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Original Article

Volatiles profiling in *Ceratonia siliqua* (Carob bean) from Egypt and in response to roasting as analyzed *via* solid-phase microextraction coupled to chemometrics

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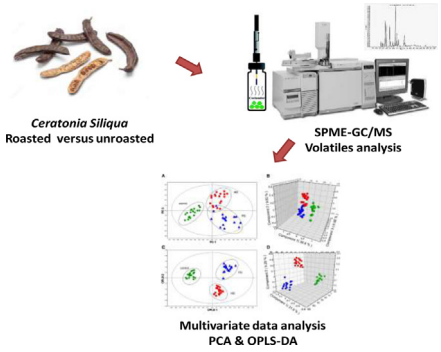
GRAPHICAL ABSTRACT



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ABSTRACT

Ceratonia siliqua is a legume tree of considerable commercial importance for the flavor and sweets industry cultivated mostly for its pods nutritive value and or several health benefits. Despite extensive studies on *C. siliqua* pod non-volatile metabolites, much less is known regarding volatiles composition which contributes to the flavor of its many food products. To gain insight into *C. siliqua* aroma, 31 volatile constituents from unroasted and roasted pods were profiled using headspace solid-phase micro extraction (HD-SPME) analyzed *via* quadruple mass spectrometer followed by multivariate data analyses. Short chain fatty acids amounted for the major volatile class at *ca.* (71–77%) with caproic acid (20%) and pentanoic acid (15–25%) as major components. Compared to ripe pod, roasted ripe pod was found less enriched in major volatile classes *i.e.*, short chain fatty acids and aldehydes, except for higher pyranone levels. Volatiles mediating for unheated and hot carob fruit aroma is likely to be related to its (*E*)-cinnamaldehyde and pyranone content, respectively. Such knowledge is expected to be the key for understanding the olfactory and taste properties of *C. siliqua* and its various commercial food products.

Introduction

Ceratonia Siliqua (Carob) is a legume tree of a well-known commercial and medicinal importance owing to its fruit (pod) enrichment in carbohydrates, dietary fibers, tannins, and phenolics. In

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the Mediterranean region, carob pod is consumed as animal or human food [1]. In terms of its health benefits, *C. siliqua* exhibits a myriad of biological effects including antibacterial, antidiarrheal, antidiabetic, anti-hypercholestrolemic, and hepatoprotective [2–5]. Additionally, Carob pods, roasted and unroasted are widely used in manufacturing of sugar syrups, molasses, and beverage [6] or as a cocoa substitute in candy products and cakes [7]. Roasting of carob pod along with sugar is thought to enhance or intensify the aroma. Since the flavor and the aroma are important aspects in the carob products, our goal was to profile its volatiles, which has scarcely been reported in the literature [8]. Steam distillation of carob fruit essential oil analyzed using GC-MS revealed for its enrichments in fatty acid and fatty acyl esters amounting for 77% of its volatile composition [8,9]. Other volatile classes found in *C. siliqua* prepared using hydro-distillation include aromatics, hydrocarbons and terpenoids [9,10].

Headspace solid phase micro-extraction (SPME) is a relatively novel technique used for volatiles extraction found superior to steam distillation, being solvent free and involving no heat application [11]. Additionally, SPME enables the enrichment of volatiles from gas or liquid samples, over a fused-silica fiber then subsequent desorption of these analytes leads to detection of less abundant volatiles [12]. One powerful feature of SPME volatiles sampling lies in preserving the true aroma without development of artifacts that might be generated with heating as in the case of steam distillation [13]. SPME has been previously applied for volatiles profiling in carob flowers revealing for its enrichment in mono- and sesquiterpenes [10]. Nevertheless, the technology has yet to be further employed for volatiles profiling in the more economical used part "pod".

Continuing our studies on Mediterranean foods flavor makeup [14,15], a report is presented herein on volatiles analysis from *C. siliqua* using SPME. The main aim of this work was to explore carob aroma using a cold SPME method for volatiles extraction and to further determine the impact of processing *i.e.*, roasting on volatile composition. To reveal for roasting effect in an untargeted manner, multivariate data analysis was applied. This study provides the most complete map for volatiles distribution in *C. siliqua* pod using SPME and its roasted product.

Experimental

Plant material, SPME, and chemicals

Ceratonia siliqua trees were grown in the semi-arid "Siwa" Oasis, Egypt and pods were collected in the full ripe stage during the month of May 2016. A voucher specimen code "6-4-2017" was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt. Roasting was accomplished by heating pods in an oven set at 120 °C for 30 min. Three to 4 biological replicates were analyzed for each sample. The fruits were stored at −20 °C till further analysis. SPME holder and fiber coated with 50 μm/30 μm Divinyl benzene/Carboxen/Polydimethylsiloxane (DVB–CAR–PDMS) was supplied by Supelco (Oakville, ON, Canada). All volatile standards *i.e.*, (*E*)-cinnamaldehyde, α-farnesene, hexanoic and benzoic acids used in the analyses were purchased from Sigma Aldrich (St. Louis, Mo., U.S.A.).

SPME volatiles isolation

The headspace volatiles analysis using SPME was explained in details as in Ref. [15,16] with few modifications. Briefly, a carob pod was dried and grounded yielding 100 mg. The grounded pod was placed inside 1.5 mL clear glass vials. (*Z*)-3-hexenyl acetate used as an internal standard (IS) being absent from the sample, dissolved in water and added to each vial at a concentration of 1 μg/vial. The vials were then immediately capped and placed on a temperature controlled tray for 30 min at 50 °C with the SPME fiber inserted into the headspace above the fruit sample. Adsorption time was 30 min. A system blank containing no fruit material was run as a control.

GC-MS volatile analysis

Three to four biological replicates for each specimen were extracted and analyzed in parallel under identical conditions to assess for biological variance SPME fibers were desorbed at 210 °C for 1 min in the injection port of a Shimadzu Model GC-17A gas chromatograph interfaced with a Shimadzu model QP-5000 mass spectrometer (Tokyo, Japan). Volatiles were separated on a DB5-MS column (30 m length, 0.25 mm inner diameter, and 0.25 μm film (J&W Scientific, Santa Clara, CA, USA). Injections were made in the splitless mode for 60 s. The gas chromatograph was operated under the following conditions: injector 220 °C, column oven 38 °C for 3 min, then programmed at a rate of 12 °C min^{-1} to 180 °C, kept at 180 °C for 5 min, and finally ramped at a rate of 40 °C min^{-1} to 220 °C and kept for 2 min, He carrier gas at 1 mL min^{-1}. The transfer line and ion–source temperatures were adjusted at 230 and 180 °C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at m/z 40–500. Volatile components were identified using the procedure fully described as in Ref. [16] and peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (RI) relative to *n*-alkanes (C6-C20), mass spectrum matching to NIST, WILEY library database with matching score above 800 and with authentic standards when available.

Multivariate data analyses

Principal component analysis (PCA) and partial least squares-discriminant analysis (OPLS-DA) were performed with the program SIMCA-P Version 13.0 (Umetrics, Umeå, Sweden). Markers were subsequently 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. The PCA was run for obtaining a general overview of the variance of metabolites, and OPLS-DA was performed to identify markers for distinguishing roasted and unroasted pods.

Statistical analysis

Paired *t*-test analysis was performed using Microsoft Excel 2013 (Microsoft Office, VA, USA) for the analysis of volatiles data. Data are represented as mean ± standard deviation SD. P value ≤ 0.05 was considered statistically significant.

Results and discussion

Volatiles analysis

The objective of this study was to assess Carob roasted pod aroma and to compare it with the unroasted pod using SPME. GC-MS analysis of *C. siliqua* samples led to the identification of 31 different volatile constituents, presented in Table 1. Detected volatiles amounted for 93% of the total volatile composition. GC chromatogram (Fig. 1) displays representative volatile profile of the roasted and unroasted pod. The qualitative volatiles composition of unroasted and roasted pods was relatively comparable, and suggesting for rather quantitative differences. Generally, *C.*