# Characterization of key aroma compounds in Burgundy truffle (proceedings)

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# Characterisation of key aroma compounds in Burgundy truffle

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## Abstract

Burgundy truffle plays an important role in the region's economic market. One of the objectives of this project is to better understand the origin and diversity of Burgundy truffle aromas.
Aroma compounds of Burgundy truffles were investigated with sensory evaluation (Quantitative Descriptive Analysis, QDA) and two physical-chemical methods:

- An analysis by Dynamic HeadSpace (DHS) coupled with Gas Chromatography - Mass Spectrometry (GC-MS) for volatile organic compounds (VOCs) identification,
- A new *in vitro* analytical method by Proton Transfer Reaction - Time of Flight - Mass Spectrometry (PTR-ToF-MS) to obtain an aroma mass fingerprint of all the samples.

With these different methods, we showed that aroma compounds of truffles are different according to varieties, harvests places and seasons.

*Keywords: Burgundy truffles, sensory analysis, GC-MS, PTR-ToF-MS*

## Introduction

Truffles have an important economic value due to their gastronomic qualities appreciated in "grande cuisine". While Périgord (*Tuber melanosporum*) and White Alba (*Tuber magnatum pico*) truffles are well-valued, Burgundy truffle (*Tuber uncinatum*) is not well-characterised in its production area.

INRAE is involved to help producers to better characterise these truffles through different research axes, especially the influence of ripeness and geographical origin on aromatic composition [1]. For this purpose, we had first to define an analytical strategy to better characterise aroma compounds in this noble fungus, and secondly, to compare aroma truffles from Burgundy and Lot regions respectively.

## Experimental

### *Truffles*

Twenty-eight truffles were analysed in this study. Burgundy truffles were harvested by truffle farmers in different places in Burgundy, but also in Lot to study aroma compounds from 2 varieties (Burgundy, *Tuber uncinatum* and Lot, *Tuber aestivum*) (Table 1).

**Table 1: Characteristics of analysed truffles.**

| Truffles numbers | Harvest dates | Harvest places |
|---|---|---|
| 221 to 227 (7 truffles) | 5th June (Summer) | Lot |
| 228 to 234 (7 truffles) | 23th June (Summer) | Burgundy, Vaubarden |
| 238 to 244 (7 truffles) | 5th October (Autumn) | Burgundy, Vaubarden |
| 245, 246, 249, 250 (4 truffles) | 22th November (Autumn) | Burgundy, Daix |
| 247, 248, 251 (3 truffles) | 22th November (Autumn) | Burgundy, Bonniere |

Aroma compounds analyses were realised on fresh truffles. After deliveries, truffles were cleaned, diced, frozen quickly with liquid nitrogen and finally grinded (grinding AICOK, model CG9100). Diced truffles (0.5 g) were transferred in amber vials for sensory analysis, while grinded truffles (1 g) were placed in 20 mL vials for GC-MS analysis. Twenty-five mg of grinded truffles were also used for PTR-ToF-MS analysis. Physical-chemical analyses were performed in triplicate.

### *Sensory analysis*

Sixteen panellists were trained during 8 sessions to recognise odour descriptors established by experts' panel. Fresh truffles were evaluated only by olfaction, during different periods (June to November). A classical descriptive profile (QDA) was made, using a continuous linear scale (noted weak to strong). The evaluated


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descriptors were: button mushroom, cep, chanterelle (girolle) mushroom, undergrowth, earthy, mouldy, nut, alcohol-pharmaceutical, spicy, smoked, animal, pungent, pepper, fruity, vanilla, bread.

### *PTR-ToF-MS analysis*

All the experiments were conducted with a PTR-ToF-MS instrument (PTR-ToF 8000, Ionicon Analytik GmbH, Innsbruck, Austria) with $H_3O^+$ as reagent ion in funnel mode. The drift-tube parameters were fixed as follows: $P_{drift}$2.3 mbar, $T_{drift}$80°C, $U_{drift}$390 V resulting in an E/N value of 92 Td (1 Td = $10^{-17}$V.cm$^2$). Mass spectra were acquired at a scan speed of 0.5 s for the mass range m/z 1-227.

Truffle vials were equilibrated at 36°C during 2 hours. The setup (Figure 1) was adapted from [2]. The truffles were rich in aroma compounds and induced a depletion of reactant ions. Hence, truffle headspace was diluted with pure air prior to be analysed with the PTR-ToF-MS.

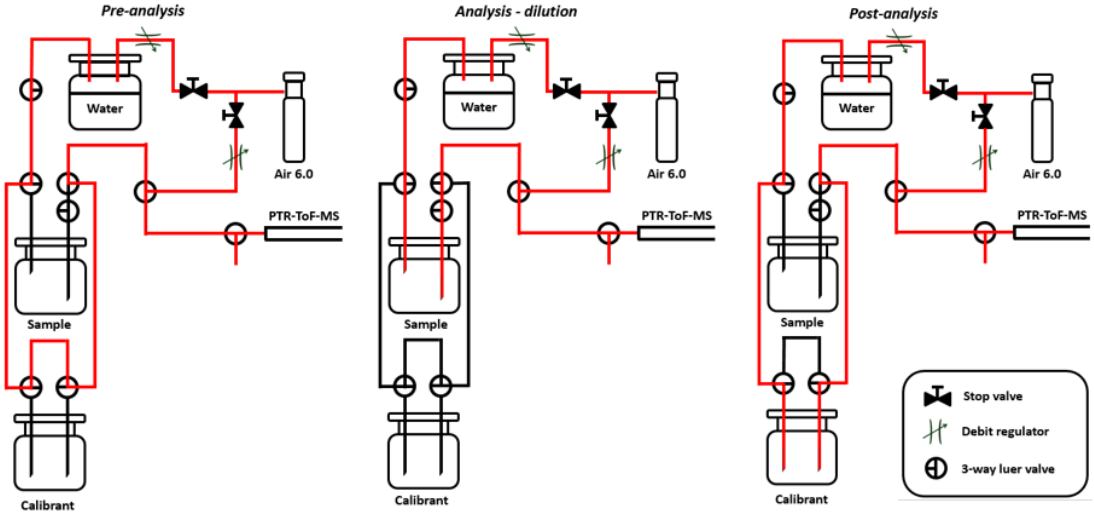


**Figure 1***: Experimental setup connected to the transfer line of the PTR-ToF-MS instrument.*

### *GC-MS analysis*

GC-MS analyses were conducted with only 14 truffles (7 Burgundy truffles, June and 7 Burgundy truffles, November) for practical reasons (low quantity available). Dynamic HeadSpace analyses (Table 2) were carried out with an automatic autosampler (Gerstel, MPS 2; Gerstel Inc., Mülheim an der Ruhr, Germany) coupled with a Gas Chromatography-Mass Spectrometer (7890A-8975c TAD Agilent Technologies, Inc., Palo Alto, CA, U.S.A). Aroma compounds released in the vapour phase were trapped on Tenax tube for DHS analyses. The aroma compounds were separated with a column DB-HeavyWax (30m, 0.25mm, 0.5µm, Agilent) with helium as carrier gas (velocity of 40 cm.s$^{-1}$). The identification of aroma compounds was validated thanks to retention index (RI) and electron ionisation (EI) mass spectrum. RI values were calculated using the Van den Dool and Kratz formula [3] from retention times of n-alkanes ($C_{10}$-$C_{30}$) on the same column, then compared with RIs from the literature and the following databases (NIST, INRAMass home database and Wiley11N17).

**Table 2: DHS parameters.**

| | DHS |
|---|---|
| Incubation | 36°C, 15 min |
| Extraction / Trapping | 20°C, 675 mL, 45 mL/min |
| Drying | 20°C, 1500 mL, 100 mL/min |
| Thermal desorption | 30°C, 100°C/min, 270°C during 5 min |
| Injection | CIS, -100°C, 12°C/s, 280°C during 5 min |

## Results and discussion

### *Sensory analysis*

A canonical variable analysis (Figure 2) and a hierarchical classification (Figure 3) on QDA data showed sensory differences between truffles. Burgundy truffles (summer) were weakly odorant, Lot truffles were characterised by forest odours (mushroom, undergrowth, earthy) and sweet odours (fruity, bread, vanilla, nut) whereas Burgundy truffles (autumn) were characterised by negative odours (animal, mouldy, undergrowth, earthy) and spicy odours (alcohol, smoked, spicy, animal).

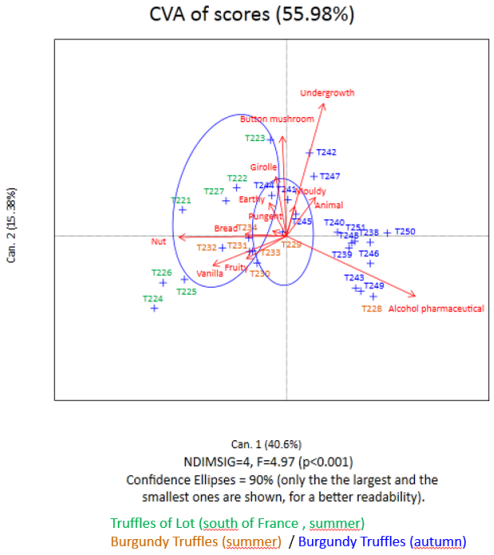


**Figure 2**: *Canonical variables analysis of truffles (QDA).*

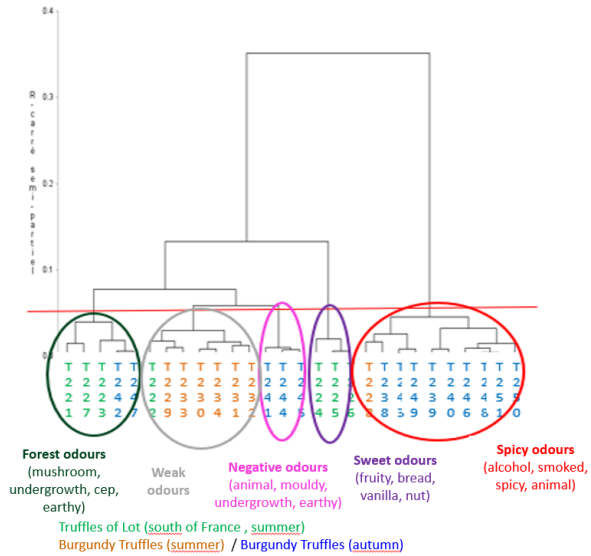


**Figure 3**: *Hierarchical classification analysis of truffles (QDA data).*

## *PTR-ToF-MS analysis*

A principal component analysis (Figure 4) on intensities and a hierarchical clustering (Figure 5) showed that truffles were separated according to places and seasons [1, 4, 5]. However, analyses of frozen mix truffles as a control showed a sample dispersion according to time from June to November (data not shown). We consequently suspected truffle conservation problems and/or an instrumental bias, and considered these results very cautiously.

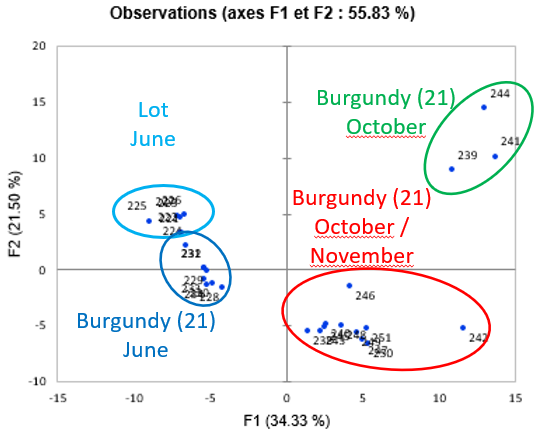


**Figure 4**: *PCA (PTR-MS intensities) of truffles from different harvests and places.*

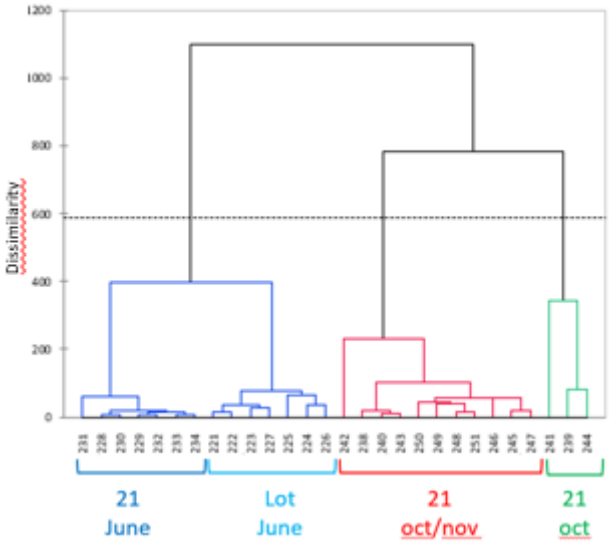


**Figure 5**: *Dendrogram of truffles (the same truffles as PCA-Figure 4).*

## *GC-MS analysis*

Results obtained by GC-MS confirmed the results obtained by PTR-ToF-MS and sensory analyses. Truffles were separated according to harvest places and seasons (Figure 6). Unfortunately, Lot truffles could not be analysed by GC-MS due to an insufficient sampling.

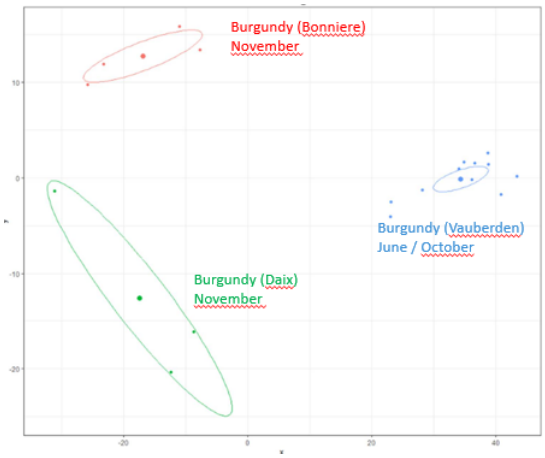


**Figure 6***: PCA of truffles from different places in Burgundy (GC-MS data; same truffles analysed by sensory analyses and PTR-ToF-MS analyses).*

GC-MS peak areas were calculated for each ion in the total ion chromatogram (TIC), for each sample, then ions of interest were highlighted, by considering a fold change higher than 3 and a p-value<$10^{-5}$). These results are presented in Table 3.

**Table 3: Compounds over-expressed, and corresponding truffles.**

| Over-expressed compounds | Truffles |
|---|---|
| Butan-2-one | Burgundy (Bonniere and Daix) / November |
| Butan-2-ol | Burgundy (Bonniere and Daix) / November |
| Pentane-2,3-dione | Burgundy (Bonniere and Daix) / November |
| 3-hydroxypentan-2-one | Burgundy (Bonniere and Daix) / November |
| 2-hydroxypentan-3-one | Burgundy (Bonniere and Daix) / November |
| 4-hydroxyhexan-3-one | Burgundy (Vauderben) / June |

Pentane-2,3-dione and butan-2-one were characteristic of truffle freshness [6]. The same results were found with the PTR-ToF-MS for the truffles harvested in October and November. The odour description of butan-2-ol is alcohol. This compound was over-expressed in GC-MS analyses of truffles from Bonniere and Daix. These truffles were also described by the descriptor alcohol in sensory analysis.

## Conclusion

Sensory analyses, PTR-ToF-MS and GC-MS succeeded in differentiating truffles according to harvest places and seasons. Burgundy truffles harvested in summer were less odorant than Burgundy truffles harvested in autumn. These results will be combined to microbiota data and genetic analyses and will bring a scientific contribution to the creation of an IGP (Indication Géographique Protégée) request.

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