



Biofenoli e aromi dell'extravergine: recenti sviluppi metodologici ed analitici

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FONDAZIONI IN RETE
PER LA RICERCA
AGROALIMENTARE



Progetto ARISTOIL, Ragusa, 7 giugno 2018





Virtù salutistiche dell'Olio Extravergine:

Azione antiossidante su lipoproteine ematiche (EFSA)

**Riduzione di fattori di rischio cardiovascolare
(ipercolesterolemia, aterosclerosi)**

Riduzione dell'ipertensione arteriosa (oleaceine)

Inibizione di eventi trombotici e micro-trombotici

**Riduzione dello stress ossidativo e mantenimento dell'integrità
cellulare (effetto anti-*ageing*)**

Azione anti-infiammatoria (oleocantale: ibuprofene-like)

Azioni positive rispetto a diabete ed obesità

Riduzione dell'incidenza del cancro ed attività antineoplastica

**Prolungamento del senso di sazietà (acido oleico e
endocannabinoidi, aromi)**



Tutto ciò è attribuito a:

Acido Oleico (composizione in acidi grassi molto simile a quella del latte umano! Stabilità ossidativa, fluidità membrane, HDL/LDL, Effetto su endocannabinoidi e sazietà, etc ...)

Biofenoli con spiccata azione biologica:

Oleocantale, con azione anti-infiammatoria simile all'ibuprofene

Oleacine, con azione ipotensiva

Secoridoidi amari, con azione di prevenzione delle malattie degenerative dell'apparato digerente (*H. Piloni*)

Idrossitirosole con azione antiossidante e anti-apoptotica

Vitamina E, azione antiossidante e protezione cardiovascolare

Fitosteroli, squalene, con azione anti-colesterolo

Sostanze volatili (aroma) ... e sazietà (obesità).



EFSA health claim on olive oil polyphenols (Commission Regulation (EU) 432/2012):

Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress.

The claim may be used only for olive oil, containing at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil (250 mg/kg).

In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.

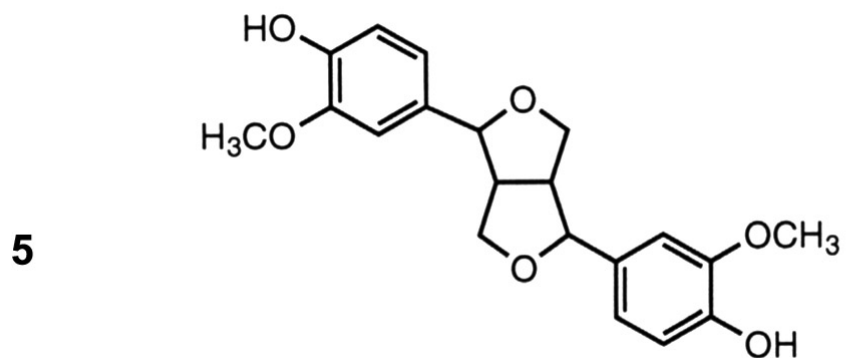
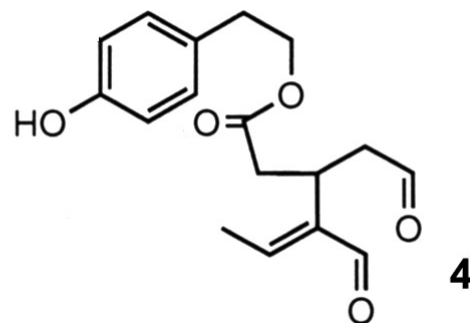
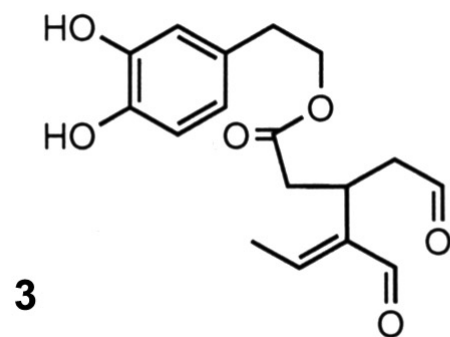
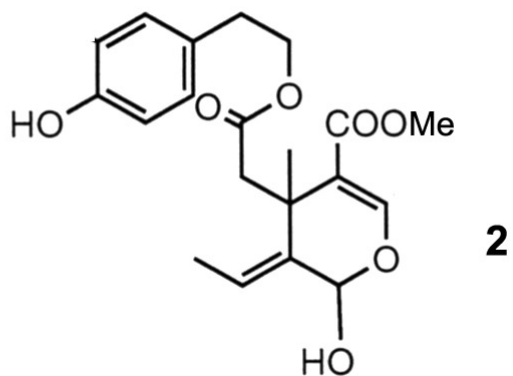
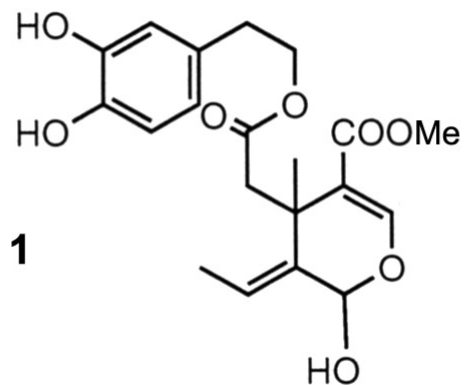


Progetto AGER 2 “COMPETITIVE”

Claims of Olive oil to iMProvE The markeT ValuE



2016-2020



I composti
biofenolici
dell'olio
extravergine
di oliva:

**nutraceutici ...
... amari!**

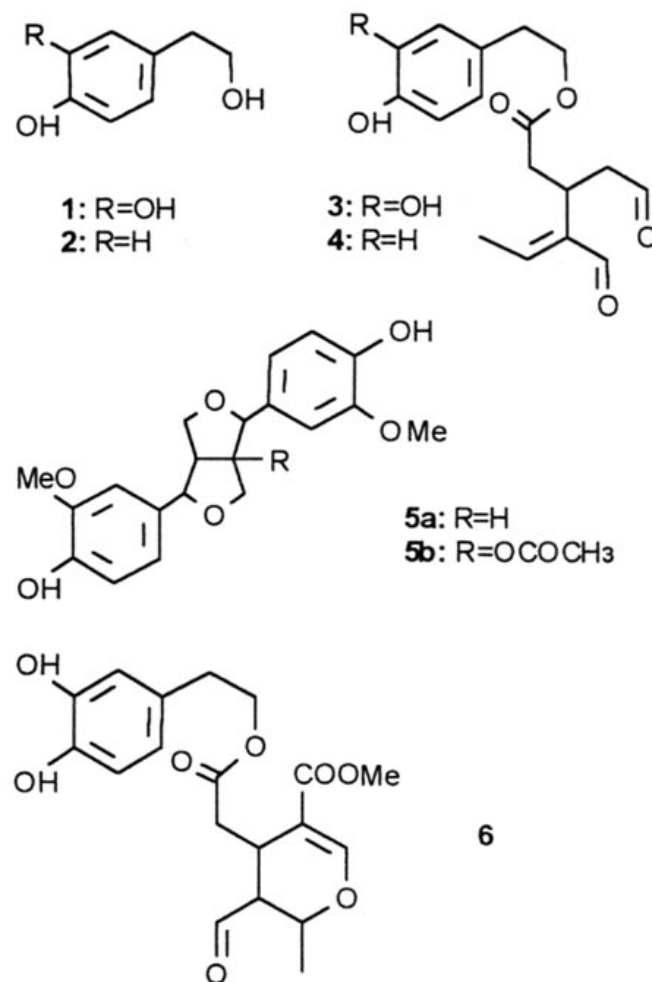
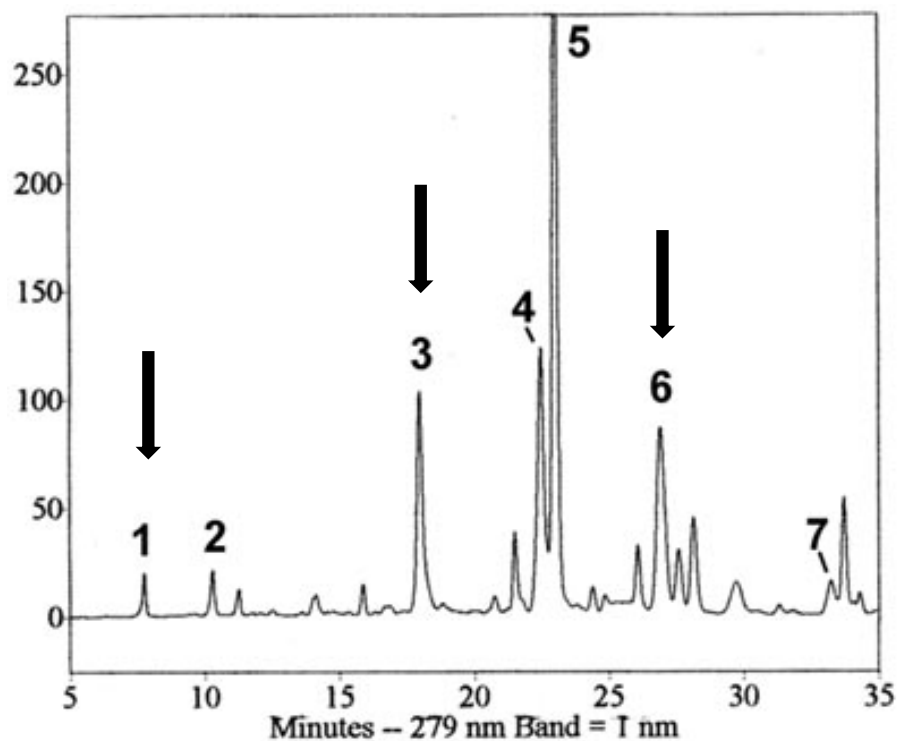
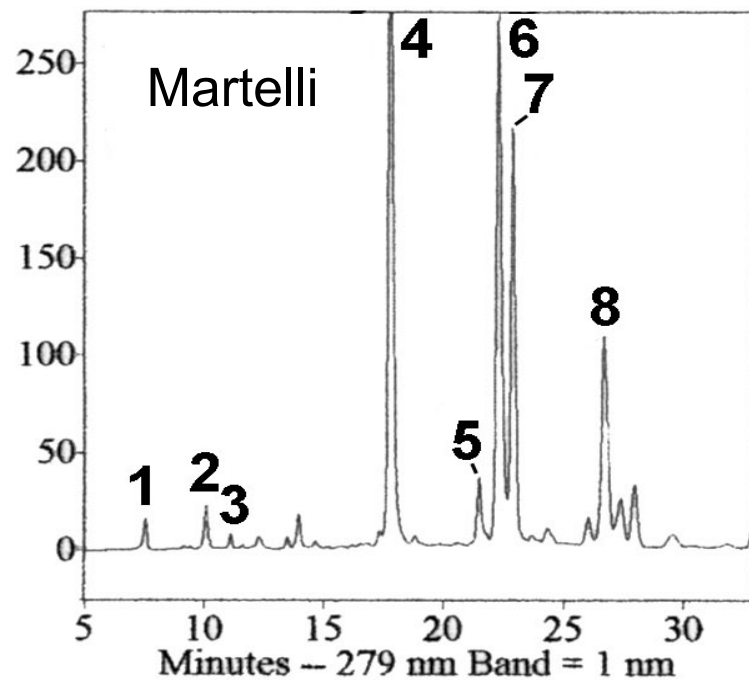
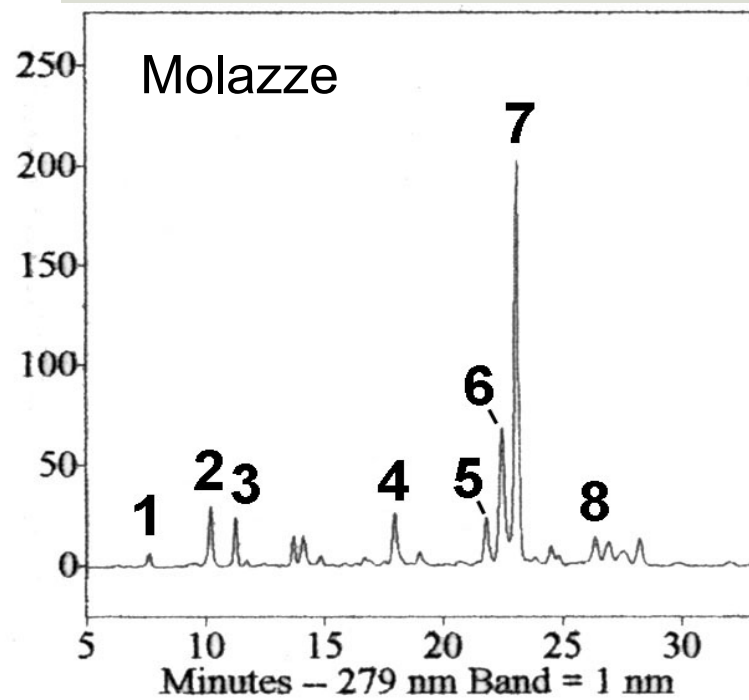


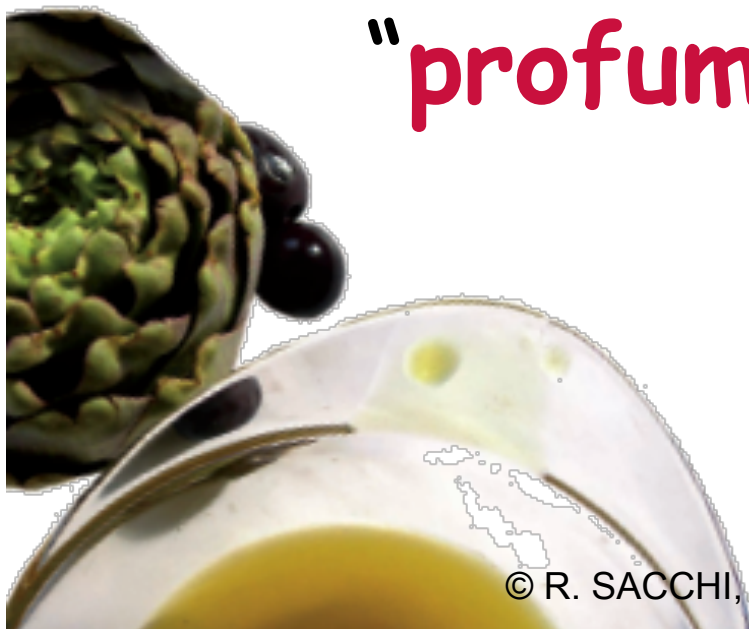
Figure 1. Chemical structures of the main phenolic compounds of virgin olive oil followed by HPLC: hydroxytyrosol (1), tyrosol (2), dialdehydic form of decarboxymethyl oleuropein aglycon (3), dialdehydic form of decarboxymethyl ligstroside aglycon (4), pinosresinol (5a), 1-acetoxypinosresinol (5b), and oleuropein aglycon (6).



Profili fenolici di oli ottenuti con
diverso sistema di frangitura
(molazze vs frangitore a
martelli)



L'olio extravergine
come
"alimento funzionale"
e
"profumo alimentare"



SACCHI, Sirena d'Oro, 2008



i) Individuazione delle **molecole volatili responsabili delle note sensoriali tipiche** degli OEVO

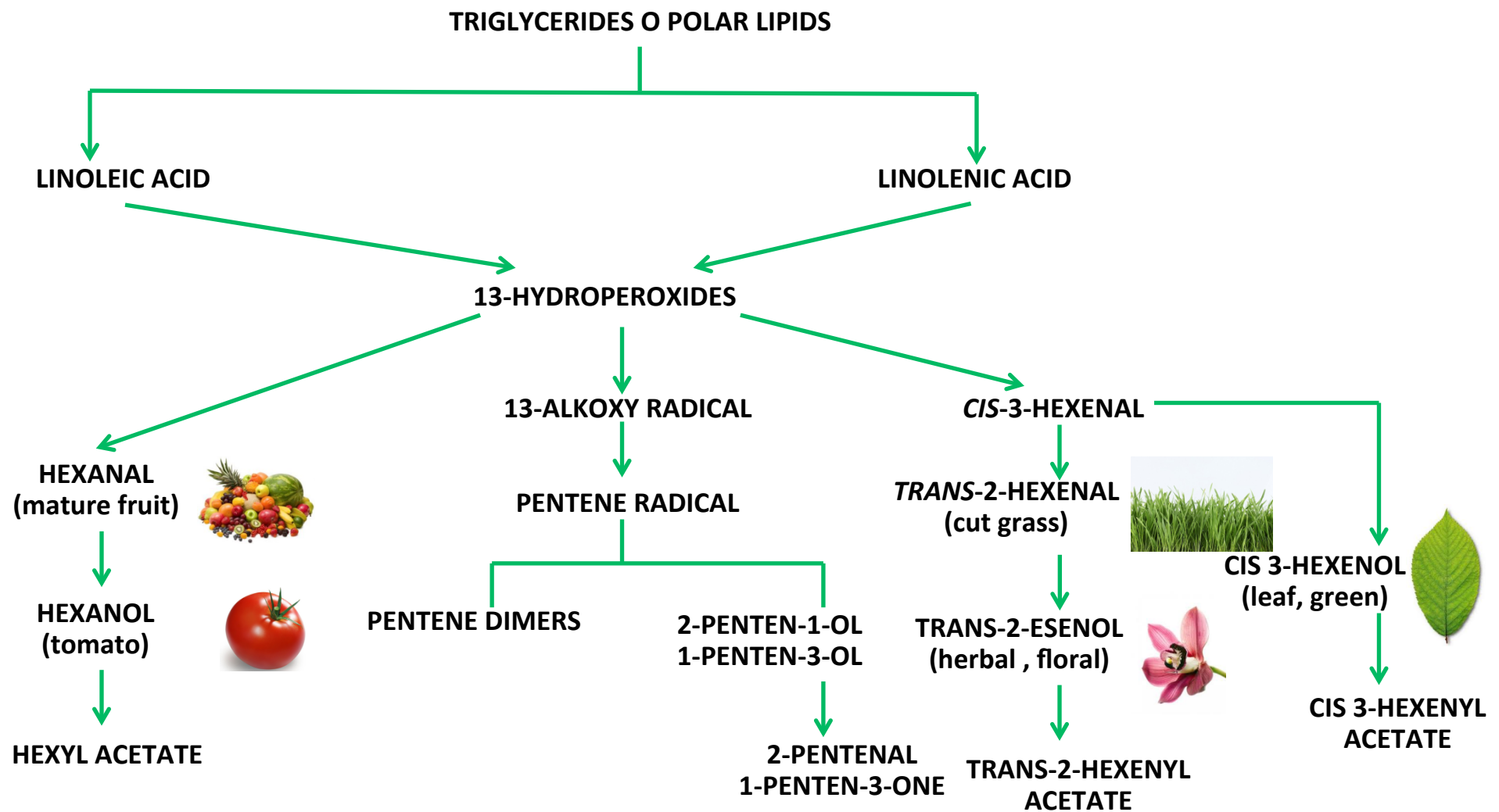
ii) **misura quantitativa accurata** dei composti volatili (SPME-GC/MS)

iii) espressione del loro **impatto odoroso** (OAV, serie aromatiche)



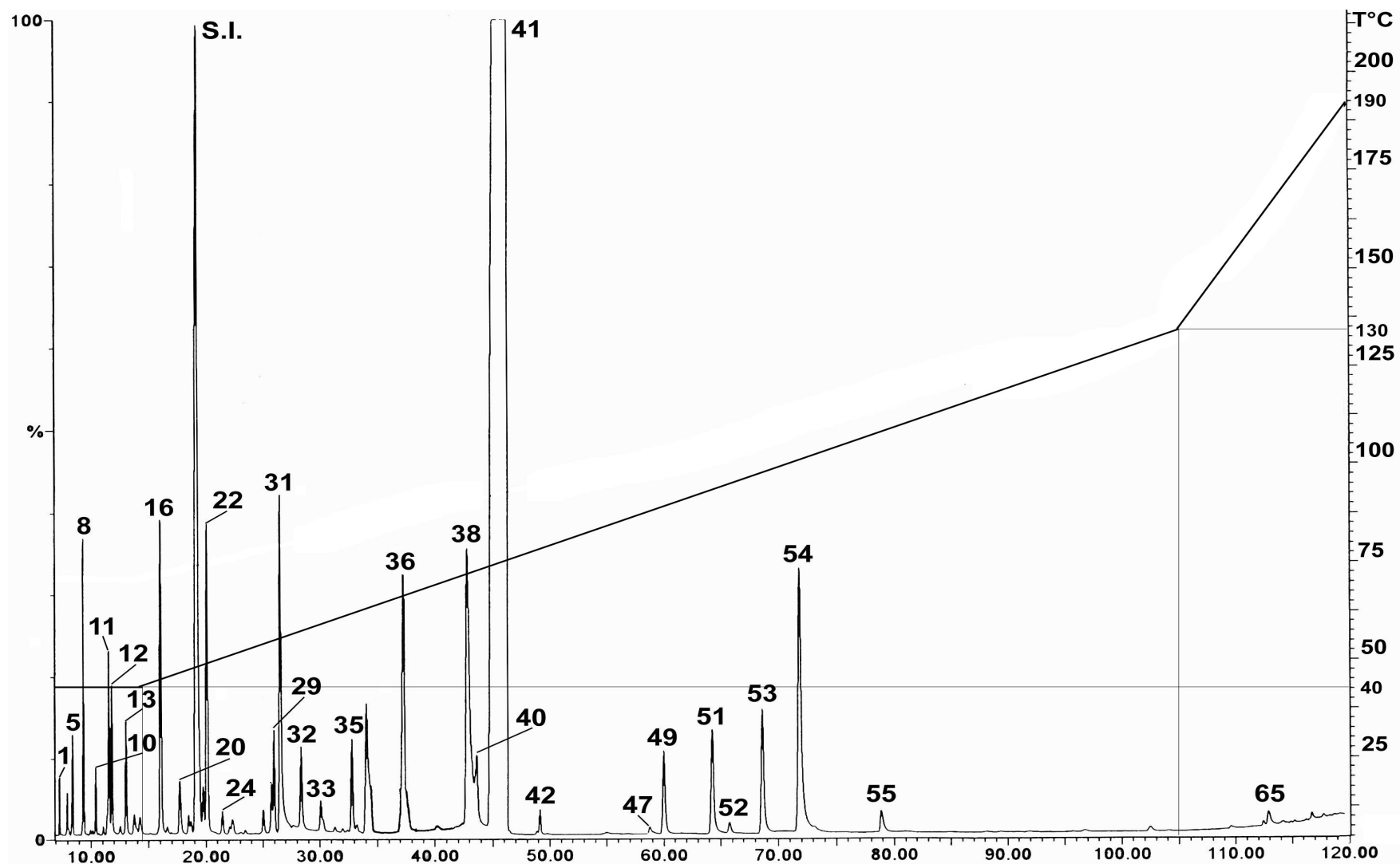


The origin of virgin olive oil fruity flavour: the Lipoxygenase (LOX) Pathway





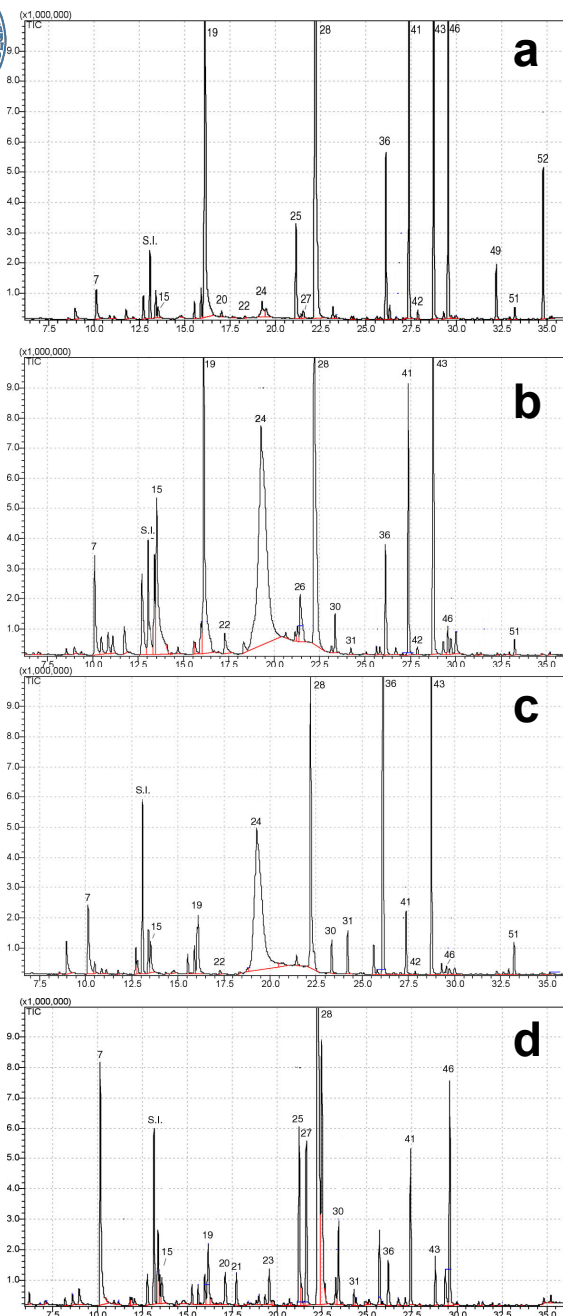
I composti volatili dell'olio (DHS-GC/MS)





Alcuni composti responsabili delle note verdi (LOX)

Picco	Composto	Descrittore (letteratura)
16	1-pentenil acetato	fruttato
31	esanale	mela, frutta
33	pentil acetato	frutta, banana, pera
38	<i>cis</i> -3-esenale	carciofo, verde, floreale
42	<i>trans</i> ,2-esenale	mandorla amara, erba
43	esil acetato	frutta dolce
49	2-penten,1-olo	verde
51	esanolo	frutta, banana
52	<i>trans</i> ,3-esen,1-olo	erba, verde
53	<i>cis</i> ,3-esen,1-olo	foglia, verde
54	<i>cis</i> ,2-esen,1-olo	erba, verde



SPME GC/MS chromatogram of the headspace of some oils:

- a) POD Bruzio,
- b) Biancolilla monovarietal oil,
- c) Ravece monovarietal oil,
- d) Minucciola monovarietal oil.

Peak identification of main volatile compounds:

7. ethanol, 15. 3 pentenone, 19. hexanal, 20. beta-pinene, 21. beta.-Phellandrene, 22. trans-2-pentenale, **23. beta.-Myrcene**, **24. cis-3-Hexenal**, **25. D-limonene**, 26. 3 methyl butanol, 27. Eucalyptol, 28. trans 2-hexenal, **30. beta-ocimene**, 31. n-Hexyl acetate, 36. cis 3 hexenil acetate, 41. hexanol, 42. trans 3 hexenol, 43. cis 3 hexenol, 46. trans 2 hexenol, 49. n-valeric acid cis-3-hexenyl ester, 51. Copaene, **52. beta-linalool**



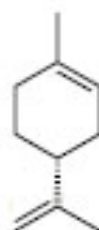
Cv. Minucciola (Penisola Sorrentina): terpeni marcatori molecolari delle note aromatiche di erbe balsamiche (rosmarino, salvia, origano) (DHS-GC/MS)



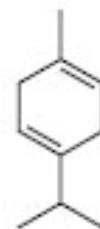
20. α -pinene



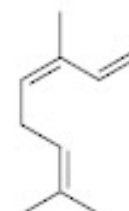
29. β -pinene



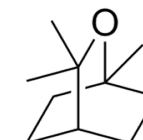
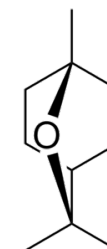
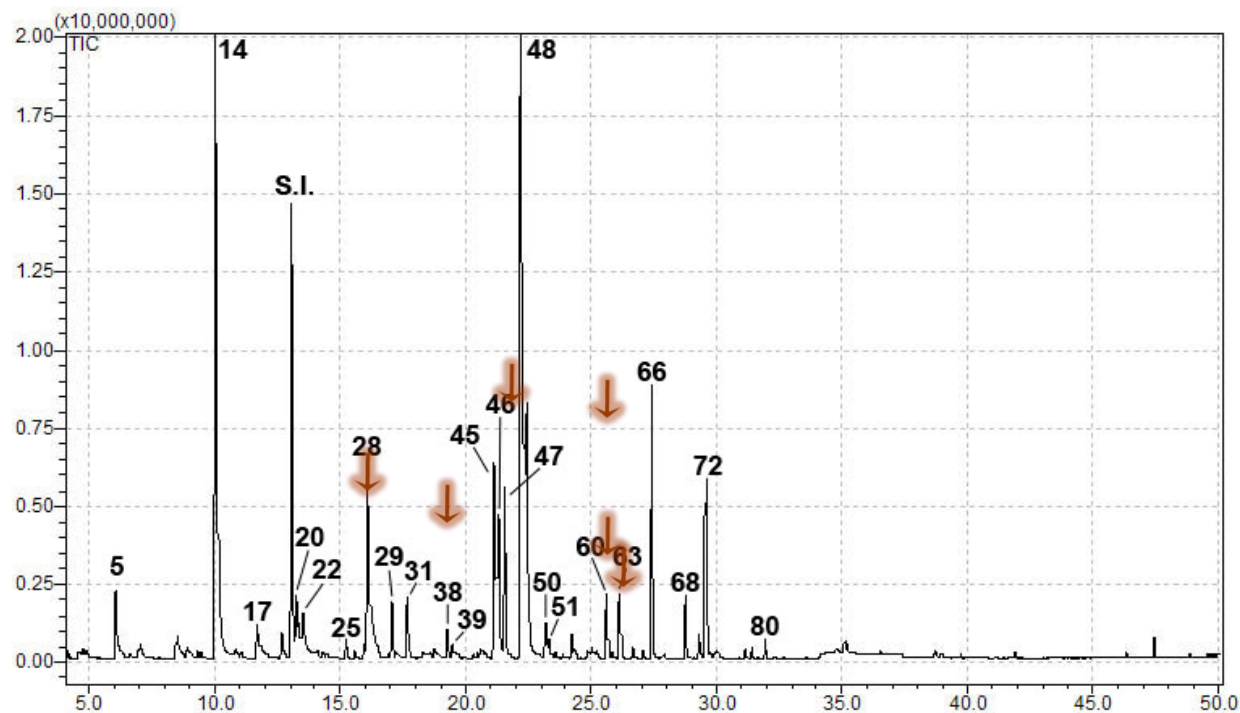
45. D-limonene



50. γ -terpinene



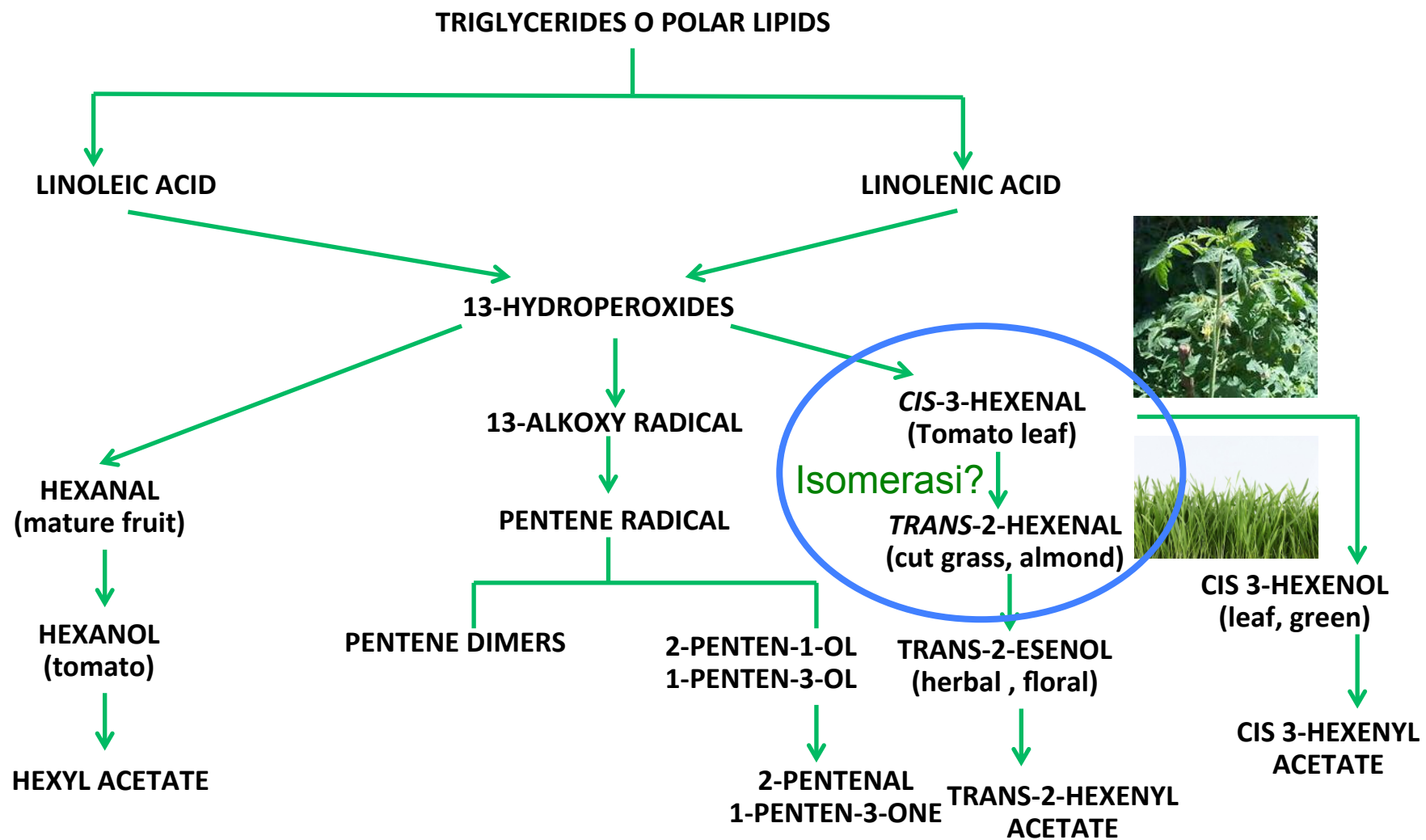
51. (Z)- β -ocimene



47. Eucaliptolo



Cis-3-hexenal and tomato leaf green flavour (molecole 'soliste' e 'orchestre di odori')





Valore Attività Odorosa (OAV):

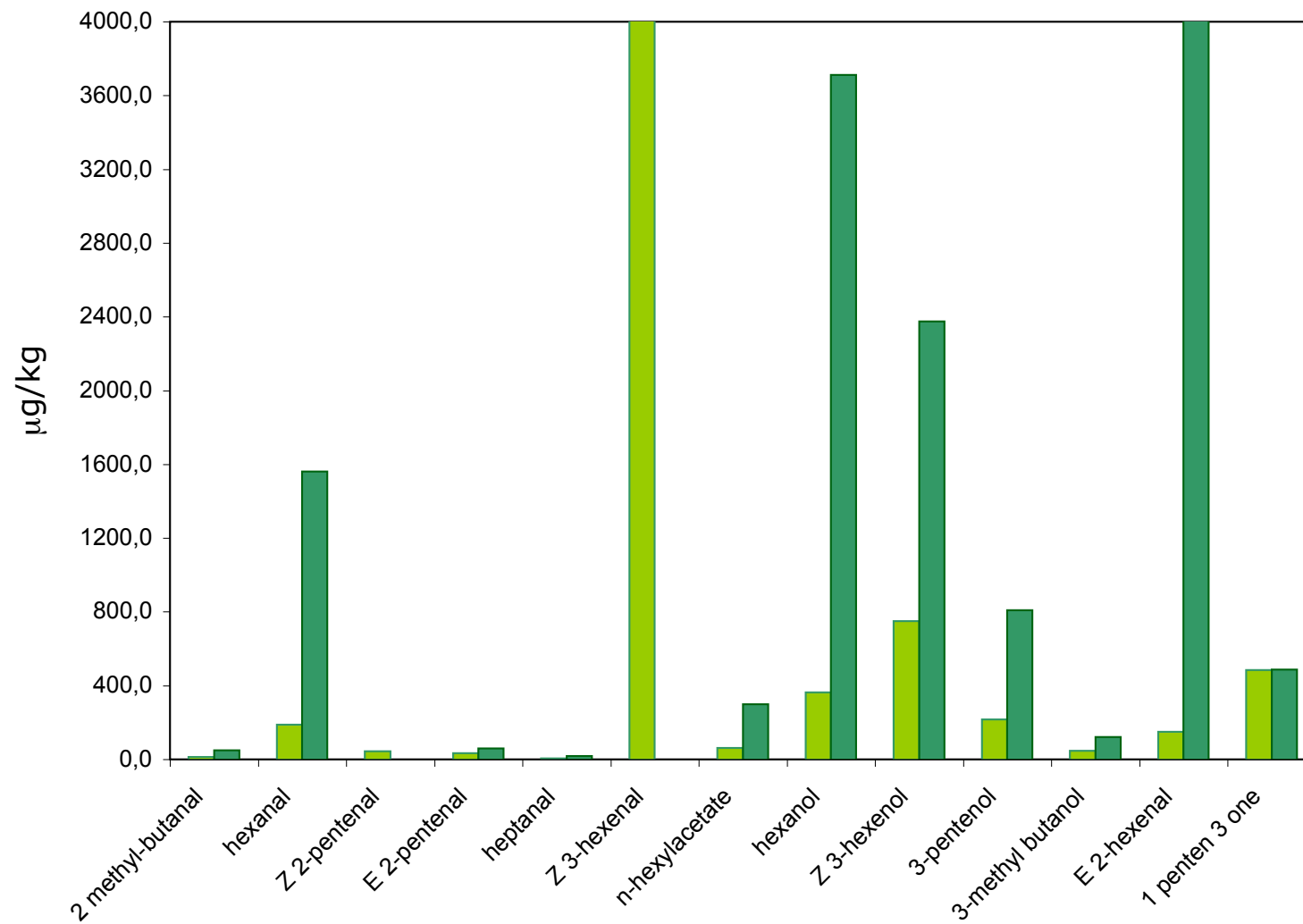
Concentrazione molecola aromatica/
/Soglia di percezione

$OAV > 1$, molecola che contribuisce all'impatto
odoroso

$OAV < 1$, molecola presente al di sotto della
concentrazione-soglia (non percepita?)

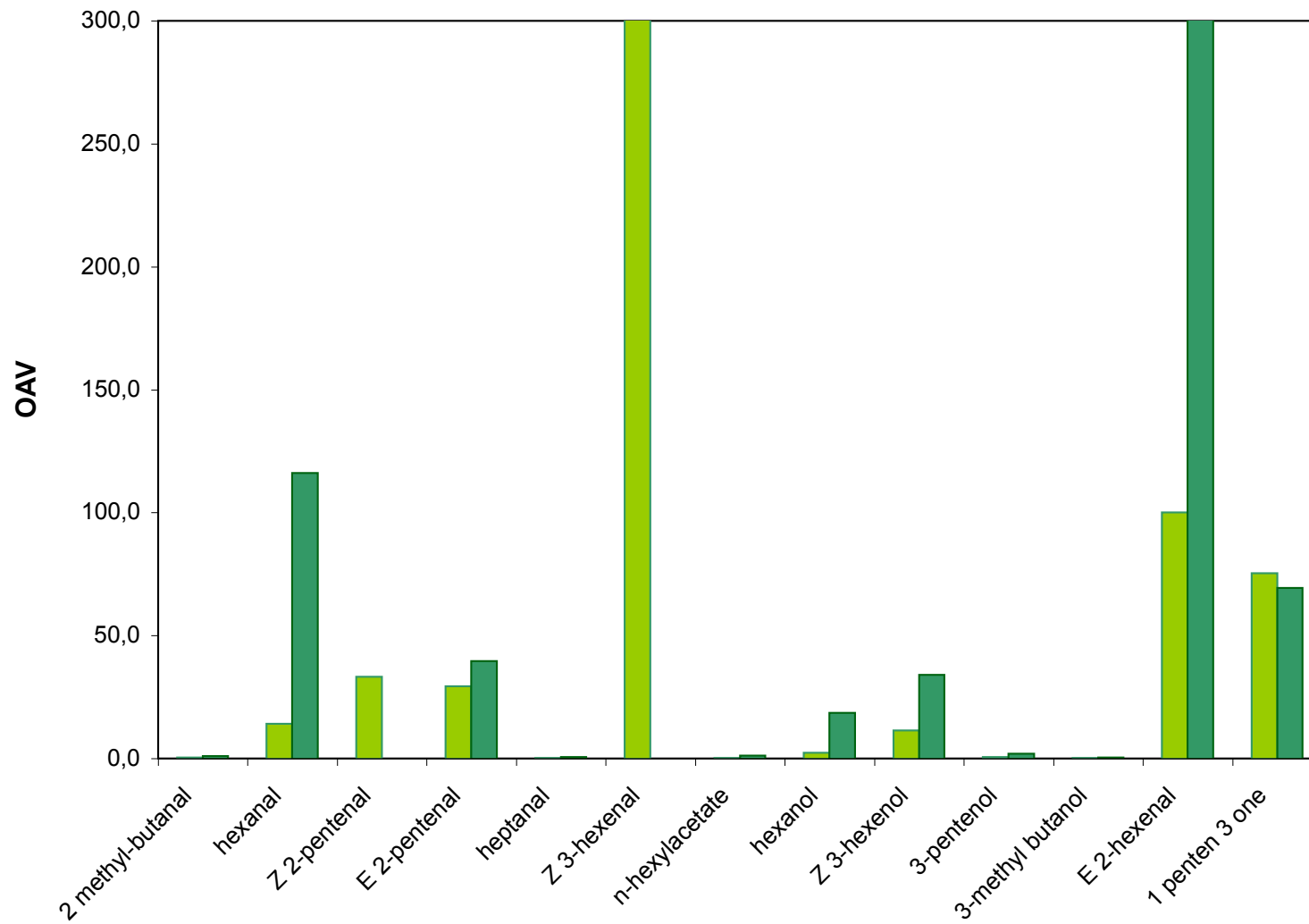


Ravece (■) vs Coratina (■)





OAV, Ravece (■) vs Coratina (■)





SPME-GC/MS Analysis of Volatiles

Extraction of volatile compound by analysis of the dynamic head space (SPME):

-2g sample of oil in VIAL 20 mL +10 μ L of S.I.(Isobutyl Acetate) for 10min at 40°C

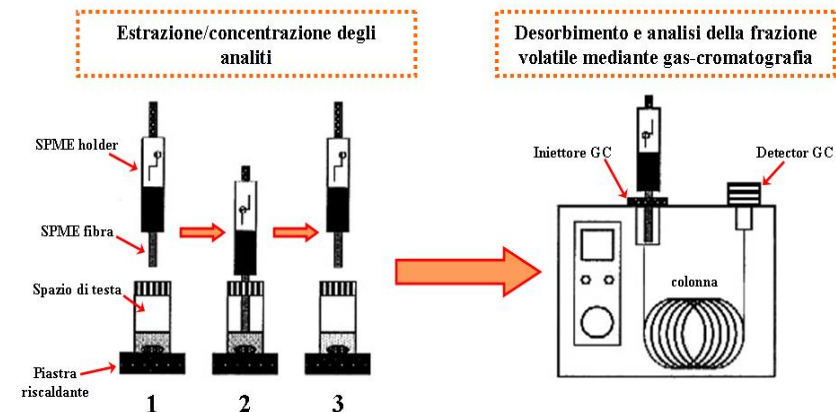
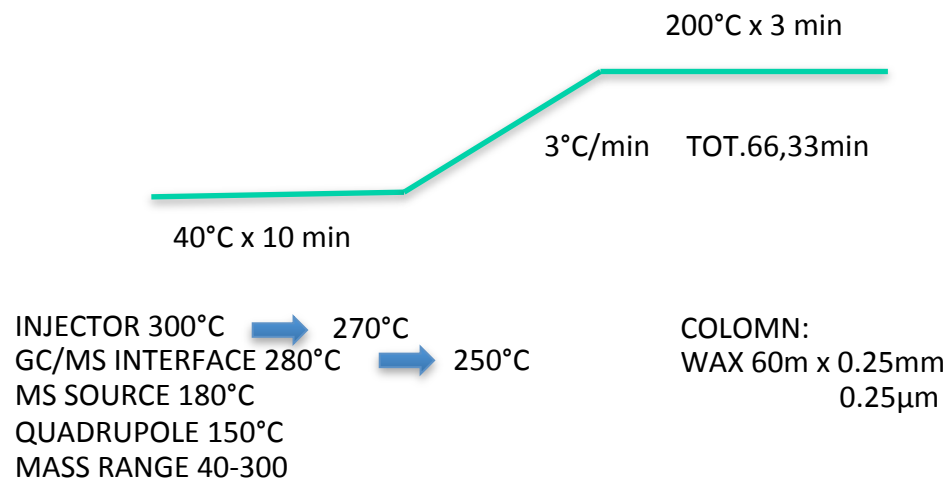
-the fiber is inserted (DVB/CAR/PDMS=grey 1cm) for adsorbment of volatile compounds for 40 min at 40°C

(Romero et al.,2015)

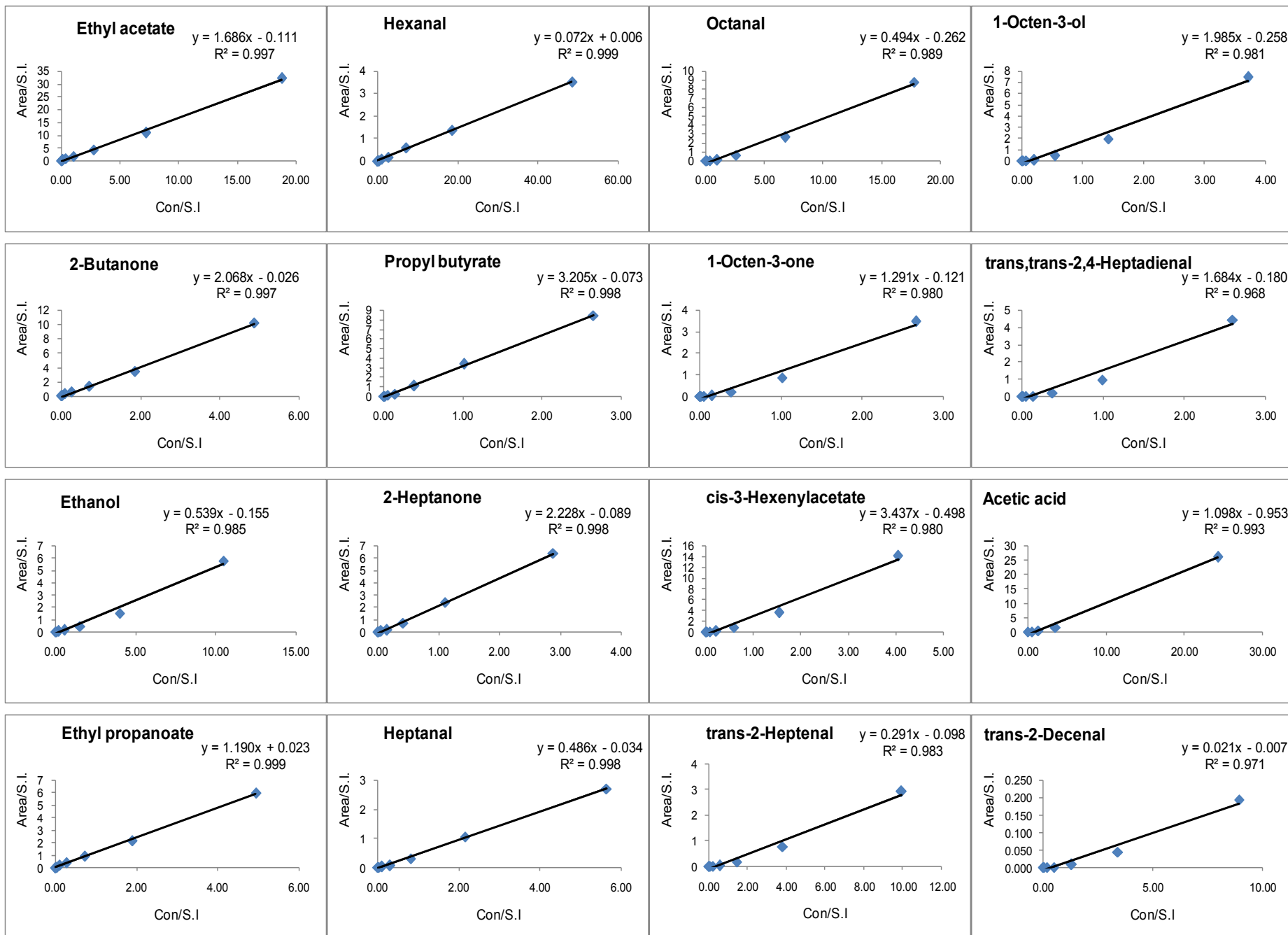
Analysis GC/MS:

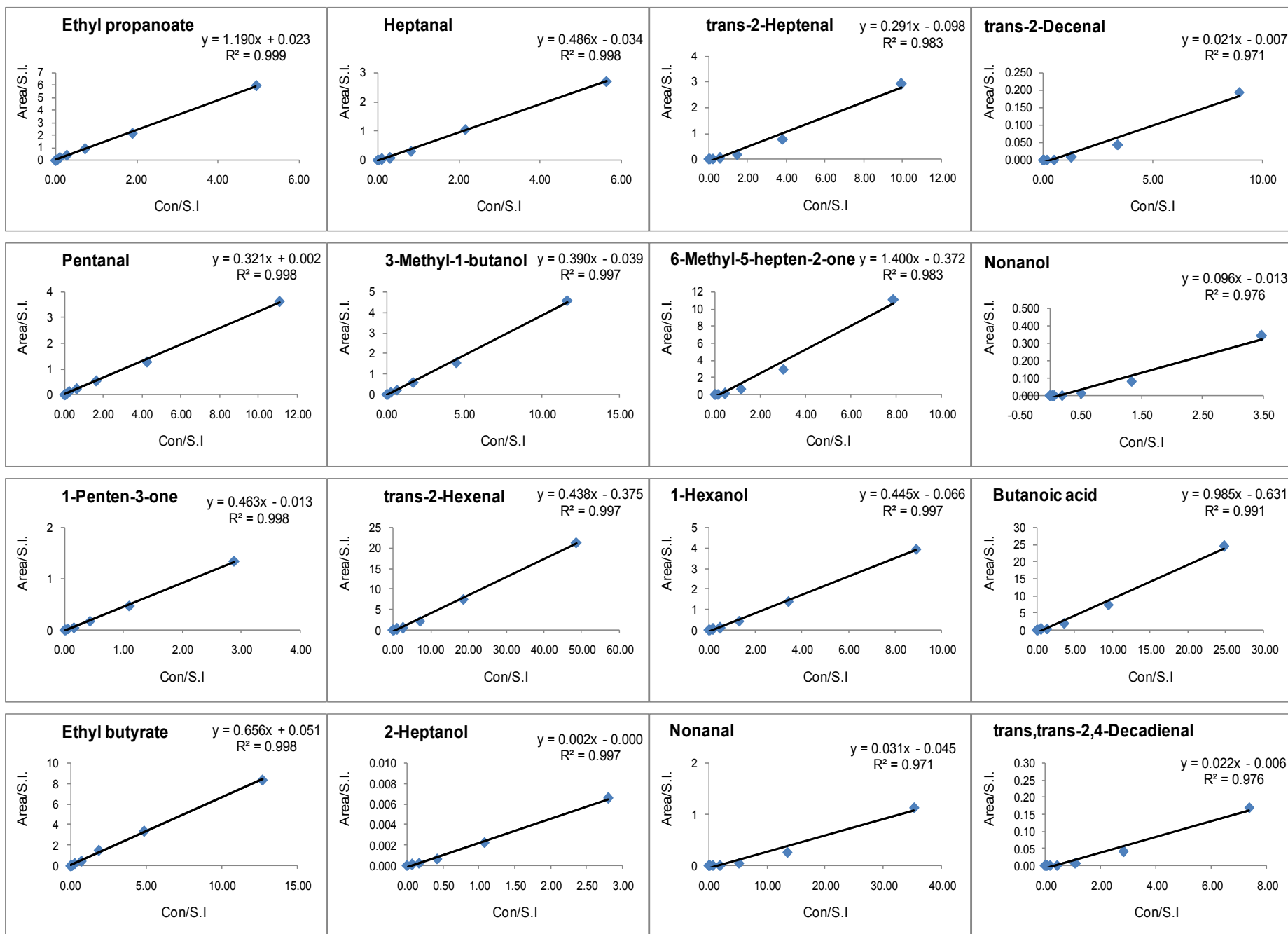
Flow= He 0.9 mL/min

Splitless



(Morales *et al.*,2005)

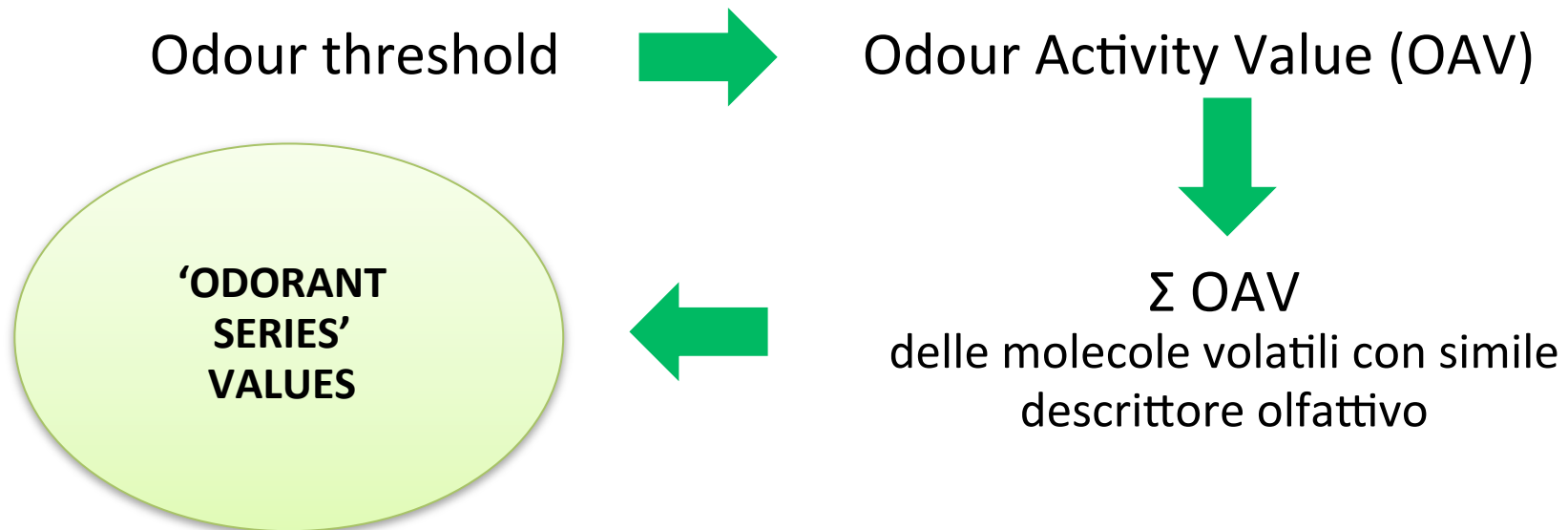




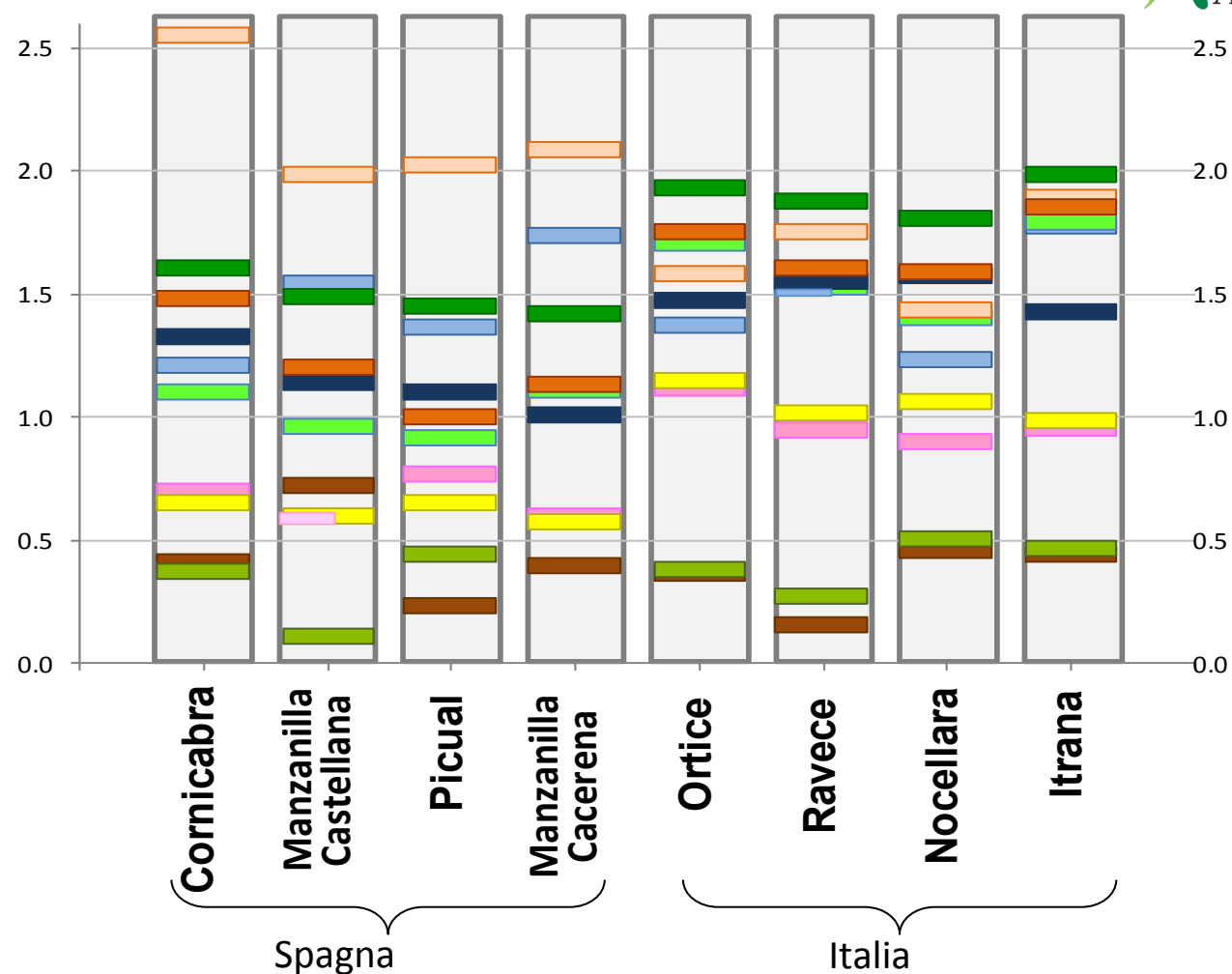


'Serie Odrose'

Somma degli OAV (concentrazione/soglia) dei composti volatili con simile descrittore di odore



Questo procedimento consente una **maggiore correlazione tra le sostanze volatili misurate strumentalmente e l'analisi sensoriale.**

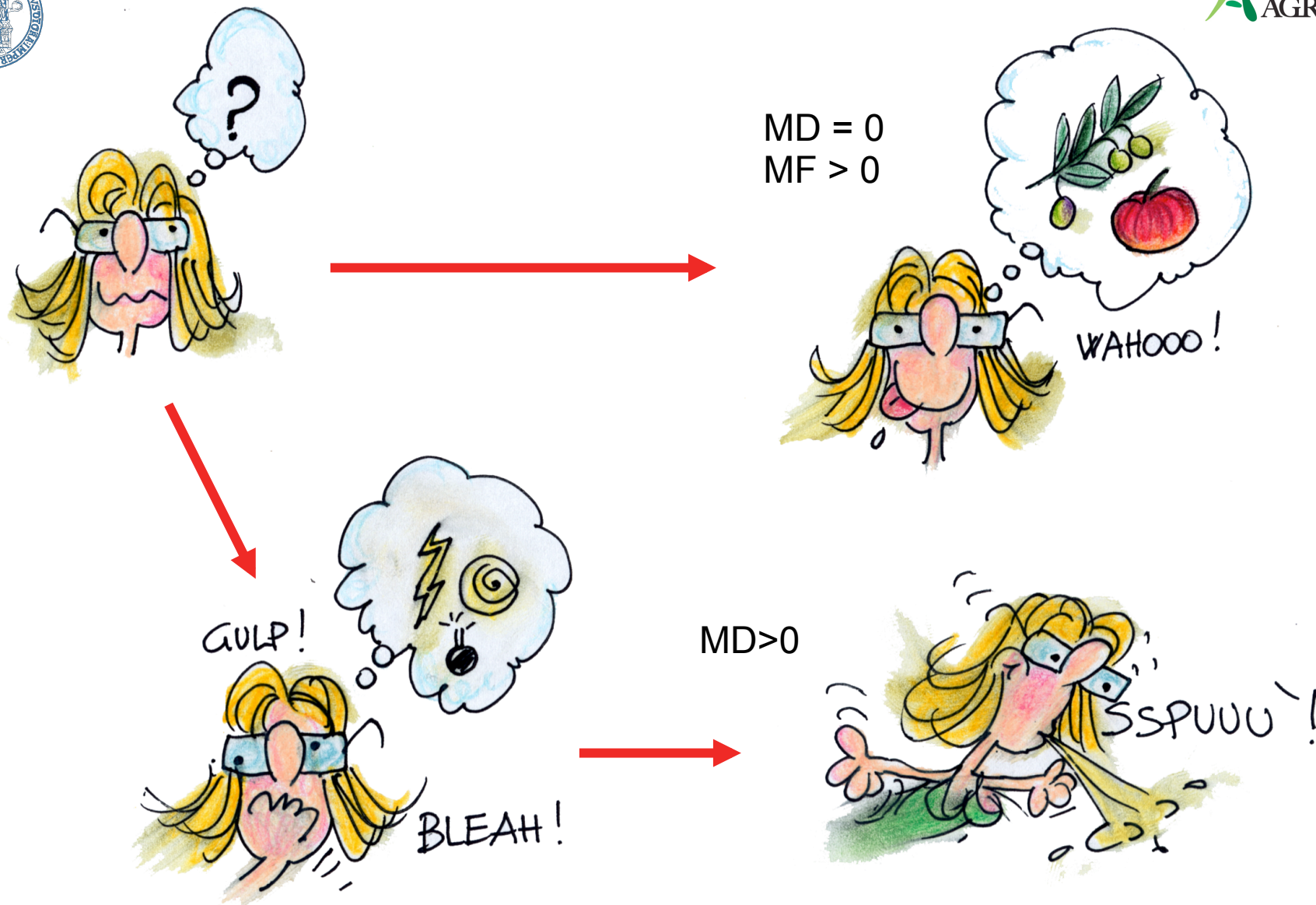


Serie odorose rappresentate come “codici a barre” (log mediana) dell’aroma degli ’OEVO.



iv) Applicazioni nella misura delle molecole volatili responsabili delle **note positive (fruttato di oliva)** e dei **difetti sensoriali** degli OEVO (qualche esempio)





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'Serie odorose' dei pregi e difetti sensoriali

Rancido

- Octan-2-ol
- Pentanal
- Hexanal
- Heptanal
- E-2-Heptenal
- Octanal
- 6-Methyl-5-hepten-2-one
- Nonanal
- 2,4-Heptadienal
- Decanal
- E-2-Decenal
- Nonanol
- 2,4-Decadienal
- Butanoic acid
- Hexanoic acid
- Heptanoic acid
- Octanoic acid
- Nonanoic acid

Riscaldo

- Ethyl acetate
- Butan-2-one
- Butyl acetate
- Ethyl propanoate
- Propyl butanoate
- 2-Heptanone
- Ethyl butanoate
- Hexanal
- 3-Methyl-1-butanol
- Butanoic acid
- Pentanoic acid

Avvinato

- Ethyl acetate
- Ethanol
- 3-Methyl-1-butanol
- Acetic acid
- Pentanoic acid
- Hexanoic acid

Muffa

- Octan-2-one
- 1-Octen-3-one
- 2-Heptanol
- 2-Octanol
- 1-Octen-3-ol
- 3-Methyl-1-butanol

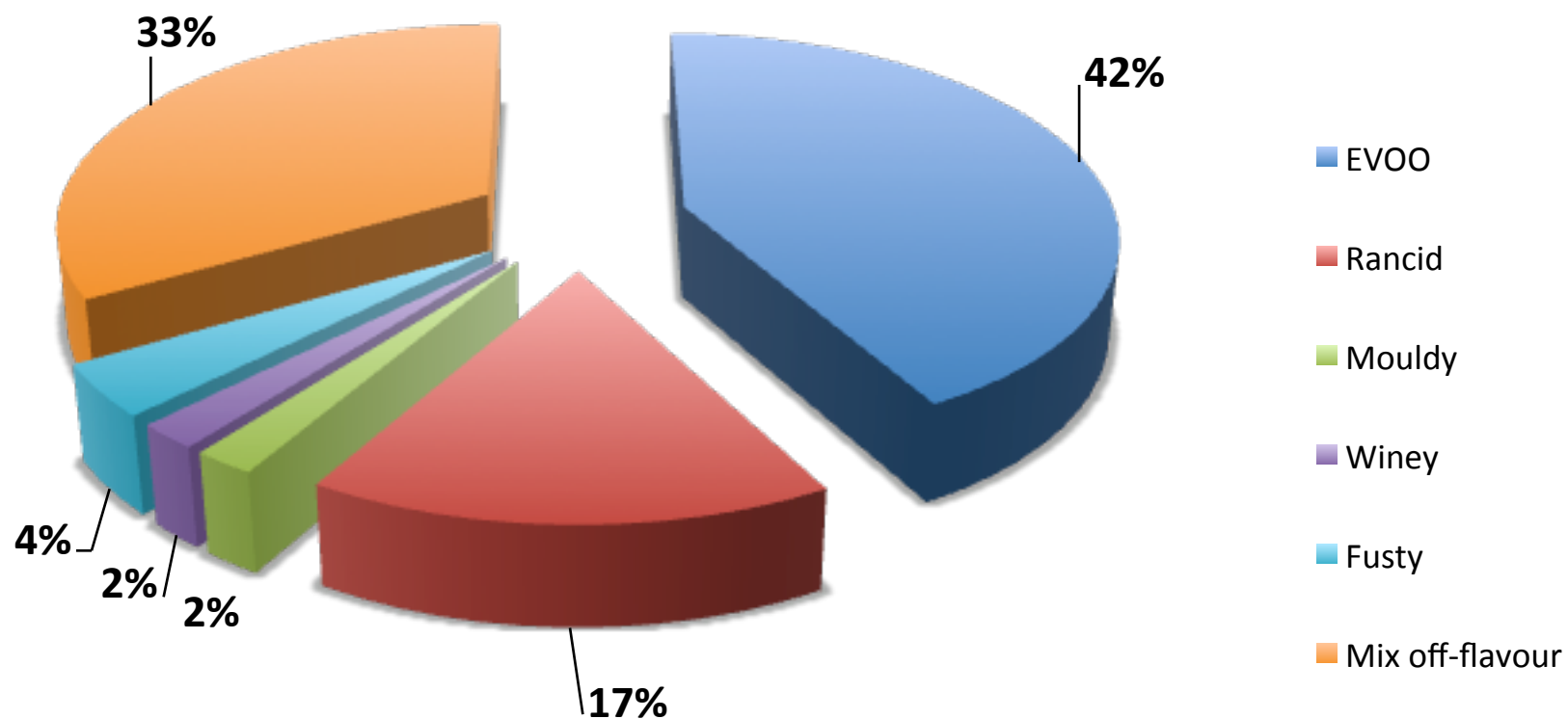
Fruttato - Verde

- 1-Penten-3-one
- E-2-Hexanal
- 1-Hexanol
- 3-Hexenyl acetate





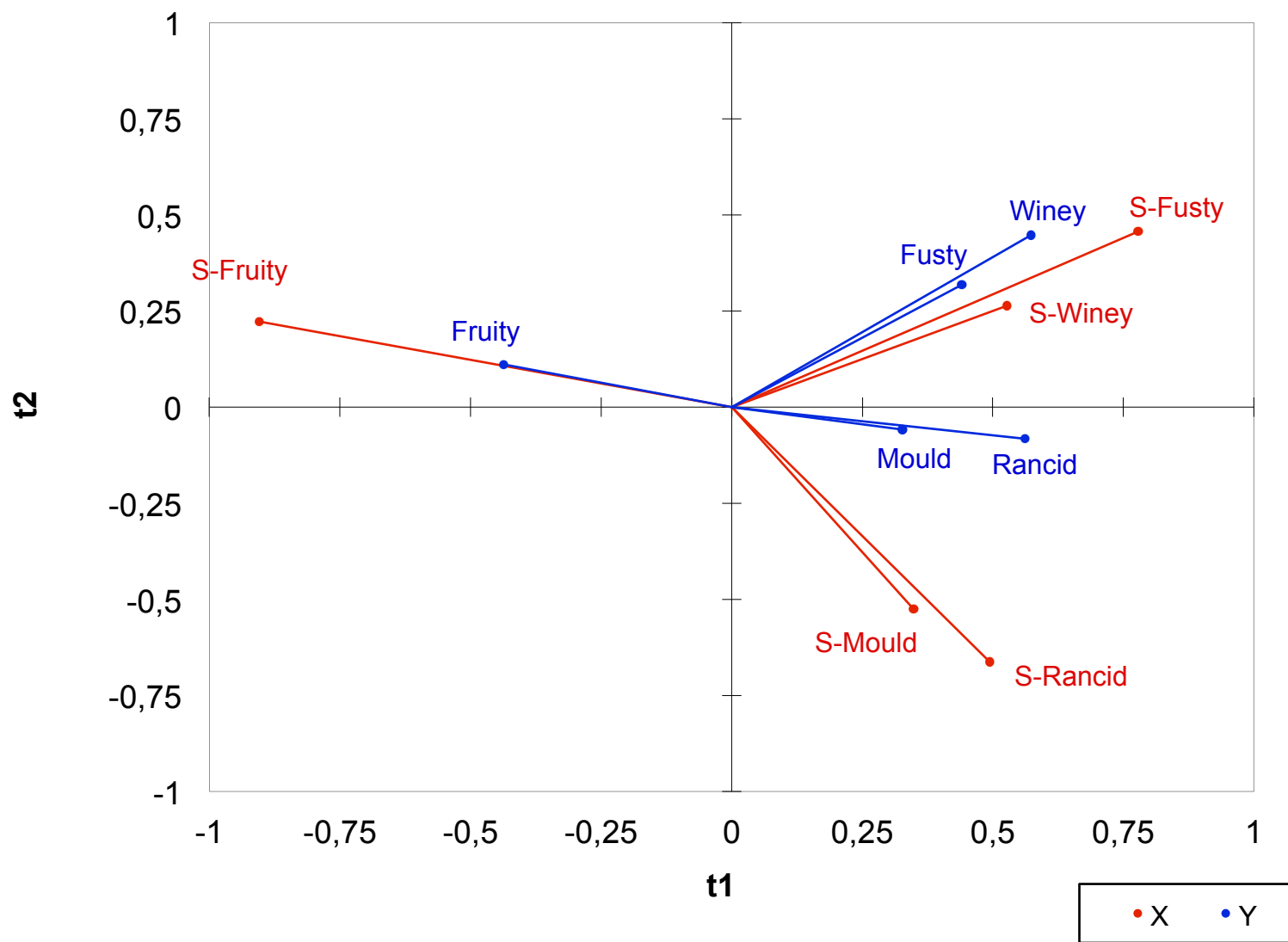
Sensory properties of 49 olive oil samples used to validate the model





PLS Analysis of aromatic series (X) vs sensory values (Y)

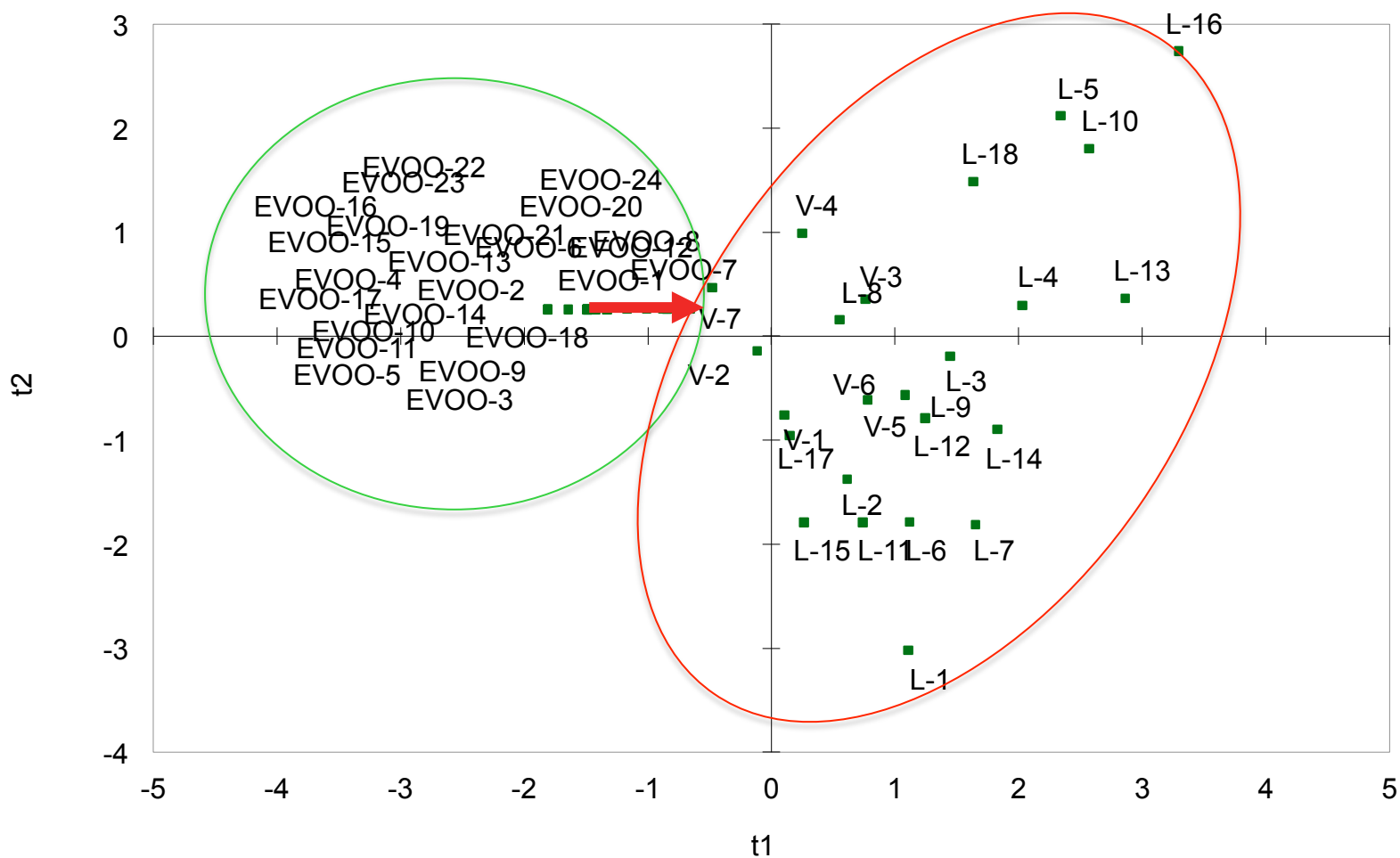
Correlazioni con t su assi t1 e t2





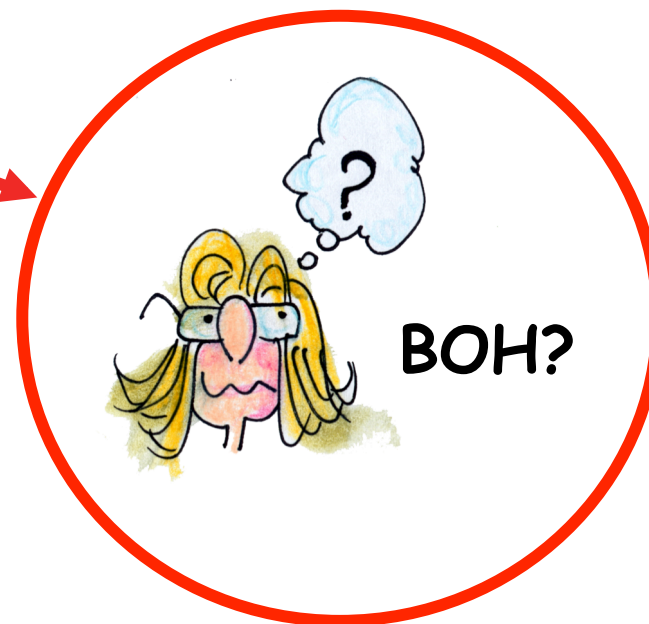
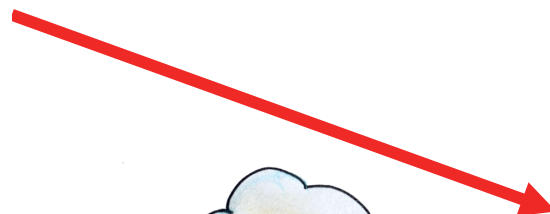
PLS of aromatic series (X) vs sensory values (Y) (samples).

Osservazioni su assi t1 e t2

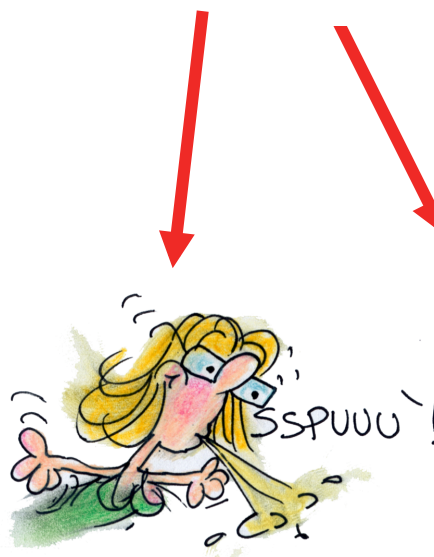




EVOO



BOH?



Lampante

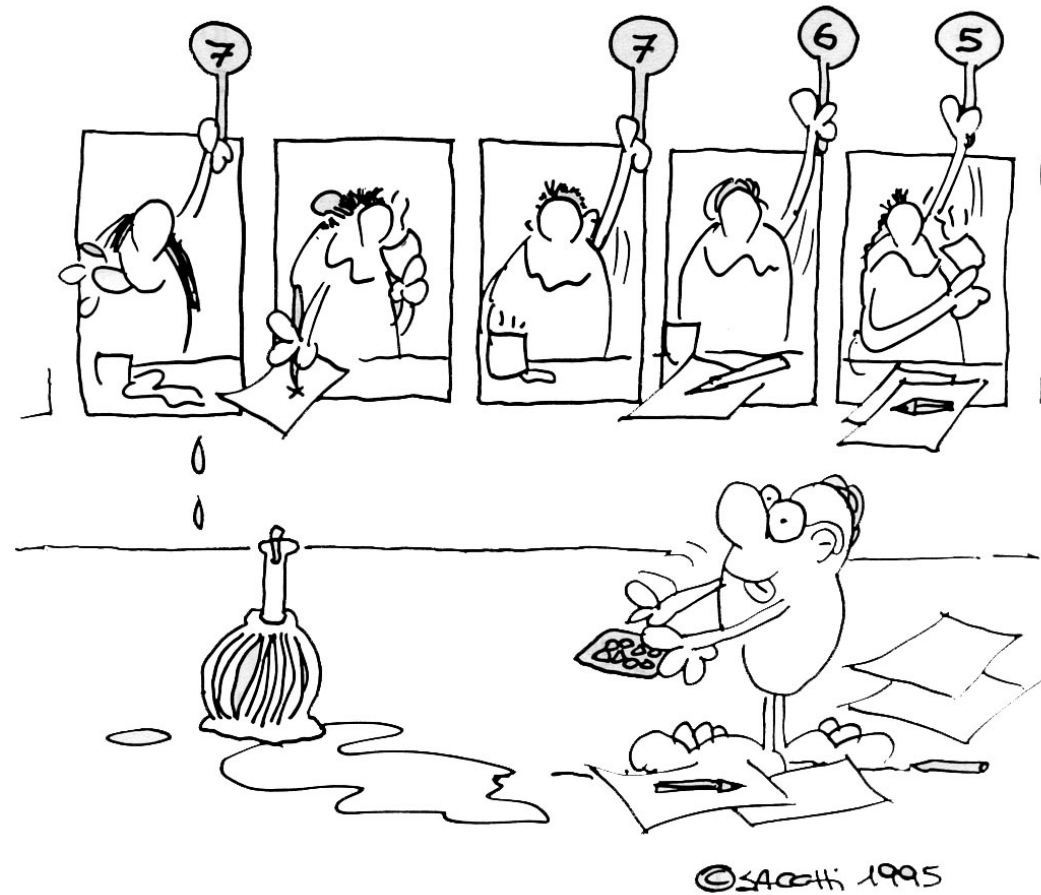


VOO

©SACCHIPISA, 2017



Panel test



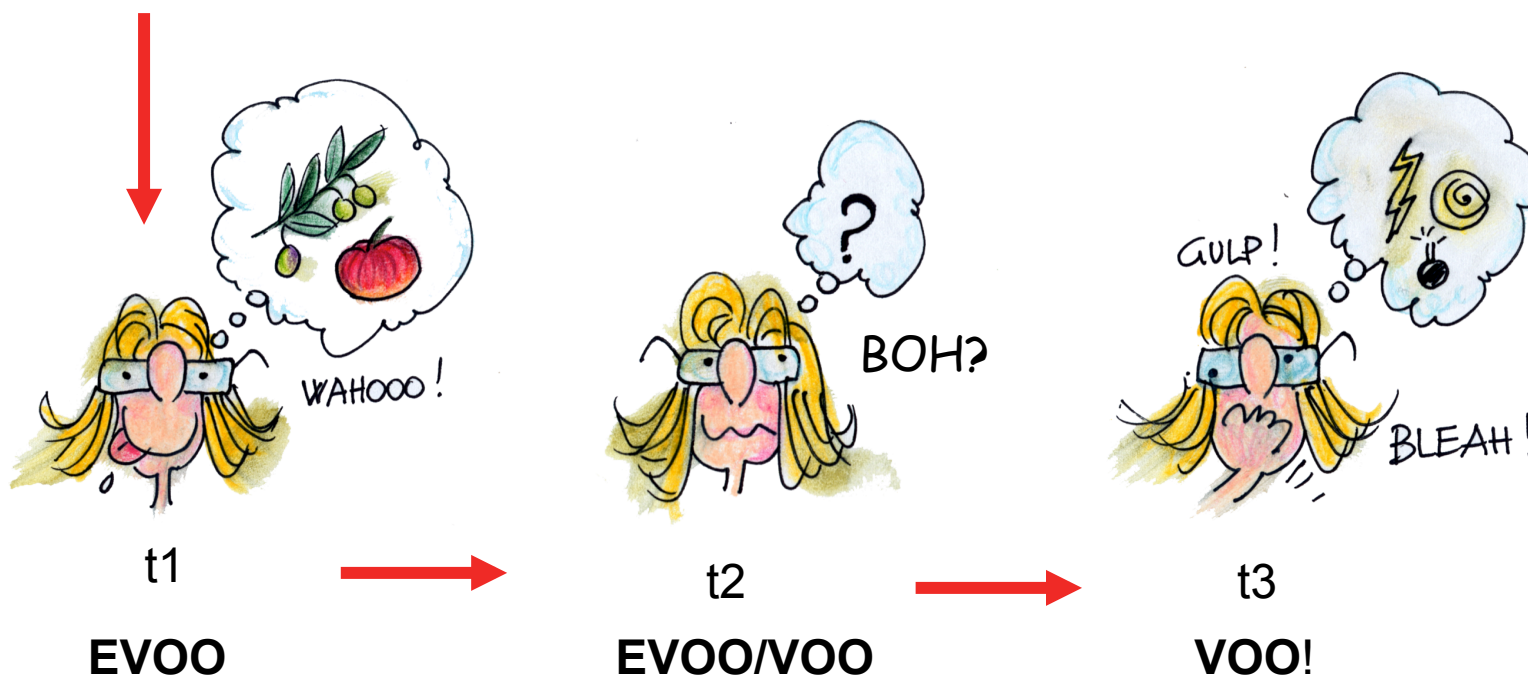
T1 MD=0 **EXTRA VERGINE**



T2 MD>0 **VERGINE**



Durante la *shelf-life* si può osservare una progressiva riduzione delle intensità del fruttato e delle note verdi, con possibile rilevazione di difetti non percepiti sugli oli appena prodotti.



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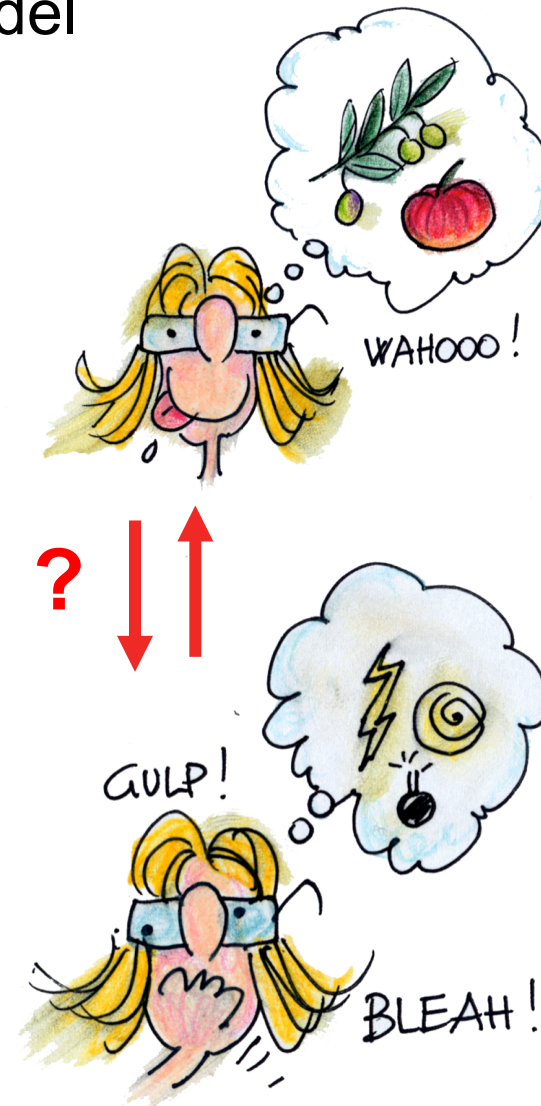
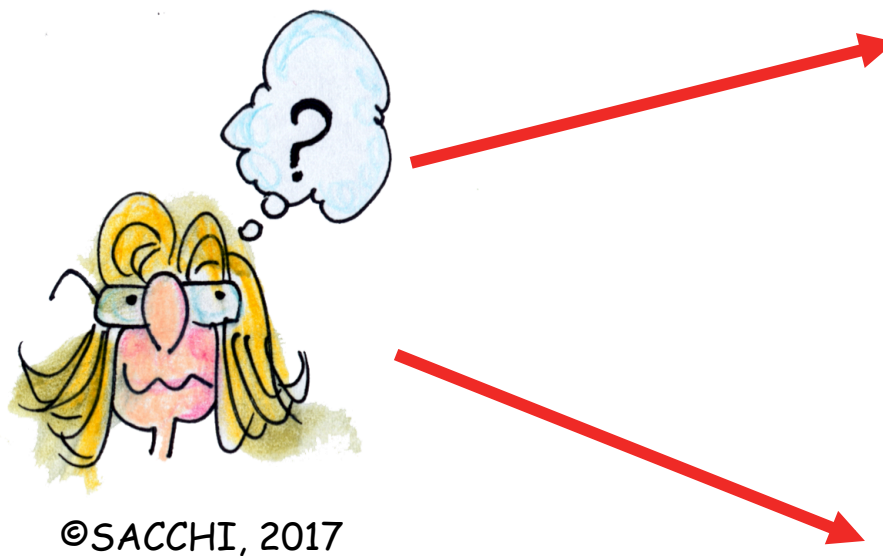


v) **effetti antagonistici e sinergici tra molecole volatili** nella percezione dei pregi e difetti dell'olio (aromi 'verdi' e difetti fermentativi)?

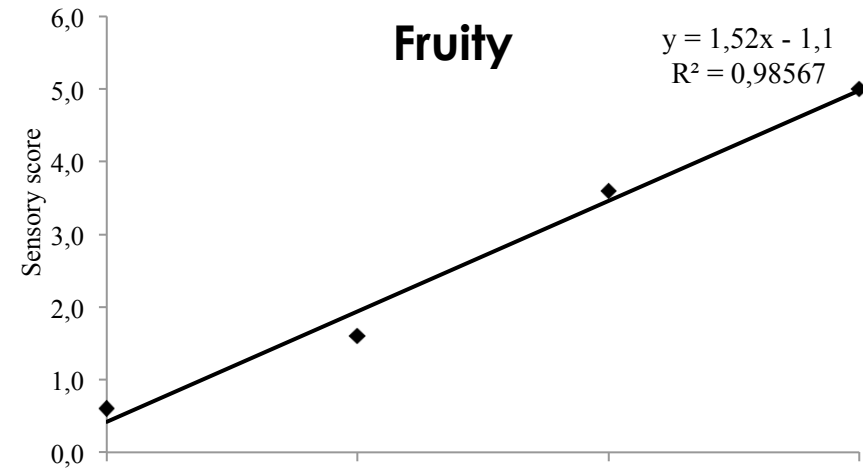
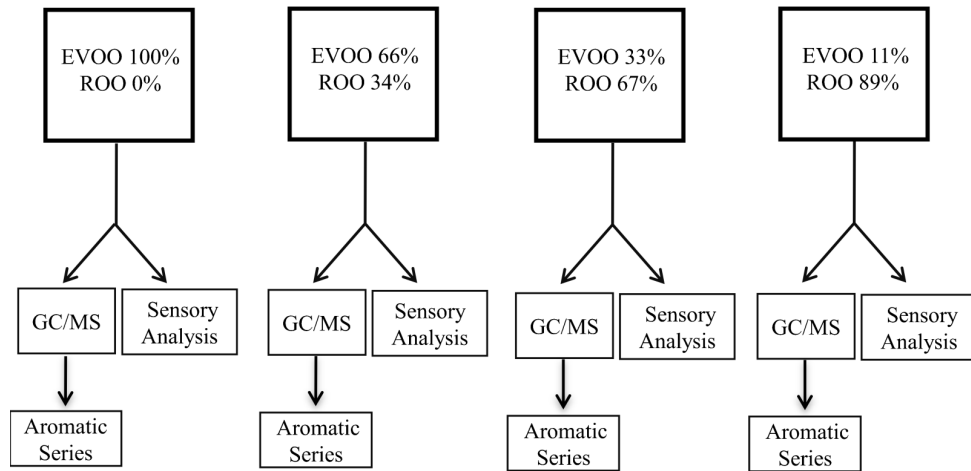


La presenza di difetti può influire sulla percezione dell'intensità del 'fruttato'?

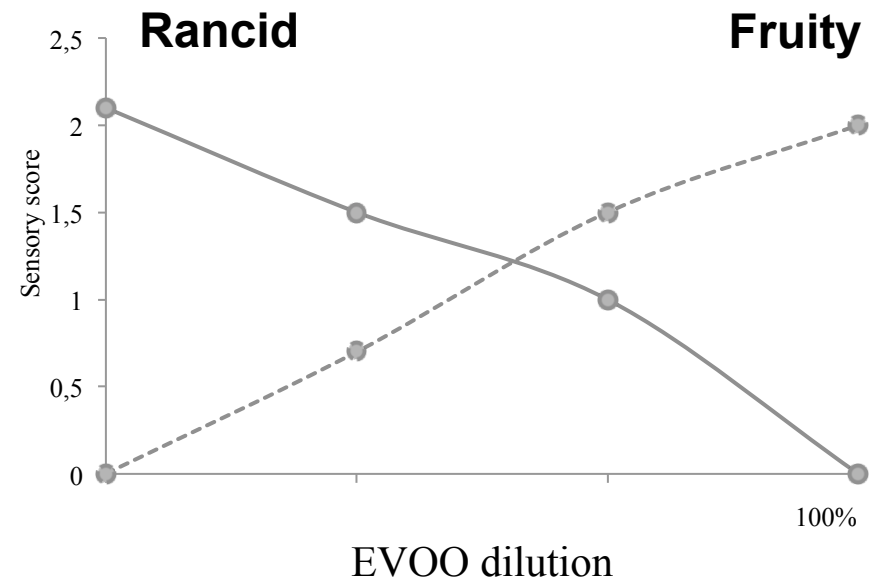
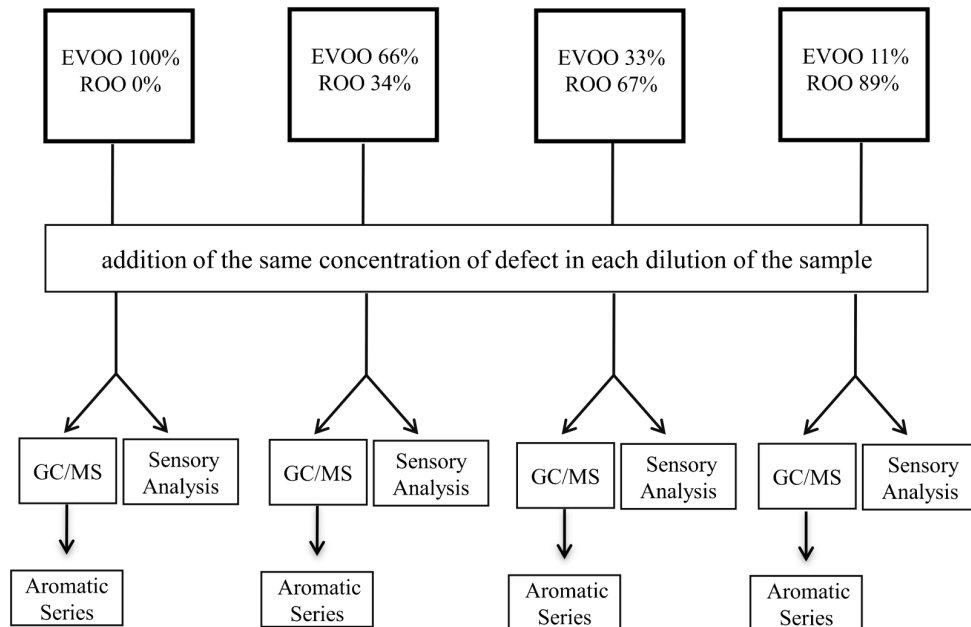
E l'intensità del 'fruttato' può mascherare i difetti?



A



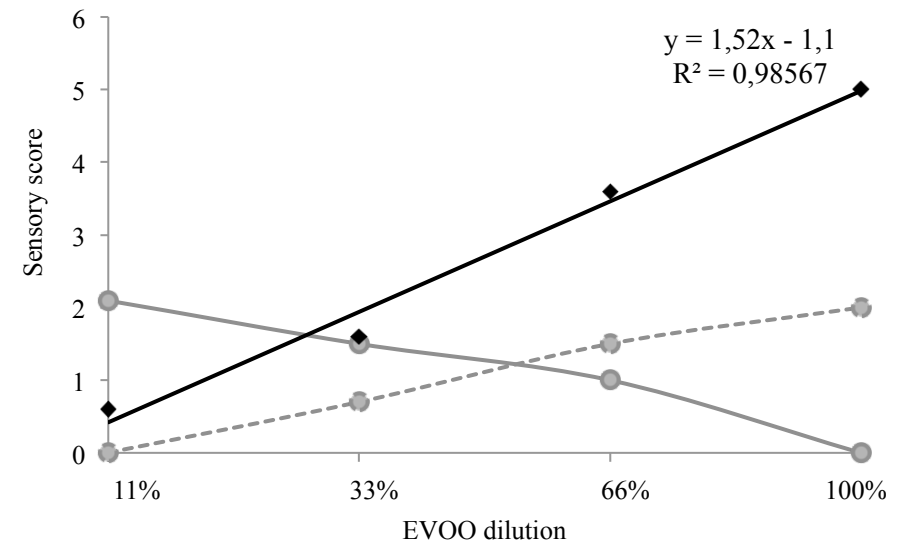
B



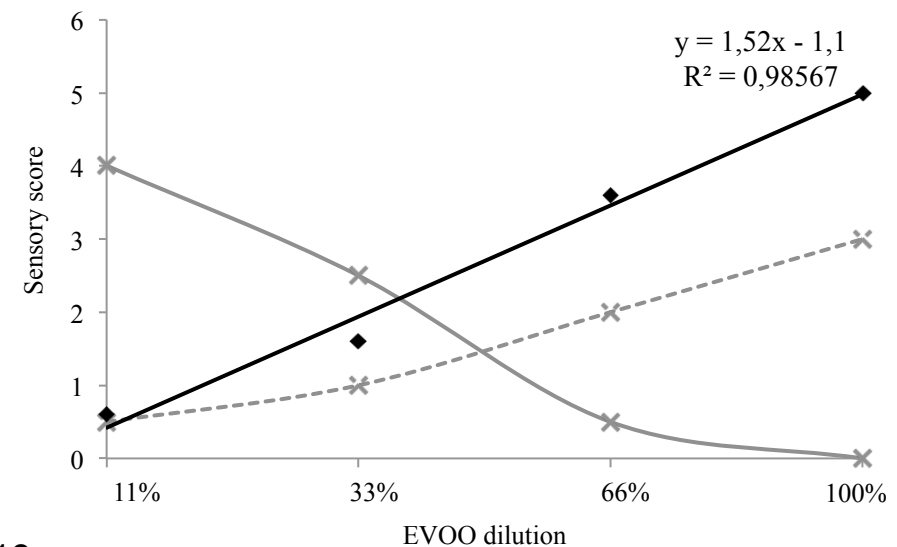


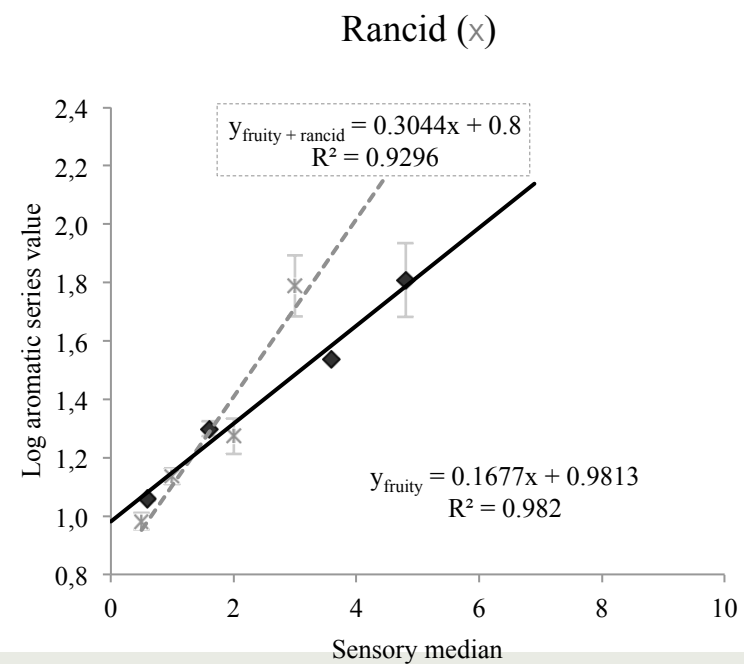
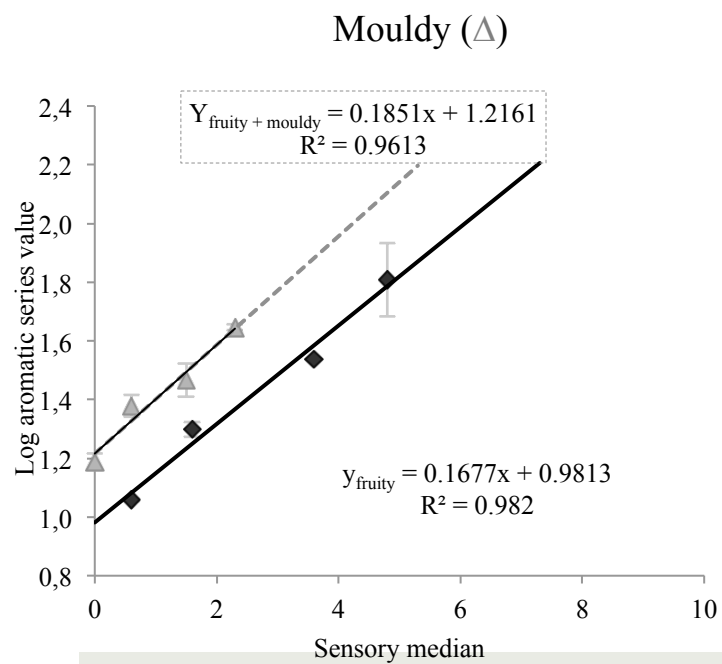
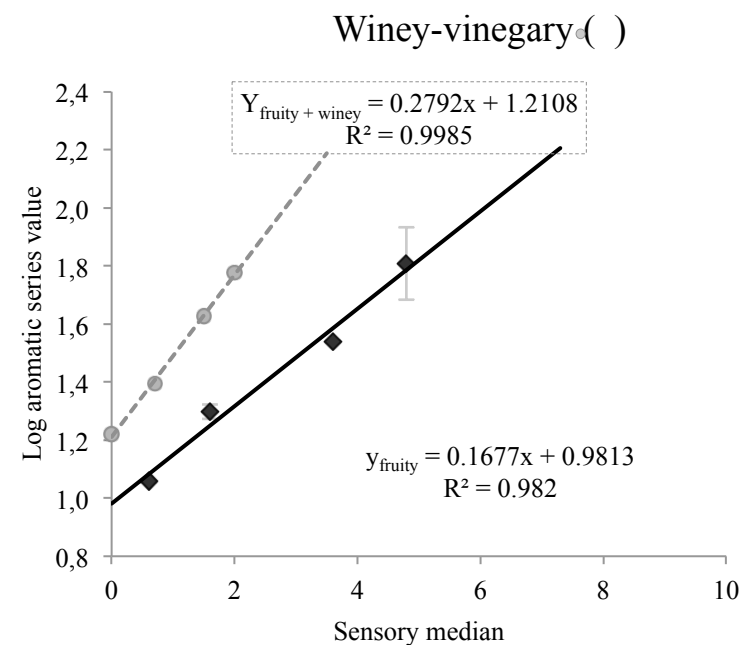
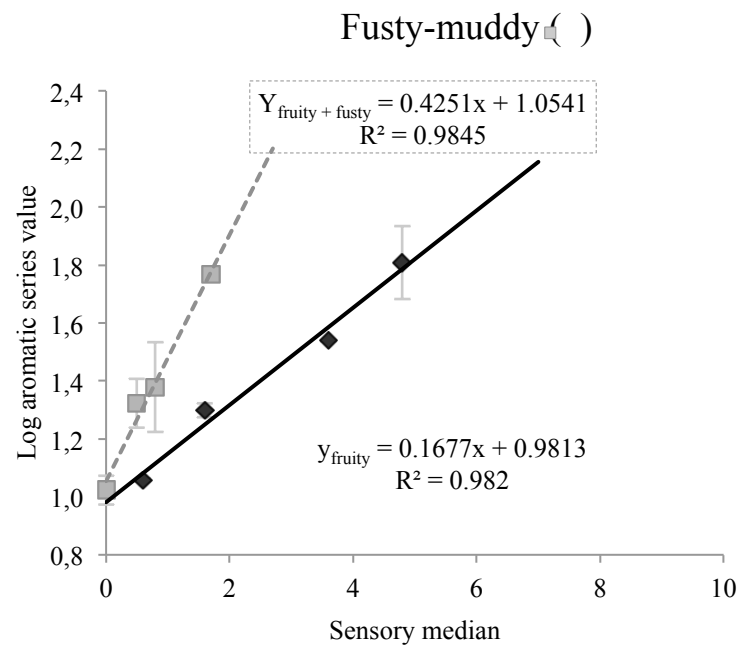
Nella risposta sensoriale si verifica un **meccanismo di antagonismo** tra intensità percepita della nota fruttata e dei difetti, con un **effetto di mascheramento** reciproco (riduzione delle intensità percepite).

Winey-vinegary (○)



Rancid (x)

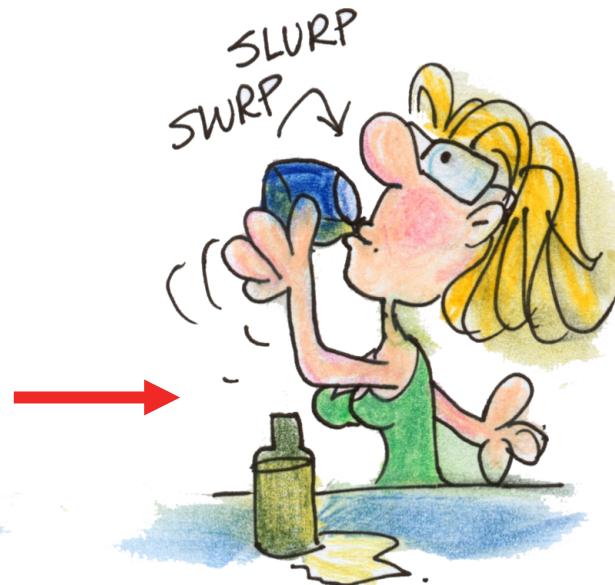




ODORE (ortonasale)



INTERAZIONI
MOLECOLE VOLATILI-**BIOFENOLI?**



AROMA (retronasale)



INTERAZIONI
AROMA-**SALIVA-BIOFENOLI?**

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vi) Interazioni aromi-saliva, saliva-fenoli e saliva-fenoli-aromi (percezione retronasale):

L'interazione con la saliva come influenza il rilascio di aroma?

Le sostanze fenoliche possono influire sul rilascio di aroma?





In vitro Retro-nasal Aroma Simulation (RAS)

SPME-GC/MS analysis (*Van Ruth et al., 2000*)...

SPME: (DVB/CAR/PDMS) (50/30 μm thickness, coating phase), 2 cm length.

Temperature: 50 °C (2min) up to 220 °C (20min) Rate: 3 °C/min.

Ion source/Analyzer: EI 70 eV (230 °C)/ Quadrupole (150 °C)

Column: Supelcowax-10 (60m, 0.32mm, 0.5 μm ; Supelco Co.)

Instruments: GC/MS: QP5050A GC + 5973N MS (Agilent Technologies)
GC: 4890D (Agilent Technologies)

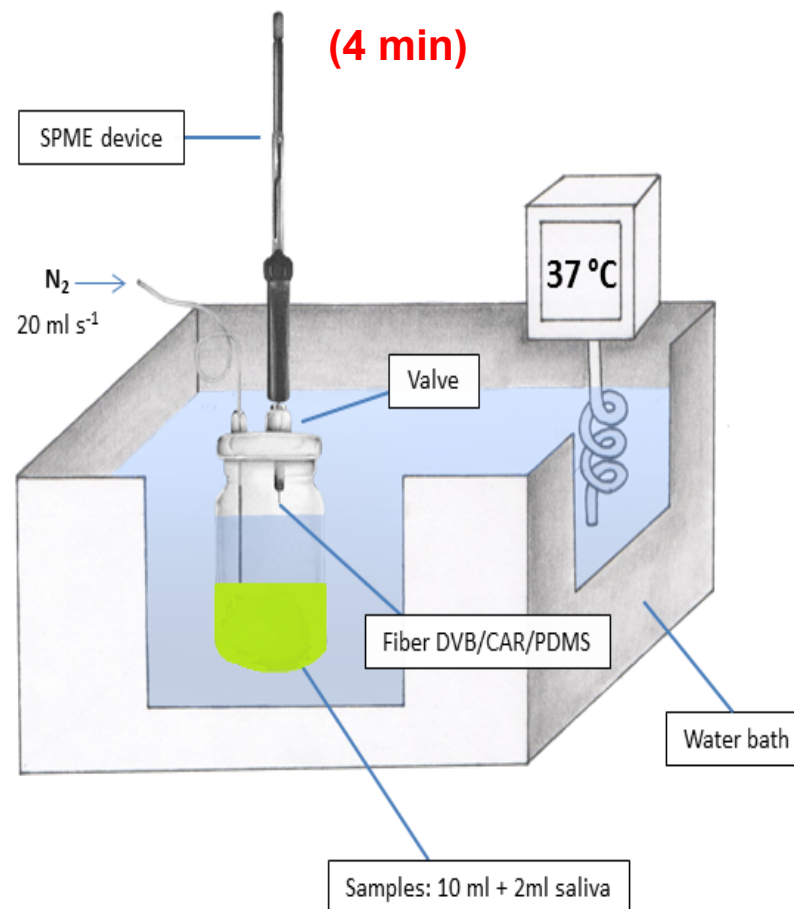
Injector: Split-splitless open at 11' and close at 25' (250°C)

Statistical analysis: Tukey test, $p < 0.05$ using XLSTAT (version 2009.3.02), using a packet add-in software for Microsoft Excel (Addinsoft Corp., Paris, France).



... coupled with RAS to simulate the retronasal aroma release

(4 min)





L'effetto della saliva sul rilascio di aroma dell'OEVO dipende anche dalla variabilità individuale, da diverse condizioni fisiologiche anche nello stesso individuo e, ad esempio, **dal BMI** (normopeso, NW; sovrappeso, OW; obesi, O).

(Genovese et al, 2018, *J.Sci.Food.Agric*)



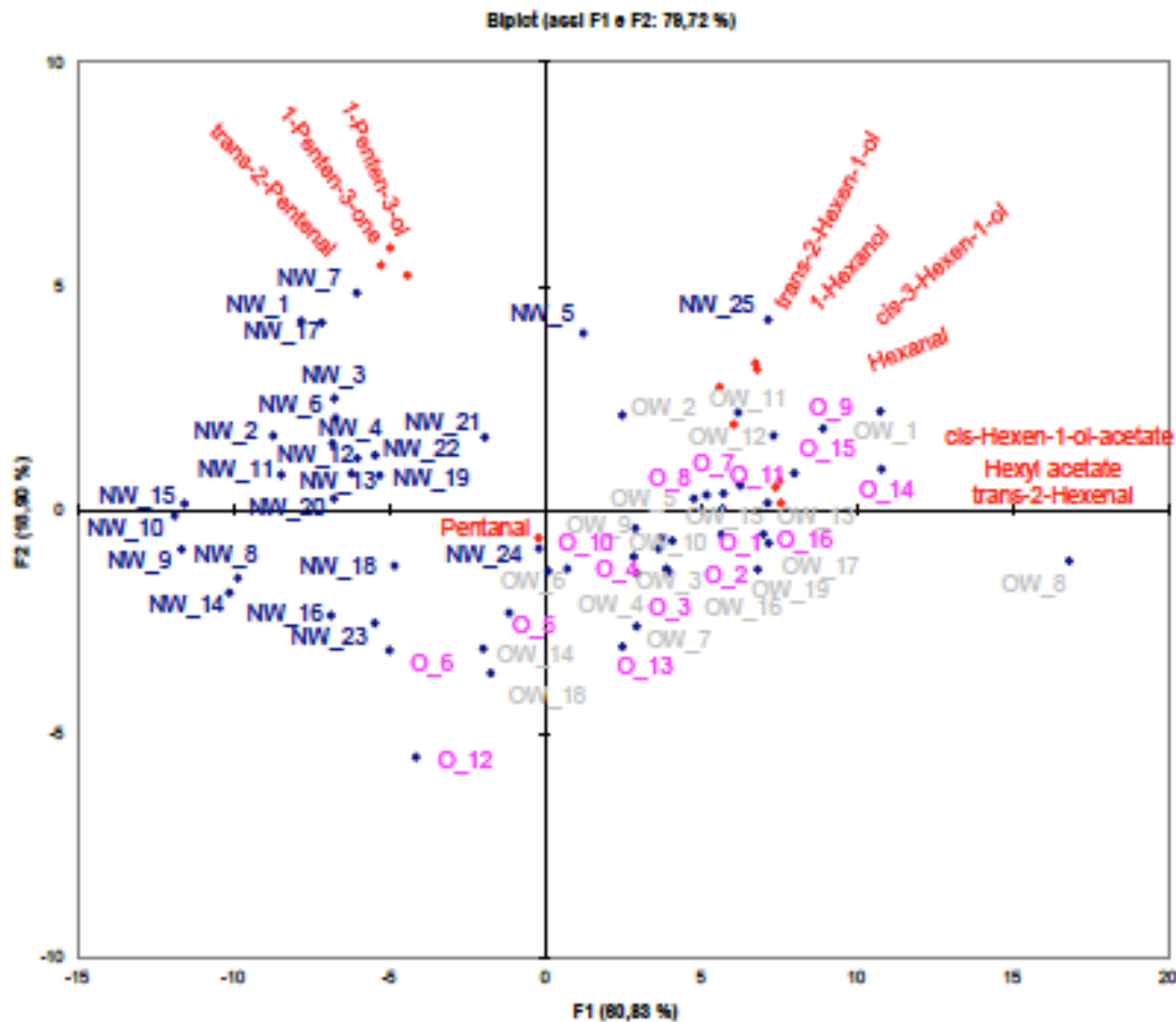
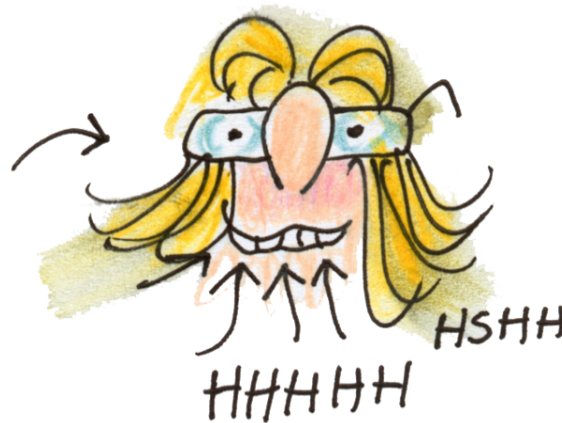


Fig.4.2 - Analisi delle componenti principali (PCA) relativa ai componenti volatili dell'EVOO rilasciati dopo interazione con la saliva di soggetti O, OW e NW.

Come percepiamo l'aroma retronasale dell'Olio?

In relazione all'**equilibrio dei componenti volatili** tra matrice lipica, fase acquosa (saliva), aria, mucosa e superficie dei recettori:

lipidic matrix $\longleftrightarrow$ aqueous matrix $\longleftrightarrow$ air $\longleftrightarrow$ mucosa $\longleftrightarrow$ receptor



Le sostanze fenoliche possono interagire con la saliva, influenzando questo equilibrio?



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Olive oil phenolic compounds affect the release of aroma compounds



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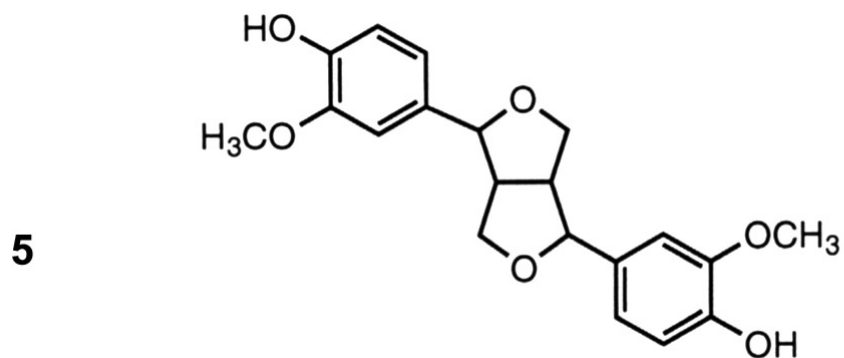
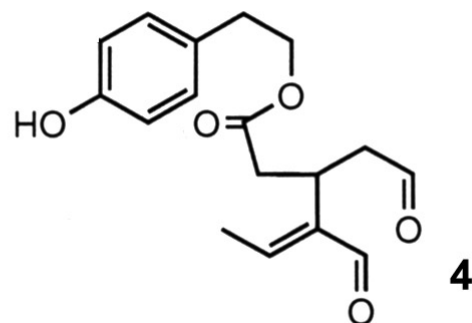
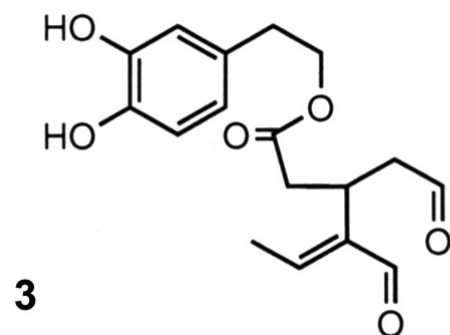
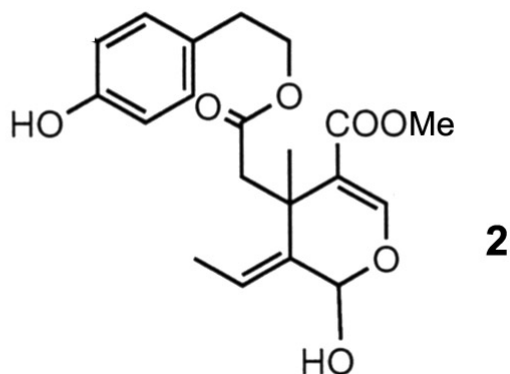
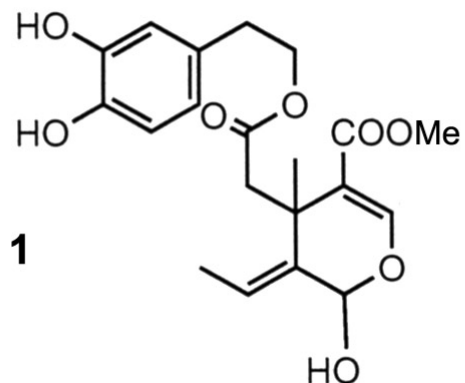
RAS

Volatile compounds

ABSTRACT

Twelve aroma compounds were monitored and quantified by dynamic headspace analysis after their addition in refined olive oil model systems with extra virgin olive oil (EVOO) biophenols to simulate EVOO aroma. The influence of polyphenols on aroma release was studied under simulated mouth conditions by using human saliva, and SPME–GC/MS analysis. While few differences were observed in orthonasal assay (without saliva), interesting results were obtained for retronasal aroma. Biophenols caused generally the lowest headspace release of almost all volatile compounds. However, only ethyl esters and linalool concentrations were significantly lower in retronasal than orthonasal assay. Saliva also caused higher concentration of hexanal, probably due to hydroperoxide lyase (HPL) action on linoleyl hydroperoxides. Epicatechin was compared to EVOO phenolics and the behaviour was dramatically different, likely to be due to salivary protein–tannin binding interactions, which influenced aroma headspace release. These results were also confirmed using two extra virgin olive oils.

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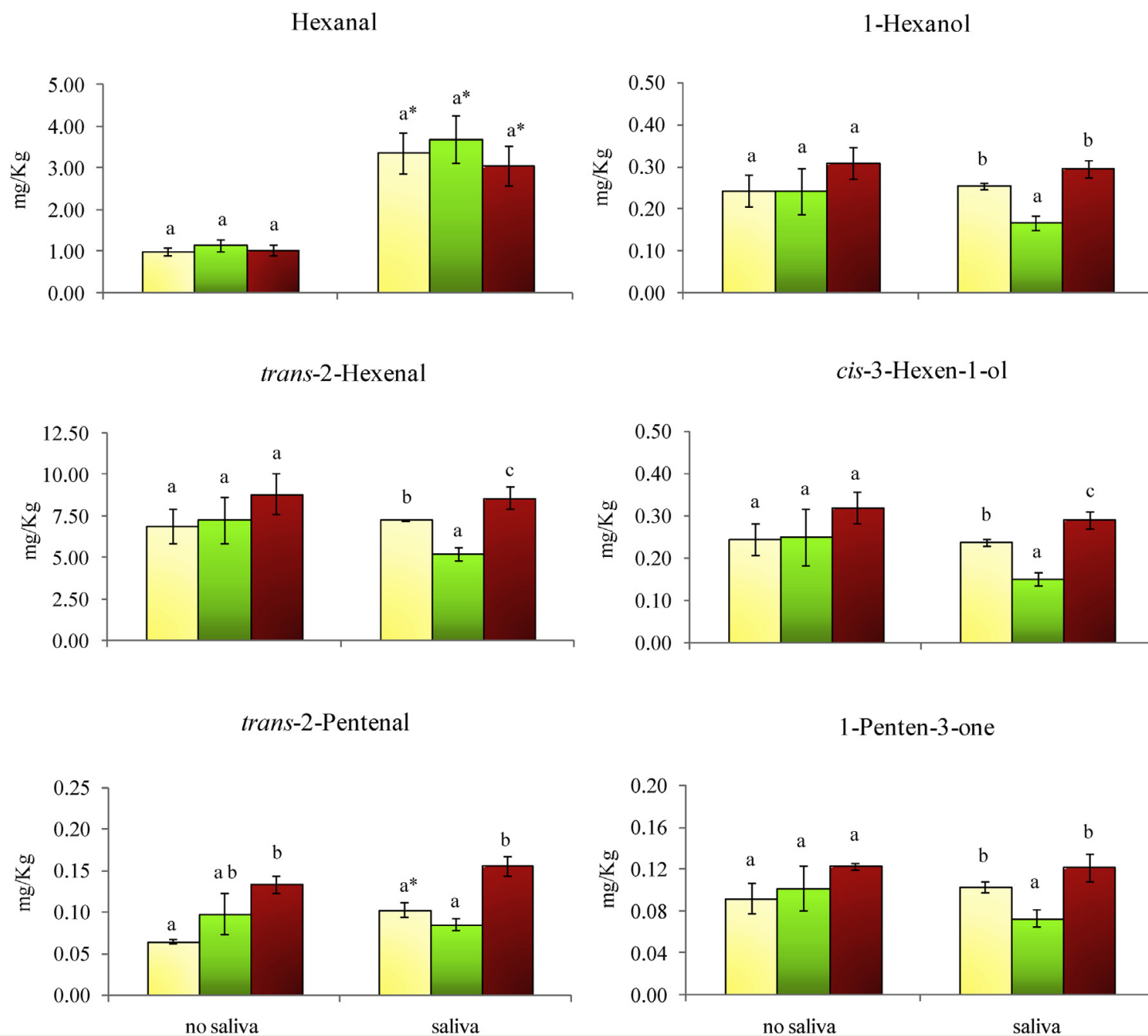
I biofenoli
dell'OEVO:
nutraceutici
...AMARI*

* Progetto AGER 2 COMPETITIVE

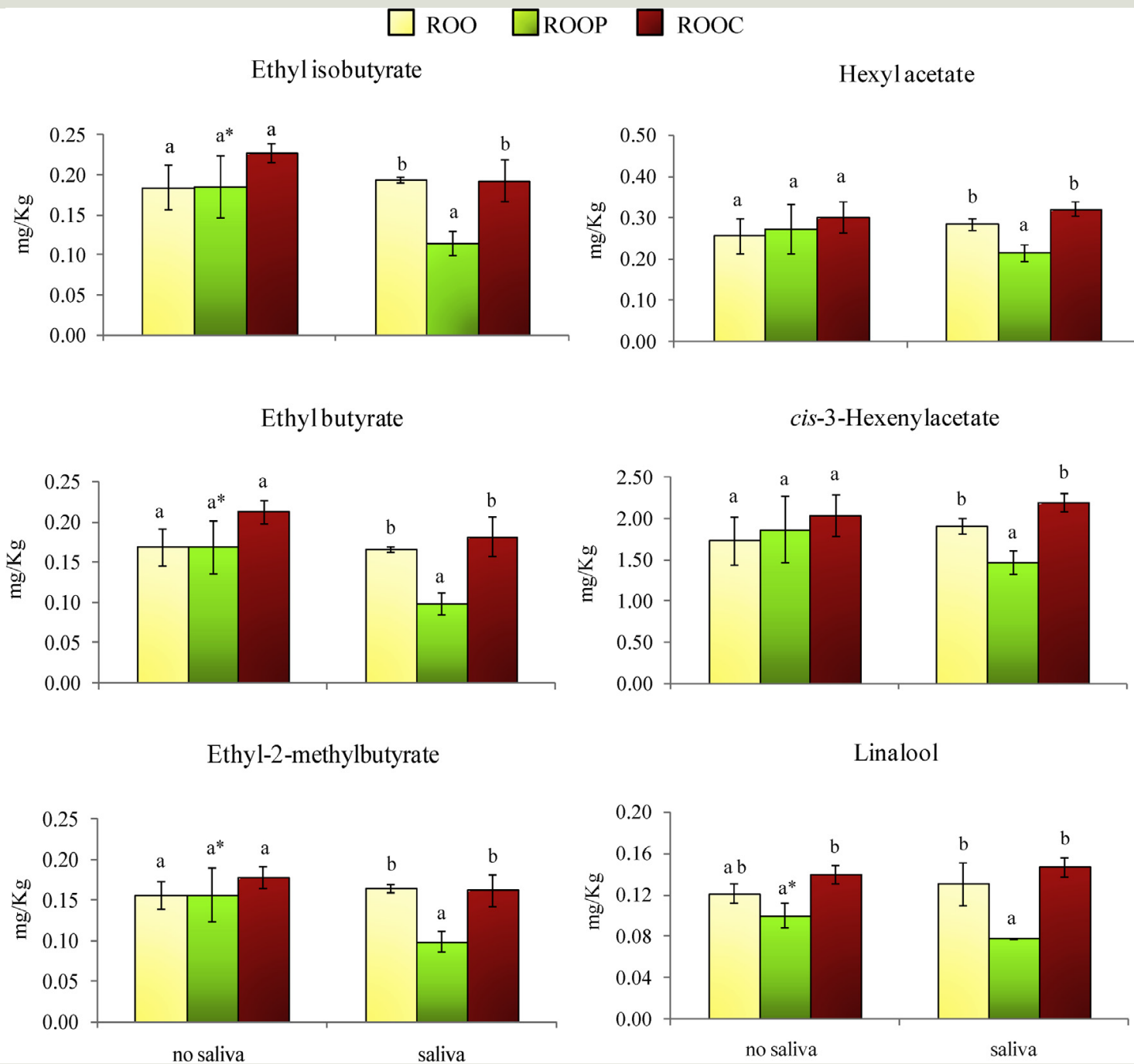


Headspace concentration of volatiles in model systems of refined olive oil (**ROO**), ROO added with VOO phenolic compounds (**ROOP**), and with added epicatechin (**ROOC**).

ROO ROOP ROOC



Headspace concentration of volatiles in model systems of refined olive oil (**ROO**), ROO added with VOO phenolic compounds (**ROOP**), and with added epicatechin (**ROOC**).





Effetto delle sostanze fenoliche sul
rilascio di aroma dell'olio, studiato
mediante **APCI-MS *in vitro* ed *in*
*vivo*** .

(Genovese et al, 2018, *in press*)





Ruolo dei Composti Fenolici sul Rilascio di Aroma Dell'olio Extravergine di Oliva

Role of Phenolic Compounds on Olive Oil Aroma Release

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THE ROLE OF PHENOLIC COMPOUNDS AND SALIVA ON OLIVE OIL AROMA RELEASE

2016-2017



In vitro APCI-MS dello spazio di testa dinamico



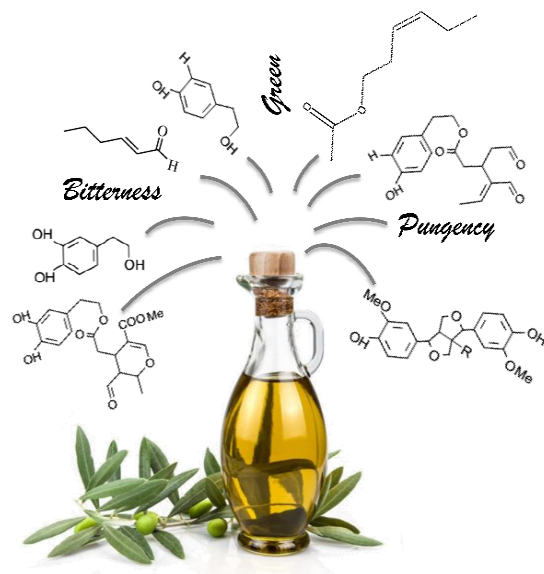


In vivo APCI/MS



Two concentrations of phenolics and one of aroma compounds were evaluated by **APCI-MS analysis**.

Soluzione modello in olio di oliva



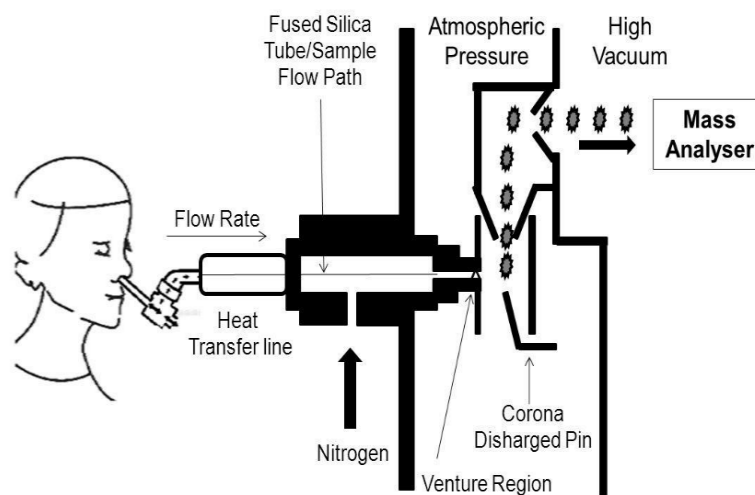
Sistemi modello in olio di oliva con diversa concentrazione di composti fenolici ed 8 componenti volatili:

P- (0 mg kg⁻¹)

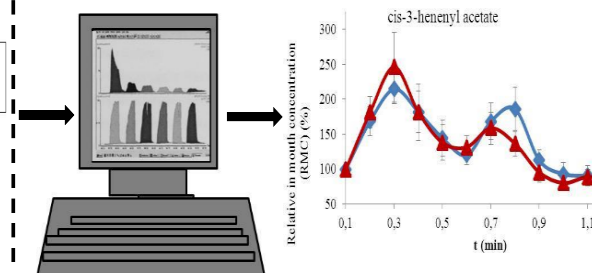
P+ (354 mg kg⁻¹)

P++ (593 mg kg⁻¹)

APCI-MS



Curva del rilascio di aroma



MS Nose interface: Micromass, Manchester, UK) fitted to a Quattro Ultima mass spectrometer (Milford, Waters)

Flow rate: 5 ml/min e (*in-vitro*) 40 ml/min (*in-vivo*)

Temperatura: Transfer line = 100 °C
sorgente ionica=75 °C

Corona discharge: 4kV

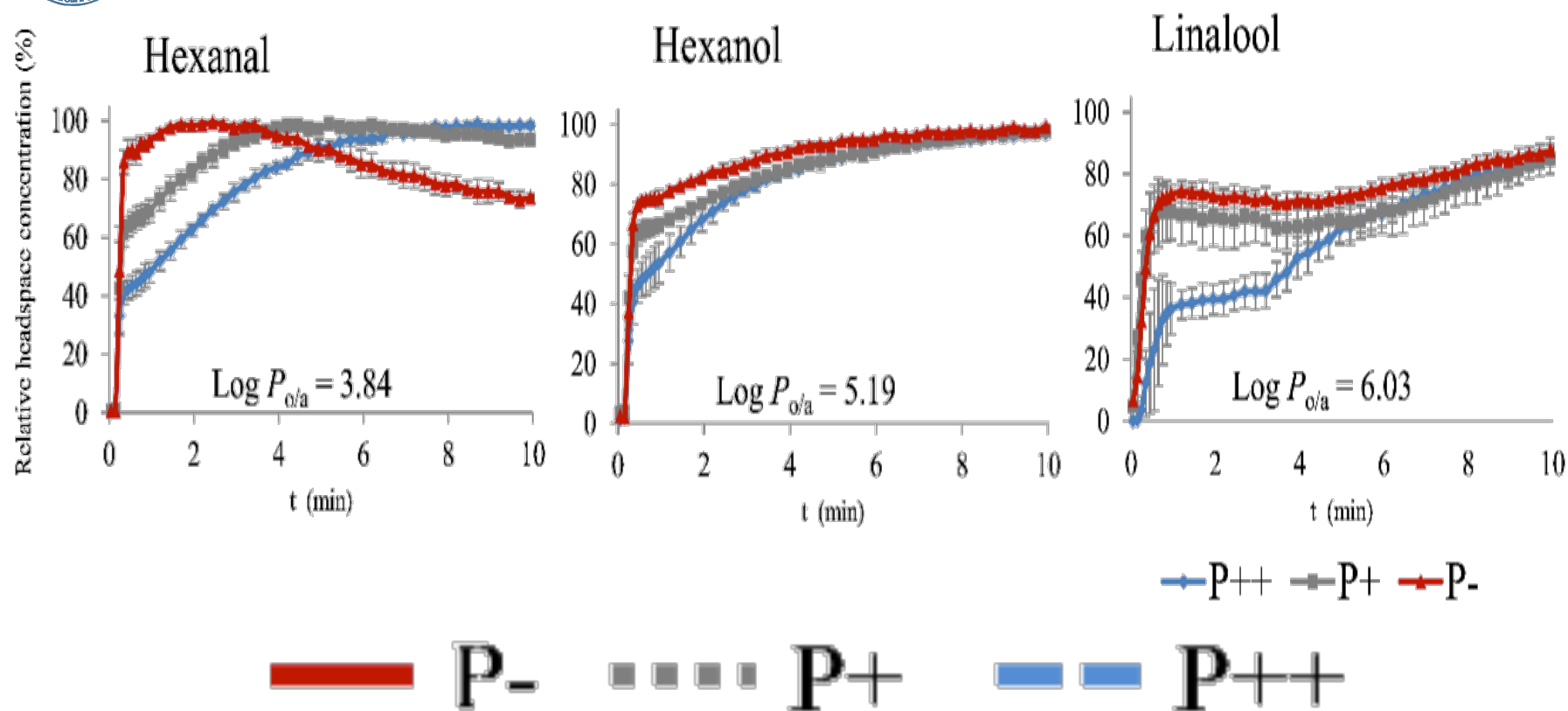
Cone voltage: 15V

Dwell time: 0,2 s (*in-vitro*) e 0,01 s (*in-vivo*)



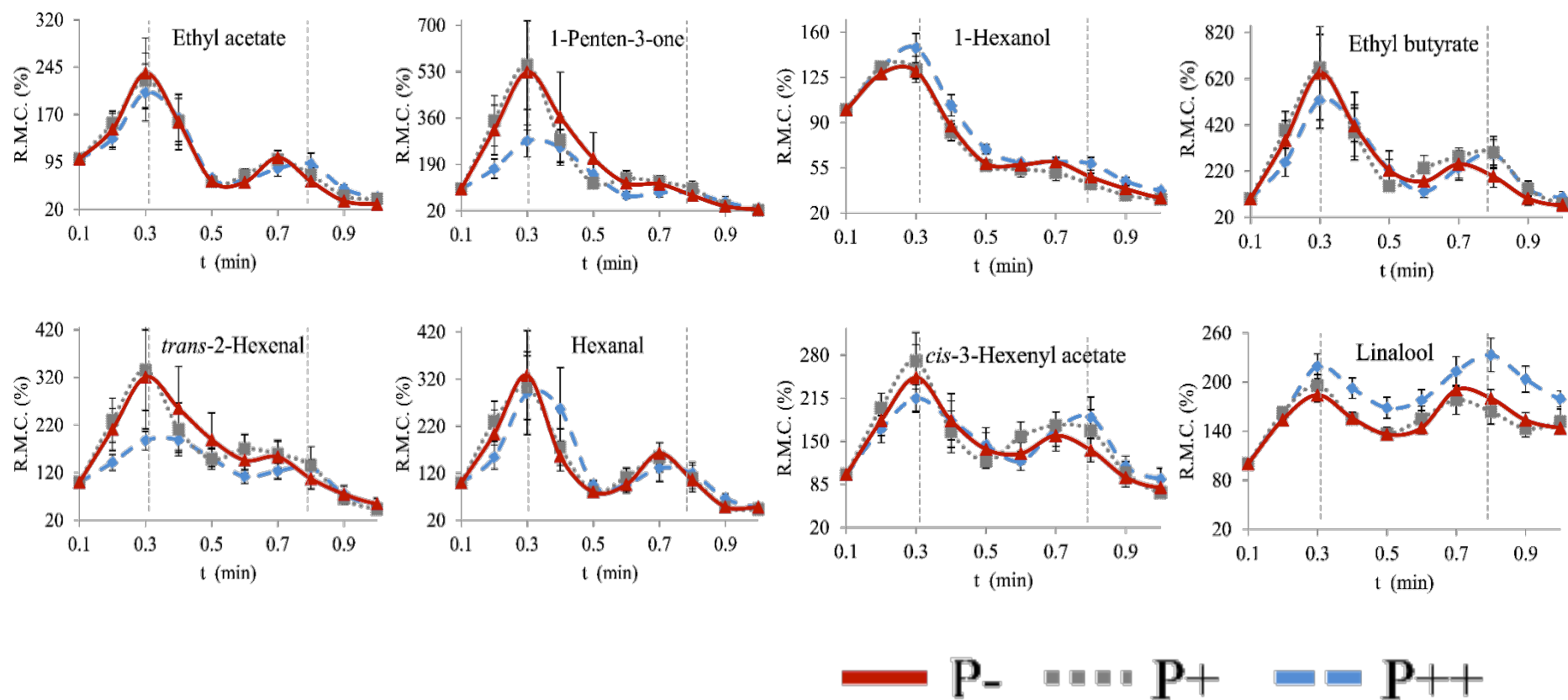
In questo studio è stato valutato **l'effetto dei composti fenolici sul rilascio di alcuni composti volatili** dell'OEVO mediante analisi APCI-MS.

Otto componenti volatili sono stati aggiunti e monitorati in condizioni dinamiche, sia *in-vitro* che *in-vivo*, in un olio di oliva rettificato, deodorato di fresco **con (P+, P++) e senza (P-)** l'aggiunta dei composti fenolici estratti in precedenza da un OEVO.



Spazio di testa dinamico di esanale (test *in-vitro*), 1-esanolo e linalolo nei sistemi modello di olio di oliva P++ (593 mg kg⁻¹), P+ (354 mg kg⁻¹) e P- (0 mg kg⁻¹). Ciascun punto è la media di 4 repliche, la barra di errore indica la deviazione standard.

I composti fenolici possono interagire con esanale, linalolo e 1-esanolo, probabilmente con legami non covalenti reversibili.



Curve del rilascio di aroma in tempo reale (test *in-vivo*) dei componenti volatili nei sistemi modello di olio di oliva P++ (593 mg kg⁻¹), P+ (354 mg kg⁻¹) e P- (0 mg kg⁻¹). Le linee tratteggiate a 0,3 e 0,8 min indicano rispettivamente la fase di deglutizione del campione e della saliva. Ciascuna curva è la media di nove ripetizioni. La barra di errore indica la deviazione standard.



Lo studio *in-vivo* ha dimostrato che, dopo la deglutizione, in presenza di fenoli il rilascio di 1-penten-3-one, *trans*-2-esenale, *cis*-3-esenil acetato ed etil butirrato è inferiore rispetto quello determinato per il campione di olio privo di fenoli.

Al contrario, il linalolo e l'1-esanolo hanno mostrato un effetto *salting-out*.

I composti fenolici dell'OEVO causano una maggiore persistenza in bocca dei composti idrofobi come linalolo e *cis*-3-esenil acetato.

Questo potrebbe portare ad una **maggiore intensità odorosa, percepita durante l'assaggio di un OEVO ricco di biofenoli, dei composti idrofobi rispetto a quelli idrofili.**

E' possibile ipotizzare una **diversa percezione del flavour e degli off-flavour in presenza di un diverso livello di biofenoli.**

UNIVERSITA' DEGLI STUDI DI NAPOI FEDERICO II



MASTER'S DEGREE IN FOOD SCIENCE AND TECHNOLOGY
DEPARTMENT OF AGRICULTURE

ROLE OF PHENOLIC COMPOUNDS ON POSITIVE AND NEGATIVE EXTRA VIRGIN OLIVE OIL OLFACTORY ATTRIBUTES

Supervisor
Ch.mo Prof.
Alessandro Genovese

Assistant Supervisor
Ch.mo Prof.
Raffaele Sacchi

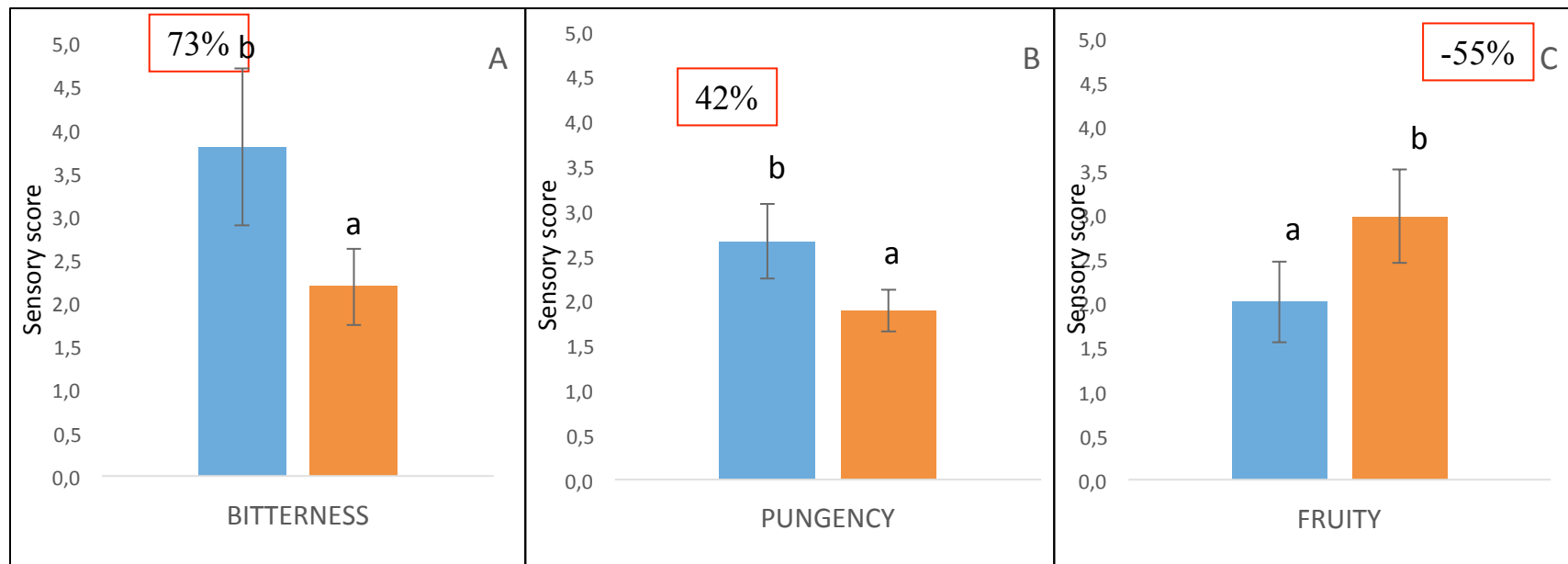
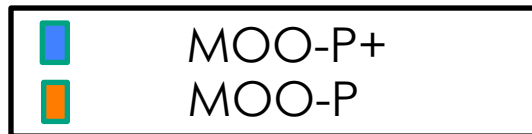
Candidate:
Ferdinando Mondola
N06/617

PORTICI, 23 ottobre 2017

© R. SACCHI, Progetto ARISTOIL, Ragusa, 7 giugno 2018



Interazioni biofenoli-proteine della saliva: effetto di mascheramento delle note aromatiche.

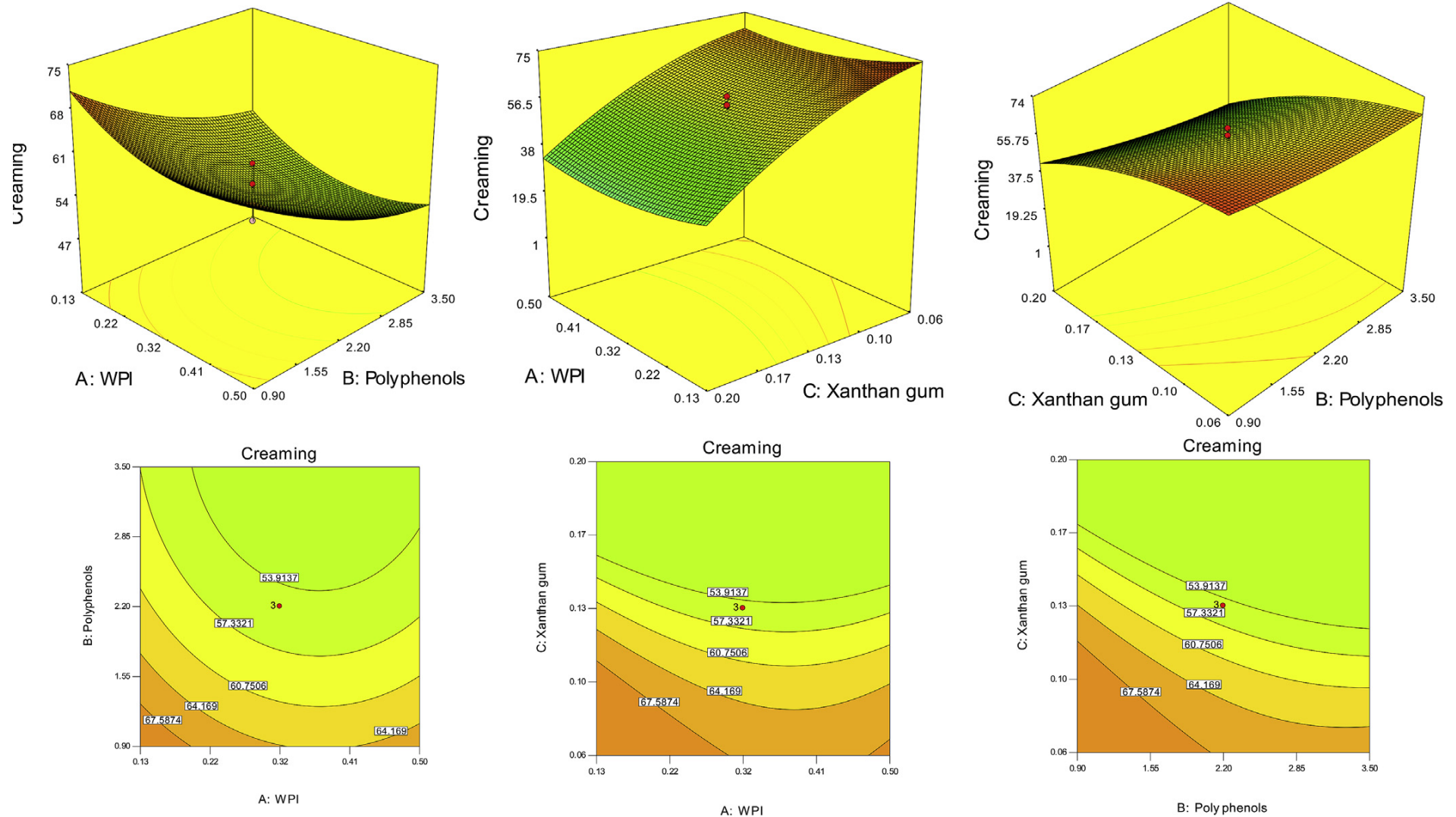


Average values of bitterness (A), pungency (B) and fruitiness (C) attributes as evaluated by 3 IOOC panels in a model EVOO (MOO-P) and in the same sample furtherly added of phenols (MOO-P+).



L'aroma 'mandorlato' della *Coratina*, 'erbaceo' della *Tonda Iblea*, di 'carciofo' e la 'foglia' del Frantoio, di 'Pomodoro verde' della *Ravece* etc., non dipende solo dalla **composizione delle sostanze volatili**, ma anche dal particolare **equilibrio con i biofenoli** che – oltre a determinare il gusto amaro/piccante dell'OEVO – possono mascherare o modificare l'impatto sensoriale di alcuni aromi influenzando sul loro rilascio nel cavo orale.

Alla base di ciò ci sono le interazioni dei biofenoli con le proteine della saliva, con le molecole volatili, ma anche quelle con le **matrici alimentari** con cui l'olio è combinato (es: proteine del siero di latte, WPI).



Response surface plots for emulsions creaming rate (%) showing the interaction effects between (a) WPI, (b) OMW phenolic compounds and (c) xanthan (Caporaso et al, 2016 (*Food Hydrocolloids*)).

vii) qualità nutrizionale dell'OEVO ed effetto saziante di alcune molecole aromatiche.



Cite this: *Food Funct.*, 2015, 6, 204

Oleic acid content of a meal promotes oleoylethanolamide response and reduces subsequent energy intake in humans

Ilario Mennella, Maria Savarese, Rosalia Ferracane, Raffaele Sacchi and Paola Vitaglione*

Animal data suggest that dietary fat composition may influence endocannabinoid (EC) response and dietary behavior. This study tested the hypothesis that fatty acid composition of a meal can influence the short-term response of ECs and subsequent energy intake in humans. Fifteen volunteers on three occasions were randomly offered a meal containing 30 g of bread and 30 mL of one of three selected oils: sunflower oil (SO), high oleic sunflower oil (HOSO) and virgin olive oil (VOO). Plasma EC concentrations and appetite ratings over 2 h and energy intake over 24 h following the experimental meal were measured. Results showed that after HOSO and VOO consumption the circulating oleoylethanolamide (OEA) was significantly higher than after SO consumption; a concomitantly significant reduction of energy intake was found. For the first time the oleic acid content of a meal was demonstrated to increase the post-prandial response of circulating OEA and to reduce energy intake at subsequent meals in humans.

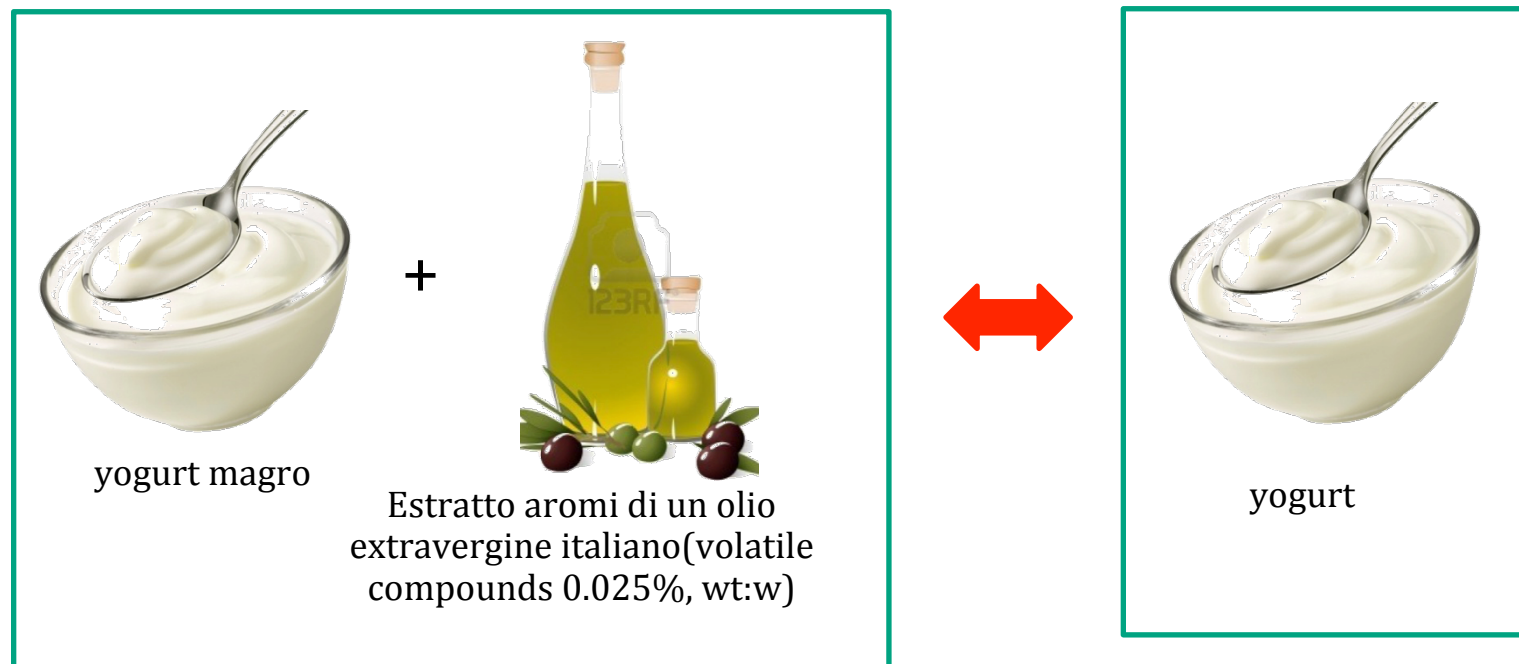
Received 4th August 2014,
Accepted 30th September 2014

DOI: 10.1039/c4fo00697f

www.rsc.org/foodfunction

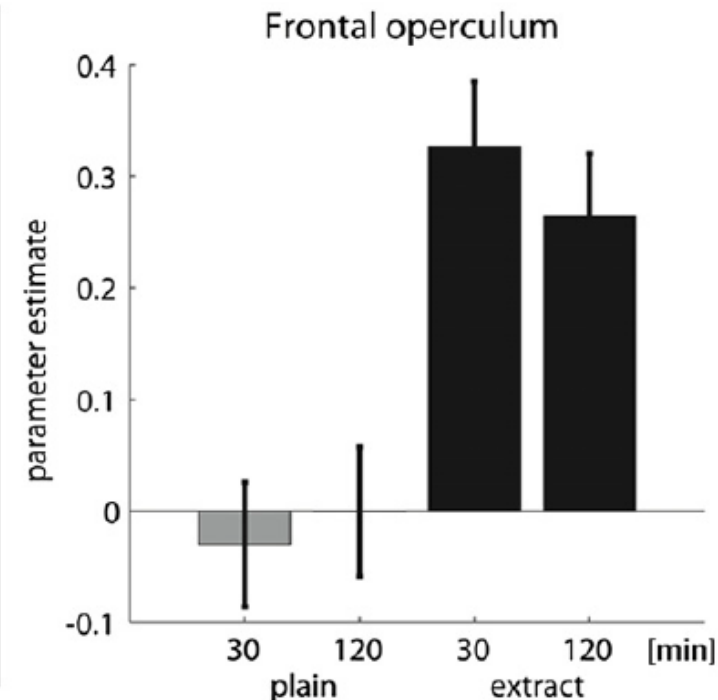
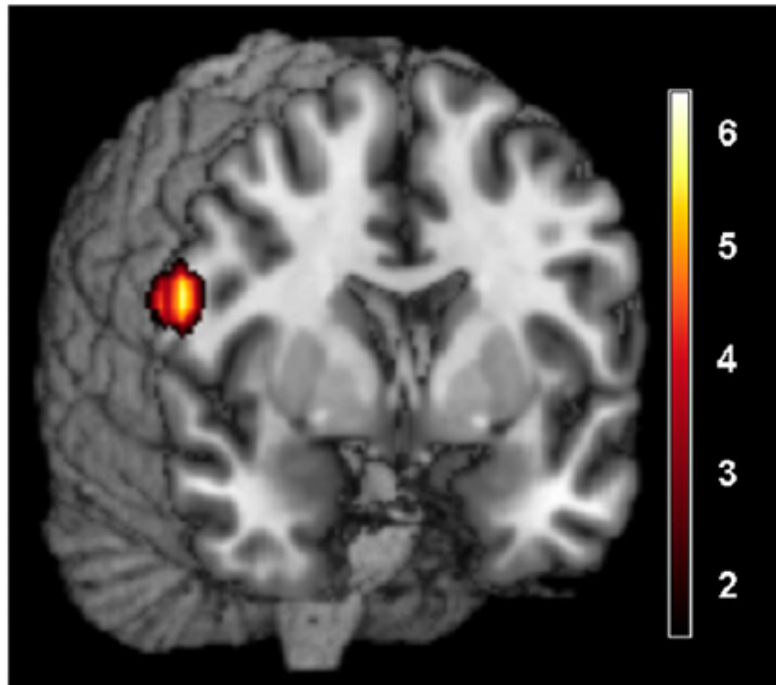
(L'olio extravergine fa aumentare gli endocannabinoidi ... e riduce la fame!)

Estratto aromatico dell'olio extravergine aggiunto allo yogurt (aroma non percepito in bocca)



(Età 28.82 ± 1.04 ; BMI 24.6 ± 0.56)

Frank et al., 2013 – Am. J. Clin. Nutr., 98, 1360–1366.

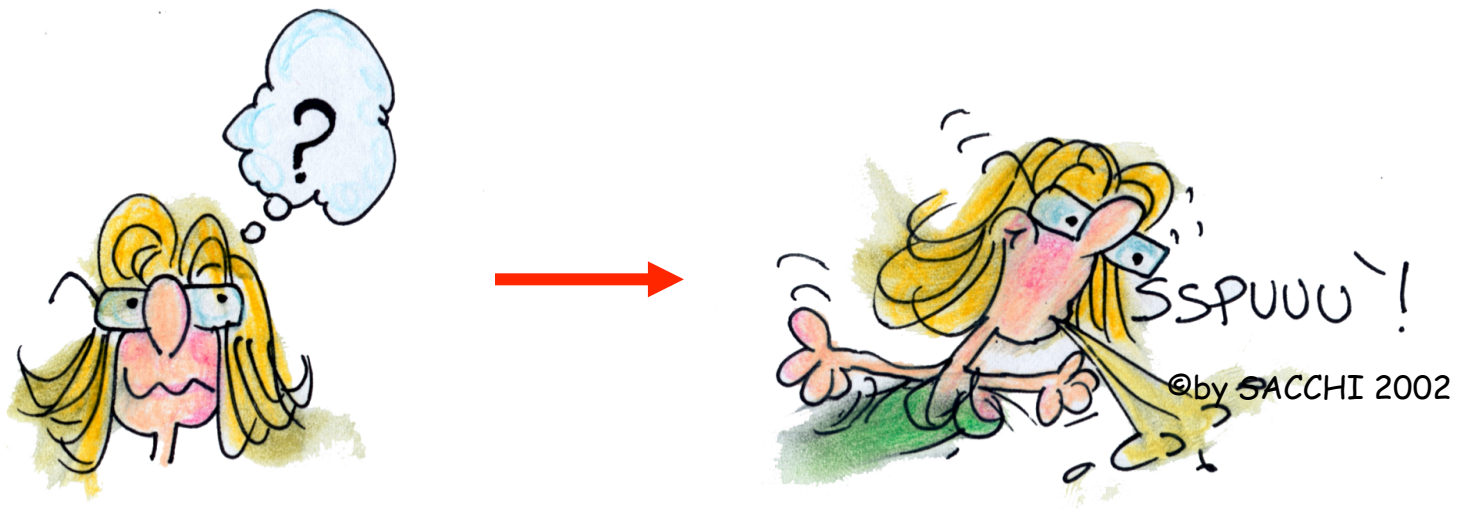


L'estratto aromatico dell'Olio Extravergine influenza il flusso sanguigno **nell'area gustativa** del cervello umano!

Frank et al (2013). Olive oil aroma extract modulates cerebral blood flow in gustatory brain areas in humans. *The American Journal of Clinical Nutrition*, 98, 1360–1366.



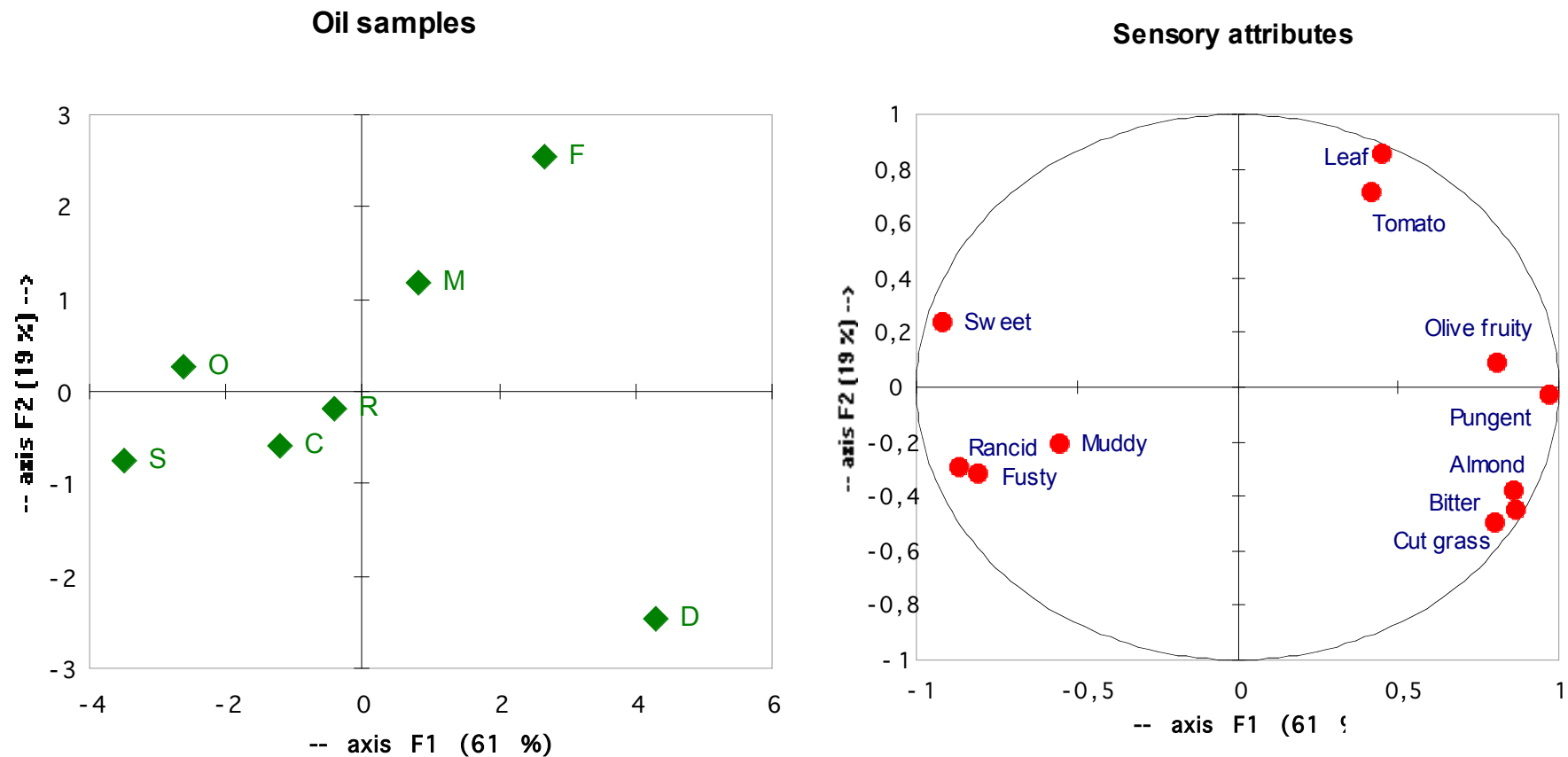
From ... the EC-FLAIR Program on “Sensory and nutritional quality of virgin olive oil” (1993-1997) up today...



How to educate consumers that
'bitter is good for you'?



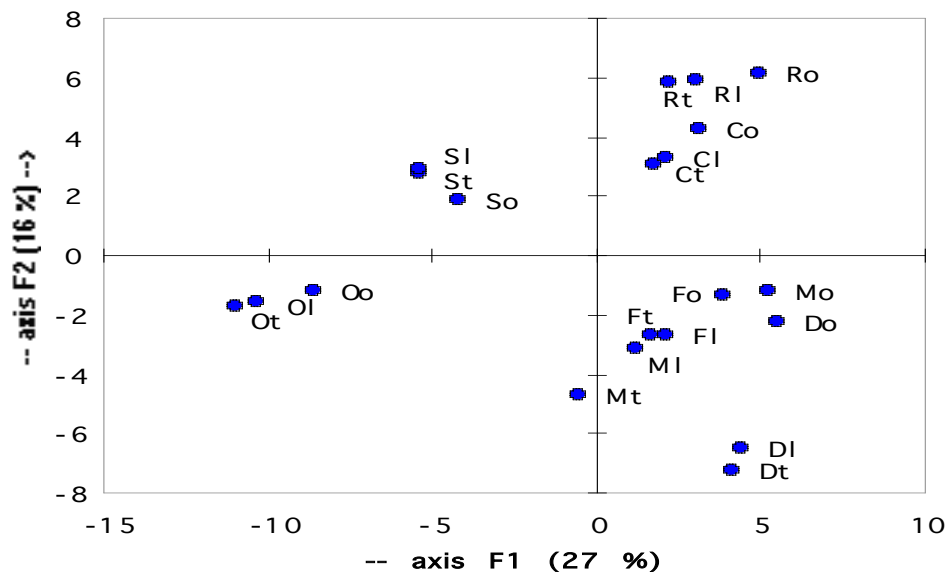
Selected olive oils used for a consumer test (Naples, 2005).



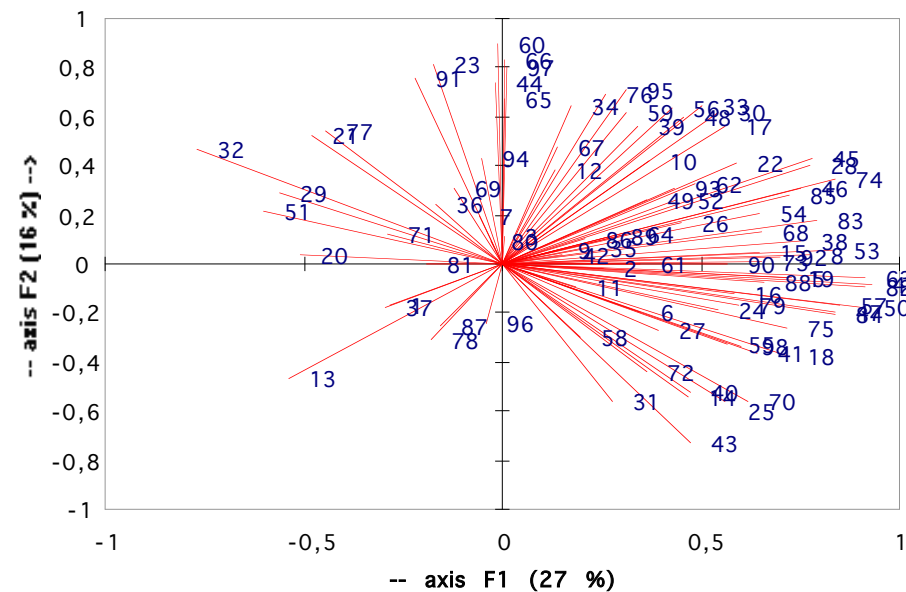


Consumer preferences measured before a nutritional information

Oil samples



98 consumers

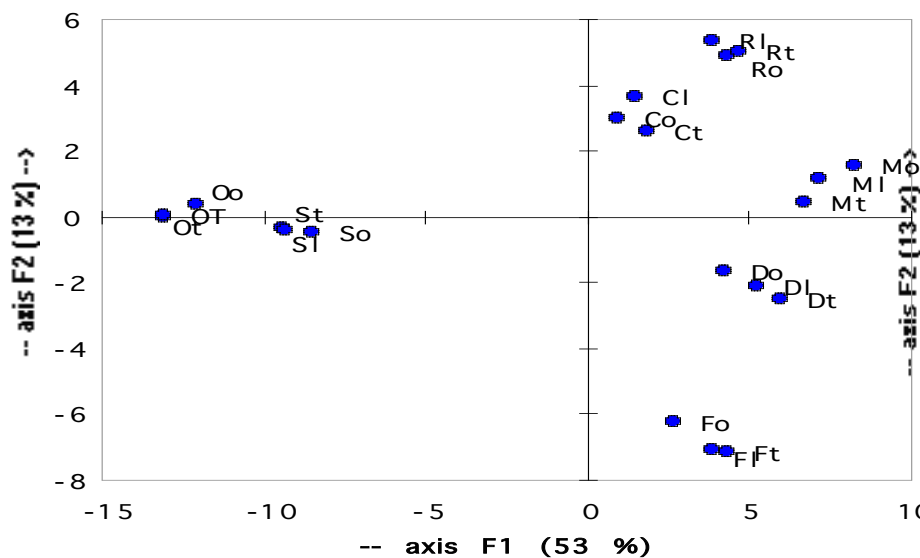


More than 50% consumers prefer low-quality virgin olive oil

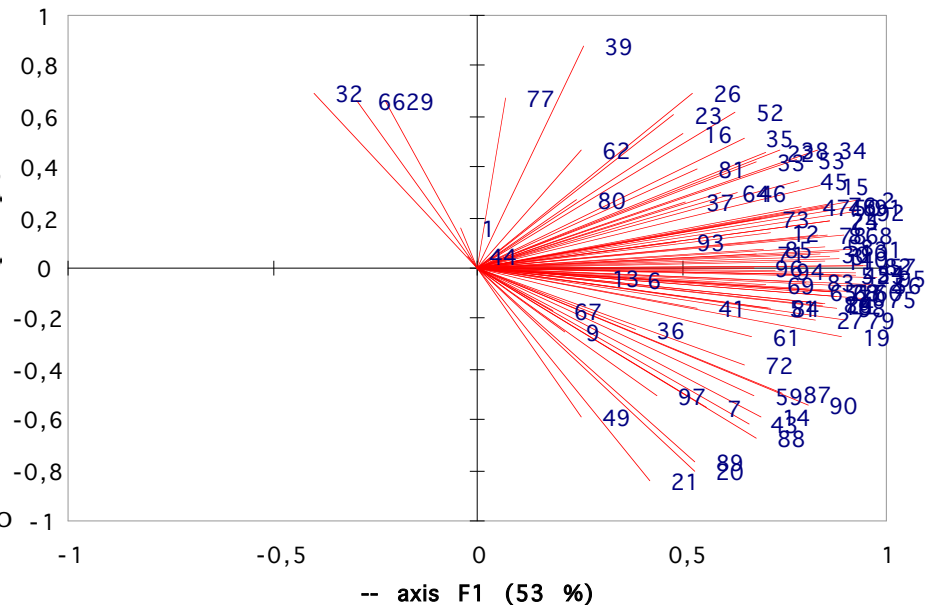


After the nutritional information (without any sensory training)

Oil Samples



98 consumers



The majority (70%) of the consumers changes the preference after a simple information on the positive effect of bitter compounds of olive oil!





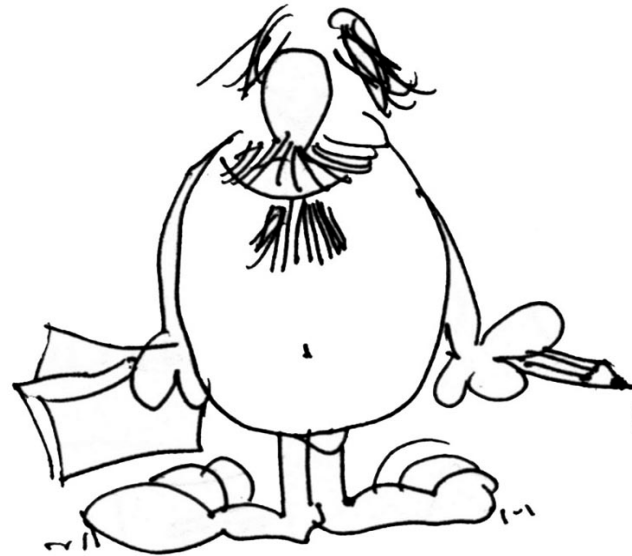
Progetto AGER 2 “COMPETITIVE”

Claims of Olive oil to iMProVE The markeT ValuE





GRAZIE PER L'ATTENZIONE!



FONDAZIONI IN RETE
PER LA RICERCA
AGROALIMENTARE





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