Non-targeted Fingerprinting of Green Arabica Coffee Volatile Organic Compounds (VOCs): HS-GC-IMS versus GCxGC-MS

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OVERVIEW

The overall quality of the green coffee (*Coffea* spp) is mainly related to germplasm quality (genetic profile, variety), post-harvest treatments e.g. microbial activity during fermentation in the case of wet processing and drying. Geographical origin and quality of green coffee can be established applying non-targeted methods by mean of different analytical approaches, followed by post-analytical chemometric treatment of data, often linked to the Artificial Intelligence-based methods. The quality of roasted coffee can be predicted considering the overall fingerprint of green coffee beans. Multidimensional comprehensive GCxGC-MS was used in the recent past to cluster green and roasted coffee beans **[1]**. Few data are available for green coffee VOCs analysis by HS-GC-IMS, particularly regarding the evaluation of VOCS during storage **[2]**.

Main aim of this work was to evaluate the volatile fraction of Arabica wet processed green coffee beans from different geographical origin, sampled by headspace-solid phase microextraction (GCxGC-TOF-MS) as well as directly analysed with HS-GC-IMS.

MATERIALS AND METHODS

Samples: Nine different green wet-processed coffee samples (*C. arabica* L., commercial lots) from 4 different geographical origins (Ethiopia (4), Brazil (3), Nicaragua (1) and Guatemala (1)) were supplied by illycaffè S.p.A (Trieste, Italy). The samples with zero primary and secondary defects, were selected on the basis of standard internal procedures of sorting and visual aspect, moisture content, screen size, and cup quality.

HS-GC-IMS: Headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) (FlavourSpec®, G.A.S., Dortmund, Germany) was used to assess the volatile composition with an untargeted fingerprinting approach. A 20 mL glass vial was filled with 1.0 g of the sample. Then samples were treated for 5 minutes at 50 ° C at 500 rpm., A 300 µL headspace sample was automatically delivered through a 70 ° C heated syringe (splitless mode). Column: MXT-5 (15 m × 0.53 mm i.d., 1 µm film thickness; Restek Corporation, Bellefonte, PA, USA). Separation condition: temperature: 40 ° C.; carrier gas: 99.999 % pure nitrogen; flow rate program: 2 mL/min for 3 minutes, followed by a 17-minute rise to 25 mL/min and a 5-minute hold. A 3H ionization source ionized the eluted nalytes before driving them to a drift tube, which was run at a constant temperature of 45 ° C and voltage of 5 kV.

GCxGC-TOF: a GCxGC-TOF-MS was used to characterize volatile molecules using headspace-solid phase microextraction analysis (SPME). A Pegasus BT 4D GCxGC-TOFMS instrument (Leco Corp., St. Josef, MI, USA) equipped with a LECO dual stage quad jet thermal modulator was used. 2.0 g of each sample were placed into a 20-mL glass vials with septa and equilibrated at 50 ° C under continuous stirring for 5 min. The volatiles were extracted by SPME, using a 50/35 µm DVB/CAR/PDMS fiber (Supelco, Inc., Bellfonte, PA, USA). The SPME fiber was preconditioned for 30 minutes at 270 ° C and reconditioned between each run to minimize carry over effects. The first dimension column was a Stabilwax-DA (Restek Corp., Bellefonte, PA) MS capillary column, with an internal diameter of 0.25 mm and a stationary phase film thickness of 0.25 mm, while the second dimension chromatographic column was a 2 m Rxi-17Sil MS (Restek Corp., Bellefonte, PA) with the same diameter and thickness of the first one. High-purity helium (99,9999%) was used as the carrier gas (flow rate: 1.4 mL/min). Temperature program: 50 ° C for 5 min, then ramped at 4 ° C/min up to 250 ° C for 5 min. The secondary column was maintained at +15 ° C relative to the GC oven temperature of the first column. Electron impact ionization was applied (70 eV). Ion source temperature: 250 ° C; mass range: 35-550 m/z, with an extraction frequency of 32 kHz. Acquisition rates: 200 spectra/s. Modulation periods (for both programs): 4s for the entire run.
Statistical analysis: Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) were performed using MetaboAnalyst 5.0 software.

RESULTS

Climatic factors directly impact the volatile organic compounds fingerprint in green Arabica coffee bean as well as coffee beverage quality **[3]**. Geographical origin is strictly correlated with different macro- and micro-climatic conditions, so representing a critical parameter for coffee quality. The traceability of green coffee can be obtained using different analytical approaches, often completing by appropriate supervised or unsupervised statistical processing of data. The analysis of volatile profile of green coffee is of great interest particularly regarding the overall sensory quality of the roasted product.

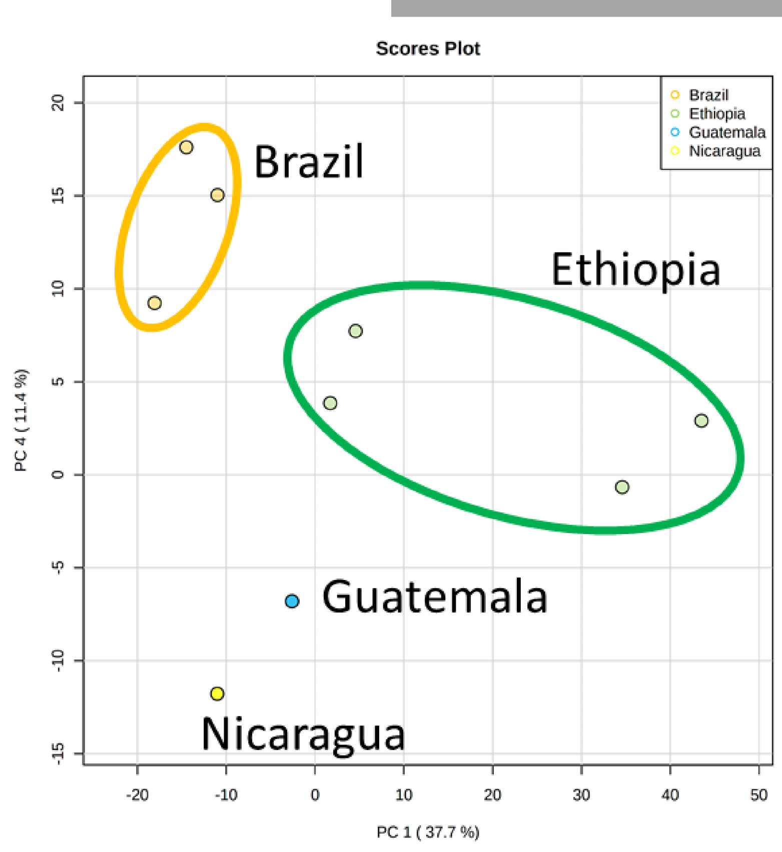

Fig. 1. Principal component analysis (PCA) obtained from GCxGC-MS data of coffee samples grouped by geographical origins.

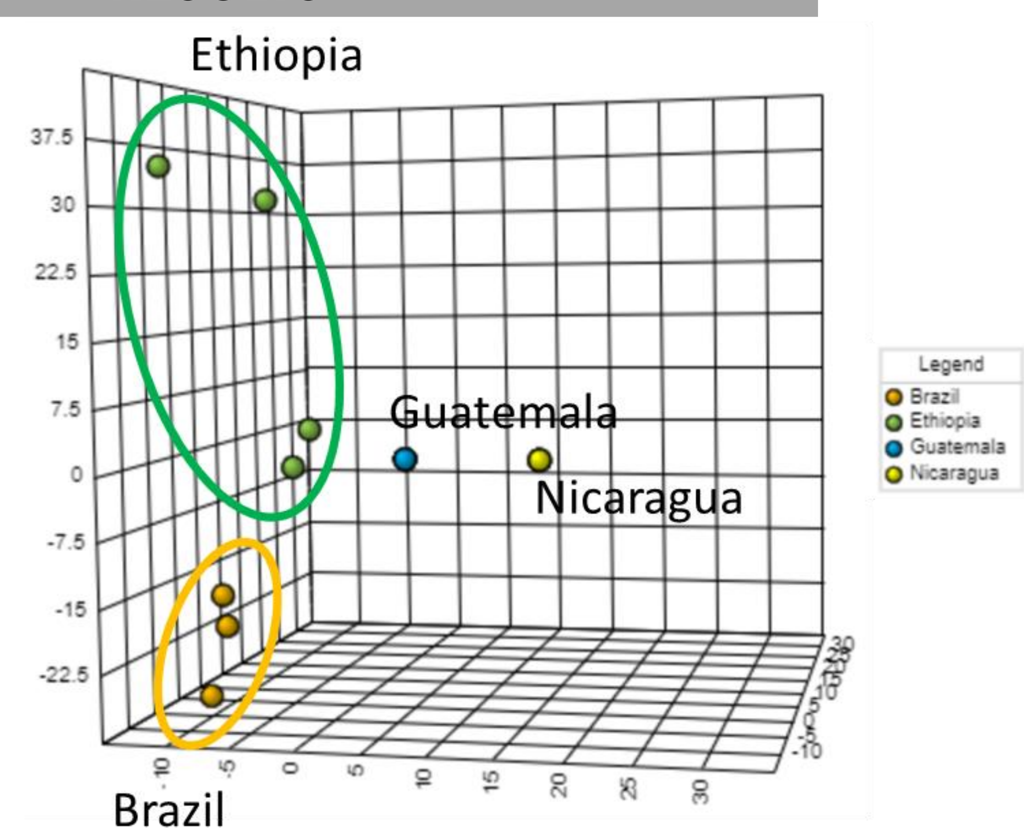

Fig. 2. Partial least square discriminant analysis (PLS-DA) obtained from GCxGC-MS data of coffee samples grouped by geographical origins.

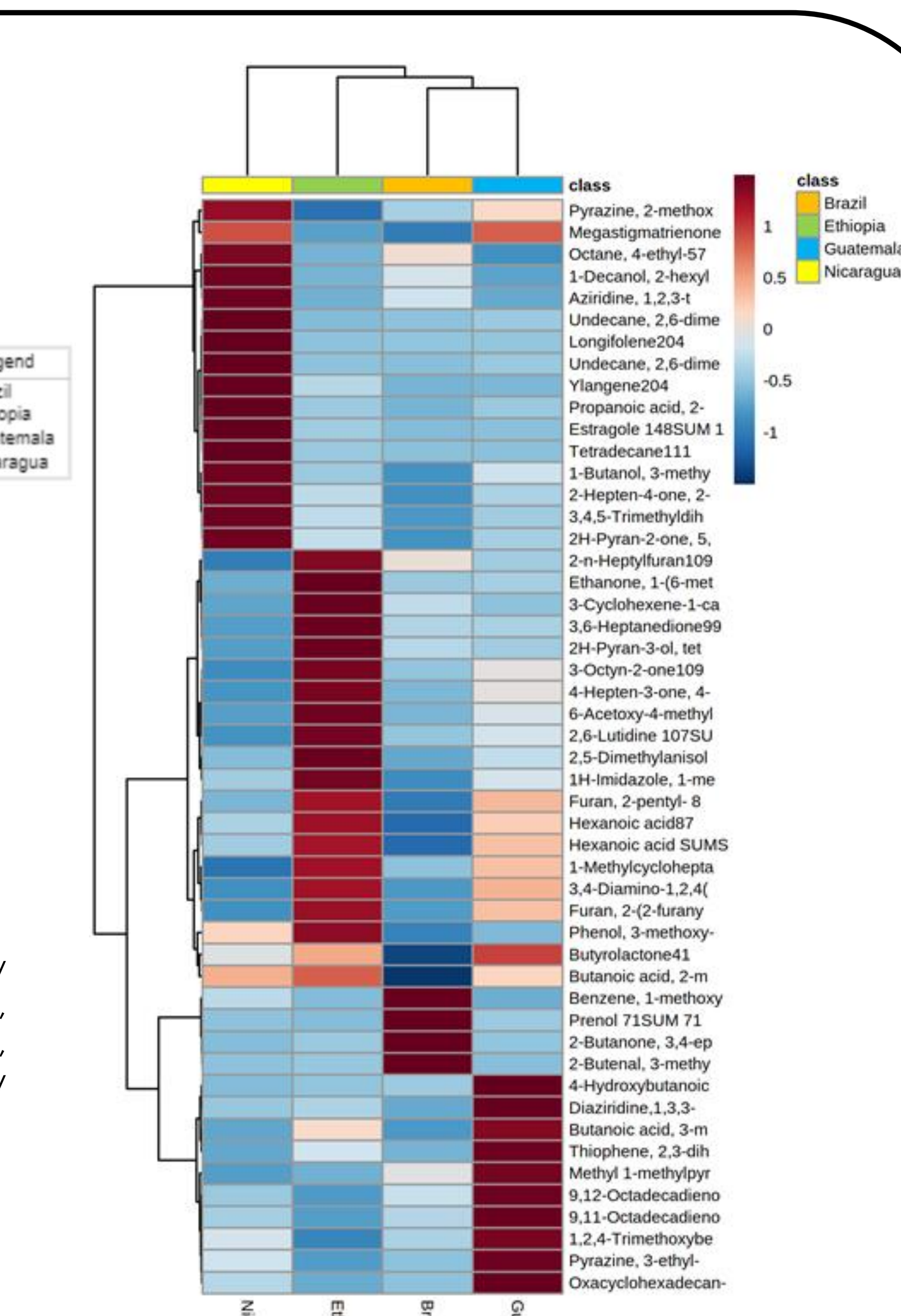

Fig. 5. Hierarchical clustering heat map of the top 50 identified molecules found in the four geographical vaieties of green cofees by GCxGC-MS analysis.

Results demonstrated that:

1. GCxGC-MS is a powerful tool useful to explore in depth the complexity of green coffee samples (e.g. the targeted identification of some key bioactive compounds within the aroma), allowing for clearly identify of some key odorants of green coffee such us methoxypyrazines, aldehydes, alcohols and hydrocarbons. The presence of raspberry-ketone (previously indicated as molecular marker of interest in green coffee, and now widely suggested as key ingredient in food supplements for weight loss) was not confirmed in these samples; however, it was clearly identified in some raspberry samples, used as positive controls.

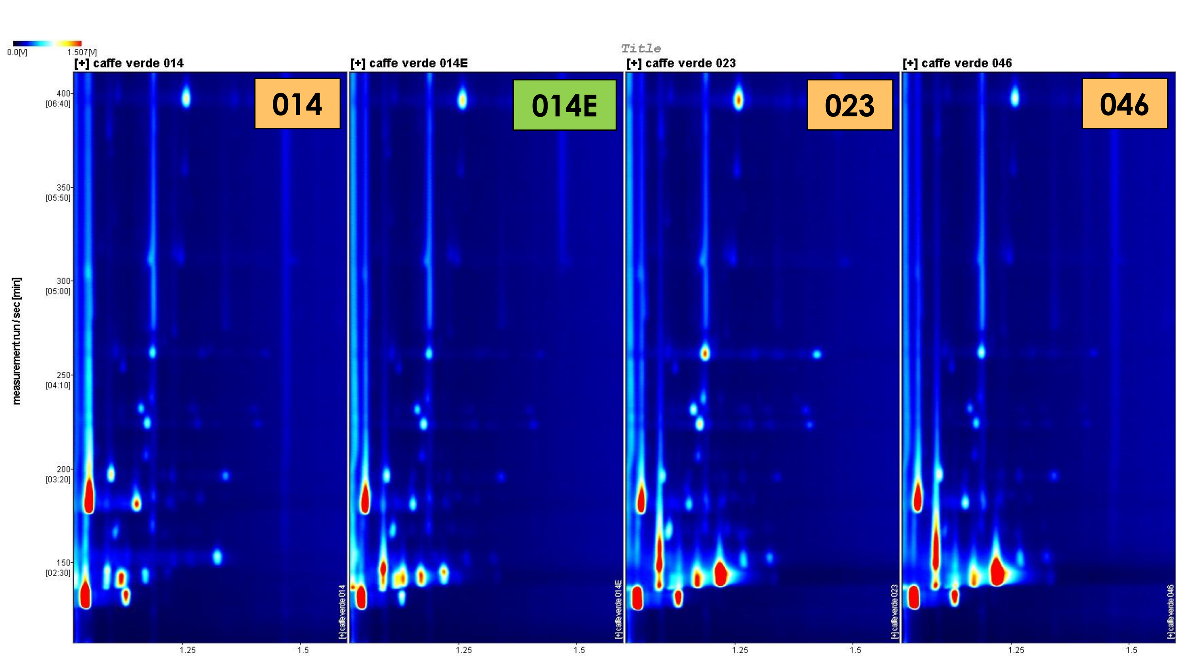

Fig. 3. HS-GC-IMS 2D spectrum of 014, 014, 023 and 046 samples.

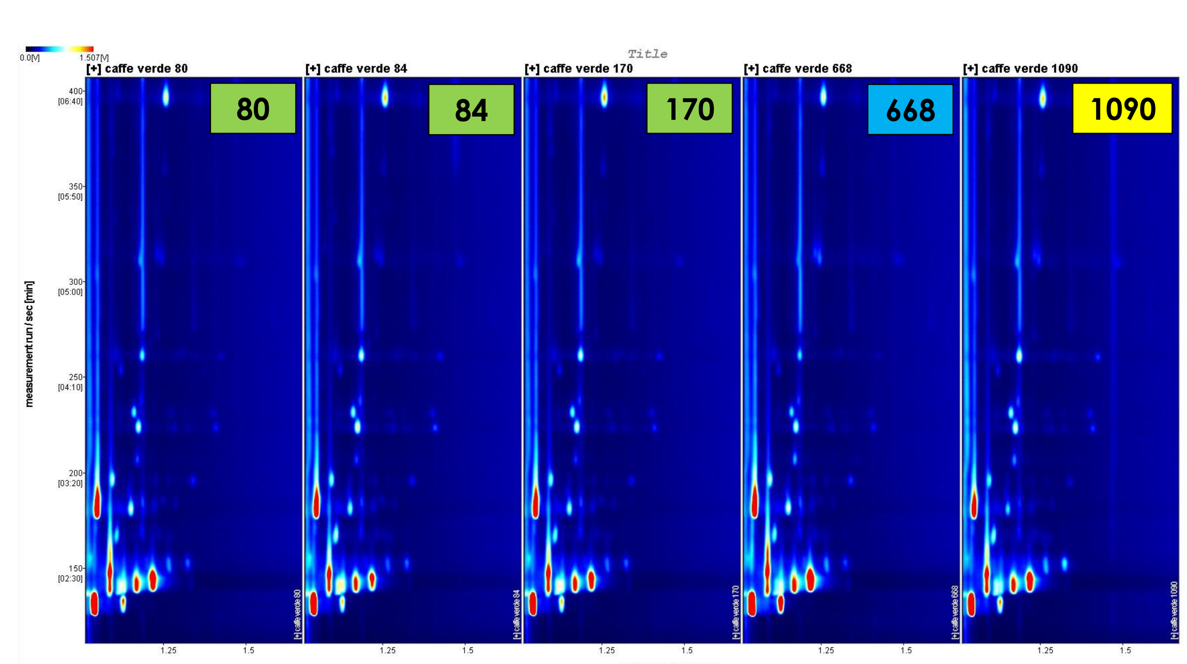

Fig. 4. HS-GC-IMS 2D spectrum of 80, 84, 170, 668 and 1090 samples.

2. the application of HS-GC-IMS on green beans (here proposed as rapid screening method compared to GCxGC-TOF) was functional to identify clusters of samples from different origin. HS-GC-IMS 2D chromatogram rapidly permitted to obtain and to compare complex patterns, avoiding any kind of sample pre-analytical handlings and processing. Also using this method, the presence of raspberry ketone was not evidenced in green coffee samples.

Finally, the comparison of these approaches, processing the overall data (and a selection of the most representative compounds) with PCA, PLS-DA and Hierarchical Clustering Heat-Map allowed us to obtain comparable clustering, also permitting to identify the geographical origin of the samples.

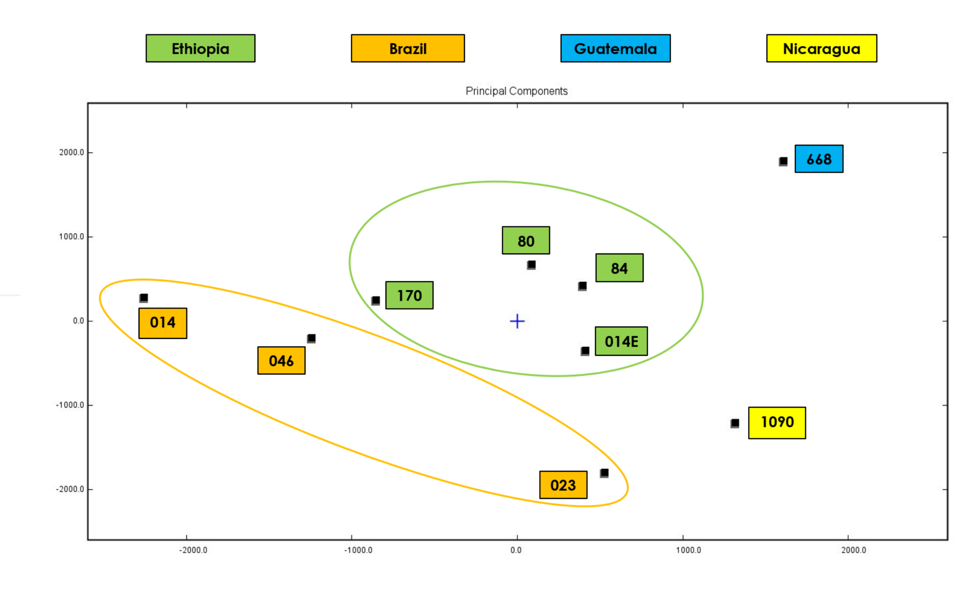

Fig. 6. PCA obtained from HS-GC-IMS data of coffee samples grouped by geographical origins.

CONCLUSIONS

These results emphasize the usefulness of the hyphenated multi-platform approach as analytical tool, preliminary to data mining. Both HS-GC-IMS and GCxGC-TOF techniques allowed to identify specific clusters of samples of known geographical origin, as well as some considerations on the precursor of aroma. In particular, HS-GC-IMS streamlined the time for the analysis, permitting a direct and rapid analysis of green beans in vials, opening new perspectives for the green coffee quality control.

References
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