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Metabolome based volatiles profiling in 13 date palm fruit varieties from Egypt *via* SPME GC–MS and chemometrics

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ABSTRACT

Dates (*Phoenix dactylifera* L.) are distributed worldwide as major food complement providing a source of sugars and dietary fiber as well as macro- and micronutrients. Although phytochemical analyses of date fruit non-volatile metabolites have been reported, much less is known about the aroma given off by the fruit, which is critical for dissecting sensory properties and quality traits. Volatile constituents from 13 date varieties grown in Egypt were profiled using SPME-GCMS coupled to multivariate data analysis to explore date fruit aroma composition and investigate potential future uses by food industry. A total of 89 volatiles were identified where lipid-derived volatiles and phenylpropanoid derivatives were the major components of date fruit aroma. Multivariate data analyses revealed that 2,3-butanediol, hexanal, hexanol and cinnamaldehyde contributed the most to classification of different varieties. This study provides the most complete map of volatiles in Egyptian date fruit, with Siwi and Sheshi varieties exhibiting the most distinct aroma among studied date varieties.

1. Introduction

Dates have for centuries been recognized as a staple food for millions in the Arab world (Vayalil, 2011). From a nutritional aspect, they are rich in carbohydrates, protein, vitamins, minerals and dietary fiber (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005; Baliga, Baliga, Kandathil, Bhat, & Vayalil, 2011). They are also rich in polyphenols that have been observed to prevent or reduce the risk of many chronic diseases (Biglari, AlKarkhi, & Easa, 2008; Michael, Salib, & Eskander, 2013). The nutritional and putative health benefits have been studied extensively in different date fruit varieties (Nasir et al., 2015; Rahmani et al., 2014; Tang, Shi, & Aleid, 2013), date fruits are recognized as an important component in the human diet (Vayalil, 2011).

The relative volatile composition, concentrations and the perception thresholds of individual compounds give each fruit their distinctive aroma (El Hadi, Zhang, Wu, Zhou, & Tao, 2013). Analysis of volatiles has various applications in food and nutraceuticals quality control including a) distinguishing between stages of maturity (Obenland, Collin, Sievert, Negm, & Arpaia, 2012) and b) differentiating between fruit of different genotypes or geographical origins (Farag & Wessjohann, 2012).

Headspace solid phase microextraction (HS-SPME) has become a standard approach for profiling volatiles in plant and/or food products (Qualley & Dudareva, 2009), as it is least prone to artifact generation among the techniques available. There are few reports of date volatile composition, especially compared to other fruits. Only two recent reports have considered the effects of maturity on the volatile profiles of six Tunisian varieties (El Arem et al., 2011, 2012). Dynamic headspace and microwave desorption identified 47 volatiles from eight Moroccan varieties (Harrak, Reynes, Lebrun, Hamouda, & Brat, 2005). Older techniques have led to partial identification of the main volatiles of Iraqi Zahdi *var.* (Jaddou, Mhaisen, & Al-Hakim, 1984) and Spanish dates (Torres, Reynes, Lebrun, & Ferry, 1996). Although Egypt was the top producer of date fruits in 2013 with 1.5 million tonnes (http://faostat3.fao.org/browse/Q/QC/E, last accessed 23.05.2016), no study has explored the volatile profile of Egyptian dates. The present study aimed to 1) explore the volatile profile of 13 Egyptian date varieties using SPME coupled to GC–MS and 2) classify and correlate date volatile profiles using chemometrics. Previously, flavonoids from 21 Egyptian date varieties have been shown to correlate well with date varieties (Farag, Mohsen, Heinke, & Wessjohann, 2014). Until recently differentiation among date varieties depended largely on

Abbreviations: SPME, solid-phase micro-extraction; MS, mass spectrometry; GC, gas chromatography; PCA, principal component analysis; OPLS-DA, orthogonal partial least square discriminate analysis.

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fruit morphometric and physical characters (Al-Khalifah, Askari, & Shanavaskhan, 2012).

2. Materials and methods

2.1. Plant material

Dates were harvested in August 2013 at the "Rutab" stage from three bunches each per date palm tree at the date orchard at Saff Agricultural Center, Giza Governorate (Egypt). The varieties were authenticated by Prof. Dr. Fayek Mahmud at the Tropical Fruits Research Department, Agricultural Research Center, Giza (Egypt). Only dates free from defect or color differences were selected. Dates were stored at −20 °C until analysis. Three biological replicates were analyzed for each cultivar.

2.2. Chemicals and material

SPME stableflex fibers coated with divinylbenzene/carboxen/p olydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) or PDMS (polydimethylsiloxane) were purchased from Supelco (Oakville, ON, Canada).

2.3. Volatiles analysis

Analysis of volatiles was done using HS-SPME, as described previously with slight modification (Farag & Wessjohann, 2012). Frozen dates (3 g) were cut in cubes (*ca.* 1–1.5 cm) using scissor and placed into screw-cap vials (20 ml) for SPME. Vials were closed rapidly and SPME fiber was released manually in the headspace above the sample at 50 °C in a temperature controlled oven for 30 min. The fiber was withdrawn using a needle and transferred to the GC–MS injection port. GC–MS analyses were performed on Shimadzu GC-17A gas chromatograph equipped with SLB-5 column (30 ml × 0.25 mm i.d. × 0.25 μm film thickness; Supelco) and coupled to Shimadzu QP5050A mass chromatograph. The temperature of injector and the interface was kept at 220 °C. The following temperature program was used; 38 °C for 3 min, then the temperature was increased to 180 °C at 12 °C min^{-1}, kept at 180 °C for 5 min, and finally ramped at a rate of 40 °C min^{-1} to 240 °C, before being kept at that temperature for 5 min. Helium was the carrier gas with flow rate of 0.9 ml/min. Splitless injection was used and the first 5 min of the analysis were considered as solvent delay and omitted from the final chromatograms. SPME fiber was prepared by leaving it in the injection port for 2 min. The QP quadruple mass spectrometer was operated in EI mode at 70 eV. The scan range was set at m/z 40–500.

2.4. GC–MS data processing and multivariate analysis

Volatile components were identified as previously described (Farag & Wessjohann, 2012), and peaks deconvoluted using AMDIS software (www.amdis.net). Identification was based on retention indices (RI) relative to *n*-alkanes (C_6-C_{20}), mass matching with NIST and WILEY library database, and/ or comparison against standards where available. A deconvolution step allowed for detection of co-eluting peaks with peak apexes separated by less than 1 s, and also enabled detection of low-abundance peaks co-eluting in the presence of other volatiles at greater concentrations. Data were prepared for multivariate data analysis by extraction using MET-IDEA software. Data were subjected to principle component analysis (PCA) and hierarchical clustering analysis (HCA) using a custom script under the R 2.9.2 environment. Partial least squares-discriminant analysis, (OPLS-DA) was performed using SIMCA-P version 13.0 software package (Umetrics, Umea, Sweden). Subsequently, markers were identified by analyzing the S-plot, which was declared with covariance (p) and correlation (pcor). All variables were mean centered and scaled to Pareto variance.

3. Results and discussion

3.1. Volatile composition of Egyptian date varieties

Two fiber types were assessed: PDMS and DVB/CAR/PDMS. The latter adsorbed more volatiles (*ca.* 108) compared with PDMS (42), as revealed by analysis of chromatograms for the "Siwi" variety using AMDIS software (data not shown). As a consequence, DVB/CAR/PDMS fibers were selected for subsequent experiments. Volatiles were identified and listed according to their biosynthetic origin, as shown in Tables 1 and S1. The constituents identified represented *ca.* 90% of the fruit aroma. Varietal differences were more obvious in terms of the amount rather than the composition of volatiles. A typical chromatogram from Abreem is shown in Fig. 1.

Fatty acid derived volatiles amounted for 51% of date aroma, with a total of 46 volatile constituents belonging to this class. Phenylpropanoid derivatives were the second most common volatiles quantitatively (*ca.* 24.7%) and qualitatively (21 volatiles). These two classes constituted *ca.* 70% of the total aroma in all varieties, except for Siwi *var.*, which differed from the others in that both classes contributed equally to its aroma with each achieving 38%. Oxygenated monoterpenes and amino acid derived volatiles constituted *ca.* 11% whereas monoterpene hydrocarbons and sugar derived volatiles occurred at only trace levels. Chemically, date fruit volatiles belonged to six major chemical classes, *viz.* alcohols/phenols, aldehydes, aromatics, ketones, esters and terpenoids, in addition to five classes of minor contribution *viz.* ethers, lactones, saturated hydrocarbons, and acids (Table S2).

Fatty acid derivatives were the most dominant volatiles for all varieties assessed, making up *ca.* half of the total volatile content except for Siwi (38%, Tables 1 and S1). Six-carbon (C_6)-derived volatiles, such as hexanol and hexanal, were the main constituents for select date varieties. Hexanol was the major volatile constituent for Abreem, amounting to *ca.* 10% of the total while hexanal amounted almost 20% of the volatiles present in six other varieties including Barhy, Hasawy, Um Aduhain, Sheikh Ali, Khesab and Sokkary Red. Hexanol and hexanal are known as "green leaf volatiles", and are responsible for the "green" aroma note of many plants (Kako, Kobayashi, & Yokogoshi, 2011). 2,3-Butanediol was the major volatile in Maktom and Kurdi varieties, amounting for 7.8% and 12.8% of the total volatiles, respectively. Although 2,3-butanediol is a well-known bacterial volatile (Audrain, Farag, Ryu, & Ghigo, 2015; Farag, Ryu, Sumner, & Pare, 2006; Ryu et al., 2003), this volatile metabolite has only recently been identified as a component of fruit volatiles (Jordan, Margaria, Shaw, & Goodner, 2003; Jordan, Shaw, & Goodner, 2001). These volatiles made up more than 40% of the fatty acid volatiles identified (Table S1) in all varieties, except Sheshi where ethyl octanoate and ethyl decanoate constituted *ca.* 21% of the aroma. It should be noted that Sheshi was the only variety where esters represented the dominant group of volatiles (Table S2). Various oxygenation levels of C6-C9 were observed, *i.e.*, alcohol, aldehyde, ketone derivatives and their unsaturated analogs; *i.e.*, octanol, octanal, octanone, octanol and octenone. These volatiles are derived from oxidative degradation of linoleic acid and other unsaturated fatty acids (El Hadi et al., 2013). Although the (date) outer surface is known to be low in fat content (*ca.* 1%, Assirey, 2015) most of the aroma appeared to be associated with lipid degradation products.

Phenylpropanoid derivatives and benzenoids were the second major group of volatile compounds with cinnamaldehyde the major constituent in Siwi (20.7%), Medjool (15.7%) and Nabtet Seif