of the edible pulp of the ripe fruit using the SPME method. The compounds, ethyl isovalerate, ethyl valerate, isoamyl isovalerate, phenol, 2,6-diterbutyl-4-methyl-phenol, phenylacetonitrile, α-bergamotene and β-farnesene were identified for the first time in the head-space of this fruit.

© 2013 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

Indigenous fruit trees contribute to the food and nutritional requirements of people in sub-Saharan Africa by providing essential nutrients such as vitamins and minerals (Chirwa and Akinnifesi, 2008). *Parinari curatellifolia* Chrysobalanaceae (Mobola plum) fruit is found in Miombo woodlands of Southern Africa (Joullain et al., 2004). The Mobola plum is an evergreen tree growing up to 20 m with a distinct mushroom shape and yields exceptional large quantities of reddish-yellow fruits, mottled with grey (National Research Council, 2008). In Malawi, the fruit is eaten directly as a snack, and its pulp is made into porridge, juices and or fritters for feeding young children and also used to make alcoholic drinks (Saka et al., 1994). It has been previously revealed that environmental conditions influence volatile formation (Vichi et al., 2003). Until now, the compounds constituting the volatile fraction of the fruit pulp of *P. curatellifolia* from Malawi remain unknown. Volatiles were isolated for the first time in the edible pulp of the ripe fruit using the SPME method which integrates sampling, extraction, concentration into a single solvent free step (Vas and Vekey, 2004). This paper presents the identity and relative abundance of flavour compounds extracted from the head-space of mobola plum using SPME.

2. Materials and methods

2.1. Collection and treatment of fruits

Ripe fruits of *P. curatellifolia* were collected from 10 trees within a radius of 700 m, from Sanga in Nkhata Bay, Malawi in September 2009. Samples were cleaned, packed in polythene bags and refrigerated at – 20 °C to minimize changes in flavour.

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

⁎ Corresponding author at: Food Science Department, Chinhoyi University of Technology, P. Bag 7724, Chinhoyi, Zimbabwe. Tel.: +263 772 988 983.

E-mail address: tsword@yahoo.com (T. Shoko).
2.2. Extraction and determination of volatiles

2.2.1. Solid phase microextraction (SPME)

An SPME (SUPELCO) device 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 from all fruits collected was mixed to a fine consistency 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 at room temperature, and then directly inserted into the injection port for 5 min for desorption (Viljoen et al., 2008). This process was repeated twice.

2.2.2. Determination of volatiles

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 peak areas 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. In calculating the percentages, the same response for all compounds was assumed.

The RI, relative retention indices calculated relative to n-alkanes.

The identity and chemical composition of the major volatile constituents of the headspace of the edible pulp of ripe P. curatellifolia using SPME method are provided in Table 1. The results revealed that eleven most abundant volatiles accounted for 99.0% of the FID response.

| Compounds       | MW (g/mol) | B.P (°C) | Peak area(%) |
|-----------------|------------|----------|--------------|
| Ethyl butyrate  | 116.16     | 120–121  | 28.7 ± 0.6   |
| Ethyl isovalerate | 130.19    | 131–133  | 19.3 ± 0.3   |
| Ethyl isovalerate | 131.19    | 144–145  | 12.4 ± 0.2   |
| Ethyl hexanoate | 144.21     | 166–168  | 3.7 ± 0.2    |
| Ethyl benzoate  | 150.18     | 211–213  | 2.5 ± 0.4    |
| Isoamyl isovalerate | 172.26    | 192–193  | 0.3 ± 0.3    |
| α-Bergamotene   | 204.35     | 259–260  | 1.1 ± 0.1    |
| β-Farnesene     | 204.35     | 206.0    | 3.0 ± 0.4    |
| 2,6-Di-ter butyl-4-methyl-phenol | 220.35 | 265 | 3.1 ± 0.3 |
| Phenol          | 108.14     | 205      | 10.5 ± 0.4   |
| Phenyl acetonitrile | 117.15   | 234      | 14.4 ± 0.5   |

3. Results and discussion

The identity and chemical composition of the major volatile constituents of the headspace of the edible pulp of ripe P. curatellifolia fruits using SPME (Mean ± SD, n = 2). The extract was qualitatively rich in esters (66.9%) were more abundant than alcohols (10.5%), hetero-atoms (14.4%), sesquiterpenes (4.1%) and phenols (3.1%). Hence, esters appear to contribute much more significantly to the total volatiles of the fruit pulp. The most abundant ester, ethyl butyrate (28.7%) was the third most abundant compound obtained in the P. curatellifolia from Thohoyandu, Venda South Africa using a different method, namely vacuum headspace concentration (Jouilain et al., 2004). In the earlier study, 2,phenylethanol and ethyl pentanoate were the most abundant. Ethyl butyrate has also been determined among the volatile components of Oiti fruit (Licaria tomentosa Benth.), Gethyllis afera and Gethyllis ciliaris fruits (Kamatou et al., 2008). Ethyl valerate also known as the green apple flavour is well known for its wide uses in the areas of food, pharmaceuticals and cosmetics industries (Raghavendra et al., 2010). Ethyl valerate and ethyl butyrate were identified in headspace volatiles of Scutellaria californica A. Gray flowers (Takeoka et al., 2008).

The extraction methods significantly determine the range and type of extracted compounds, evidently, dynamic extraction has greater sensitivity than a static equilibrium process (Jouilain et al., 2004). This work has thus expanded the range of volatiles obtainable from the headspace of the fruit: ethyl isovalerate, ethyl valerate, isoamyl isovalerate, phenol, 2,6-diter butyl-4-methyl-phenol, phenylacetonitrile, α-bergamotene and β-farnesene. The PDMS fibre used on the SPME was responsible for the sample-headspace-fibre equilibrium; this probably accounted for the type of compounds extracted. The results also seem to show that lower molecular weight esters were easier to extract using the SPME than higher molecular weight esters. For example, ethyl hexanoate (144.21 g/mol), ethyl benzoate (150.18 g/mol) and isoamyl isovalerate (172.26 g/mol) accounted for 3.7%, 2.5% and 0.3% respectively. Ethyl butyrate, having the lowest molecular weight (116.16 g/mol) was the most abundant ester extracted. Further, ethyl isovalerate (boiling point of 131 °C–133 °C) is more volatile than ethyl valerate (144 °C–145 °C). Consequently, ethyl isovalerate gave a larger peak area (19.3%) than ethyl valerate (12.4%). Furthermore, the real relative abundances of the volatiles played a very important role in their extraction. The present results will inform future work on establishing the effect of provenance on the headspace volatiles of the pulp.

Acknowledgements

We thank the Carnegie Regional Initiative in Science Education (Carnegie-RISE) through the Southern Africa Biochemistry and Informatics for Natural Products (SABINA) network for funding and a fellowship to Tinotenda Shoko and the ACP EU through the POL-SABINA network for a study attachment at the University of Pretoria. We also thank Prof A. Viljoen and Dr G. Kamatou of Tshwane University of Technology for the technical support.

References

Chirwa, P.W., Akinnifesi, F.K., 2008. Ecology and Biology of Uapaca kirkiana, Strychnos coccoides and Sclerocarya birrea in Southern Africa. In: Akinnifesi, F.K., Leakey, R.R.B., Ajayi, O.C., Sileshi, G., Tchoundjou, Z., Matakalal, P., Kwasiga, F., (Eds.), Indigenous Fruit Trees in the Tropics: Domestication, Utilisation and Commercialisation. CABI, UK, p. 322.

Jouilain, D., Casazza, A., Laurent, R., Portier, D., Guillamon, N., Pandya, R., Le, M., Viljoen, A., 2004. Volatile flavor constituents of fruits from Southern Africa: Mobolua Plum (Puninia curatellifolia), Journal of Agricultural and Food Chemistry 52, 2322–2325.

Kamatou, G.P.P., Viljoen, A.M., Oitz, T., Baser, K.H.C., 2008. Headspace volatiles of Gethyllis afera and G. ciliaris fruits (“kukumakranka”), South African Journal of Botany 74 (4), 768–770.

National Research Council, 2008. Lost Crops of Africa, Volume III: Fruits. The National Academies Press, Washington D.C. 265.

Raghavendra, T., Sayania, D., Madamwar, D., 2010. Synthesis of the green apple ester ethyl valerate in organic solvents: effects of immobilisation and reaction parameters. Journal of Molecular Catalysis B: Enzymatic 63 (1–2), 31–38.

Saka, J.D.K., Month, J.D., Maghembe, J.A., 1994. Nutritional value of edible fruits of indigenous wild trees in Malawi. Forest Ecology and Management 64 (2–3), 245–248.

Takeoka, G.R., Lan, D., Rodriguez, D.M., Patterson, R., 2008. Headspace volatiles of Scutellaria californica A. Gray flowers. Journal of Essential Oil Research 20, 169–171.
Vas, G., Vekey, K., 2004. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. Journal of Mass Spectrometry 39, 233–254.

Vichi, S., Pizzi, L., Conte, L.S., Buxaderas, S., Lopez-Tamames, E., 2003. Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: characterisation of virgin olive oils from two distinct geographical areas of northern Italy. Journal of Agricultural and Food Chemistry 51 (22), 6572–6577.

Viljoen, A.M., Kamatou, G.P.P., Baser, K.H.C., 2008. Headspace volatiles of marula (Sclerocarya birrea subsp. caffra). South African Journal of Botany 74, 325–326.

Williamson, J., 1975. Useful Plants of Malawi. Montfort Press, Limbe.