Correlation between the volatile compounds and the organoleptic characteristics of Extra Virgin Olive Oils

Perugia 16/06/2022

TASK FORCE -- Italian Association of the Oil Industry (ASSITOL)

The task force has composed by five companies and four laboratories

COMPANIES

1. Carapelli Firenze Spa
2. Colavita Spa
3. Costa d'Oro Spa
4. Oleificio RM Srl
5. Salov Spa

LABORATORIES

1. Carapelli Firenze
2. Chemiservice
3. Coteca
4. Soremartec

Soremartec and Chemiservice labs until 2019

ASSITOL PROFESSIONAL PANEL

ASSITOL 1 – c/o Costa d'Oro Spa
ASSITOL 2 – c/o Carapelli Firenze Spa
ASSITOL 3 – c/o Agridè Srl

The work has started at the end of 2018, due to the pandemic, in 2020 the work was interrupted and resumed in 2021 to improve the first results obtained.

Goals of ASSITOL Task Force

The method of analysis of the panel test, published since the birth of the EC Regulation 2568/1991, in those years, surely has improvement the production of extra virgin olive oils.

From 1991 to today, due to the increasing demand for extra virgin olive oils and the globalization of production, the panel test has created any problem in the classification of ''commercial'' extra virgin olive oil.

The goal of the ASSITOL Task Force is to find an instrumental analytical system that can communicate with the panel test, in order to obtain the best possible result for a correct classification of the product present on the market.

The choice made in this research is to obtain a result not dependent by the panel test and the content of volatile compounds, **but that to obtain a congruent analytical system between panel test and chemical analysis of volatile compounds.**

Analytical System

Considering the works present in bibliography, we have chosen the analysis by Solid Phase Microextraction (SPME) whit GC/MS detection

- SPME analysis (dynamic headspace) is influenced by the type of FIBER, by the absorption conditions (temperature / time) and by the concentration of the analytes
- During the absorption phase there is competition between the analytes, so the conditions must be the same for all laboratories
- The calibration of the system must be done with the greatest number of analytes that can be present in the matrix
- To have a good linearization it is necessary to use at least three internal standards distributed along the entire chromatographic run
- It is advisable to initially check the linearity of response by making mixtures between EVOO and Lampant olive oil at different concentrations

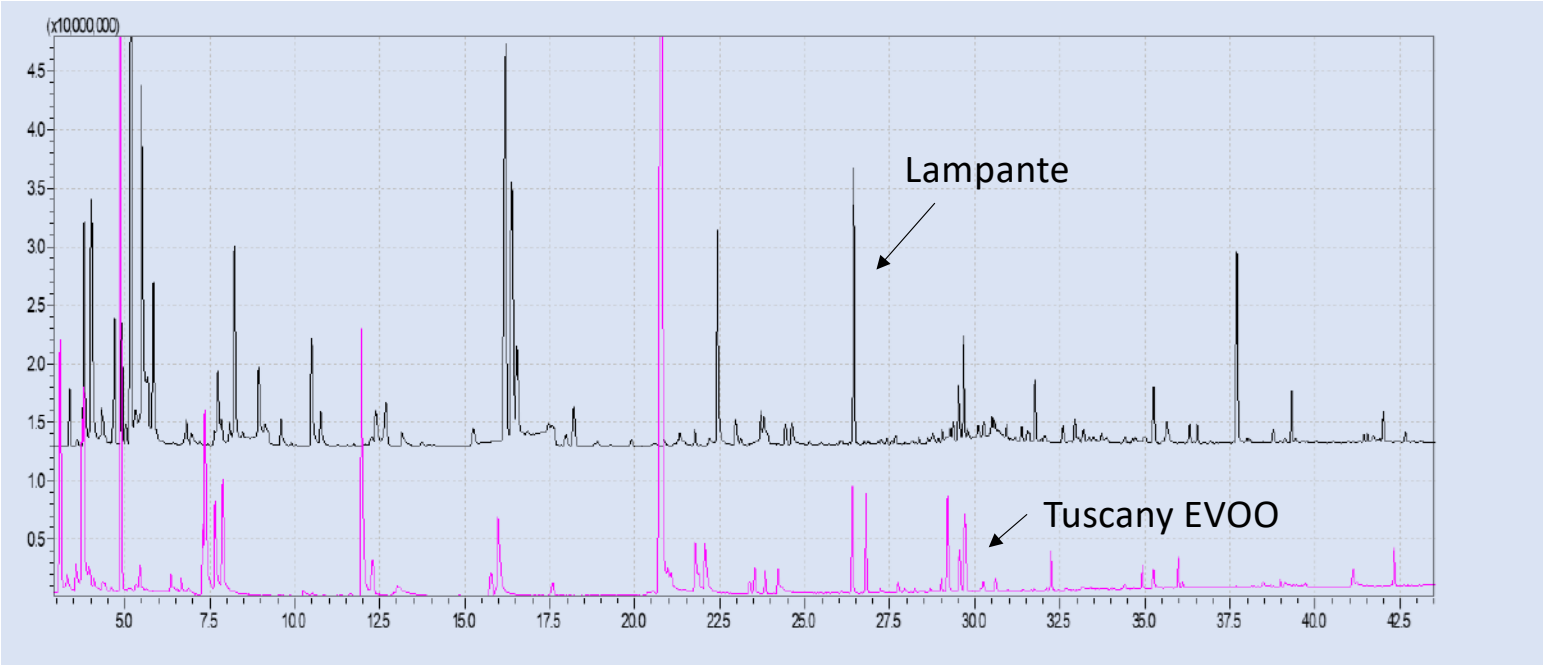
Analytical System

FIBER: DVB/CAR/PDMS

Incubation Temperature (°C): 45
Pre Incubation tiume (min): 5
Conditioning time (min): 20
Sample Desorption time (min): 2

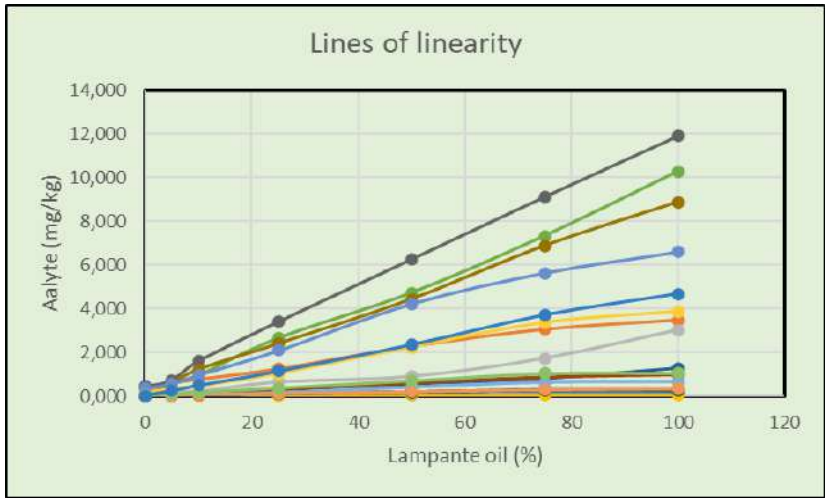
The cromatographic condition must be the better than obtain a good separation of the volatile compounds.

The detection is in Single Ion acquisition



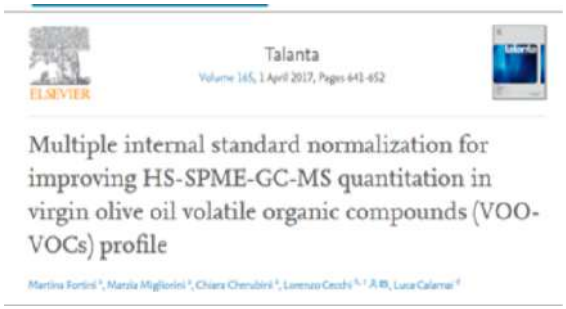
Linearity test between laboratories

	Average of laboratories (mg/kg)									
Analyte/(% of lampante)	0	5	10	25	50	75	100	m	q	R^2
1-octen-3-one	0,028	0,041	0,046	0,059	0,085	0,116	0,152	0,001	0,031	0,993
2-Decenal	0,404	0,619	0,786	1,259	2,277	3,056	3,476	26,001	0,144	0,977
2-methyl-1-butanol	0,039	0,047	0,065	0,093	0,145	0,225	0,284	0,078	0,036	0,995
2-octanone	0,017	0,023	0,021	0,030	0,040	0,058	0,062	0,169	0,019	0,960
3-methyl-1-butanol	0,000	0,049	0,069	0,102	0,194	0,322	0,330	4,310	-0,019	0,947
4-ethyl phenol	0,041	0,634	0,956	2,654	4,723	7,323	10,257	21,836	0,199	0,999
4-ethylguaiacol	0,037	0,078	0,132	0,293	0,562	0,809	1,274	0,118	0,004	0,990
6-methyl-5-epten-2-one	0,008	0,071	0,112	0,327	0,621	0,885	0,994	0,914	-0,012	0,971
Acetic acid	0,445	0,729	1,600	3,401	6,262	9,116	11,892	9,091	0,389	0,999
Butanoic acid	0,022	0,567	1,212	2,392	4,429	6,867	8,856	1,502	-0,930	0,999
E-2-nonenal	0,028	0,035	0,052	0,096	0,165	0,252	0,240	0,021	0,037	0,921
E-2-octenal	0,035	0,048	0,061	0,112	0,193	0,282	0,282	0,989	0,007	0,942
Ethyl acetate	0,036	0,056	0,093	0,150	0,402	0,625	0,643	3,025	-0,018	0,946
Ethyl propanoate	0,027	0,036	0,071	0,106	0,203	0,300	0,349	0,571	0,033	0,990
Guaiacol	0,132	0,304	0,223	0,642	0,902	1,728	3,012	8,876	0,060	0,952
Hexanal	0,165	0,309	0,444	1,027	2,229	3,357	3,862	7,695	0,066	0,981
Nonanal	0,354	0,569	0,965	2,086	4,216	5,630	6,590	2,695	0,369	0,978
Octanal	0,047	0,109	0,186	0,385	0,718	1,049	1,063	0,099	0,107	0,958
Octane	0,037	0,244	0,492	1,140	2,349	3,712	4,674	2,826	-0,028	0,992



Choice of marker

Markers have been associated with the main defects of virgin olive oils by data of literature, but especially by the contribution of Carapelli Firenze Spa, which made its data collected over several years available to the Task Force.



Positive charatteriscs
1-penten-3-ol
E-2-hexenal
E-2-hexenol
E-2-pentenal
E-2-pentenol
Ethyl vinyl ketone
Hexanol
Hexyl acetate
Z-2-pentenol
Z-3-hexenal
Z-3-hexenol
Z-3-hexenyl acetate

Vinegary
Acetic acid
Ethanol
Ethyl acetate

Musty
1-octen-3-ol
1-octen-3-one
2-methyl-1-butanol
2-octanol
2-octanone
3-methyl-1-butanol
6-methyl-5-epten-2-one
Octane

Rancid
2-Decenal
Butanoic acid
E-2-heptenal
E-2-nonenal
E-2-octenal
Hexanal
Hexanoic acid
Nonanal
Octanal

Fusty/Muddy
4-ethyl phenol
4-ethylguaiacol
Ethyl butanoate
Ethyl propanoate
Guaiacol
Methyl propanoate

12 Markers for positive attributes
28 Markers for negative attributes

Work has started using 7 internal STD.

We have tried to use only 3 internal STD and have obtained a correct quantification of the markers.

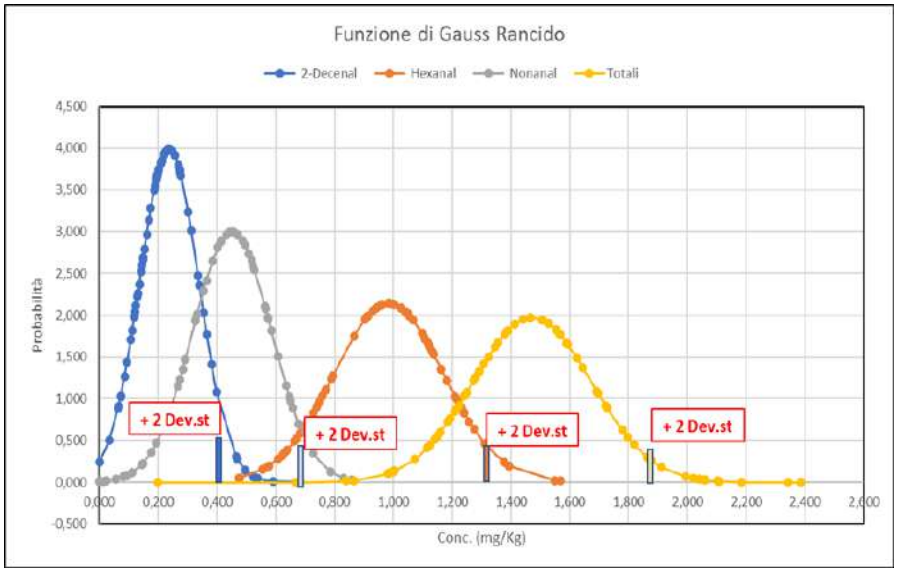
STD1: 4-Methyl-pentan-2-ol
STD2: Octan-3-one
STD3: 3,4-Dimethylphenol

Marker optimization and threshold

To verify the choice of the markers and their correlation with the defects of VOO we have used the three ASSITOL professional panel recognized by MIPAFF.

ASSITOL 1 – c/o Costa d'Oro Spa
ASSITOL 2 – c/o Carapelli Firenze Spa
ASSITOL 3 – c/o Agridè Srl

First Step – Analysis of EVOO Verified without defects → Determination of the threshold of the markers



Num.	***Rancid***
1	2-Decenal
2	Butanoic acid
3	E-2-heptenal
4	E-2-nonenal
5	E-2-octenal
6	Hexanal
7	Hexanoic acid
8	Nonanal
9	Octanal
10	Total Rancid

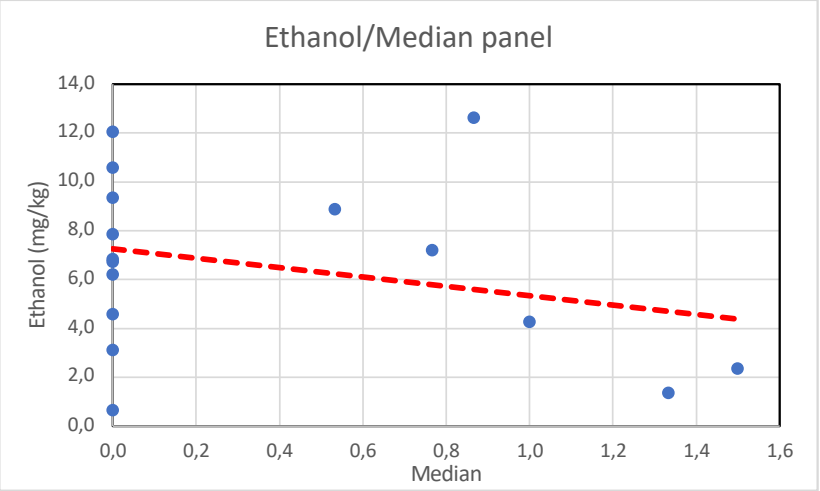
Only 2-Decenal, Hexanal and Nonanal were present in EVOO without defects, the other markers were below 0,1 mg/kg.
We have considered also the total rancid = sum of the threshold of the markers

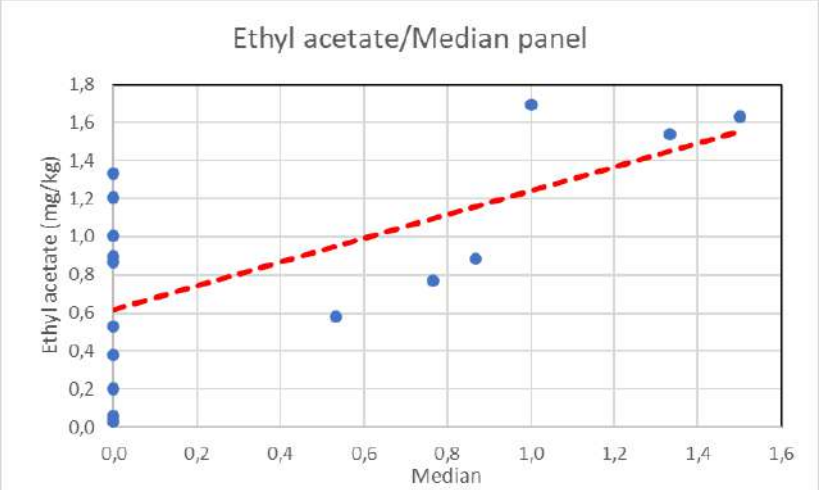
Threshold = Average + 2*St. Dev.

ANOMAL DATA ➡ Discordance between markers and panel test

Num.	*Vinegary*
1	Acetic acid
2	Ethanol
3	Ethyl acetate
4	Total Vinagry

Vinegary			
Sample	***Averaage Median***	***Ethanol***	***Ethyl acetate***
C1	1,3	1,360	1,542
C2	0,0	6,723	0,528
C3	0,0	12,059	0,897
C4	0,0	6,832	0,381
C5	0,0	10,584	1,002
C6	0,5	8,873	0,580
C7	0,0	3,112	0,028
C8	0,0	4,588	0,060
C9	0,8	7,191	0,767
C10	0,9	12,632	0,883
C11	1,0	4,256	1,691
C12	0,0	7,864	1,204
C13	0,0	6,206	1,333
C14	0,0	0,644	0,202
C15	1,5	2,351	1,632
C16	0,0	9,356	0,870

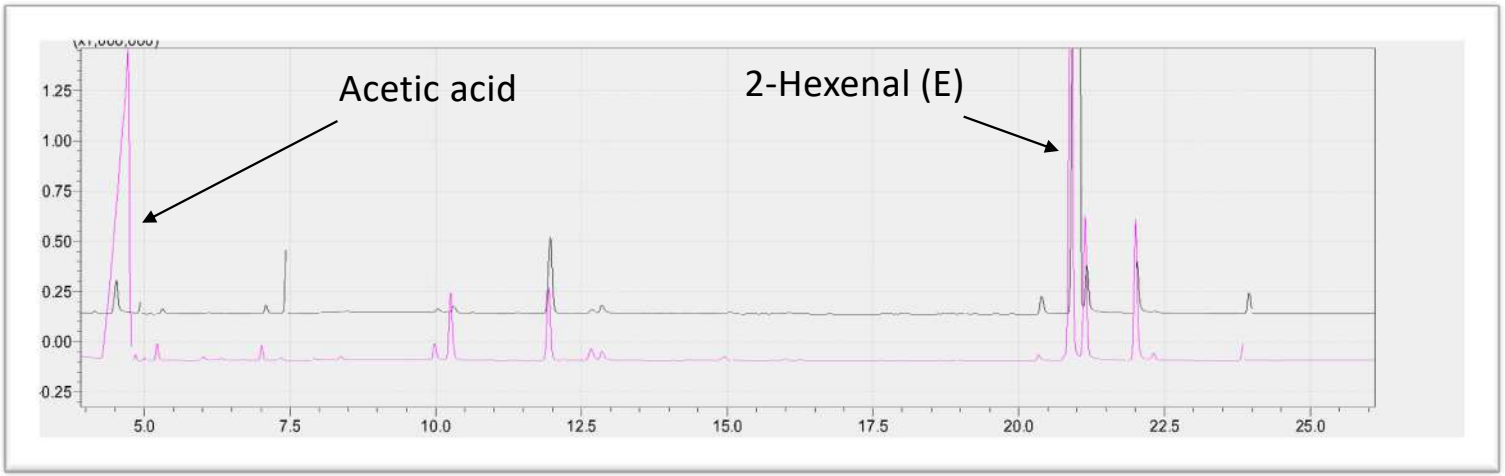




Choice of most representative volatile compounds for EVOO organoleptic characteristics

Positive attributes

1	2-Hexenal, (E)-
2	2-Hexen-1-ol, (E)-
3	1-Hexanol
4	Acetic acid, hexyl ester
5	3-Hexen-1-ol, (Z)-
6	3-Hexen-1-ol, acetate, (Z)-

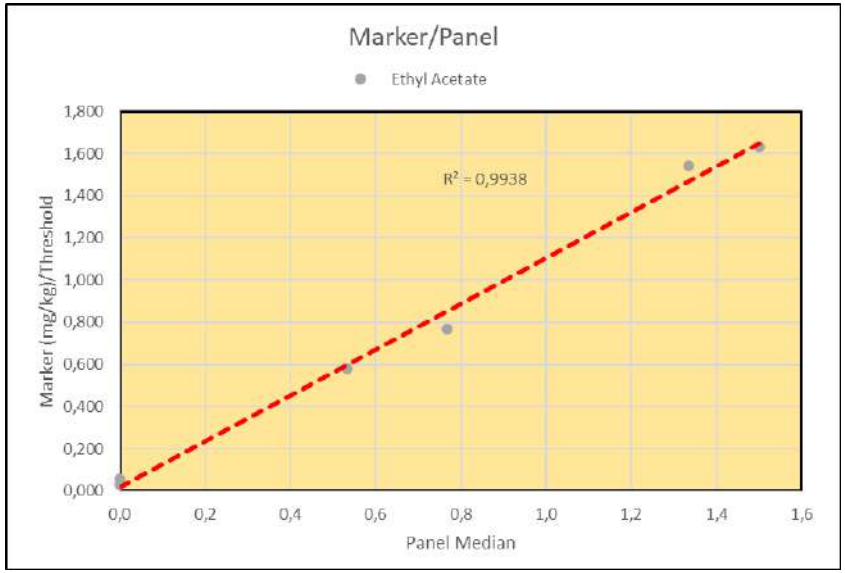


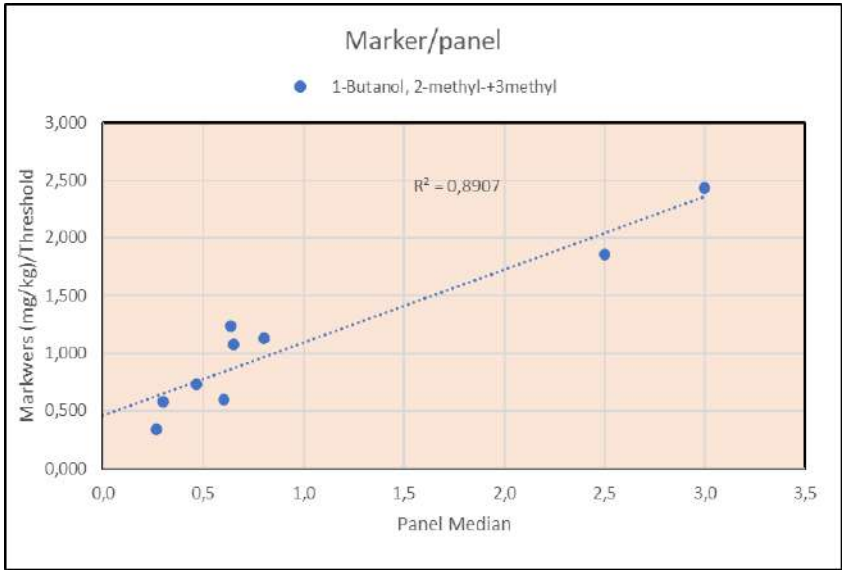
Negative attributes

1	Acetic acid
2	Ethyl Acetate
3	1-Octen-3-ol
4	1-Octen-3-one
5	1-Butanol, 2-methyl-
6	2-Octanone
7	1-Butanol, 3-methyl-
8	5-Hepten-2-one, 6-methyl-
9	Octane
10	2-Decenal, (E)-
11	2-Heptenal, (E)-
12	2-Nonenal, (E)-
13	2-Octenal, (E)-
14	Hexanal
15	Hexanoic acid
16	Nonanal
17	Octanal
18	Phenol, 4-ethyl-
19	Phenol, 4-ethyl-2-methoxy-
20	Butanoic acid, ethyl ester
21	Propanoic acid, ethyl ester

Mathematical regressions between markers and panel tests

The regression were found between the ratio of the markers content (mg/kg) and their threshold in EVOO versus the median of panel test in EVO





Medians calculated by the regression

Example for the rancid defect

Median Calculated = {[marker(mg/kg)/trheshold]-q}/m
Where: m – slope q - intercept

Marker	m	q	mg/kg	Mediana Calcolata
Hexanal	0,362	0,181591	1,150	**1,8**
Hexanoic acid	1,110	-0,64539	0,300	**1,8**

To have the same median, the content of Hexanal must be four times that of Hexanoic Acid

COMPARISION BTEWEEN PANEL TEST AND VOLATILE ANALYSIS

On 17 EVOO purchased on the market, including top-of-the-range, commercial and first price oils, a comparison was made between official and professional panel test and analysis of volatile compounds

Panel test analysis

Sample	Panel	Fusty/Muddy	Musty	Vinegary	Rancid	Category	Other defects
1	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	**1,1**	**V**	
	3	**0,9**	**1,0**	0,0	0,0	**V**	
	4	**1,7**	0,0	0,0	0,0	**V**	
2	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	3	**1,4**	0,0	0,0	0,0	**V**	
	4	0,0	0,0	0,0	0,0	EV	
3	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	0,0	0,0	0,0	EV	
	4	0,0	0,0	0,0	0,0	**V**	Hay-wood: 1,3
4	1	0,0	0,0	0,0	**2,5**	**V**	
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	**1,8**	0,0	**1,3**	**V**	
5	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	0,0	0,0	0,0	EV	
6	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	0,0	0,0	0,0	EV	
7	1	0,0	0,0	0,0	0,0	**V**	Oxidation: 2
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	0,0	0,0	**1,1**	**V**	
8	1	**2,5**	0,0	0,0	0,0	**V**	Oxidation: 1
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	**1,0**	0,0	0,0	**V**	
9	1	0,0	0,0	0,0	**1,5**	**V**	
	2	0,0	0,0	0,0	**1,3**	**V**	
	3	**1,3**	0,0	0,0	0,0	**V**	
	5	0,0	0,0	0,0	0,0	EV	
	6	0,0	0,0	0,0	**1,6**	**V**	

Sample	Panel	Fusty/Muddy	Musty	Vinegary	Rancid	Category	Other defects
10	1	0,0	0,0	0,0	**2,5**	**V**	
	2	0,0	0,0	0,0	0,0	EV	
	3	**1,0**	0,0	0,0	0,0	**V**	
	5	**1,7**	0,0	0,0	0,0	**V**	
	6	0,0	0,0	0,0	0,0	EV	
11	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	0,0	0,0	**1,0**	**V**	
	5	0,0	0,0	0,0	0,0	EV	
	6	0,0	0,0	0,0	0,0	EV	
12	1	**2,0**	0,0	0,0	**1,0**	**V**	
	2	0,0	0,0	0,0	0,0	**V**	Dirty: 1,4
	3	0,0	**1,1**	0,0	0,0	**V**	
	4	0,0	0,0	0,0	0,0	**V**	Burnt:1,3
13	1	**2,0**	0,0	0,0	0,0	**V**	
	2	0,0	0,0	0,0	0,0	**V**	Dirty: 1,3
	3	0,0	0,0	0,0	**1,0**	**V**	
	4	0,0	0,0	0,0	**1,4**	**V**	
14	3	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	1	0,0	0,0	0,0	0,0	EV	
	4	0,0	0,0	0,0	0,0	EV	
15	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	0,0	0,0	0,0	EV	
16	1	0,0	0,0	0,0	0,0	EV	
	2	0,0	0,0	0,0	0,0	EV	
	3	0,0	0,0	0,0	0,0	EV	
17	1	0,0	0,0	**1,0**	0,0	**V**	
	2	0,0	0,0	0,0	0,0	**V**	Dirty: 1,3
	3	0,0	**1,2**	0,0	0,0	**V**	

COMPARISION BTEWEEN CLASIFICATION OF PANEL TEST AND VOLATILE ANALYSIS

	Panel Category		*Volatile Category*	
Sample	*EVOO*	*EVO*	*EVOO*	*EVO*
1	1	3	0	3
2	3	1	3	0
3	3	1	3	0
4	1	2	0	3
5	3	0	3	0
6	3	0	3	0
7	1	2	0	3
8	1	2	2	1
9	1	4	0	3
10	2	3	0	3
11	1	4	3	0
12	0	4	0	3
13	0	4	0	3
14	4	0	3	0
15	3	0	3	0
16	3	0	3	0
17	0	3	1	2

Highlighted in green → Congruent results

Highlighted in green → Incongruent results

For the panel test there are 8 congruent results

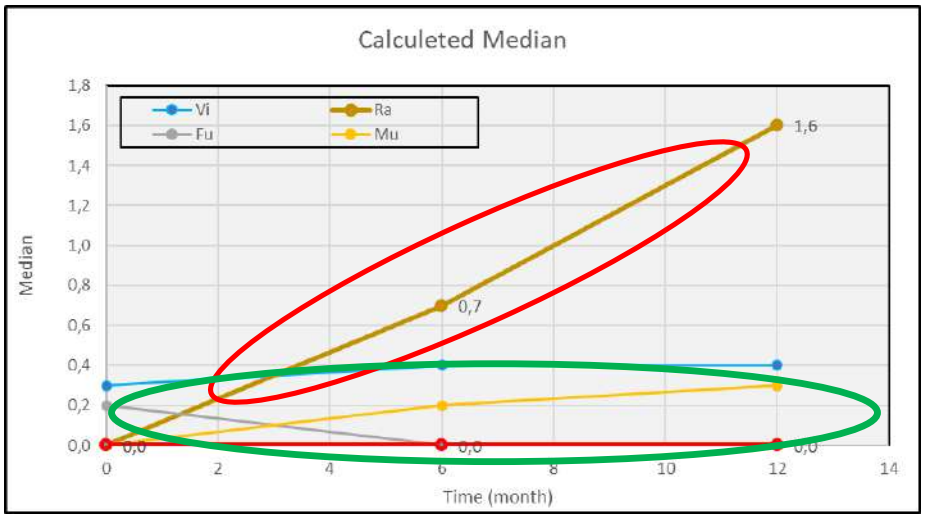
For volatile analysis there are 15 congruent results

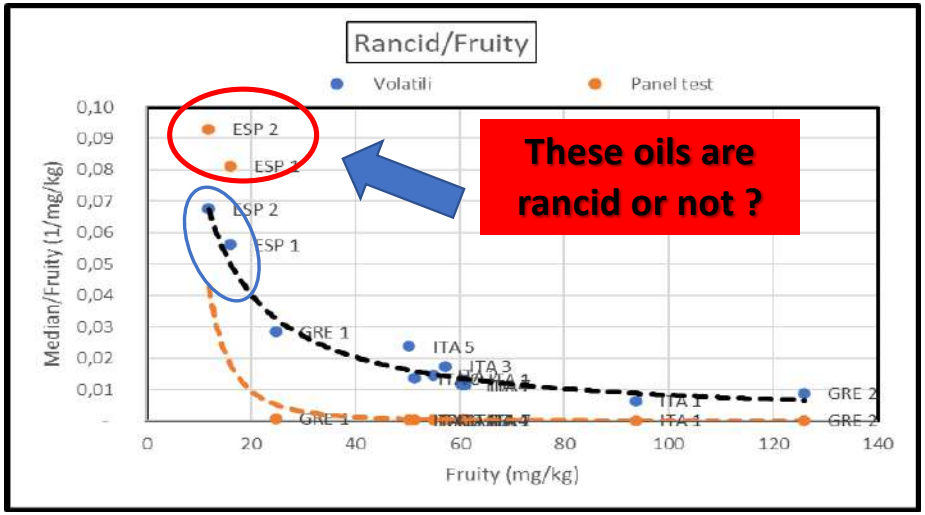
These conclusions don't want say that the volatile compounds analysis is better than panel test, but at the moment, that the volatile compounds analysis has a better reproducibility of the panel test

Two interesting works with volatile compounds

First
Variation of volatile compounds during aged of the EVOO

Second
Influent of the fruity in the valuation of the rancid





The work is in progress, there are many things to made.

We like to open a collaboration with other societies and laboratories, to improve this interesting work.

We think that an open collaboration has necessary to obtain a analytical system to combine at the panel test, to have a good method for the classification the oils with the organoleptic analysis.

THANK FOR YOUR ATTENTION