SUPPLEMENTARY MATERIAL

SPME-GC-MS analysis of commercial henna samples (*Lawsonia inermis* L.)

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Abstract

The aim of this work was to provide a characterization of volatile constituents from different commercial batches of henna (*Lawsonia inermis*) leaves of different geographic origin. Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) was used for the purpose. A total of 72 components were identified by GC-MS in the headspace of different henna samples which proved to differ considerably from each other, because they were characterized by different classes of components, mainly aliphatic compounds (9.0-64.7%), terpenoids (5.8-45.5%), and aromatics (7.9-45.2%), with alkanes (0.9-18.5%), aldehydes (2.1-18.8%) and carboxylic acids (3.1-29.3%), monoterpenes (3.4-30.0%) and sesquiterpenes (0.8-23.7%), and phenyl propanoids (0.6-43.1%), being the most abundant, respectively. Major representatives of these groups were *n*-hexadecane (0.5-4.7%), (2*E*)-hexenal (0.5-11.7%) and acetic acid (2.8-24.5%), limonene (0.8-14.7%), carvol (3.8-7.1%), geranyl acetone (1.4-7.9%) and (*E*)-caryophyllene (3.3-8.4%), and (*E*)-anethole (0.6-35.0%), respectively. We assume that factors such as the manufacturing process, the storage conditions, and the different geographic origin of the samples may contribute to such variability.

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3. Experimental

3.1. Materials and Chemicals

Six samples of commercial henna were analyzed in this study (Table S1). All the samples were finished products, with the exception of sample 6, which was a batch of dried and cut leaves. All products differed in the place of origin, trade name, and the color they give the after application. A mixture of aliphatic hydrocarbons (C8–C30) in *n*-hexane were purchased from Sigma Aldrich (Milan, Italy).

3.2. HS-SPME procedure

Four hundred mg of powder sample were placed in 4 ml vial. The fiber used was the three phases DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) (divinylbenzene/carboxen/polydimethylsiloxane) 50/30 μm (2 cm) with intermediate polarity. The equilibration time was 10 min at 70 °C in a water bath under magnetic stirring. The absorption phase consisted in exposing the SPME in the headspace of the vial at 70 °C for 30 min, allowing the volatile compounds to be adsorbed on the SPME fiber. The third desorption phase occurred in the GC-MS for 2 min at 260 °C.

3.3. HS-SPME coupled GC-MS analysis

GC-MS analysis was carried out on a GC Agilent (Santa Clara, CA, USA) 6890 N Network System coupled to a 5973 N Mass Selective Detector. GC-MS instrument employed the following conditions: an HP-5-MS capillary column (30 m $\times$ 0.25 mm $\times$ 1 μm) (J&W Scientific, Folsom, CA, USA) composed of 95% dimethyl polysiloxane and 5% phenyl, operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2 μl was employed in splitless mode for 2 min. Injector temperature 260 °C. The oven temperature was programmed from 30 °C (isothermal for 2 min.), with an increase of 5 °C/min to 220 °C. The solvent delay was 0.00 min. Transfer line at 300 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 29 to 450 Da. Total GC

running time was 40 min. A mixture of aliphatic hydrocarbons (C8–C30) in *n*-hexane (Supelco) was loaded into the SPME fiber and injected using the above temperature programme in order to calculate the retention indices of each extracted compound using HS-SPME. All GC/MS analyses were conducted in triplicate. Data were analyzed using MSD ChemStation software (Version G1701DA D.01.00, Agilent, PA, CA). Semi-quantitative determinations were carried out by peak area normalization without using correction factors.

3.4. Identification of components

We identified the constituents in headspace of henna samples using the approach of the International Organization of the Flavour Industry (IOFI, <http://www.iofi.org/>).

Interactive combination of chromatographic linear retention indices showed them to be consistent with those reported in literature for apolar stationary phases (Adams 2007; NIST 08 2008; FFNSC 2 2012). It also involved the use of MS data, through computer matching with the WILEY275, NIST 08, ADAMS, FFNSC 2 libraries as well as one we developed based on the analyses of reference oils and commercially available standards. Formic acid, acetic acid, hexanal, benzaldehyde, 1-octen-3-ol, *n*-decane, *p*-cymene, limonene, γ -terpinene, linalool, camphor, terpinen-4-ol, α -terpineol, *n*-dodecane, *n*-decanal, β -cyclocitral, (*E*)-anethole, *n*-tridecane, carvacrol, eugenol, *n*-tetradecane, (*E*)-caryophyllene, α -humulene, (*E*)- β -ionone, *n*-pentadecane, caryophyllene oxide, *n*-hexadecane, *n*-heptadecane, *n*-octadecane were also identified comparing retention index and mass spectrum of the chromatographic peak with that of the authentic compound purchased from Sigma-Aldrich (Milan, Italy).

3.5. Principal Component Analysis

To reveal the relationship among different henna samples according to the headspace volatile composition, and to identify the main constituents influencing variability, the

composition data matrix of six samples (72 variables x 6 samples = 432 data) was analysed using principal component analysis (PCA) with STATISTICA 7.1 (Stat Soft Italia srl, 2005, www.statsoft.it). Eigenvalues were calculated using a covariance matrix among 72 chemical compounds as input, and the two-dimensional PCA biplot, including both samples of different origin and volatile constituents was generated.

References

Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL: Allured Publishing Co.
 [FFNSC 2] Flavors and Fragrances of Natural and Synthetic Compounds. 2012. Mass Spectral Database. Japan: Shimadzu Corps.
 [NIST 08] National Institute of Standards and Technology. 2008. Mass spectral library (NIST/EPA/NIH). Gaithersburg, MD: Author.

Figure S1. HS-SPME-GC-MS chromatograms of Henna samples. Every chromatogram corresponds to one henna sample as listed in Table S1.

Table S1. Main information on Henna samples investigated

N.	Product Name	Brand	Origin	Batch
1	Henne rosso mogano	Brisighello	Iran	n° 882/11
2	Mohini	Mohini	India	n° 2013-A
3	Henne rosso naturale foglie polvere	Minardi e figli s.r.l	Iran	C-02091324101
4	Zarqa henna	Manzoor& Co.	Pakistan	#-5255
5	Sahara	Tazarine	Morocco	n°115615
6	Henne rosso naturale foglie	Minardi e figli s.r.l	Morocco	PM 10111109021