Fingerprinting of high quality cocoa by two-dimensional comprehensive gas chromatography - time-of-flight mass spectrometry and tandem ionization

ABSTRACT SUBMISSION FORM

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Summary: *Comprehensive two-dimensional gas chromatography (GC×GC) coupled with time of flight mass spectrometry (TOF-MS) is a powerful technique for detailed analysis (profiling and fingerprinting studies) of medium-to-high complexity mixtures of volatiles. In this study the characteristic volatiles fingerprints of "high quality" cocoa are investigated by advanced pattern recognition approaches on MS signals from tandem ionization (70 and 12 eV) detection.*

Keywords: *Theobroma cacao L.; combined untargeted and targeted fingerprinting; comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry and tandem ionization*

1 Introduction

Cocoa, produced from cocoa beans (Theobroma cacao L. Malvaceae family), is a crop of great economic relevance as raw ingredient for chocolate manufacturing. Cocoa and chocolate are consumed worldwide and their popularity is related to pleasant sensory properties, although, recent evidences of health benefits open new market perspectives and uses as functionalized food(s) [1, 2].

Theobroma cacao L. is a tree crop native to tropical forests of American continent. Recent studies on cocoa germoplasm [3], defined 10 major genetic clusters or groups, i.e. Marañon, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purŏs, Nacional and Guiana. This new classification reflects accurately the genetic diversity available and overcomes the traditional in Criollo, Forastero or Trinitario.

Cocoa quality and economic value are related to its unique and complex flavour. The sensory profile (aroma, taste, mouth feeling, and texture) is a key-factor for premium quality products suited to consumer preferences. Flavours develop from complex biochemical and chemical reactions occurring at post-harvesting and vary with genotype, geographical origin, farming practices, and technological processing [4]. Above all, post-harvest treatments and, in particular, fermentation [5] and roasting [6] are key steps in the formation of the characteristic cocoa aromas.

The present study investigates volatile organic compounds (VOCs) peculiar signatures from commercial grade, high-quality cocoa with novel pattern recognition strategies that combine untargeted and targeted fingerprinting on GC×GC-TOF-MS and tandem ionization (*UT fingerprinting*) [7]. Samples are from different origins and stages of processing. Advanced pattern recognition is tested to validate its effectiveness in highlighting the chemical information encrypted in VOCs signatures. Furthermore tandem ionization data (70 and 12 eV) are mined to explore different issues such as origin/process characteristics and sensory profile(s) quality.

2. Experimental

Samples: cocoa samples were selected by confectionery experts for their peculiar sensory characteristics. Origins: Mexico, Ecuador, Colombia and Sao Tomè (harvest 2015).
Four technological stages: raw, roasted, steamed and nibs after removal of shells. Processing was by hot-air roasting in a Bühler AG apparatus (Uzwil, Switzerland) by Guido Gobino srl (Turin, Italy) in two batches.

Head Space Solid Phase Micro Extraction sampling: samples were frozen in liquid nitrogen and ground up to 300 µm (Grindomix GM200, Retsch, Haan, Germany); precisely

weighted (0.500 g) in headspace glass vials (20 mL) for HS-SPME sampling.
SPME DVB/CAR/PDMS d_f 50/30 µm - 2 cm (Supelco, Bellefonte, PA, USA). Standard-in-fiber procedure was before sampling for 30 min at 50°C. Fibre thermal desorption into the S/SL injection port for 5 minutes at 250°C.
GC×GC-TOF-MS instrument set-up: Agilent 6890 GC coupled with Markes Bench-TOF Select™ (Markes International, Llantrisant, UK) operating in tandem ionization (70 eV and 12 eV). Transfer line 270°C; Ion source 250°C. Thermal modulation by two-stage KT 2004 loop-type modulator (Zoex Corporation, Houston, TX) cooled with liquid N_2 and controlled by Optimode™ V.2 (SRA Instruments, Italy). Hot jet pulse time 250 ms, modulation time 4s.
Column set: ^{1}D SolGel-Wax (100% PEG) (30 m × 0.25 mm d_c, 0.25 µm d_f) SGE and ^{2}D OV1701 (86% PDMS, 7% phenyl, 7% cyanopropyl) (1 m × 0.1 mm d_c, 0.10 µm d_f), from J&W. Carrier gas: He, const flow 1.3 mL/min. Oven from 40°C (1 min) to 200°C at 3°C/min and to 250°C at 10°C/min (5 min).
Data acquisition and data elaboration: data were acquired by TOF-DS and processed by GC Image 2.7 (GC Image, LLC Lincoln NE, USA).

3. Results

UT fingerprinting work-flow: Untargeted and Targeted (UT) fingerprinting was by template matching approach [8]. The approach establish reliable correspondences between the same chemical entities across multiple chromatograms by matching MS signatures.
Targeted analysis focused on 190 compounds identified by matching EI-MS fragmentation pattern at 70 eV (NIST MS Search algorithm) with those in commercial and in-house databases. Linear Retention Indices (I^T_S) were adopted as additional constants.
Untargeted analysis was based on peak-regions features [8, 9] and performed by GC Image Investigator™ R 2.7 (GC-Image Lincoln NE, USA) by also including all targeted peaks. This process [7] aligned a feature template to each of the 64 chromatograms using a set of registration peaks. The resulting data matrix for untargeted and targeted reliable peak-regions was 64 × 558 at 70 eV and 64× 1037 at 12 eV. Response data from all cross-aligned peaks were used for multivariate analysis (MVA) and supervised discrimination approaches.
Targeted fingerprinting: Heat-map based on Normalized 2D volumes for targeted analytes (mean and centering normalization) and hierarchical clustering indicated that (Ecuador origin) raw and roasted cocoa have distinctive VOCs patterns; different from those of steamed and grinded cocoa (**Figure 1**).
UT fingerprinting results: when the VOCs pattern is fully exploited including also untargeted chemical entities, the fingerprinting informs about samples origins and/or technological stage. The availability of tandem Ionization signals enable cross-validation of results and makes more consistent analytes identification.

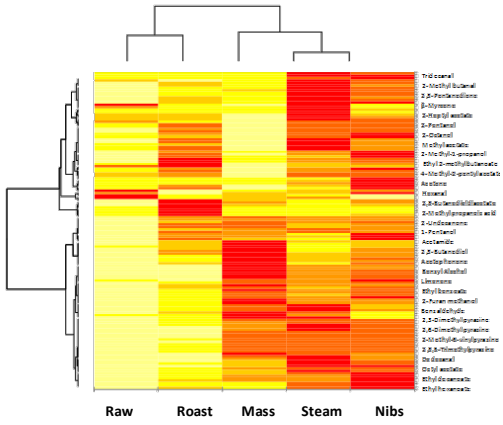

Figure1

Soft and hard ionization provides complementary information on analytes structure (**Figure 2**).

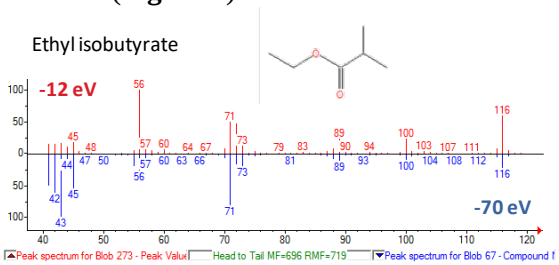

Figure2

4. Conclusions

Complex fraction of volatiles could be effectively investigated to exploit all chemical information they encrypt by "multi" dimensional analysis. Mass Spectrometry with tandem ionization adds extra-dimensions of great potential for reliable and consistent fingerprinting.

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