Phytochemistry 99 (2014) 61–72

Contents lists available at ScienceDirect

Phytochemistry

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

Metabolomics in melon: A new opportunity for aroma analysis

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ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form 6 December 2013
Available online 10 January 2014

Keywords:
Cucumis melo
Volatile organic compound (VOC)
Aroma
Amino acid
Thermal desorption (TD)
Solid phase micro extraction (SPME)
Gas chromatography mass spectrometry (GC–MS)
Proton-nuclear magnetic resonance spectroscopy (^{1}H NMR)
Metabolomics

ABSTRACT

Cucumis melo fruit is highly valued for its sweet and refreshing flesh, however the flavour and value are also highly influenced by aroma as dictated by volatile organic compounds (VOCs). A simple and robust method of sampling VOCs on polydimethylsiloxane (PDMS) has been developed. Contrasting cultivars of *C. melo* subspecies *melo* were investigated at commercial maturity: three cultivars of var. Cantalupensis group Charentais (cv. Cézanne, Escrito, and Dalton) known to exhibit differences in ripening behaviour and shelf-life, as well as one cultivar of var. Cantalupensis group Ha'Ogan (cv. Noy Yisre'el) and one non-climacteric cultivar of var. Inodorus (cv. Tam Dew). The melon cultivar selection was based upon fruits exhibiting clear differences (cv. Noy Yisre'el and Tam Dew) and similarities (cv. Cézanne, Escrito, and Dalton) in flavour. In total, 58 VOCs were detected by thermal desorption (TD)-GC–MS which permitted the discrimination of each cultivar via Principal component analysis (PCA). PCA indicated a reduction in VOCs in the non-climacteric cv. Tam Dew compared to the four Cantalupensis cultivars. Within the group Charentais melons, the differences between the short, mid and long shelf-life cultivars were considerable. ^{1}H NMR analysis led to the quantification of 12 core amino acids, their levels were 3–10-fold greater in the Charentais melons, although they were reduced in the highly fragrant cv. Cézanne, indicating their role as VOC precursors. This study along with comparisons to more traditional labour intensive solid phase micro-extraction (SPME) GC–MS VOC profiling data has indicated that the high-throughput PDMS method is of great potential for the assessment of melon aroma and quality.

Introduction

The melon (*Cucumis melo* L.) belongs to the Cucurbitaceae family, which contains numerous species differing greatly in fruit size (several grams to kilograms), shape (round to elongated) and organoleptic properties (bitter to sweet) (Stepansky et al., 1999a). Climacteric *C. melo* cultivars (Hadfiel et al., 1995) such as the var. *cantalupensis* (groups Ha'Ogan and Charentais) are highly prized for their sweet, refreshing, and aromatic flesh, whereas non-climacteric *C. melo* such as the var. *inodorus* melons lack aroma (Stepansky et al., 1999a, 1999b). The aroma of melon fruit is dictated by the content of volatile organic compounds (VOCs) (Aubert and Bourger, 2004; Berger, 1991; Buttery et al., 1982; Fallik et al., 2001; Kourkoutas et al., 2006; Yabumoto et al., 1977). From a chemical perspective VOCs represent a heterogeneous group of compounds, with aromatic, hetero-aromatic, branched- and straight-chain backbones, with diverse chemical groups as for example hydroxyl, carboxyl, carbonyl, amine, ester, lactone, and thiol functions (Schwab et al., 2008). The detection of VOCs has classically been achieved by means of Gas chromatography–mass spectrometry (GC–MS) (Dewulf et al., 2002) since GC lends itself to the separation of sample components based upon volatility and thus no form of chemical derivatisation is required. VOC samples are introduced to the GC–MS via a number of methods including both solid phase

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0031-9422/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2013.12.010

micro-extraction (SPME) (Beaulieu and Grimm, 2001; Song et al., 1997) and direct thermal desorption (TD) (Riazanskaia et al., 2008; Xu et al., 2010).

Contrasting test materials for this investigation, which was performed as part of the EU Frame Work VI META-PHOR project (http://www.meta-phor.eu/) (Allwood et al., 2009; Biais et al., 2009; Hall et al., 2008; Moing et al., 2011), were provided by the Israel Agricultural Research Organisation (ARO), two green fleshed melon cultivars, *C. melo* var. *cantalupensis* group Ha'Ogan cv. Noy Yisre'el (henceforth called Noy Yisre'el), and a non-aromatic fruit, *C. melo* var. *inodorus* cv. Tam Dew (henceforth called Tam Dew) (Stepansky et al., 1999a), and by France CEFEL who provided three orange fleshed *C. melo* var. *cantalupensis* group Charentais melon cultivars known as (and henceforth called) Cézanne, Escrito, and Dalton, which exhibit differences in ripening behaviour and shelf-life (Aubert and Bourger, 2004). Cézanne is the most aromatic cultivar but has a very short shelf-life, Escrito has a mid shelf-life and is less aromatic, Dalton has the longest shelf-life and is considered to be the least aromatic (Aubert and Bourger, 2004; Dumoulin and Odet, 1998). Previous GC–TOF/MS and ^{1}H NMR spatial analysis of extracted polar metabolites in Cézanne and Escrito melon fruit indicated that the inner mesocarp was hypoxic and produced ethanol, it also contained high concentrations of specific sugars and amino acids (Biais et al., 2009, 2010), which are known to be key precursors for the VOCs responsible for fruit aroma (Gonda et al., 2013; Schwab et al., 2008).

Melon VOCs have been extensively investigated in many varieties, especially with regard to how aroma profiles alter during fruit ripening (Beaulieu and Grimm, 2001; Buttery et al., 1982; Fallik et al., 2001; Homatidou et al., 1992; Kourkoutas et al., 2006; Schieberle et al., 1990; Vallone et al., 2013; Wang et al., 1996; Yabumoto et al., 1977). Other investigations have focused upon the aroma profiles of antisense-ACC oxidase expressing plants that revealed massive reductions in VOCs (Ayub et al., 1996; Bauchot et al., 1998; Flores et al., 2002). Although aroma profiles have been previously investigated in Charentais melon varieties (Homatidou et al., 1992; Bauchot et al., 1998; Flores et al., 2002), only one previous study has focused upon the aroma profiles of Charentais with respect to shelf-life (Aubert and Bourger, 2004). The aim of this study was firstly to develop a simple and inexpensive screening method of sampling melon VOCs that could also be appropriate for application to other fruits, and secondly to validate this method by investigating the aroma variability of a series of diverse melon cultivars. Subsequently, ^{1}H NMR was applied for the quantification of amino acids, since they are known to act as precursors for many volatile constituents that contribute to aroma (Gonda et al., 2013; Schwab et al., 2008). While the fruits used are equivalent to supermarket availability, they were grown under different conditions between the countries of origin. Consequently, the analysis of the melon fruits was considered first as a whole and second as two independent comparisons. The first comparison focused on the two diverse Israeli cultivars and posed the question whether PDMS derived data are able to discriminate typical climacteric and non-climacteric varieties. The second comparison focused on the closely related French Charentais melons since they posed more of an analytical challenge to discriminate and also posed the question as to which VOC components differed between the short, mid and long shelf-life fruit.

Results & discussion

A reliable and robust simplified method of passively sampling melon VOCs

The first aim of the study was to develop a simple and reproducible screening method for the ambient sampling of melon VOCs, that could be applied to the future analyses of any potential fruit or plant derived foodstuff. PDMS membrane was selected, since the sample collection routine simply involved the gentle warming of a set volume of melon homogenate within a sealed vial to liberate VOCs that were subsequently trapped upon three technical replicate PDMS membranes suspended from the vial lid (Fig. 1). Fears were raised that such a simplified method of VOC trapping would not be reproducible, therefore a set volume of 0.0001% 1-pentanol was added to the melon homogenate to serve as an internal standard. Based upon the peak area in single ion monitoring mode, the 1-pentanol signal was stable throughout the experiment for all blanks, technical and biological replicate samples regardless of the melon cultivar (standard deviation = 6.76; standard error = 1.01; Fig. 2). Therefore confidence was installed that the passive melon VOC sampling technique was not only easy to perform but was also highly reproducible.

PDMS is a suitable sorbent material for trapping a wide range of melon VOCs

Analysis of the PDMS trapped VOCs was performed by means of TD-GC–MS. In total 58 compounds were detected, 47 of which were putatively identified with high confidence (Table 1). These compounds fell into many classes, most significantly perhaps were the esters ($n = 19$), alcohols ($n = 6$), sulfur compounds ($n = 3$), and aldehydes ($n = 2$), although several other classes were detected including ketones, alkanes, akenes and alkynes. A greater number of aromatic compounds were detected when we applied a standard SPME approach (Verhoeven et al., 2011) to an identical sample set (Supplementary Fig. 1). However, the larger number of detected VOCs compared to the PDMS approach most likely relates to differences in the extraction protocol (15 × larger sample volumes and the use of inorganic salts) rather than trapping efficiency or detection sensitivity. The supplementary SPME data also confirm the presence of many of the identified VOCs detected using the PDMS approach, boding well for its application as a rapid screening approach. Comparisons of our PDMS and SPME data for the same compounds also reveals some differences in varietal distribution which again can be attributed to relative differences in trapping efficiencies by the different adsorbents (Supplementary Figs. 1 and 2). On the basis of the major VOC groups (alcohols, esters, sulfur compounds and aldehydes), 22 out of a total of 30 compounds detected via the PDMS method were also detected in the total of 75 SPME compounds of the same respective chemical classes, indicating the complementary nature of the two methods as well as the greater sensitivity of SPME.

Within this study a greater number of VOCs were detected by TD-GC–MS than in a previous study applying GC–FID (Flame In-

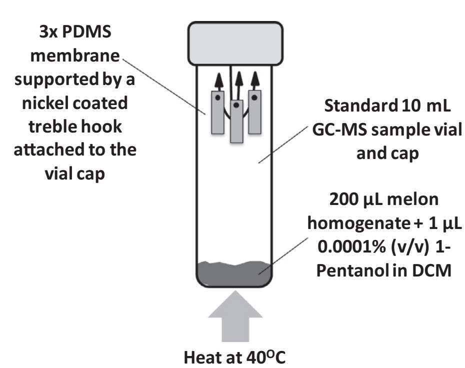

Fig. 1. Sampling of melon volatile organic compounds using PDMS membranes. An illustration as to how sampling melon VOCs upon three technical replicate PDMS membranes is achieved.