GC-O and Olfactoscan approaches to reveal premature aging markers in Chardonnay wine

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Introduction

Molecular markers of wine oxidation, such as sotolon or Strecker's aldehydes that induce respectively nut or curry and boiled vegetables or wilted rose odors, can be perceived as a default by consumers. These odor-active compounds are especially formed during the premature aging of wine, but it is likely that several contributing compounds are still unknown as is their combined contribution.

AIM: To identify the markers of oxidation in Chardonnay wine by Gas Chromatography Olfactometry (GC-O) and to study the impact of these markers on the complex wine aromatic buffer using the Olfactoscan approach.

Material and methods

Subjects selection and training

- 13 selected subjects on the basis of their results at 3 tests : ETOC[1], Bourdon, odor description
- 2 training sessions on GC-O (molecules added in standard wine, standard wine extracted by SPE[2])

GC-O & Olfactoscan experiments

- Wine : Bourgogne Hautes Côtes de Nuits blanc 2019 (**HCN**) taken after malolactic fermentation
- Wine oxidized with H_2O_2
- SPE extraction on non-oxidized HCN (**NOhcn**) and on oxidized HCN (**Ohcn**)

- **Classical GC-O without background (figure 1.a)**
- NOhcn and Ohcn extracts
- HP 6890A GC + FID equipped with a sniffing port
- DB-Wax, 30 m, 0.32 mm, 0.5 µm. Injection volume: 1 µl

- **Olfactoscan** (OLFhcn) **with NOhcn as odor background (figure 1.b)**
- Ohcn extract

a

b

c

Figure 1: GC-O & Olfactoscan devices (a & b) and olfactory port (c)

Responses were collected at the sniffing port during 40 min with the Gerstel ODP recorder® and the odorous zones were determined with detection frequency.

Three methods to determine odorous zones (OZ) based on odor events (OE)

1st method – Manual (reference method)
- Assessment by a single expert of GC Linear Retention Index (LRI) related to LRI of OE
- OZ when detection frequency of OE ≥4

2nd method – Manual + Automatic
- Use of an excel spreadsheet to calculate Sliding Sums (SlS) on OE, recoded as a binary variable, to draw aromagrams
- OZ when detection frequency of OE ≥4

3rd method – Automatic
- Use of a Matlab program[3] transposed in R based on mathematical calculations to determine automatically aromagrams and OZ with OE
- OZ when detection frequency of OE ≥4

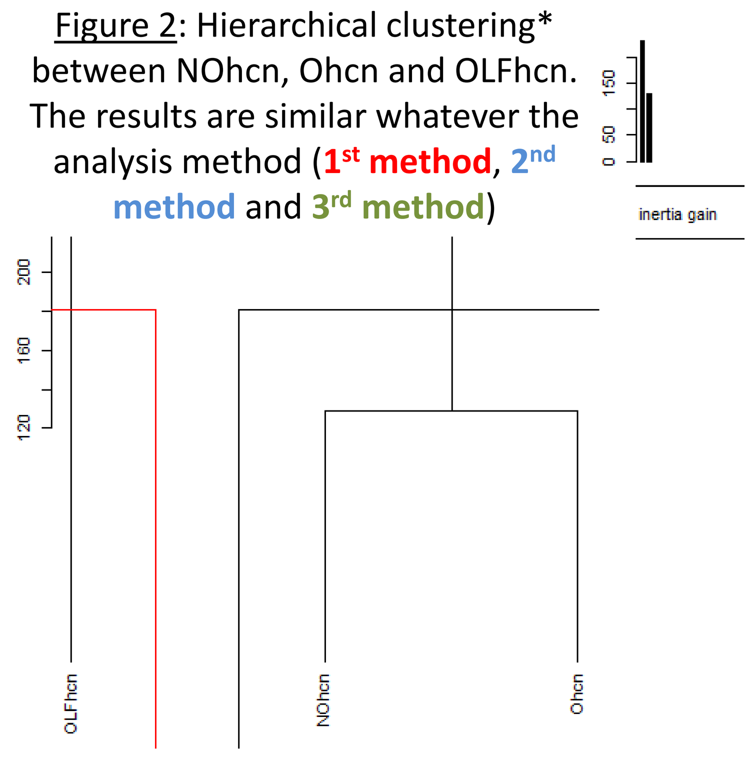
Results and discussion

Common results

Table 1: Determination of categories of odorants according to their behavior in the different conditions (Nohcn, Ohcn and OLFhcn) and the percentage of representation in the three methods (**1st method**, **2nd method** and **3rd method**)

Categories	Representation in 1st method (%)	Representation in 2nd method (%)	Representation in 3rd method (%)
DISPODOR T : Odorants detected in NOhcn, absent in Ohcn and OLFhcn	19.44	12.56	20.45
DISPODOR T & OLF : Odorants detected in NOhcn and OLFhcn, absent in Ohcn → positive odor reduced by oxidation or odorant in too low concentration in oxidized wine	11.11	21.26	6.82
SYN : Key odorants detected only in OLFhcn → synergy with background odor	25.00	17.39	25.00
OXMASK : Key odorants detected in Ohcn and masked by OLFhcn	6.94	6.28	9.09
OXOLF : Key odorants detected in Ohcn and maintained within OLFhcn	8.33	10.14	4.55
MALEXT : Odorants detected in NOhcn and Ohcn, absent in OLFhcn → not well extracted and masked by background odor	11.11	7.73	18.18
WINE : Odorants detected in NOhcn, Ohcn and OLFhcn	18.06	23.67	15.91

Figure 2: Hierarchical clustering* between NOhcn, Ohcn and OLFhcn. The results are similar whatever the analysis method (**1st method**, **2nd method** and **3rd method**)



* : Hierarchical Clustering (HC) were done with the FactoMineR Package and the HCPC function
** : Factorial Correspondence Analysis (FCA) were done with the FactomineR Package and the CA function

- **7 types of behavior have been revealed but only 3 (SYN, OXMASK and OXOLF) are related to wine oxidation. Only SYN is highly represented in the 3 methods, showing the high impact of the complex aromatic buffer on the odor activity of compounds from the oxidized Ohcn wine as revealed by the Olfactoscan technique.**
- **Figure 2 confirms that the Olfactoscan technique (red) brings different information compared to GC-O (black).**

Specific results

Table 2: Example of final table summarizing the LRI of the OZ, the identified odorants, their LRI and main descriptors given by the participants, and the compounds "behavior" categories (*here are shown 10 odorants among the 73 from the first method*)

HCN					
N°	LRI OZ	Odorants	LRI odorant	Descriptors	Category
1	983	methyl butanoate	981	red fruits	WINE
2	1014	2-methyl-2-butanol	1009	plastic, medicine	DISPODOR T
3	1050	ethyl butyrate	1034	strawberry, raspberry, forest fruit	MALEXT
4	1070	butyl acetate	1071	strawberry, wine	WINE
5	1085	hexanal	1078	red fruits, raspberry	MALEXT
6	1112	isoamyl acetate	1118	spicy, vinegar	SYN
7	1135	(E)-2-pentenal	1126	sweet, banana	WINE
8	1155	/	/	background odor	SYN
9	1165	1-penten-3-ol	1159	sulfur, acid, unpleasant	DISPODOR T & OLF
10	1171	1,1-ethanedithiol	1174	unpleasant, sulfur, rotten eggs	DISPODOR T

Table 3: Balance sheet of the number of identified molecules, the percentage of significant OZ and the percentage of categories related to oxidation in the 3 methods

Methods	Number of identified odorants	Significant OZ (%)	Categories linked to oxidation (%)
1st method	73	79.5 %	41.0 %
2nd method	84	29.9 %	32.0 %
3rd method	44	95.5 %	38.6 %

- **Several molecules have been identified with a large overlap for the 3 methods.**
- **The 3rd method has the highest percentage of significant OZ and is the closest to the usual 1st method.**

Comparison of methods

Figure 3: Factorial correspondence analysis** to compare the 3 analysis methods for the 3 conditions (NOhcn, Ohcn and OLFhcn) (contribution to dim1+dim2 = 40 %)

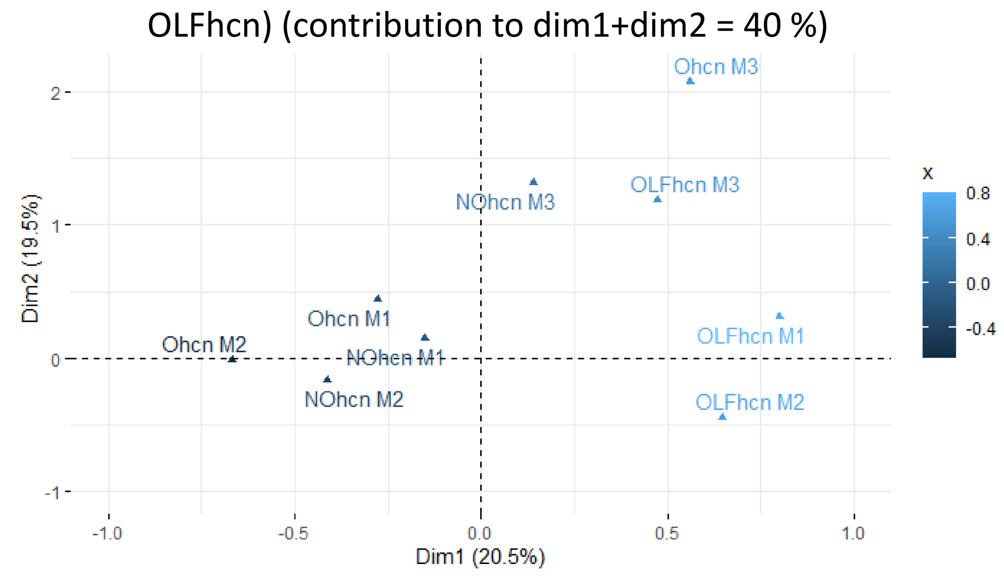
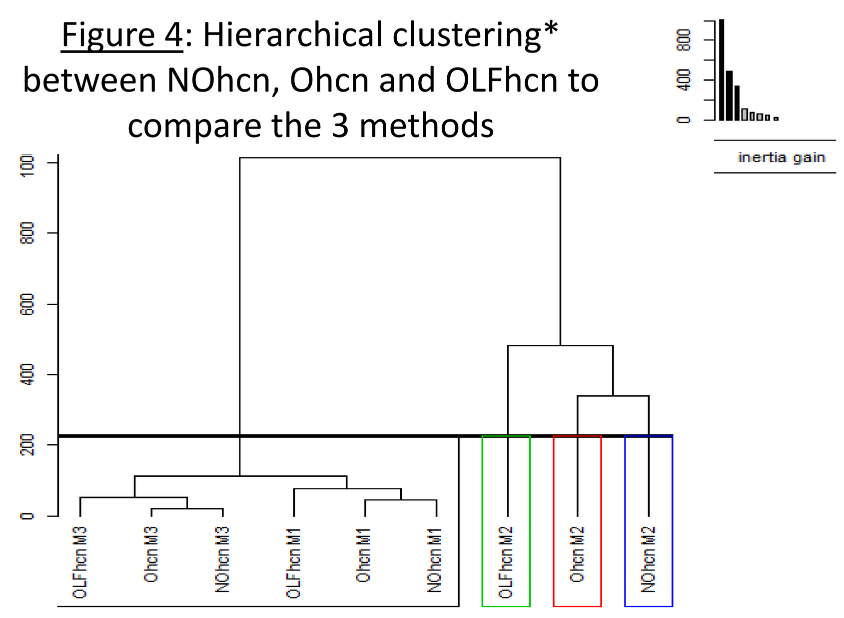


Figure 4: Hierarchical clustering* between NOhcn, Ohcn and OLFhcn to compare the 3 methods



- **FCA and HCA highlighted that the 1st and the 3rd analysis methods provided similar results which are rather different from the 2nd method.**
- **Whatever the method, Olfactoscan provided different results compared to GC-O. Method 2 discriminate the most the 3 conditions (NOhcn, Ohcn and OLFhcn).**

Conclusion

- Specific odorant markers of a Chardonnay wine oxidation have been identified whilst the Olfactoscan approach pointed out that some markers are revealed and other masked by the wine odor aromatic buffer.
- Several GCO data analysis methods, from manual to fully automatic, have been compared to analyze GCO and Olfactoscan data. The automatic method (**3**) provided very similar results compared to the usual manual procedure (**1**) but was found easier and faster and avoid human bias.
- Complementary experiments on 2 other wines (Bourgogne Chitry blanc 2018 and Chablis 1er Cru Côte de Jouan 2018) are still to be analyzed to confirm and/or to complete those results.

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[1] Thomas-Danguin *et al.* 2003 – Rhinology, Volume 41
[2] Ma *et al.* 2017 – J. Agric. Food Chem., Volume 65
[3] Blanquet *et al.* 2017 – Chemometrics and Intelligent Laboratory Systems, Volume 167