Società Chimica Italiana

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Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

2nd MS-Wine Day
MS for grapes, wine, spirits

FONDAZIONE EDMUND MACH

High resolution mass approaches for wine and oenological products analysis

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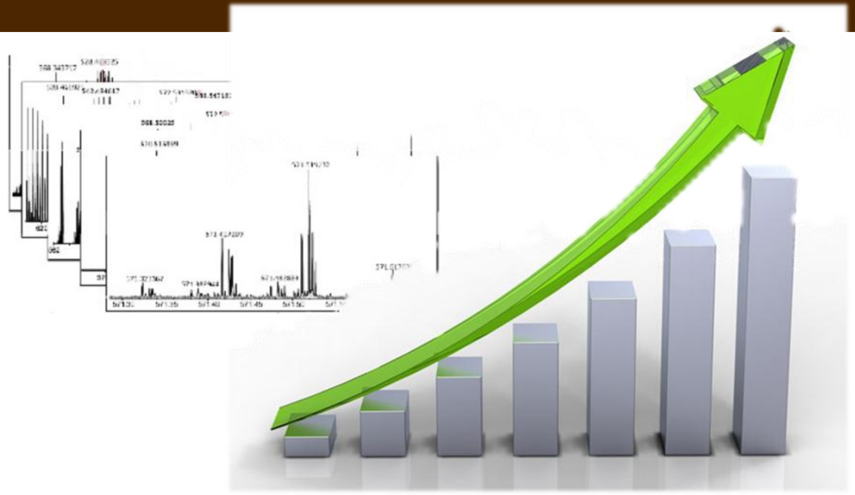
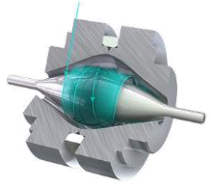
High resolution mass analysis

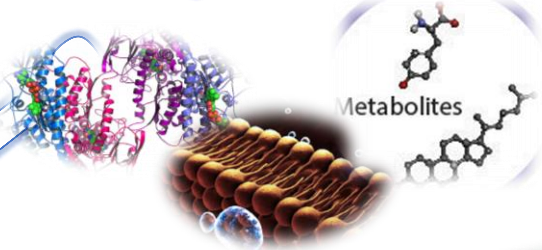


- Over 2,400 studies on LC-HRMS in the last 20 years;
- Almost 50% of them on Orbitrap.



Main topic

- clinic and forensic toxicology
- omic sciences
- food safety and control
- environmental pollution



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High resolution mass analysis

Non-targeted screening (**NO** reference standard)	Suspect screening (**NO** reference standard)	Targeted analysis (reference standard)
	Suspect ions list	Targeted ions list
Automated peak detection by exact mass filtering	Exact mass (*m/z*) filtering	Exact mass (*m/z*) filtering
Non-targeted ions list		
Generation of elemental formulae fit by isotopic pattern distribution		
Molecular structure search in databases		
Matching of measured RT with predicted RT (theoretical $logK_{ow}$) of databse hits	Matching of measured RT with predicted RT(theoretical $logK_{ow}$) of suspects	Matching of measured RT with RT of reference standards
Matching of measured MS/MS fragmentation with that predicted for database hits	Matching of measured MS/MS fragmentation with that predicted for suspects	Matching of measured MS/MS fragmentation with that of reference standards
List of likely present unknowns	**List of likely present suspects**	**Quantification of targets**

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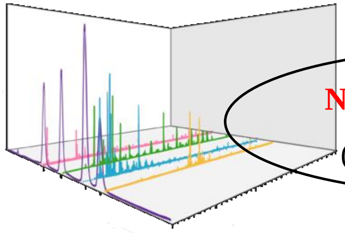
High resolution mass analysis



Non-targeted screening

(**NO** reference standard)

Automated peak detection by **exact mass** filtering

Non-targeted ions list

Generation of **elemental formulae** fit by isotopic pattern distribution

Molecular **structure search** in databases

Matching of measured **RT** with predicted RT (theoretical **$logK_{ow}$**) of database hits

Matching of measured **MS/MS** fragmentation with that predicted for database hits

List of likely present unknowns

- Absence of any *a priori* information about analytes;
- Detection criteria (e.g. product scan or NL);
- Mass accuracy: < 5 ppm;
- Relative isotopic ratio accuracy: < 5%;

- Reduced number of recorded experimental spectra;
- Limited comparability of different source ionization;
- Ion suppression can affect mass accuracy and number of unknows;
- Risk of false negatives (e.g. loss during sample preparation).

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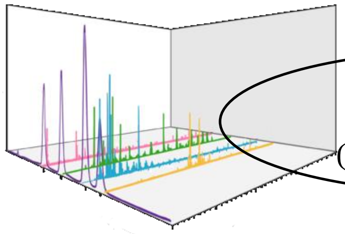
High resolution mass analysis



Suspect screening

(**NO** reference standard)

Suspect **ions list**

Exact mass (*m/z*) filtering

Matching of measured **RT** with predicted RT (theoretical **logK_{ow}**) of suspects

Matching of measured **MS/MS** fragmentation with that predicted for suspects

List of likely present suspects

- Absence of reference standards, but specific information available;
- Exact mass from molecular formula of analytes of interest;
- Mass accuracy: < 5 ppm;
- Relative isotopic ratio accuracy: < 5%;

- Reduced number of recorded experimental spectra;
- Limited comparability of different source ionization;
- Ion suppression can affect mass accuracy and number of suspects;
- Risk of false negatives.

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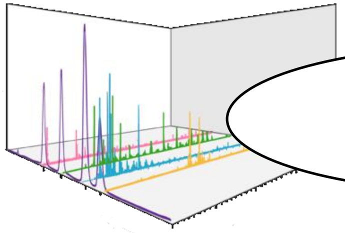
High resolution mass analysis



Targeted analysis

(reference standard)

Targeted **ions list**

Exact mass (m/z) filtering

Matching of measured **RT** with RT of **reference** standards

Matching of measured **MS/MS** fragmentation with that of **reference** standards

Quantification of targets

- Identification and quantification through reference standards;
- No limits in the number of targeted compounds to be identified in the same analytical run;
- No risks of false negative thanks to method validation with reference standards;
- Mass accuracy: < 5 ppm;
- Relative isotopic ratio accuracy: < 5%;

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HRMS applications

International monovarietal wines

↑

Glycosidic profiling

↑

Non-targeted screening

Tannins ← Glycosylated low-molecular-weight phenolic profiling ← **Suspect screening**

Targeted analysis → Free low-molecular-weight phenolic enrichment → Oak aged-wines

Free and glycosylated low-molecular-weight phenolic profiling

↓

Grapes & wines

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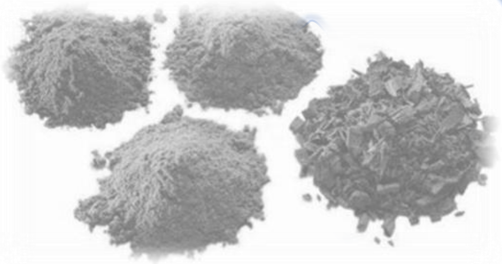
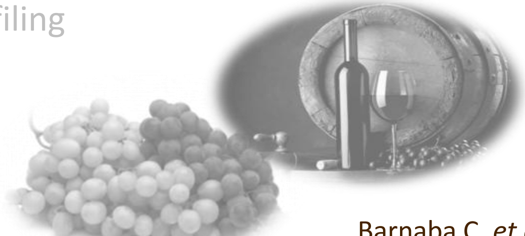


Free and glycosylated low-molecular-weight phenolic profiling

↓

Grapes & wines



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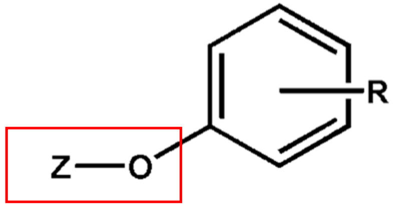
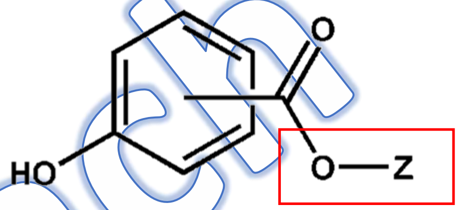
Non-targeted screening analysis

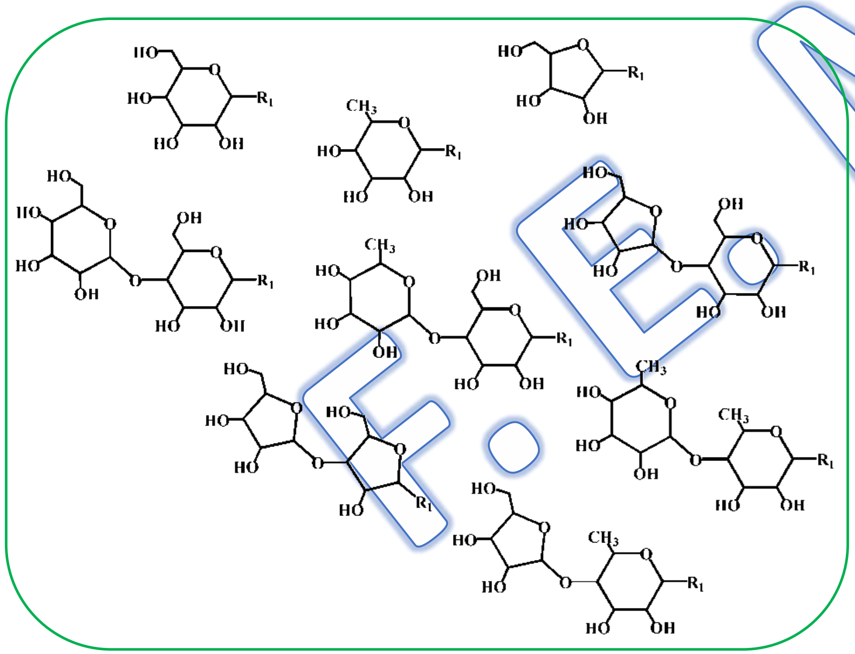
Glycosides



Sugar esters



Z =



Glycosylation:

- Increases compound water solubility;
- Protects hydroxyl/phenolic groups from oxidation;
- Decreases toxicity of phitotoxins;
- Facilitates compound membrane transports;
- …

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Neutral Loss experiment

Chromatographic separation

- ✓ Accucore™ Polar Premium LC C18
- ✓ Flow rate: 0.300 ml/min;
- ✓ Run time: 55 min.

Mass analysis

Full MS/AIF/NL dd-MS^2

- ✓ Full MS resolving power: 140,000 FWHM;
- ✓ AIF and dd- MS/MS resolving power: 17,500 FWHM;

NEUTRAL LOSS

($\Delta m/z$ = 10 ppm)

- ✓ m/z 132.04225
- ✓ m/z 146.05790
- ✓ m/z 162.05282
- ✓ m/z 264.08451
- ✓ m/z 294.09508
- ✓ m/z 324.10564

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Neutral Loss experiment: tentative identification

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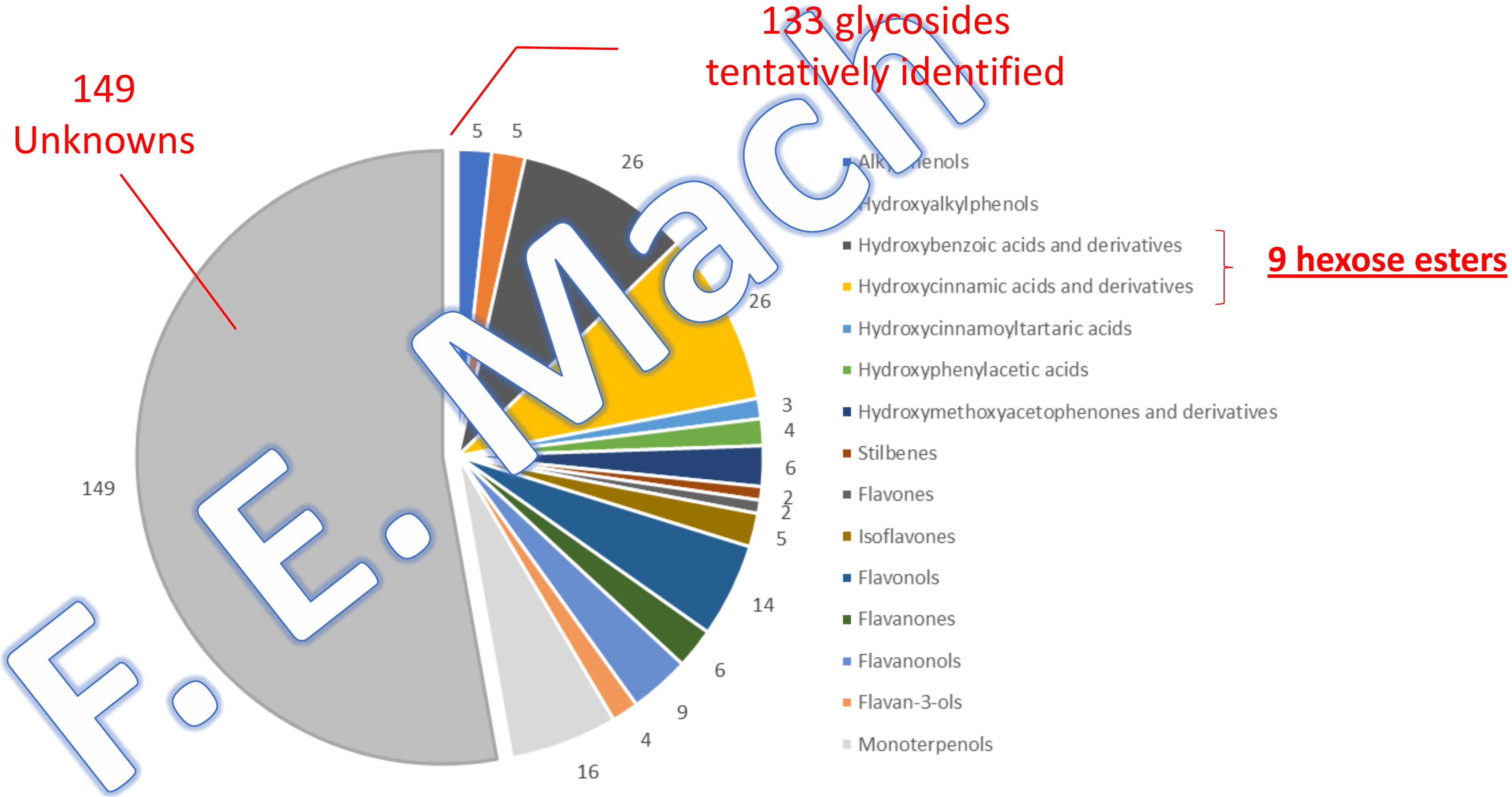
282 glycosylated compounds detected:



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HRMS applications

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Free and glycosylated low-molecular-weight phenolic profiling

↓

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Suspect & targeted screening analysis

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Free low-molecular weight phenolic compounds (N=56)

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Compounds	[M – H]⁻ (m/z)	RT (min)	NCE	MS/MS fragments	LOQ (µg mL^{-1})
gallic acid	169.0142	5.60	45	125.0244	0.0001
protocatechuic acid	153.0193	5.76	50	109.0294	0.0001
p-carboxyphenol acid	137.0244	6.14	40	93.0646	0.0001
gentisic acid	153.0193	6.21	45	109.0295, 108.0217	0.0001
hydroxytyrosol	153.0557	6.28	50	123.0437, 95.0487	0.0005
vanillic acid	167.0350	6.42	40	152.0114, 123.0452	0.0001
syringic acid	197.0455	6.57	35	182.0216, 166.9984	0.0001
caffeic acid	179.0350	6.60	40	135.0452	0.0001
homovanillic acid	181.0506	6.79	45	137.0617, 122.0373	0.0010
tyrosol	137.0608	6.79	40	119.0502, 106.0426	0.0001
protocatechuic aldehyde	137.0244	7.10	60	108.0216, 93.0344	0.0001
pirocatecolo	109.0295	7.28	80	108.0202, 91.0176	0.0005
p-coumaric acid	163.0401	7.37	35	119.0502, 93.1266	0.0001
salicylic acid	137.0244	7.72	60	93.0346, 122.0374	0.0001
phenol	93.0345	7.73	100	65.0382	0.1050
catechin	289.0717	7.89	35	245.0805, 221.0812	0.0051
ferulic acid	193.0506	8.17	40	178.0268, 149.0608	0.0001
aesculetin	177.0193	8.48	50	133.0296, 105.0345	0.0001
sinapinic acid	223.0611	8.54	30	208.0373, 179.0714	0.0005
homovanillic alcohol	167.0714	8.78	35	152.0477, 122.0375	0.0051
epicatechin	289.0718	9.67	40	245.0805, 221.0812	0.0001
vanillin	151.0401	9.86	40	136.0152, 108.0202	0.0001
coniferyl alcohol	179.0714	10.11	35	164.0478, 121.0296	0.0107
4-methylcatechol	123.0451	10.18	100	108.0214, 90.0591	0.0005
syringaldehyde	181.0506	10.42	40	166.0269, 151.0035	0.0008
isopropiovanillone	179.0714	10.55	40	164.0477, 121.0295	0.0054
scopoletin	191.0350	10.66	40	176.0112, 148.0166	0.0010

Compounds	[M – H]⁻ (m/z)	RT (min)	NCE	MS/MS fragments	LOQ (µg mL^{-1})
acetovanillone+isoacetovanillone	165.0557	10.69	40	150.0321, 122.0371	0.0001
isopropiosiringone	209.0819	10.81	35	194.0581, 179.0348	0.0011
acetosyringone	195.0662	11.00	30	180.0426, 165.0190	0.0001
isoacetosiringone	195.0662	11.24	30	180.0426, 165.0190	0.0011
syringol	153.0557	11.32	50	138.0321, 123.0087	0.0129
coniferylaldehyde	177.0556	11.51	35	162.0320	0.0001
sinapinaldehyde	207.0663	11.64	35	192.0427, 177.0193	0.0010
tryptophol	160.0767	11.87	70	142.0659, 130.0660	0.1102
o-vanillina	151.0401	12.09	40	136.0152, 123.0083	0.0010
methyl vanillate	181.0506	12.13	40	166.0268, 151.0036	0.0005
(*m*+*p*)-cresol	107.0502	12.27	60	79.0551, 65.7207	0.1010
4-ethylcatechol	137.0608	12.30	35	122.0374	0.0005
o-cresol	107.0502	12.41	60	82.5568	0.1170
vanillyl ethyl ether	181.0870	12.67	30	166.0633, 153.0656	0.0010
guaiacol	123.0451	12.85	70	108.0215, 105.0346	0.0110
4-methylsyringol	167.0713	12.87	20	152.0478, 137.0243	0.0101
4-vinylphenol	119.0502	13.60	100	91.0550, 93.0346	0.0112
ethyl vanillate	195.0662	13.69	40	180.0415, 130.9911	0.0006
3.4-xylenol	121.0658	13.73	90	119.0503, 96.9445	0.0100
4-vinylguaiacol	149.0608	14.00	20	134.0375, 87.0088	0.0055
ellagic acid	300.9989	14.00	60	229.0149, 185.0071	3.03
4-ethylphenol	121.0658	14.22	90	106.0423, 83.9854	0.1022
4-methylguaiacol	137.0608	14.37	35	122.0374	0.0105
4-ethylguaiacol	151.0764	14.58	10	136.0529, 121.0293	0.0009
4-allyl syringol	193.0870	14.85	10	178.0632, 163.0399	0.0202
eugenol	163.0764	15.11	30	148.0529	0.0087
isoeugenol	163.0764	15.47	30	148.0529, 118.9925	0.0102

Glycosylated low-molecular weight phenolic compounds (N=7)

Compounds	[M – H]⁻ (m/z)	RT (min)	NCE	MS/MS fragments	LOQ (µg mL^{-1})
acetovanillone-glu [h]	327.1085	8.40	20	165.0557; 150.0321	0.2800
aesculetin-glu [b]	339.0722	6.8	35	177.01933; 133.0296	0.1100
orcinol-glu [f]	285.0980	6.83	40	123.0452; 108.0214	0.0500
p-hydroxybenzaldehyde-all [f]	283.0823	6.08	100	121.0295; 108.0218	0.1000
salicylic acid-glu [f]	299.0772	5.84	20	137.0244; 93.0344	0.0100
scopoletin-glucoside [h]	353.0878	8.60	20	191.03498; 176.0112	0.1800
vanillic acid-glucoside [h]	329.0878	5.42	20	167.03498; 152.0114	0.2100

Barnaba et al., J. Chromatography A (2015), 1423, 124-135.

HRMS applications

International monovarietal wines

↑

Glycosidic profiling

↑

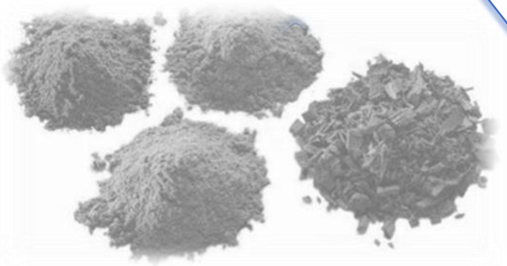
Non-targeted screening

Suspect screening

Targeted analysis

Tannins ← Glycosylated low-molecular-weight phenolic profiling ← Suspect screening

Targeted analysis → Free low-molecular-weight phenolic enrichment → Oak aged-wines



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Free and glycosylated low-molecular-weight phenolic profiling

↓

Grapes & wines

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Suspect & targeted screening analysis: grapes

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Hybrid grape varieties

Cabernet Cantor

Prior

Solaris

Muscaris

Vitis vinifera grape varieties

Merlot

Chardonnay

Barnaba et al., Food Res. Intern. (2017), *in press*.

Barnaba C. *et al.*, 2° MS-Wine Day 2017

Suspect & targeted screening analysis: wines

- ✓ Primitivo di Manduria (DOP);
- ✓ Negroamaro (IGP).

Primitivo di Manduria

- 2006
- 20
- 2011
- 2012
- 2013
- 2014

12 months in French and American oak barrels

Negroamaro

- 2007
- 2010
- 2011
- 2012
- 2013
- 2014

Barnaba et al., Food Chem (2016), 206, 260-266.

HRMS applications

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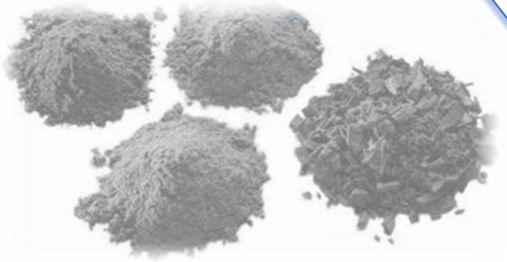

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Free and glycosylated low-molecular-weight phenolic profiling

↓

Grapes & wines

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Targeted screening analysis: oak-aged wines

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Pressurized cold water (NT)

Chemical treatment (KOH) (CT)

Ozone (OT)

Guzzon et al., J. Food Sci. Technol (2017), 54, 810-821.

Wines were oak-aged for <u>97 days</u>

HRMS applications

FONDAZIONE EDMUND MACH

International monovarietal wines

↑

Glycosidic profiling

↑

Non-targeted screening

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Targeted analysis

Tannins ← Glycosylated low-molecular-weight phenolic profiling ← Suspect screening

Targeted analysis → Free low-molecular-weight phenolic enrichment → Oak aged-wines

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Free and glycosylated low-molecular-weight phenolic profiling

↓

Grapes & wines

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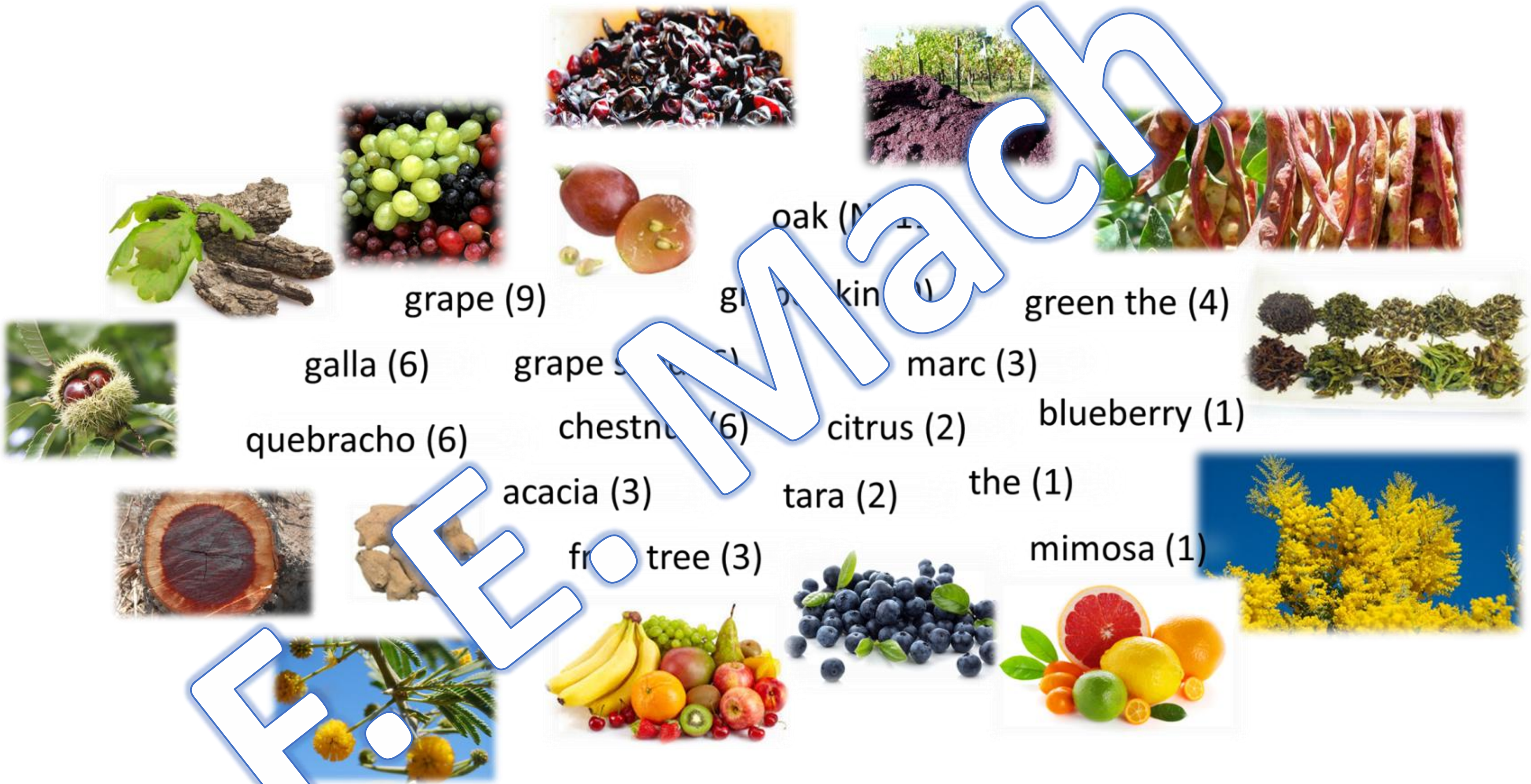
Suspect screening analysis: tannins



<u>Suspect</u> screening analysis: tannins

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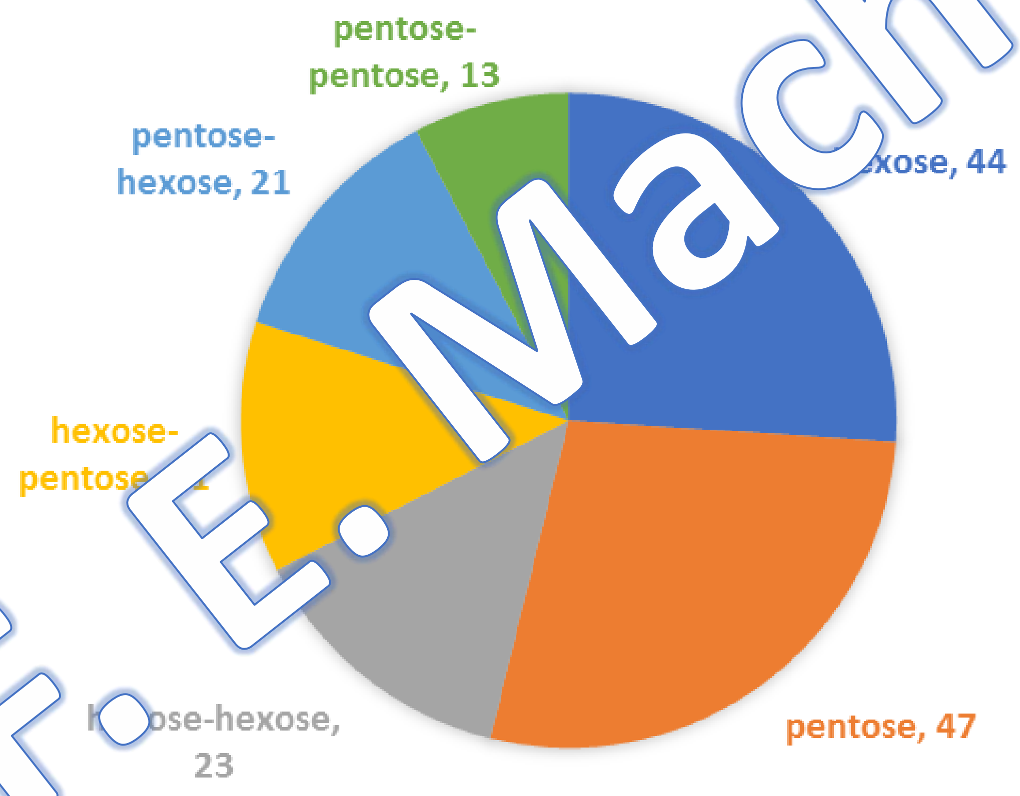
169 glycosylated low-molecular weight phenolic compounds tentatively identified:



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