Characterization of the key aroma compounds in Burgundy truffle

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Introduction

Truffles are a fungus of the genus *Tuber*. Some of them have an important economic value due to their gastronomic qualities appreciated in "grande cuisine". Perigord truffle (*T. melanosporum* or Black truffle) and White Alba truffle (*T. magnatum pico*) are actually the noblest ones. While these two species are well-valued, Burgundy truffle (*T. uncinatum*) is less well-characterized in its production area and truffle producers encounter some difficulties to sell their harvest at its fair value.
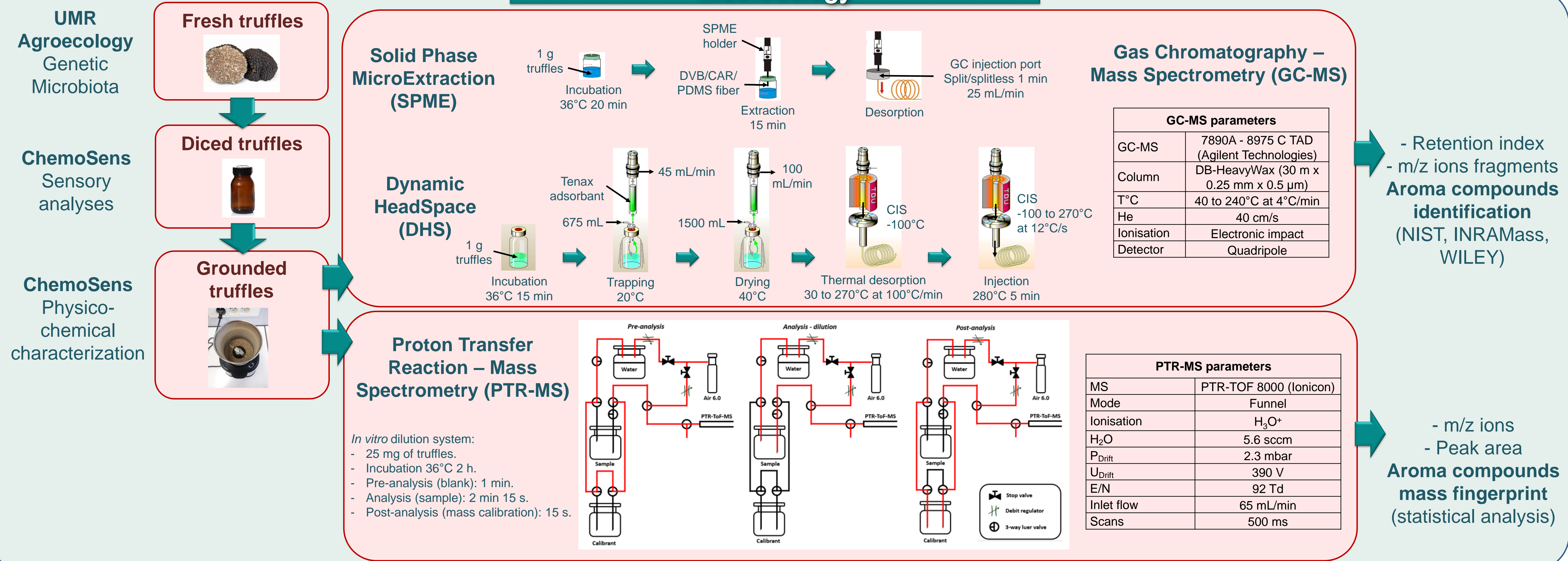
Peridium
Gleba

INRA of Dijon is strongly involved in the BIJOU project (truffe de **B**ourgogne, un produit de qual**I**té à forte valeur a**JOU**tée), in order to help the professionals to better characterize the Burgundy truffle through different research axes. Because maturation and geographical origin have been reported to influence the aromatic composition of truffles [1], the results should provide answers to, at least, 3 questions:
  (i)   Is it possible to highlight specific aromatic notes/compounds in Burgundy truffle compared to truffles from other regions? [2,3]
  (ii) Which gleba color is required for a quality harvest (related to ripening stage)?
  (iii) In which area is harvested the quality Burgundy truffle?

The first objective of this large project is to define an analytical strategy to characterize volatile aroma compounds (VOC) in Burgundy truffles.

Methodology

UMR Agroecology
Genetic
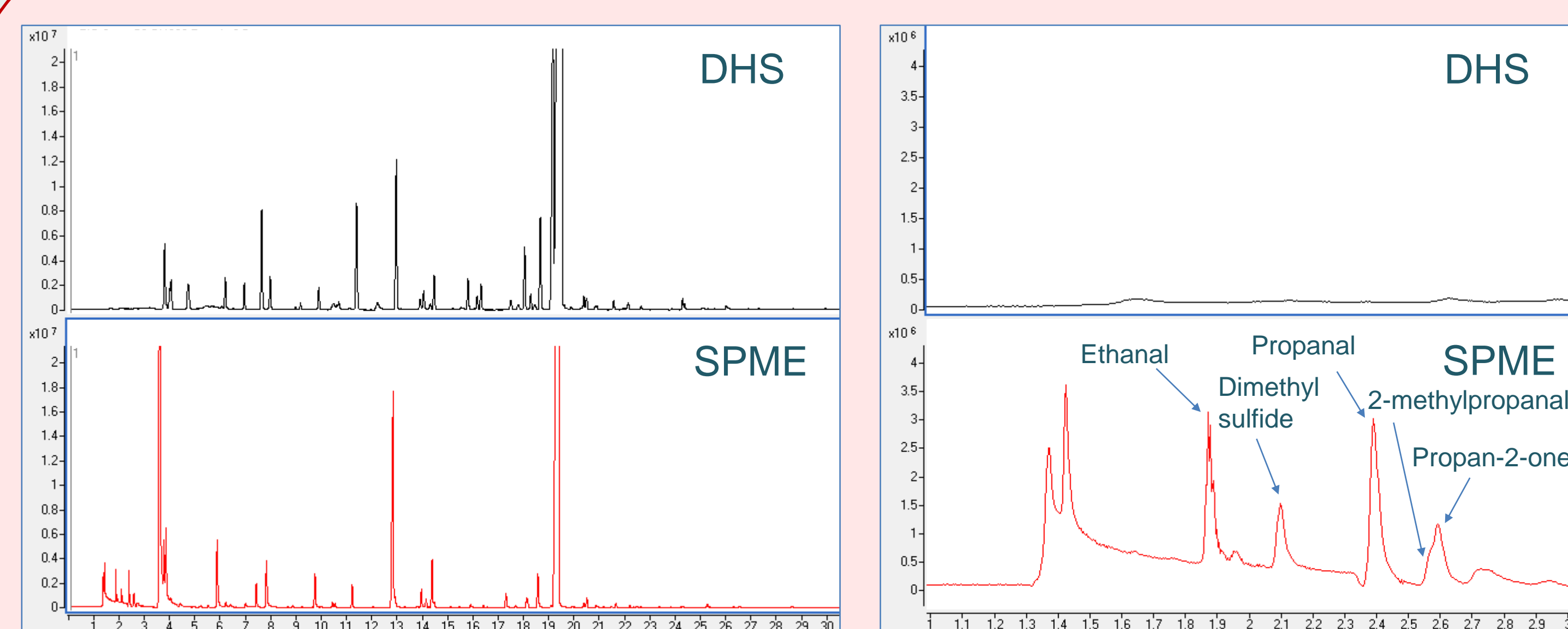Microbiota

ChemoSens
Sensory analyses

ChemoSens
Physico-chemical characterization

Fresh truffles

Diced truffles

Grounded truffles

Solid Phase MicroExtraction (SPME)

1 g truffles → Incubation 36°C 20 min → SPME holder / DVB/CAR/PDMS fiber → Extraction 15 min → Desorption → GC injection port Split/splitless 1 min 25 mL/min

Dynamic HeadSpace (DHS)

1 g truffles → Incubation 36°C 15 min → Tenax adsorbant 45 mL/min 675 mL Trapping 20°C → 100 mL/min 1500 mL Drying 40°C → CIS -100°C Thermal desorption 30 to 270°C at 100°C/min → CIS -100 to 270°C at 12°C/s Injection 280°C 5 min

Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS parameters	
GC-MS	7890A - 8975 C TAD (Agilent Technologies)
Column	DB-HeavyWax (30 m x 0.25 mm x 0.5 µm)
T°C	40 to 240°C at 4°C/min
He	40 cm/s
Ionisation	Electronic impact
Detector	Quadripole

- Retention index
- m/z ions fragments
Aroma compounds identification
(NIST, INRAMass, WILEY)

Proton Transfer Reaction – Mass Spectrometry (PTR-MS)

Pre-analysis   *Analysis - dilution*   *Post-analysis*

In vitro dilution system:
- 25 mg of truffles.
- Incubation 36°C 2 h.
- Pre-analysis (blank): 1 min.
- Analysis (sample): 2 min 15 s.
- Post-analysis (mass calibration): 15 s.

Stop valve
Debit regulator
3-way luer valve

PTR-MS parameters	
MS	PTR-TOF 8000 (Ionicon)
Mode	Funnel
Ionisation	H_3O^+
H_2O	5.6 sccm
P_{Drift}	2.3 mbar
U_{Drift}	390 V
E/N	92 Td
Inlet flow	65 mL/min
Scans	500 ms

- m/z ions
- Peak area
Aroma compounds mass fingerprint
(statistical analysis)

Results

DHS *vs* SPME

DHS
SPME

Figure 1 – TIC overlay for DHS and SPME analyses from 0 to 30 min.

DHS
SPME

Ethanal
Dimethyl sulfide
Propanal
2-methylpropanal
Propan-2-one

Figure 2 – TIC overlay (zoom from 1 to 3 min) for DHS and SPME analyses.

Table 1 – VOC identification classified by chemical classes.

Chemical classes	DHS	SPME
Aldehydes	22	19
Alcohols	17	12
Ketones	16	13
Acids	8	1
Sulfur compounds	2	3
Lactones	2	0
Furans	1	1
Hydrocarbons	1	1
Phenols	1	0
Total	**70**	**50**

✓ More VOC are identified by DHS analysis (**Fig. 1**) and intensities are often more important.

✓ An exception occurs for the elution range from 1 to 3 min (**Fig. 2**), where 5 chemical compounds are not found in the DHS analysis.

✓ The chemical classes expected in truffles (**Table 1**) are well represented in the DHS extraction, with on average 28 % more compounds identified compared to the SPME extraction.

PTR-MS methodological development

Pre-analysis   Analysis without dilution
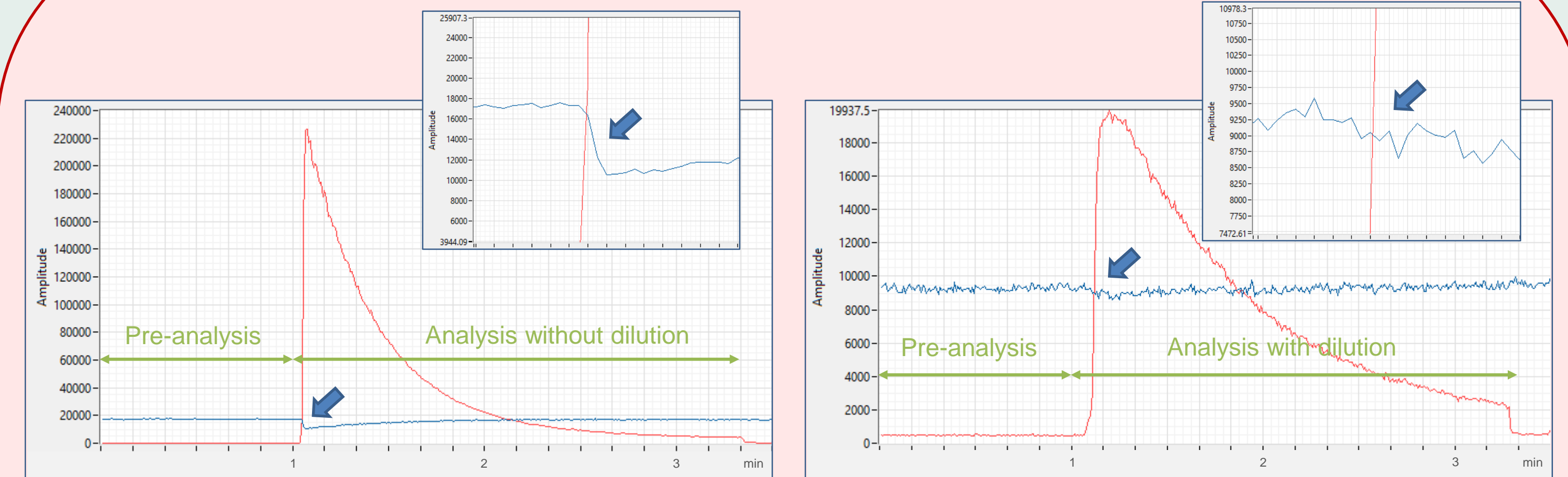Pre-analysis   Analysis with dilution

Figure 3 – TIC for H_3O^+ ions (blue) and acetone ions (red) released by 25 mg of grounded truffles. Measurement without (left) and with optimised dilution system (right).
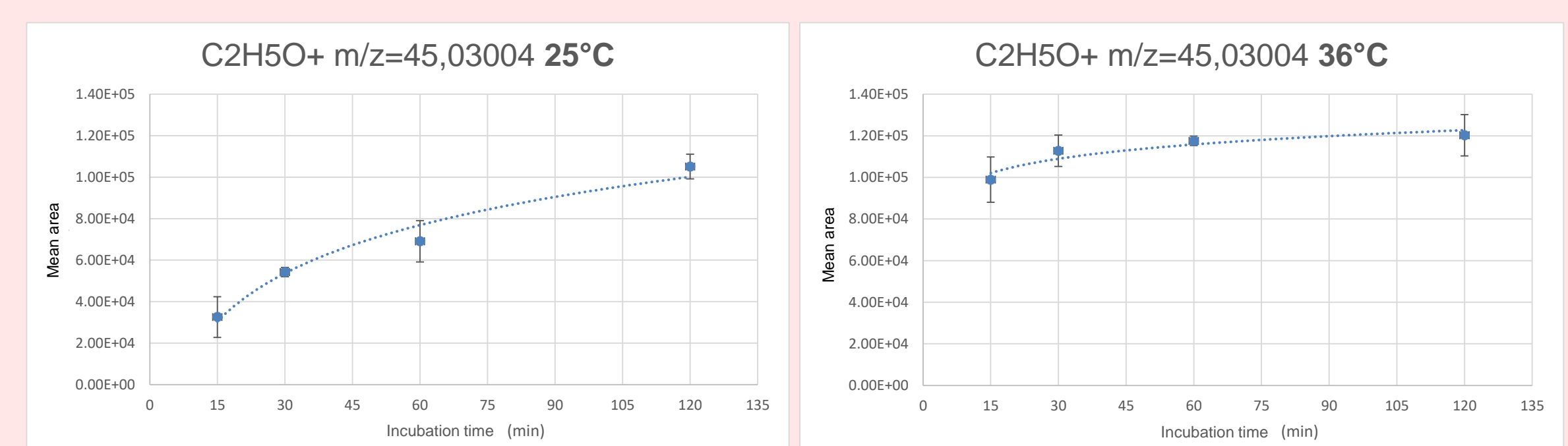
C2H5O+ m/z=45,03004 **25°C**
C2H5O+ m/z=45,03004 **36°C**

Figure 4 – Cinetic analysis of the incubation time (15, 30, 60 and 120 min) according to incubation temperature (25°C and 36°C). Example with the ion $C_2H_5O^+$ (m/z = 45).

✓ The blue arrows in **Fig. 3** shows the hydronium ions depletion when aroma compounds are released from the headspace of the vial. Optimisation of a dilution system with humidified air at 1500 mL/min avoids to decrease H_3O^+ ions for a better ionisation of the VOC.

✓ Ion $C_2H_5O^+$ was chosen as a representative aroma compound in truffle (**Fig. 4**). Peak integration enables peak area calculation. A temperature of 36°C during 2 h allows to reach equilibrium between grounded truffles and headspace in the vial. ANOVA analysis on 9 different ions confirmed this result (*data not shown*).

Conclusion & Perspectives

These preliminary results allowed to set up an extraction and an analysis method by DHS GC-MS and PTR-MS to better characterize the truffle aroma.
Between June and December 2019, truffles from different localizations (Burgundy and south of France) will be harvested and analyzed by this approach. Physico-chemical results will then be combined to microbiota, genetic and sensory analyses. This work will bring a scientific contribution to the creation of an IGP (Indication Géographique Protégée) request.

(1) Vita, F., et al. (2015). "Volatile organic compounds in truffle (*Tuber magnatum Pico*): Comparison of samples from different regions of Italy and from different seasons." Sci Rep 5(12629).
(2) Gioacchini Anna, M., et al. (2005). "Solid-phase microextraction gas chromatography/mass spectrometry: a new method for species identification of truffles." Rapid Communications in Mass Spectrometry 19(17): 2365-2370.
(3) March, R. E., et al. (2006). "Volatile compounds from six species of truffle – head-space analysis and vapor analysis at high mass resolution." International Journal of Mass Spectrometry 249-250: 60-67.

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