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Circular Economy as a New Model for the Exploitation of the Agroindustrial Biomass

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Circular Economy as a New
Model for the Exploitation of
the Agroindustrial Biomass

Circular Economy Approach

Circular economy refers to the material aspects of integrated socio-economic and environmental systems.

In July 2014 the EU Commission adopted a Circular Economy Package, including the Communication COM 398 ***"Towards a circular economy: a zero waste programme for Europe"*** according to the issue of *turning waste into resources*.

It requires changes through value chains, from product design to new business, market models and new consumer behavior.

Circular *Economy Approach*

"ZERO WASTE"

Based upon this perspective, a new concept of waste and by-products within the agroindustrial system can be suitable developed.

The present study aims to be only an initial approach to the Circular Economy in the agri-food sector.

Contents

-The Circular Economy approach

-Case study

-Final Remarks

SWEET CHESTNUT

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

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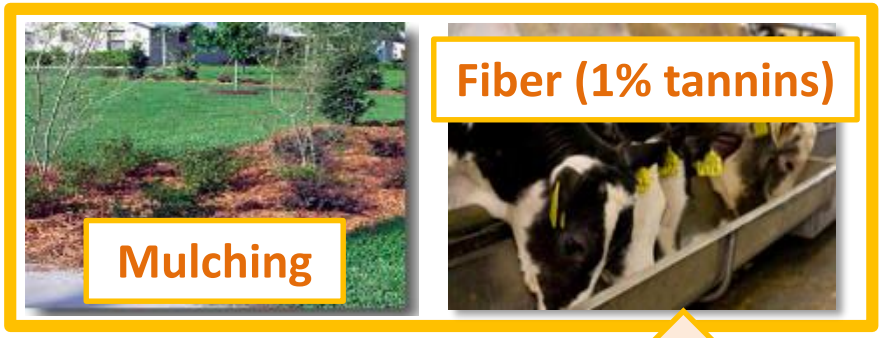

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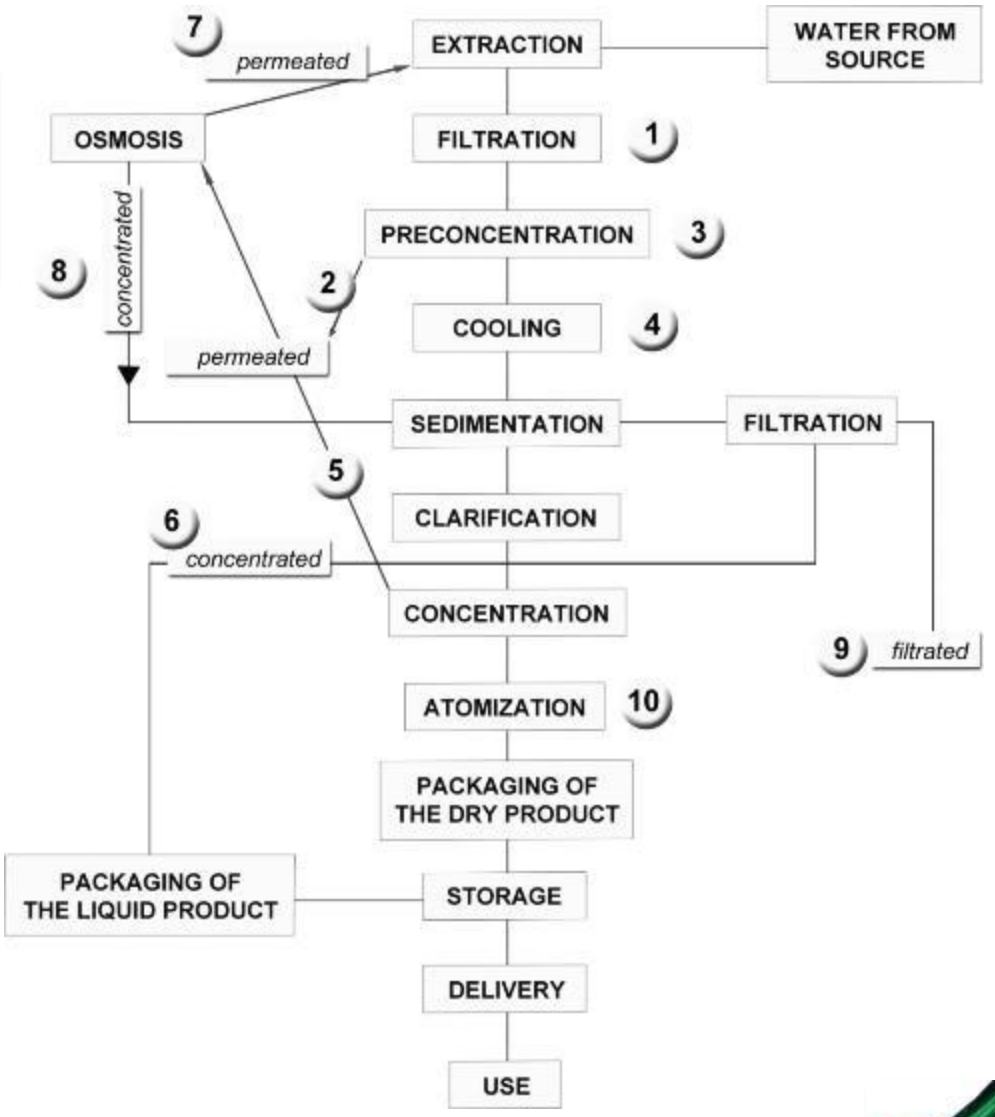
SAVIOTAN® NUTRACEUTICAL FOR CROPS

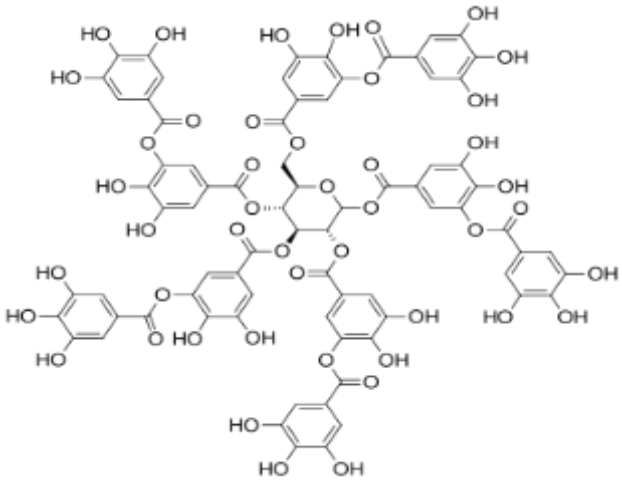
B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

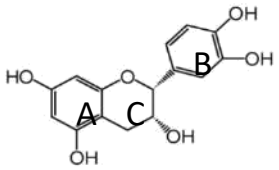
SWEET CHESTNUT: Kilo-scale extraction plant



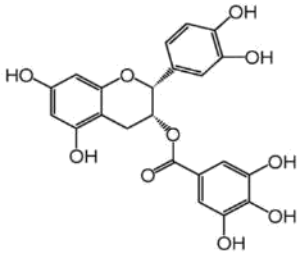
TANNINI AS NATURAL ANTIMICROBIALS

CASTAGNO



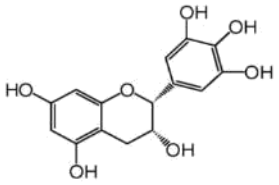


(-)-Epicatechin (EC)

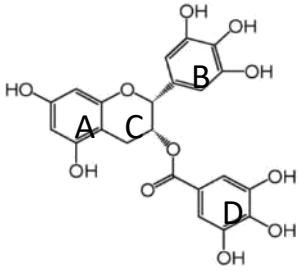


(-)-Epicatechin-3-gallate (ECG)

GRAPE SEEDS



(-)-Epigallocatechin (EGC)



(-)-Epigallocatechin-3-gallate (EGCG)

GREEN THE

Sweet Chestnut: lab scale extracts of bark, leaves and cupules

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

Sweet Chestnut bark aqueous extract	mg/mL ext	mg/g bark
Monogalloyl glucose I	0.007	0.286
Gallic acid	0.034	1.377
Monogalloyl glucose II	0.000	0.000
Gallagic derivatives*	0.005	0.190
Ellagitannins with ellagic acid units**	0.006	0.238
Ellagitannins with HHDP- or NHTP- units**	0.001	0.022
Ellagic acid	0.004	0.173
Total	**0.057**	**2.286**

* Calibrated as gallic acid; ** Calibrated as ellagic acid

Sweet Chestnut: lab scale extracts of bark, leaves and cupules

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

Sweet Chestnut leaf aqueous extract	mg/mL ext	mg/g
Monogalloyl glucose I	0.046	1.821
Gallic acid	0.045	1.815
Monogalloyl glucose II	0.008	0.334
Gallagic derivatives*	0.023	0.918
Ellagitannins with ellagic acid units**	0.000	0.000
Ellagitannins with HHDP- or NHTP- units**	0.022	0.865
Ellagic acid	0.025	0.986
Flavonoids***	0.028	1.114
Total	**0.168**	**6.739**

*Calibrated as gallic acid;
** Calibrated as ellagic acid;
*** Calibrated as kaempferol-3-glucoside.

Sweet Chestnut: lab scale extracts of bark, leaves and cupules

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

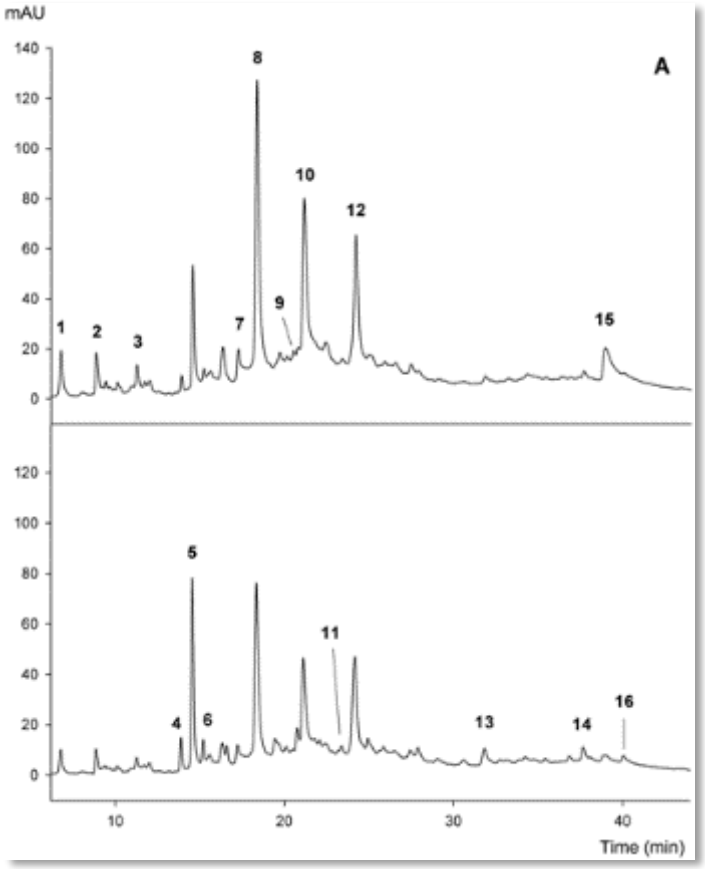
Sweet Chestnut cupules aqueous extract	mg/mL ext	mg/g
Monogalloyl glucose I	0.000	0.000
Gallic acid	0.005	0.208
Monogalloyl glucose II	0.001	0.033
Gallagic derivatives*	0.000	0.000
Ellagitannins with ellagic acid units**	0.000	0.000
Ellagitannins with HHDP- or NHTP- units**	0.000	0.000
Ellagic acid	0.003	0.129
Total	**0.009**	**0.370**

* Calibrated as gallic acid; ** Calibrated as ellagic acid

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

SWEET CHESTNUT

Quali-quantitative analysis HPLC/DAD/ESI-MS
Individual compounds: Kilo-scale commercial fractions



peak	compound	content in commercial liquid fraction			content in commercial dry fraction		
		mg/g	µmol/g	RSD %	mg/g	µmol/g	RSD %
1	vescalin	3.57	5.65	1.9	8.68	13.73	4.6
2	castalin	3.21	5.08	0.6	8.83	14.0	4.9
3	pedunculagin I	3.31	4.22	1.2	10.1	12.9	4.4
4	monogalloyl glucose I	2.51	7.57	1.3	4.69	14.1	2.4
5	gallic acid	1.53	9.02	1.9	13.6	79.8	2.1
6	monogalloyl glucose II	2.68	8.08	4.7	5.03	15.2	4.3
7	roburin D	4.03	4.36	1.2	9.61	10.4	3.4
8	Vescalagin	26.7	28.6	2.4	45.2	48.4	3.6
9	dehydrated tergallagic-*C*-glucoside	1.77	2.88	1.6	6.03	9.82	4.5
10	Castalagin	21.0	22.5	1.8	39.7	42.5	3.6
11	digalloyl glucose	3.79	7.84	1.2	12.2	25.3	2.0
12	*O*-galloyl-castalagin isomer	20.8	19.2	1.9	32.0	29.5	2.2
13	trigalloyl glucose	5.03	7.91	1.4	12.1	19.0	3.1
14	tetragalloyl glucose	4.72	5.99	3.2	9.18	11.6	2.4
15	ellagic acid	2.50	8.26	0.8	7.80	25.8	8.7
16	pentagalloyl glucose	3.67	3.90	2.0	0.00	0.00	-
	total tannins	**111**	**151**	**1.0**	**225**	**372**	**2.5**

M. Campo, P. Pinelli and A. Romani, *Nat. Prod. Commun.*, **2016**, *11(3)*, 409.

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

C5 Monitoring of the economic benefits deriving from the recycling of the spent vegetable biomass after the extraction of the high quality standardised polyphenolic molecules at laboratory level

Sweet Chestnut extraction plant

- **Into autoclave**: 20 mc (7 tons) biomass to be extracted

- 330 Kg spray dried (**yield: 4.7%**)

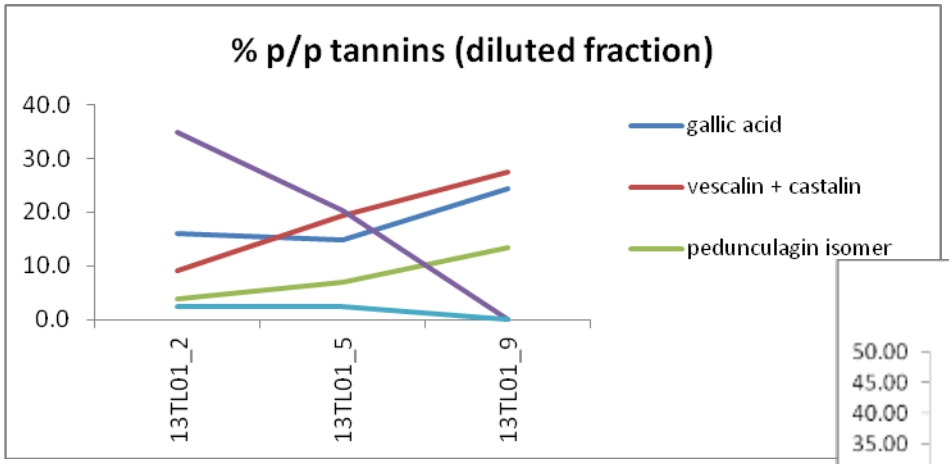
- **Process: 4080 Kcal/Kg powder** → 1346400 Kcal each batch

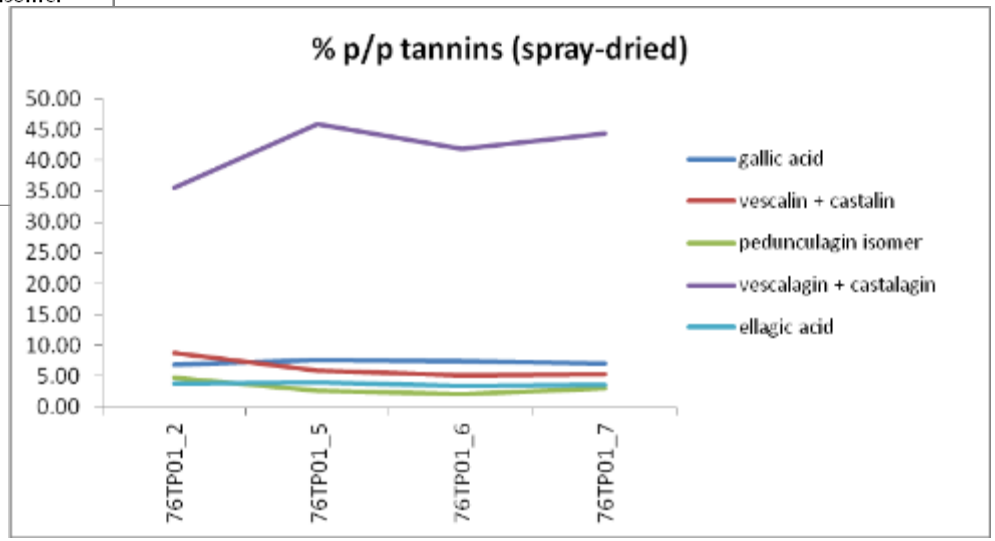
- **Biomass calorific value**: mix pressed post-extraction spent residue+ 1/8 wood dust from chipper **2000 Kcal/Kg**

- **Biomass for energy**: 10 tons each batch → 2000*10000 Kcal energy delivered: energy from biomass can power the whole process.

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

B3 Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale.





Sweet Chestnut fractions: chemical stability

- Each process stream was monitored
- The less concentrated fractions are chemically unstable due to hydrolysis of the high MW tannins
- The spray dried fractions is chemically stable after > 12 months at room temperature

B3 Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale.

In vitro antioxidant/antiradical activity

Measured values of GAE, EC_{50} and TE. d: density (g/mL); GAE: Gallic Acid Equivalents (g gallic acid/mmol total tannins by HPLC/DAD); EC_{50}: polyphenolic concentration inhibiting DPPH· activity to 50% (μM); TE: Trolox Equivalents (μmol/g sample).

	d	GAE	EC_{50}	μmolTE/g
1	1.01	0.636	0.695	
2	1.01	0.413	2.007	
3	1.06	1.182	0.444	
4	1.04	0.938	1.000	
5	1.03	0.565	1.429	
6	1.25	1.319	1.516	450.4
7	1.00	0.250	2.399	
8	1.07	0.616	1.510	
9	1.08	0.052	0.545	
10		1.210	0.586	3050

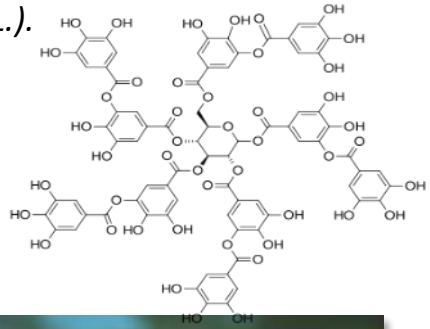
Sweet Chestnut: Antioxidant/antiradical activity

- Folin-Ciocalteu
- DPPH·
- ORAC (commercial fractions)

M. Campo, P. Pinelli and A. Romani, *Nat. Prod. Commun.*, **2016**, *11(3)*, 409.

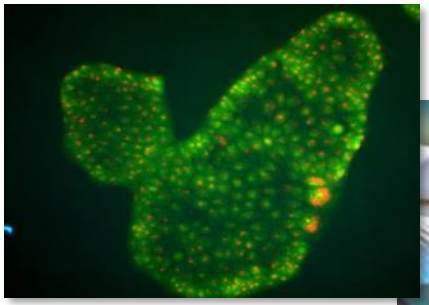
Antimycotic activity

Attività antimicotica di tre differenti estratti nei confronti di ceppi selezionati di lieviti:
***A**. Scorza di melagrana (Punica granatum L.); **B**. Corteccia di castagno (Castanea sativa Mill.); **C**. foglie di mirto (Myrtus communis L.).*



ADWB in YPD - 25°C		Diameter of inhibition area (mm)		
		A	B	C
concentrations (mg/mL) corresponding to 5.00 mM of total polyphenols		6.41	9.8	5.26
Species	**Strain**			
C. albicans	6133		16	
C. albicans	6157		18	
C. glabrata	7212	33	25	21
C. glabrata	3828	30	23	20
P. guilliermondii	6140			
C. parapsilosis	6150		17	
C. tropicalis	3982			
C. zeylanoides	6163			
Cl. lusitaniae	6142			
Cl. lusitaniae	6148		14	
I. orientalis	6782	20		
K. marxianus	6141		16	
S. cerevisiae	6173	19	22	19
S. cerevisiae	6497	28 R	24 R	21 R
S. cerevisiae	6500	18	21	12
Y. lipolitica	6053			
Cr. laurentii	3883	25 R		
Cr. laurentii	4272		36	30
Cr. laurentii	6265			
Fil. neoformans	3428	25 R	18 R	
Fil. neoformans	6010	24 R	19 R	18 R
Fil. neoformans	6225	25	24	21
Fil. neoformans	6981	33	34	25
Fil. neoformans	6982	29	24	25
P. wickerhamii	8879	19 R	24 R	

Feed applications SAVIOTAN FEEDS



In vitro Antimycotic activity

EU project LIFE13 ENV IT 000461 / "EVERGREEN"

"Environmentally friendly biomolecules from agricultural wastes as substitutes of pesticides for plant diseases control"

Final meeting 24 months – September 23, 2016

Prof. Annalisa Romani - PHYTOLAB – DiSIA – University of Florence

Dr. Margherita Campo - INSTM - National Interuniversity Consortium of Materials Science and Technology

Sweet Chestnut: aqueous extract of bark from pilot plant

Bark extract from pilot plant	mg/mL
Gallotannins*	0.024
Ellagitannins with ellagic acid units**	0.014
Ellagictannins with HHDP- or NHTP- units**	0.014
Procyanidins***	0.036
Ellagic acid	0.032
Total tannins	**0.118**

*Calibrated as gallic acid;
** Calibrated as ellagic acid;
*** Calibrated as catechin.

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

New Formulations

GEL FORMULATION with grape seed post-oil extraction residue:

Release of condensed tannins into the ground to obtain antimicrobial and nematostatic/nematicidal effects

In a phase of characterization and optimization (laboratory + industrial scale)

The formulation may be enriched with other natural extracts.

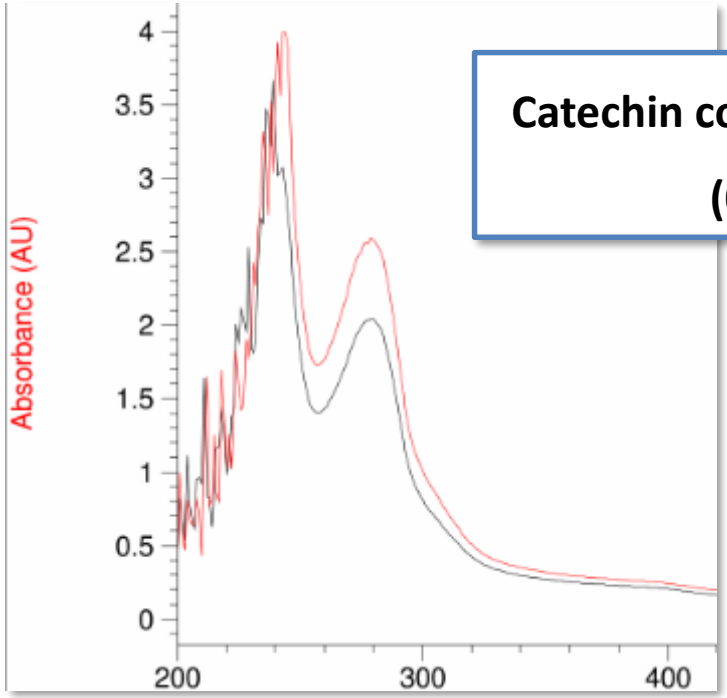
Dried grape skin from wine-making process

"EVERGREEN" - LIFE13 ENV/IT/000461

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

New Formulations

GEL FORMULATION with grape seed post-oil extraction residue:



Catechin content: 7.4 mg/g gel

(0.7% p/p)

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

New Formulations

- **FORM. 1 (liquid)**: TC 2%, O 1% in water (dilute 1:10 for use)
- **FORM. 2 (liquid)**: TC 1.5%; O 1%; V 0.3% in water (dilute 1:10 for use)
- **FORM. 3 (gel)**: TC 0.2%, O 0.1% in water + hydrophilic polymer
- **FORM. 4 (gel)**: TC 0.15%; O 0.1%; V 0.03% in water + hydrophilic polymer

TC: **Sweet Chestnut** aqueous extract, liquid fraction 12% p/p hydrolyzable tannins;

O: liquid fraction of ***O. europea*** leaves 3.22% p/v polyphenols (1.13% hydroxytyrosol);

V: commercial dry **grape seeds** extract 82% p/p condensed tannins.

Provided to:
- DiSPAA- University of Florence (Prof. Tegli)
- INSTM
- ASTRA Innovazione e Sviluppo
- CEBAS-CSIC

B.2 Demonstration of the qualitative and quantitative yields of extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste at laboratory scale

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D Communication/Dissemination actions

Romani A., Vita C., Campo M., Scardigli A. Recupero di sottoprodotti agroindustriali: un modello di economia circolare. Rivista delle Tecnologie Agroalimentari - Sistemi per produrre. Anno XXVII n.5 giugno/luglio 2016. p. 50-55.

Campo M., Pinelli P., Romani A. (2016) Hydrolyzable Tannins from Sweet Chestnut Fractions Obtained by a Sustainable and Eco-friendly Industrial Process. *Natural Product Communications*, 11(3), 409-415.

Romani A., Campo M., Scardigli A., Biancalani C., Cerboneschi M., Tegli S. Natural standardized polyphenolic fractions for innovative green formulations in agronomics. Polyphenols Communications 2016, Acts of the XXVIII International Conference on Polyphenols, July 11-15, 2016, Vienna – Austria.

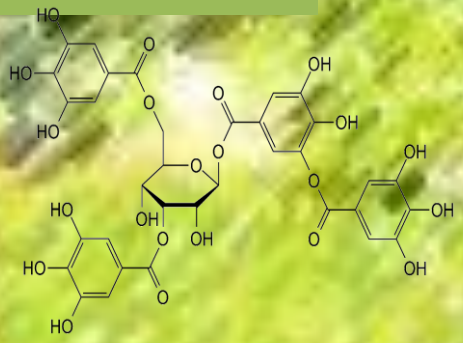
"EVERGREEN" - LIFE13 ENV/IT/000461

Project LIFE13 ENV/IT/000461

"Environmental friendly biomolecules from agricultural wastes as substitutes of pesticides for plant diseases control"

"EVERGREEN"

Start:
01/10/2014
End:
31/09/2016



Final Remarks

The recovery of biomolecules and the production of energy, can be a continuous process of interaction between high technology and environmental and economic sustainability, making this kind of multifunctional platform highly innovative and consistent with the principles of the circular economy, as well as with the development of a new business.

Case Study

The case study concerns the exploitation of Olea Europaea L., Cynara and by-products (leaves, stems and pulps) as new source for energy, bioactive antioxidant and antimicrobial compounds (polyphenols), in order to established a multifunctional platform in the Apulia region

Aim of the research

The present paper belongs to a research in progress and aims to implement a multifunctional platform, by which the agrifood chain and the life style towards a ***"zero waste" model*** can be suitable developed. The platform as new source for energy and polyphenols has to be established in Apulia, a region of the South of Italy.

Multifunctional platform for the production of antioxidant extracts and bioenergy from byproducts of *Olea europaea* L.

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

OLEA: Kilo-scale extraction plant

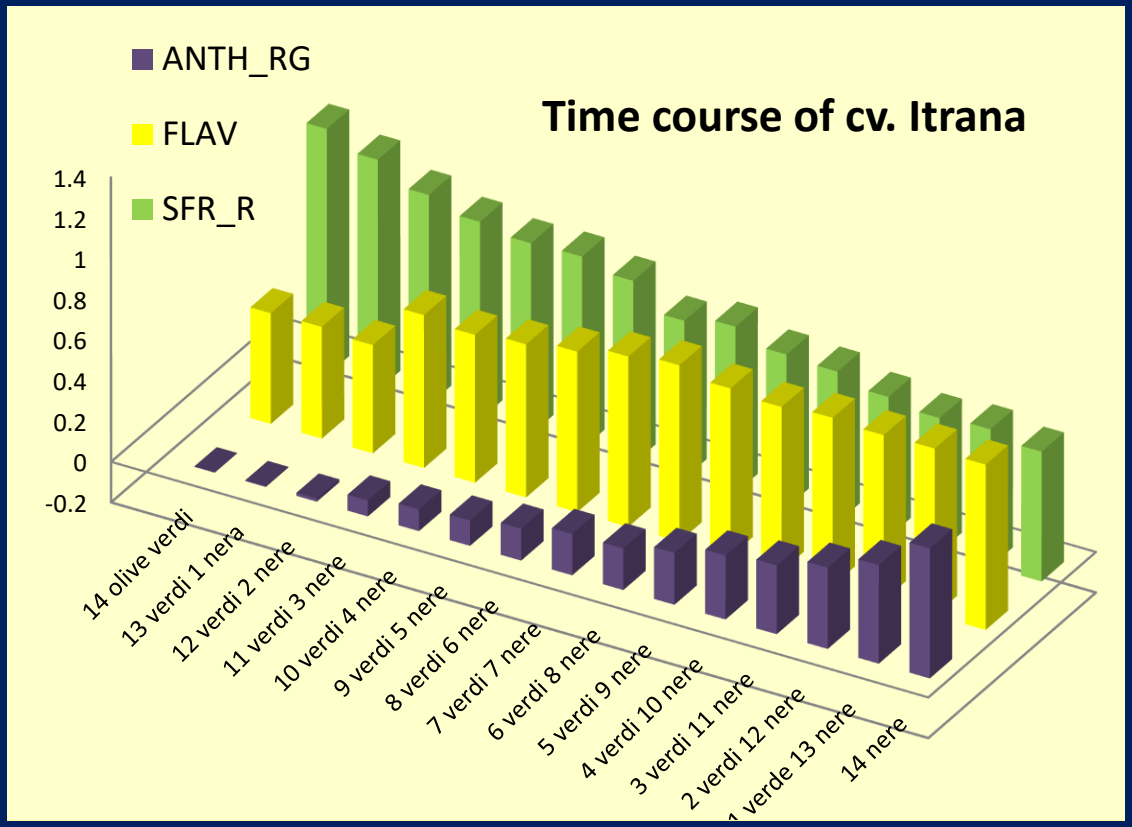
Polyfunctional platform for the production of antioxidant extracts and biogas from byproducts of *Olea europaea* L.

Phenolea [R], new biophenolic fractions from different tissues of *Olea europaea* L. Romani A., Scardigli A., Ieri F., Banelli L., Pinelli P., Franconi F. *XXVth* International Conference of Polyphenols, *Polyphenols Communcations 2010.* **2010**, 2, T4. 75.

"EVERGREEN" - LIFE13 ENV/IT/000461

Application of Multiplex on olive fruits: anthocyanin, flavonols and chlorofyll assessment during ripening

Non destructive evaluation of “phenolic maturity” and technological ripening



Extra Virgin Olive Oil: Functional properties

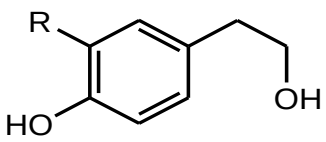
- Antioxidant
- Antinflammatory
- Antimicrobic
- Platelets anti-aggregant
- Anti-cancer

Extra virgin olive oil is the only food for which a healthy claim has been authorized by the European Food Safety Agency (**EFSA**)

Owen et al, THE LANCET Oncology Vol 1 October 2000; Romeu et al, 2016; Beauchamp et al, 2005; Romero et al, 2007; Widmer et al, 2013; Pampaloni et al, 2014.

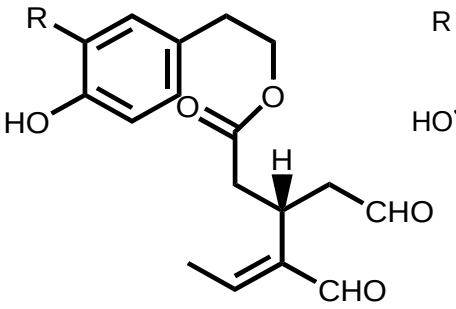
Sottoclassi CMP (Composti Minori Polari)

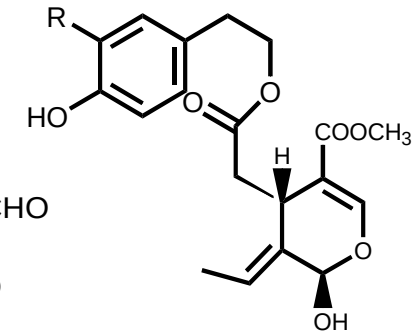
< 2% olio extravergine oliva



Fenoli semplici

Secoiridoidi





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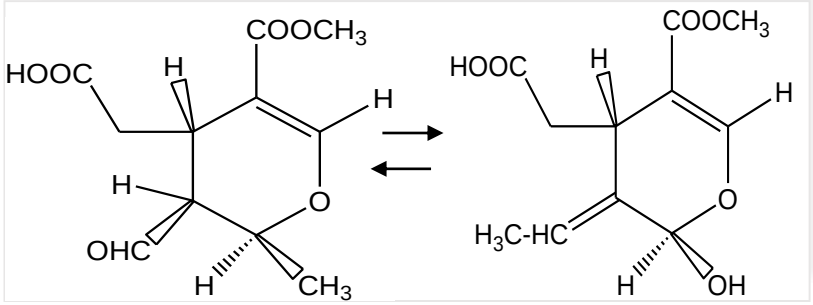

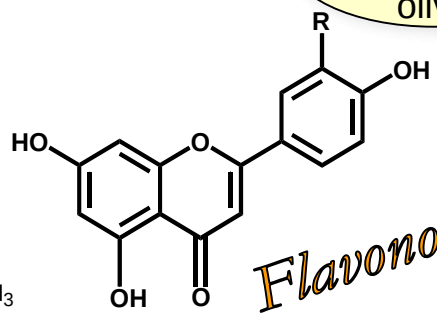

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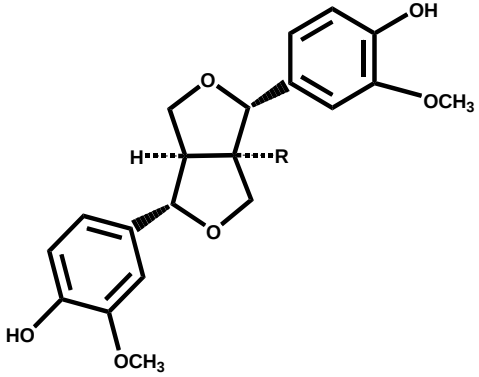

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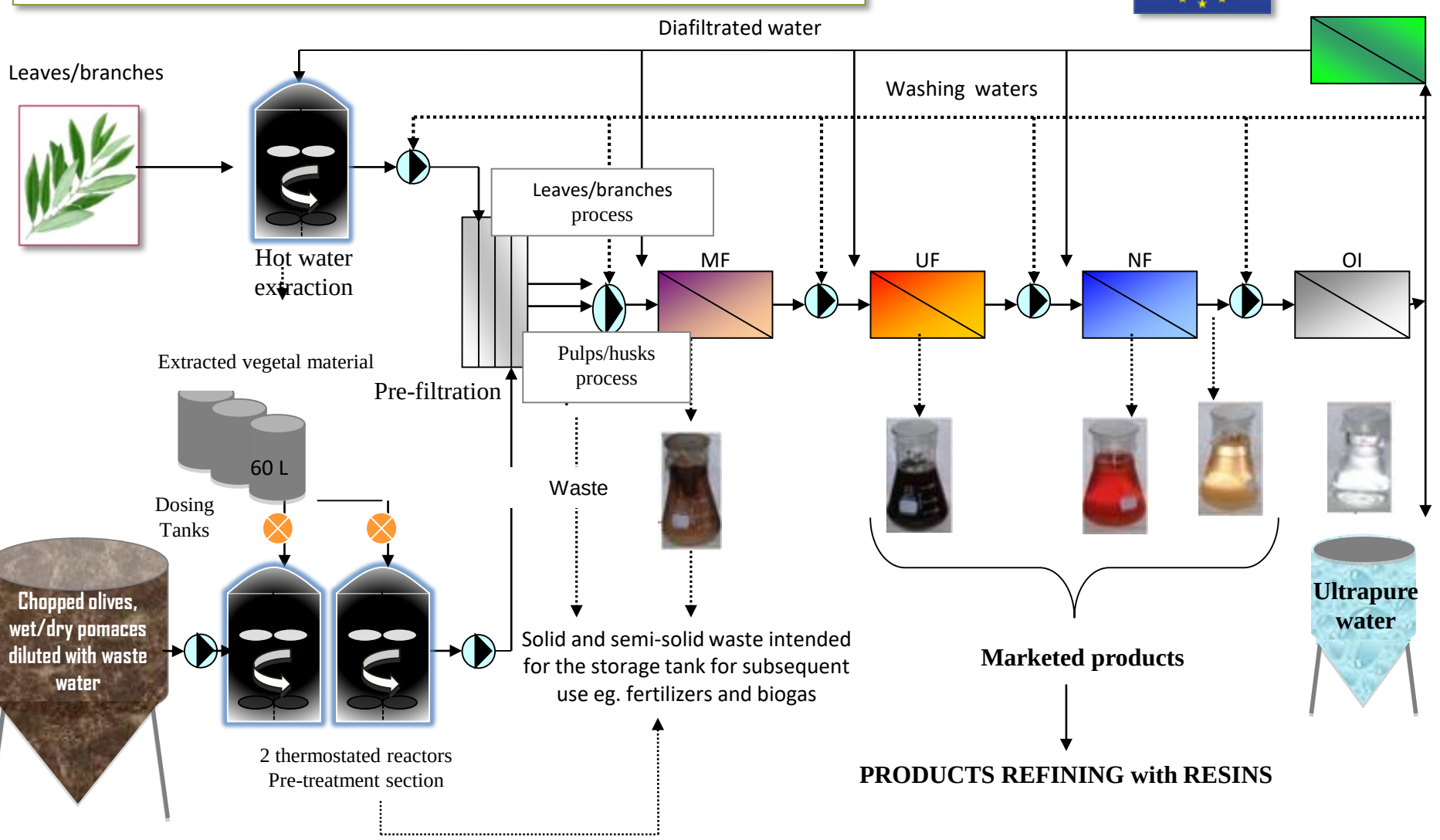
OLEA: Kilo-scale extraction plant



Treatment plant for leaves/branches/pulp and dry/wet husks diluted with waste water

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

OLEA

	Plant Fractions (g/L)				Concentrated Fractions (mg/g)			Spray Dried (mg/g)	
	GL Olea CMF	GL Olea CNF	GL Olea CRO	DL Olea CRO	Soft Extract Olea OH-Tyr	Soft Extract Olea GL	Soft Extract Olea DL	Olea GL	Olea DL
1	0.29 ± 0.10	4.69 ± 0.67	6.18 ± 0.58	3.63 ± 0.64	279.89 ± 18.24	24.69 ± 3.47	25.21 ± 1.56	23.55 ± 0.03	15.98 ± 0.96
2	2.74 ± 1.75	25.13 ± 8.88	26.62 ± 8.14	2.44 ± 1.74	nd	164.19 ± 1.47	11.09 ± 0.45	78.18 ± 16.70	25.41 ± 11.20
3	0.82 ± 0.28	4.05 ± 1.33	4.15 ± 0.45	1.05 ± 0.37	0.51 ± 0.04	28.34 ± 0.43	7.54 ± 0.40	16.98 ± 1.17	9.30 ± 4.46
4	0.03 ± 0.02	0.24 ± 0.13	0.30 ± 0.67	0.21 ± 0.12	7.83 ± 0.25	1.42 ± 0.06	4.30 ± 0.31	1.26 ± 0.88	1.49 ± 0.61
5	0.15 ± 0.09	0.56 ± 0.18	0.83 ± 0.13	0.29 ± 0.21	nd	1.27 ± 0.01	1.00 ± 0.41	4.38 ± 1.63	3.08 ± 1.05
6	0.09 ± 0.03	0.99 ± 0.31	0.83 ± 0.23	0.71 ± 0.49	1.69 ± 0.17	6.76 ± 0.10	5.85 ± 1.05	4.13 ± 0.44	2.27 ± 0.37
7	nd	3.18 ± 1.16	nd	nd	nd	17.48 ± 0.01	2.65 ± 0.23	nd	nd
Tot.	**4.12 ± 2.12**	**38.84 ± 10.31**	**38.91 ±8.24**	**8.33 ± 2.51**	**289.93 ± 18.70**	**244.15 ± 5.54**	**57.63 ± 4.42**	**128.48 ± 20.84**	**57.53 ± 18.66**

CMF = Concentrate of Microfiltration; CNF = Concentrate of Nanofiltration; CRO = Concentrate of Reverse Osmosis; GL = Green Leaves; DL = Dried Leaves; nd = not detected. 1. Hydroxytyrosol der.; 2. Secoiridoid der.; 3. Elenolic acid der.; 4. Hydroxycinnamic der.; 5. Flavonoids; 6. Verbascoside; 7. Lignans.

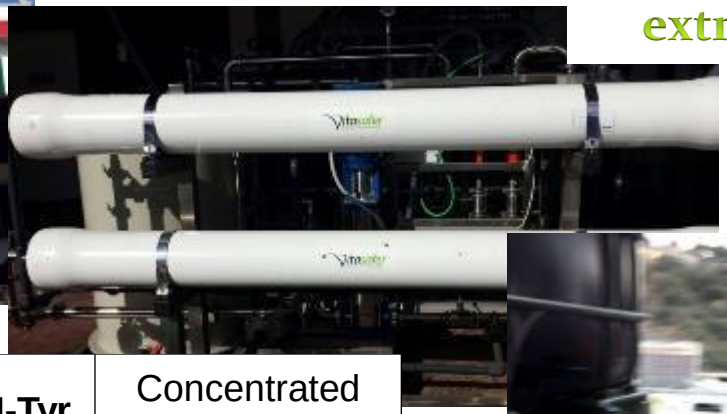
A. Romani, A. Scardigli and P. Pinelli, *Int. J. Food Sci. Tech.*, **2016**, *Submitted*.

C. Biancalani, M. Cerboneschi, F. Tadini-Buoninsegni, M. Campo, A. Scardigli, A. Romani and S. Tegli, *PLoS ONE* **2016** ***In Press***.

"EVERGREEN" - LIFE13 ENV/IT/000461

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

OLEA: Kilo-scale extraction plant



Soft Extract Olea OH-Tyr	Concentrated fraction (mg/g)
Hydroxytyrosol derivatives	279.89 ± 18.24
Secoiridoid der.	nd
Elenolic acid der.	0.51 ± 0.04
Hydroxycinnamic derivatives	7.83 ± 0.25
Flavonoids	nd
Verbascoside	1.69 ± 0.17
Lignans	nd
Total Polyphenols	**289.93 ± 18.70**

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

Olive leaves extract	mg/g	µmol/g
hydroxytyrosol glycol	2.352	13.834
hydroxytyrosol glucoside	17.373	54.978
hydroxytyrosol	2.194	14.245
tyrosol	0.319	2.313
oleoside (demethyl elenolic acid glucoside)	7.067	18.119
demethyl elenolic acid diglucoside	13.463	24.390
elenolic acid glucoside	4.605	11.399
elenolic acid glucoside derivative	2.905	7.191
caffeic acid derivatives	0.475	2.638
p-cumaroyl acid derivatives	0.422	2.571
aesculin	0.483	1.421
verbascoside	4.726	7.573
verbascoside isomer	1.969	3.155
luteolin 7-*O*-glucoside	1.262	2.817
pinoresinol	5.339	14.913
acetoxy pinoresinol	12.131	29.160
oleuropein	149.158	276.219
oleuropein derivative	13.993	25.865
total polyphenols	**240.234**	**512.801**

Lab scale extracts of olive leaves and paste

Olive paste extract	mg/g extract
Hydroxytyrosol derivatives	267.00
Secoiridoid der.	0.48
Elenolic acid der.	traces
Hydroxycinnamic derivatives	9.23
Total Polyphenols	**276.71**

C. Biancalani, M. Cerboneschi, F. Tadini-Buoninsegni, M. Campo, A. Scardigli, A. Romani and S. Tegli, *PLoS ONE*, **2016** ***In Press***.

B3 Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale.

Olea fractions stability

Total polyphenols content (mg/g)	Phenolea FF	Phenolea OH-Tyr
T0	248.07	303.14
T12	240.23	276.70

Stability of Olea fractions at time 0 (T0) and at 12 months (T12) at room temperature.

In vitro antioxidant/antiradical activity

The **Phenolea fractions** antiradical activities, evaluated using a DPPH solution (3.16 10^{-4} mM), show the following **EC_{50} values**: 6.757 µM for Phenolea F, 5.440 µM for Phenolea S, and 12.195 µM in the case of Phenolea OH-TYR.

The antiradical activity of the **grape seeds** extract has also been measured by the *in vitro* assay with stable radical DPPH• and the **EC_{50}** was 1.675 µM.

Considerations about the polyphenols market

Polyphenols could be employed as ***active principles*** in

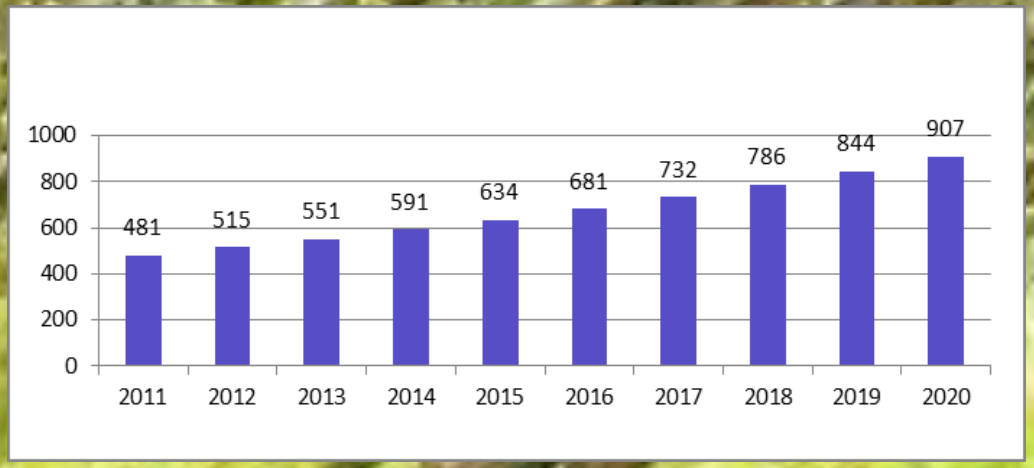
- Agronomy
- Cosmetics
- Foods
- Feed
- Pharmaceutical applications.

The most commonly used polyphenols are resveratrol, gallic acid, tannins and others extracted from cocoa, grapes, apples, olives, pomegranate and cranberries.

The hydroxytyrosol is less used, but has an increasing market potential. It comes from Olea and Cynara, two crops cultivated in the region Apulia

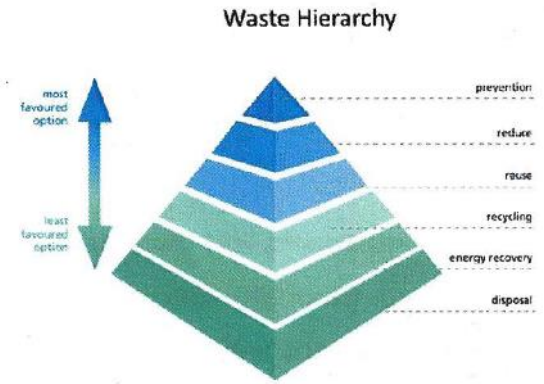
Considerations about the polyphenols market

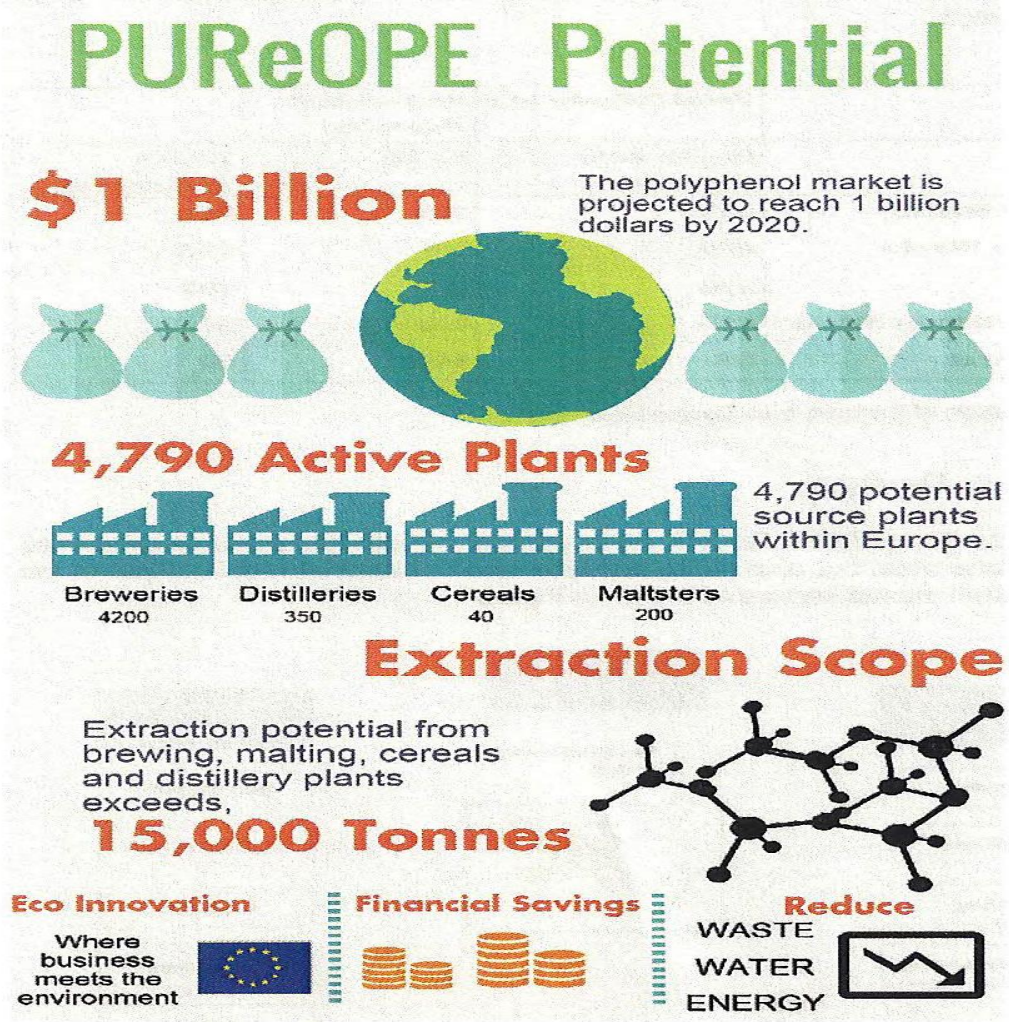
Globally the polyphenols market rapidly grew in the last years and it is expected to reach 900 million Euros by 2020



Global Polyphenols market (Million Euros) (2011-2020)

Considerations about the polyphenols market

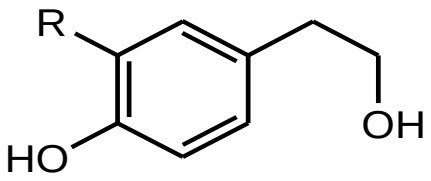




FOOD BioEnergy

RedoxPhenol™

HYDROXYTYROSOL



- **It helps to protect cells from oxidative stress caused by free radicals responsible for cell aging.**
- **Promotes normal and proper cognitive and nervous functions.**

Effects of Phenolea Complex polyphenols on brain nerve growth factor and brain derived neurotrophic factor in a mouse model.

Luigi Tarani, Mauro Ceccanti and Marco Fiore, 2014

Phenolea® Active contains Oleocanthal - a natural anti inflammatory compound featuring an intensity and a profile very much alike to that of Ibuprofen, with which it shares pharmacological activities. Both of the Oleocanthal enantiomorphs, such as Ibuprofen, cause an inhibition dose-dependant from the activities of the COX-1 and COX-2 without effects on the lipoxygenase
(Table 1).

Oleocantale Ibuprofene

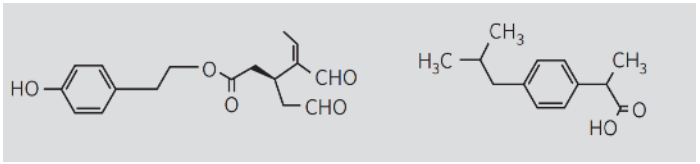


Table 1 | Selective inhibition of COX enzymes by oleocanthal enantiomers

Agent	Concentration (μM)	COX-1 (%)	COX-2 (%)	15-LO (%)
(−)Oleocanthal	100	83.5 ± 3.5	70.9 ± 8.6	0.4 ± 0.8
	25	56.1 ± 3.2	56.6 ± 9.5	0.0 ± 0.0
	7	24.6 ± 7.3	14.5 ± 2.3	0.0 ± 0.0
(+)Oleocanthal	100	68.0 ± 15.2	69.6 ± 3.9	3.5 ± 5.5
	25	54.5 ± 4.6	41.3 ± 15.9	0.7 ± 1.0
	7	24.6 ± 7.5	6.1 ± 4.2	0.0 ± 0.0
Ibuprofen	25	17.8 ± 2.3	12.7 ± 3.6	0.2 ± 0.3
	7	0.0	1.3	ND
Indomethacin	25	90.1	89.8	0.1 ± 0.9
	7	86.6	66.3	0.5 ± 0.1
NDGA	25	ND	ND	63.1 ± 0.8
	7	ND	ND	52.5 ± 1.1
caffeic acid	25	ND	ND	25.2 ± 2.2
	7	ND	ND	19.8 ± 1.3

Percentage inhibition of cyclooxygenases 1 and 2 (COX-1, COX-2) and 15-lipoxygenase (15-LO) by three different concentrations of oleocanthal and of ibuprofen are presented as mean ± s.e.m. for three independent experiments. ND, not determined. Indomethacin was used as a positive (inhibitory) control in the cyclooxygenase assays and *nor*-dihydroguaiaretic acid (NDGA) and caffeic acid were used as positive (inhibitory) controls in the lipoxygenase assay. The concentrations for 50% inhibition (IC_{50}; calculated by least-squares regression analysis of inhibition versus concentration) for the natural (−) oleocanthal are 23 μM and 28 μM for COX-1 and COX-2, respectively; IC_{50} values for (+) oleocanthal are 25 μM and 40 μM for COX-1 and COX-2, respectively; IC_{50} values for ibuprofen are 5 μM and 223 μM for COX-1 and COX-2, respectively[13]. (For methods, see supplementary information.)

RedoxPhenol

Gary K. Beauchamp, Russell S. J. Keast, Diane Morel, Jianming Lin, Jana Pika, Qiang Han, Chi-Ho Lee, Amos B. Smith, Paul A. S. Breslin. '**Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil**'. *Nature* 437 (2005) 45-46.

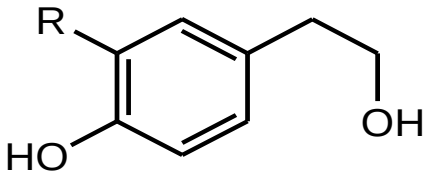
ArtroPhenolea™

Artrophenolea is a food supplement with natural extracts with physiological effect in liquid formulation containing **Phenolea® Active (*Oleocanthal)* – Devil's Claw (*Harpagenine)***

Natural anti inflammatory properties

Artrophenolea™'s liquid formulation eases the absorption, fastening the action of its natural components.

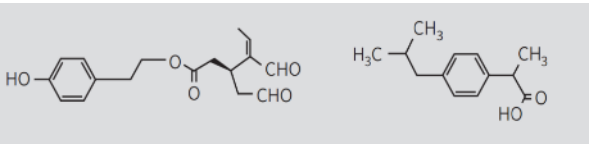
Recommended dosage: 5 ml per day to take preferably far from meals.



Apporti medi di componenti caratterizzanti	Per 5 ml
Artiglio del diavolo e.s.	1,5 g
apporto in arpagoside	25 mg
Phenolea® Active OHTY	100 mg
apporto in polifenoli tot.	10 mg
apporto in Idrossitirosolo	6 mg

ArtroPhenolea™

Oleocantale Ibuprofene



Ingredients

Olea europaea L. Phenolea® Active OHTY (*Oleocanthal)*

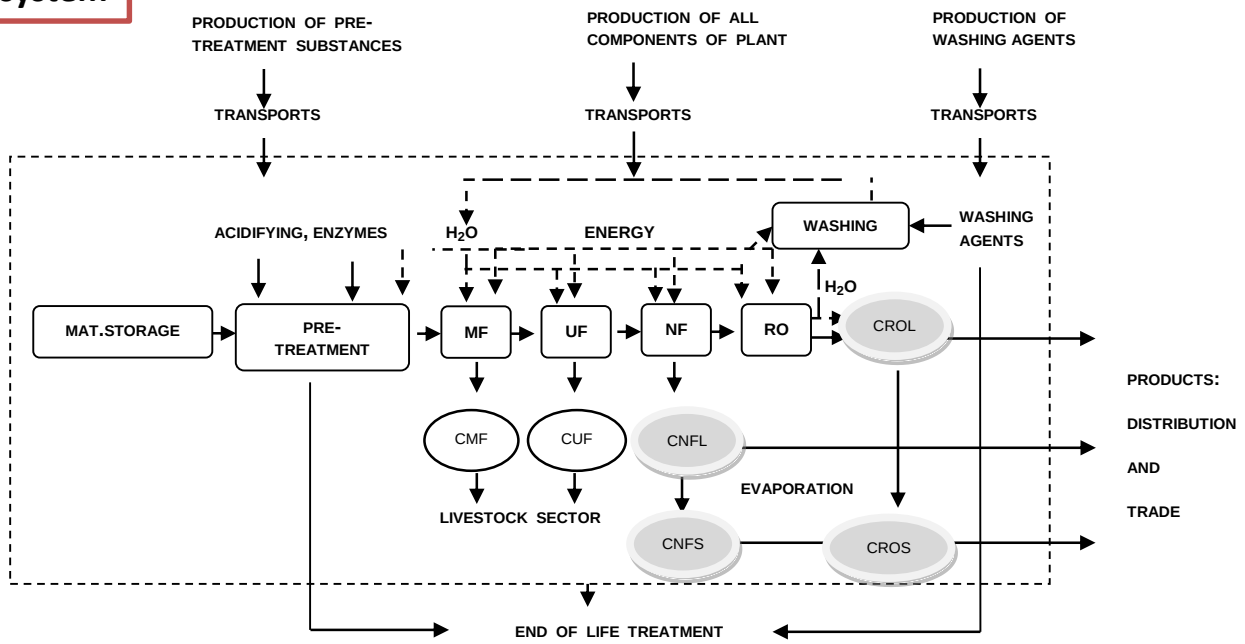
Devil's Claw (*Harpagenine)*

The polyphenols perform their protective action by interacting with different molecular mechanisms responsible for signal transduction within the cell, modulating the expression of pro-inflammatory genes such as cyclooxygenase, lipoxygenase, nitric oxide synthetase and various cytokines, mainly through the interaction with the transcription factor NFK-B.

The anti-inflammatory activity test of **Phenolea® Active Complex** on cytokine IL10, carried out at the **Cellular Biology and Neurobiology Institute, CNR**, Rome, confirmed that the phytocomplex inhibits the inflammatory action in animal model.

Life Cycle Assessment: Olea plant

Flow chart of the system



LEGEND

MAT.:MATRICES (pulps and water) from biphasic olive mill	**MF**: MICROFILTRATION	**UF**: ULTRAFILTRATION	**NF**: NANOFILTRATION	**RO**: REVERSE OSMOSIS
CMF: CONCENTRATE OF MICROFILTRATION (CO-PRODUCT 1)	**CUF**: CONCENTRATE OF ULTRAFILTRATION (CO-PRODUCT 2)	**CNFL**: CONCENTRATE OF NANOFILTRATION LIQUID (PRODUCT 1)	**CROL**: CONCENTRATE OF REVERSE OSMOSIS LIQUID (PRODUCT 2)	
CNFS: CONCENTRATE OF NANOFILTRATION SOLID (PRODUCT 3)	**CROS**: CONCENTRATE OF REVERSE OSMOSIS SOLID (PRODUCT 4)			

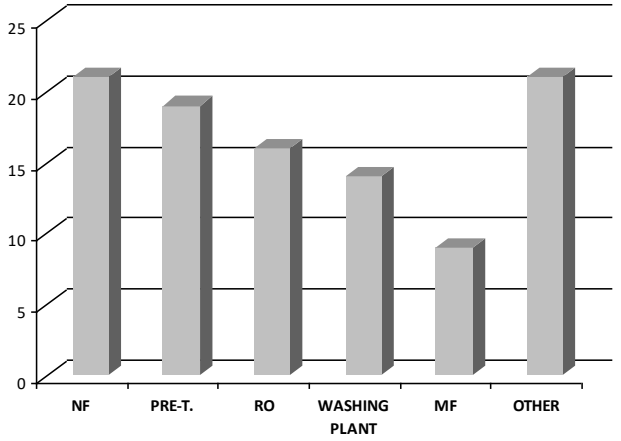
Olivieri G., P. Neri, F. Bandini, A. Romani, Atti del congresso The AGS Annual Meeting 2007, Barcelona, Spain.
Olivieri G., P.Neri, A.Romani, Journal of Commodity Science, Technology And Quality 2010 A, 49 (II) 95 -103.
Olivieri G., Romani A., Falconi F. LCA food VII Conference on Life Cycle Assessment in the agri-food Sector, Bari, 22-24 Settembre 2010 B, ISBN 978-88-88793-29-0.

Life Cycle Assessment: Olea plant

Characterization results in LCIA (equivalent units) [FU: 1l and 1kg]

IMPACT POTENTIAL	UM	PRODUCTS FROM OLEA EUROPAEA L. MATRICES			
		CNFL (1l)	CNFS (1kg)	ROL (1l)	ROS (1kg)
Carcinogens	kg C_2H_3Cl	5,74E-03	2,76E-02	5,62E-03	2,97E-02
Non-Carcinogens	kg C_2H_3Cl	7,86E-03	4,19E-02	7,95E-03	4,57E-02
Respiratory inorganics	kg PM2.5	7,19E-04	3,61E-03	7,59E-04	4,02E-03
Ionizing radiation	Bq C-14	2,13E+01	1,09E+02	2,30E+01	1,23E+02
Ozone layer depletion	kg CFC-11	9,37E-08	4,84E-07	9,85E-08	5,29E-07
Respiratory organics	kg ethylene	2,82E-04	1,21E-03	2,73E-04	1,34E-03
Aquatic ecotoxicity	kg TEG water	6,44E+01	3,35E+02	6,55E+01	3,59E+02
Terrestrial ecotoxicity	kg TEG soil	1,36E+01	6,44E+01	1,38E+01	7,03E+01
Terrestrial acid/nutri	kg SO_2	1,55E-02	7,88E-02	1,64E-02	8,80E-02
Land occupation	m^2org.arable	3,62E-03	1,55E-02	3,39E-03	1,67E-02
Aquatic acidification	kg SO_2	5,27E-03	2,74E-02	5,69E-03	3,07E-02
Aquatic eutrophication	kg PO_4 P-lim	3,95E-05	1,97E-04	4,12E-05	2,18E-04
Global warming	kg CO_2	8,68E-01	4,53E+00	9,34E-01	5,06E+00
Non-renewable energy	MJ primary	1,44E+01	7,46E+01	1,53E+01	8,30E+01
Mineral extraction	MJ surplus	3,32E-02	1,95E-01	3,35E-02	2,14E-01

Processes average contribution in LCIA weighting (%)

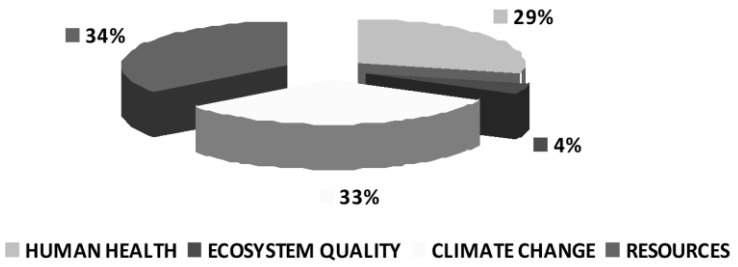


Life Cycle Assessment: Olea plant

Weighting results in LCIA (Pt) [FU: 1l and 1kg]

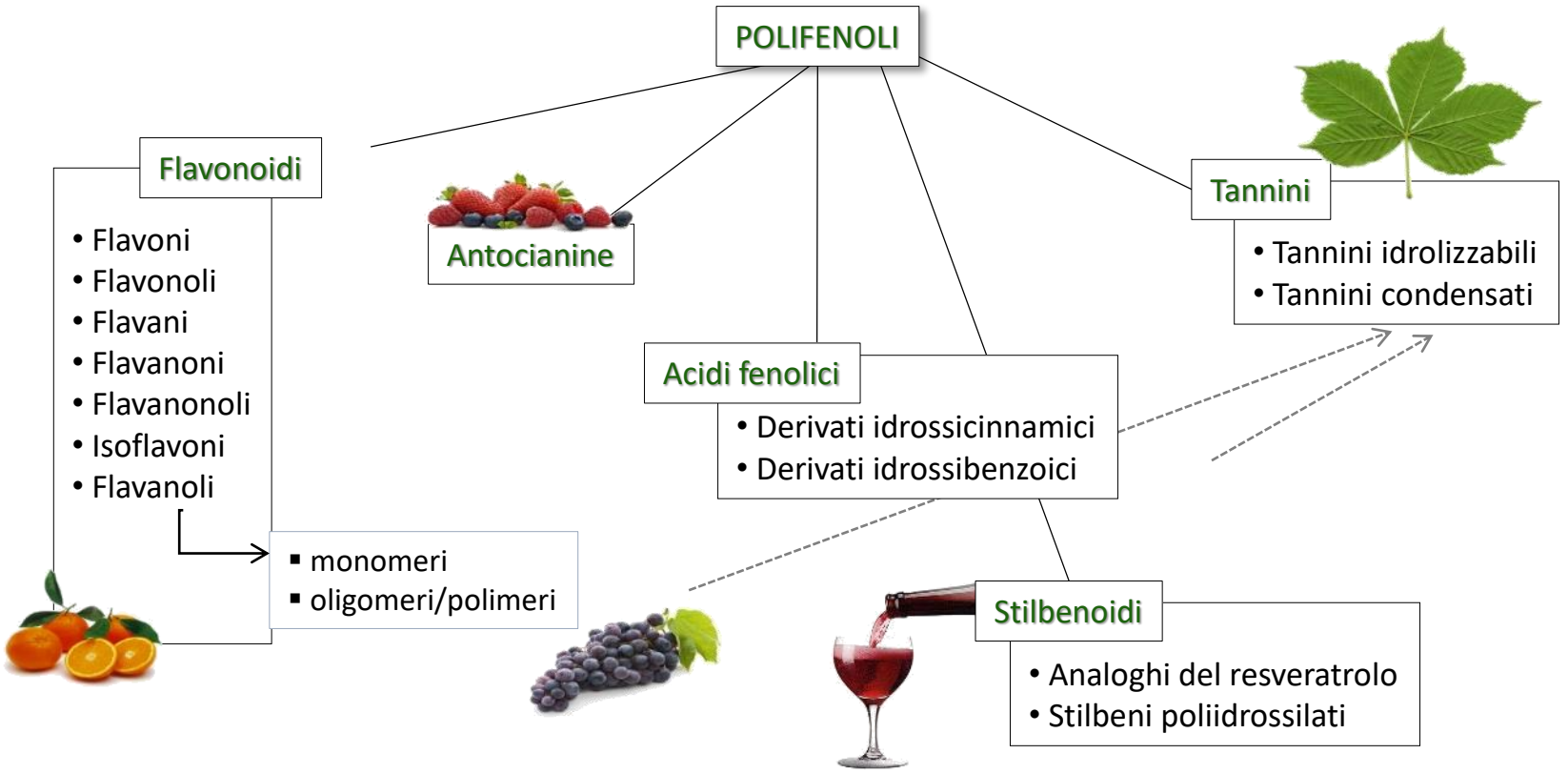
DAMAGE CATEGORY	UM	PRODUCTS FROM OLEA EUROPAEA L. MATRICES			
		CNFL	CNFS	CROL	CROS
TOTAL	Pt	0,00027	0,0014	0,00029	0,0015
HUMAN HEALTH	Pt	7,7E-05	0,0003875	8,1E-05	0,00043
ECOSYSTEM QUALITY	Pt	9,56E-06	4,56E-05	9,69E-06	4,99E-05
CLIMATE CHANGE	Pt	8,8E-05	0,00046	9,4E-05	0,00051
DEPLETION OF RESOURCES	Pt	9,4E-05	0,00049	0,0001	0,00054

Weighting results in LCIA (% Pt) [FU: 1l and 1kg]

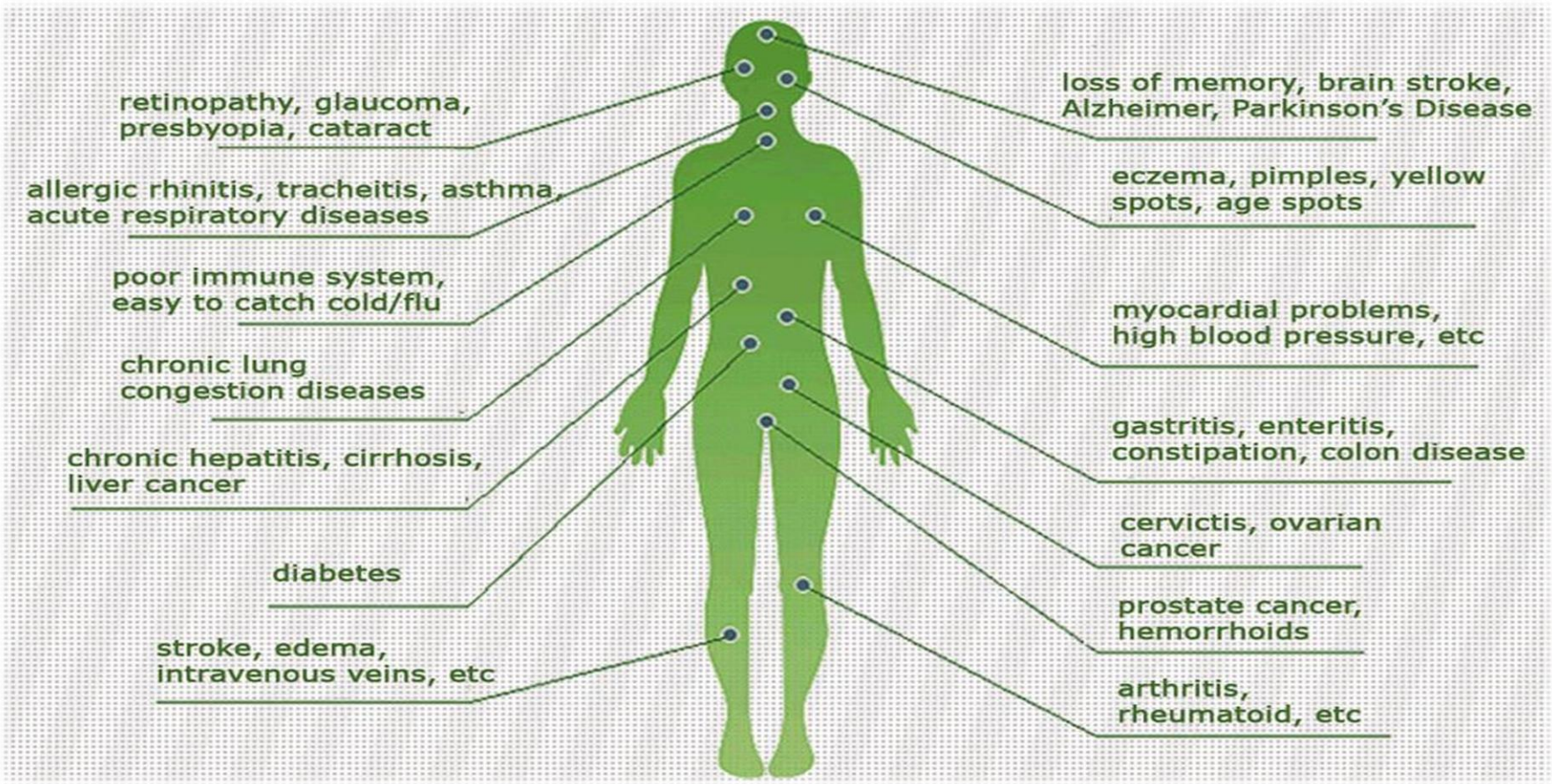


The result of impact assessment showed that the **energy consumptions** are the most significant impacts, therefore next aims could be to improve the system to reduce the use of energy from non renewable resources. Hence, the categories where impact are more significant are **Climate Change** and **Depletion of Resources**.

Diet and natural antioxidants : Polyphenols



Oxydative stress diseases



Case Study

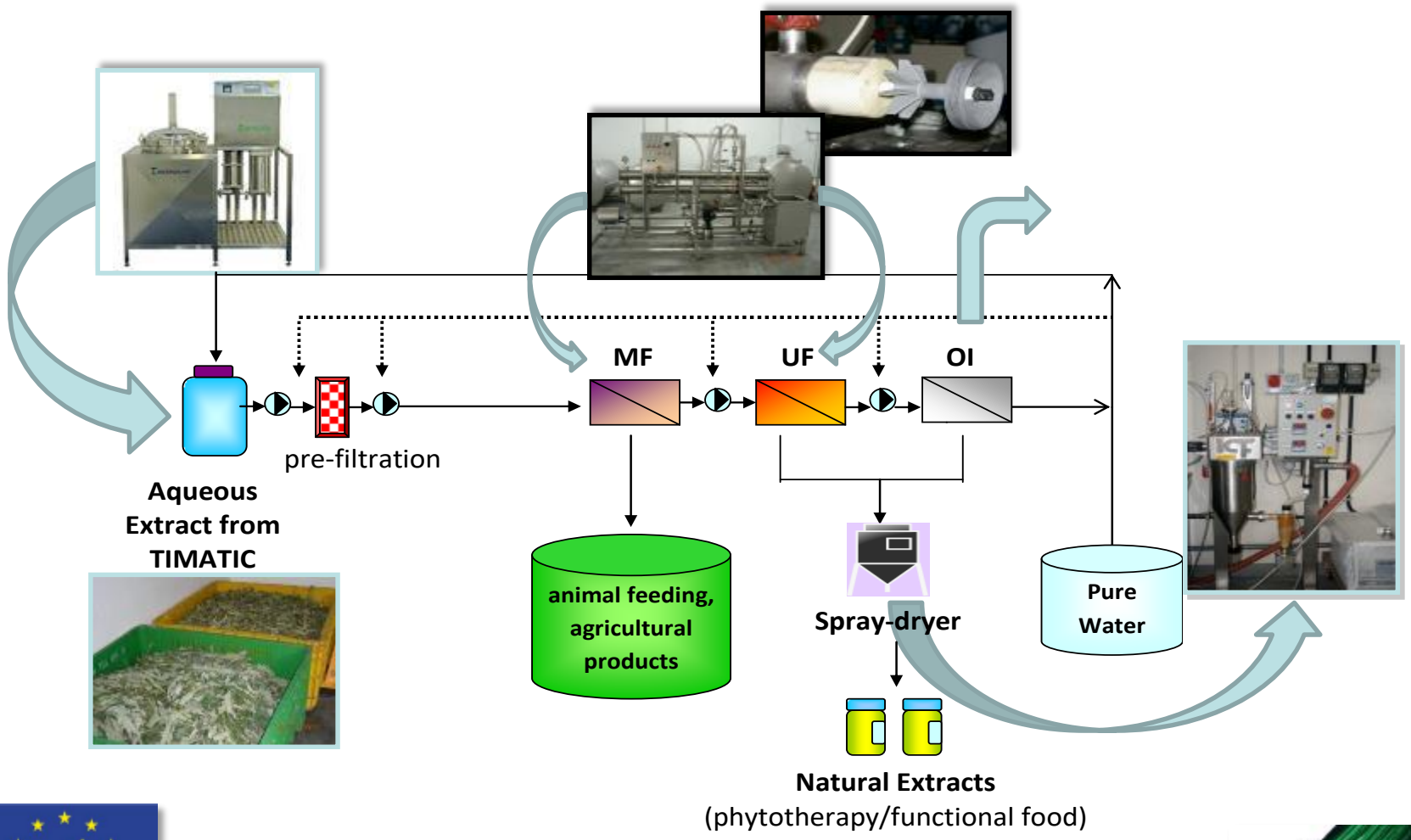
Development of Environment-friendly extraction process

This innovative process uses new sustainable technologies based on membrane separation system.

They are physical technologies (PCT/IT/2009/09425529 Olea europaea L.; PCT/IT/2008/000135 Cynara scolymus L.), defined as BAT (Best Available Technology) and recognized from Environmental Protection Agency.

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

Cynara Active^{R} , nutraceutical standardized extracts from *Cynara scolymus* L.



B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

Lab scale extracts of artichoke leaves

Artichoke leaves extract	mg/g	µmol/g
monocaffeoyl quinic acids	2.133	6.026
dicaffeoyl quinic acids	4.688	9.085
clorogenic acid	16.350	46.188
cynarin	0.000	0.000
p-cumaroyl quinic acid	0.000	0.000
luteolin derivative	2.362	8.260
total polyphenols	**25.534**	**69.559**

C. Biancalani, M. Cerboneschi, F. Tadini-Buoninsegni, M. Campo, A. Scardigli, A. Romani and S. Tegli, *PLoS ONE*, **2016** ***In Press***.

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

CYNARA

	CUF Cynara GL mg/L	CRO Cynara GL mg/L	Cynara GL Soft extract mg/g	Cynara GL Spray Dried mg/g
MCC	1.07 ± 0.58	65.19 ± 13.28	6.61± 1.34	14.23± 0.48
DCC	2.81 ± 1.19	3.96 ± 5.60	7.64± 0.69	7.63± 0.20
Chlorogenic acid	2.04 ± 0.47	34.00 ± 7.38	11.93± 1.72	12.36± 0.03
Cynarin	0.50 ± 0.43	28.94 ± 14.89	1.62± 0.01	4.41± 0.34
Flavonols	0.23 ± 0.06	10.11 ± 5.39	1.09± 0.27	3.48± 0.56
Total Polyphenols	**6.57 ± 1.92**	**142.21 ± 9.58**	**28.90± 4.02**	**42.10± 0.42**

CUF = Concentrate Ultrafiltration; CRO = Concentrate Reverse Osmosis; GL = Green Leaves.

HPLC/DAD quantitative analyses of different plant fractions from *Cynara* leaves (CUF and CRO), and two concentrated fractions from CRO: soft extract and spray dried from green leaves. Data are mean values of triplicate analyses (±SD).

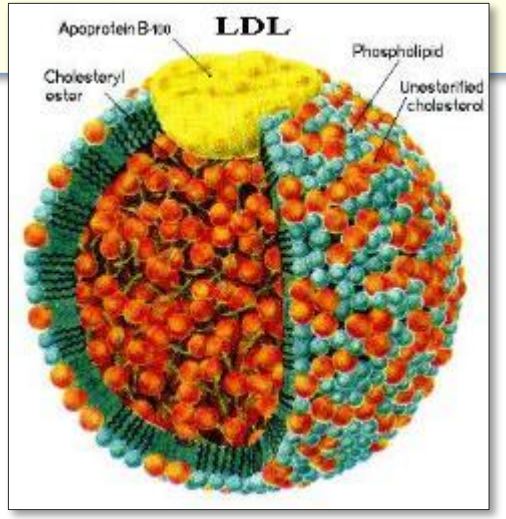
A. Romani, A. Scardigli and P. Pinelli, *Int. J. Food Sci. Tech.*, **2016**, *Submitted*.

C. Biancalani, M. Cerboneschi, F. Tadini-Buoninsegni, M. Campo, A. Scardigli, A. Romani and S. Tegli, *PLoS ONE*, **2016** ***In Press***.

September 23, 2016 - "EVERGREEN" - LIFE13 ENV/IT/000461

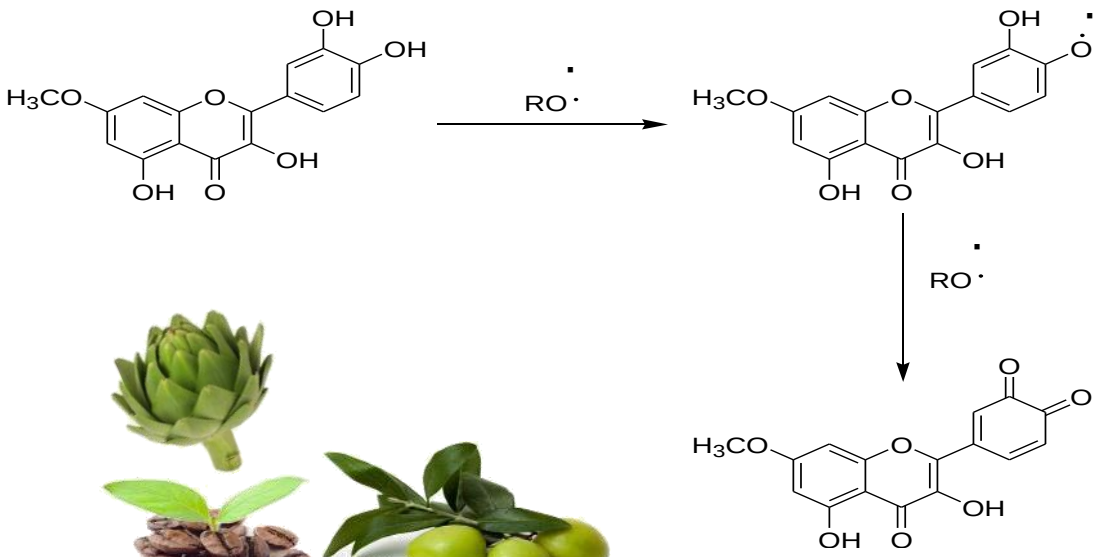
COLESTEROL AND OXIDATIVE STRESS

TEST IN HUMANS



- Isolate LDL (Low Density Lipoproteins) in Human plasma and antioxidant test.

Cynara antioxidants and antiradical compounds
New nutraceuticals for colestrerol decrement



Case Study

Three commercial products are obtained through this process: a liquid fraction, a concentrate paste, and a powder, with different hydroxytyrosol concentration. The market prices of the enriched products strictly depends on their hydroxytyrosol content, increasing with its higher concentration (title)

Case Study

Cloosing the loop

After the extraction of bioactive fractions, residues of Cynara and Olea can be used as animal feed, compost or other agricultural or agro industrial products and/or exploited as energy source.

In particular two kinds of lignocellulosic residue provide the thermal energy (about 0.202 Toe) need for the extraction process that by 77 tons of virgin biomass produces the dry extract with a 4.7% yield.

Case Study

Cloosing the loop

It has to be underlined the ecological and economical sustainability of the process

- Agro industrial residues as input
- Water as solvent
- Waste re-used in the same process and/or in external one
- Energy consumption reduced

Final Remarks

These biomolecules are suitable for innovative uses in many sectors, like agronomy, textile/dyeing, cosmetics, foods and phytotherapy, that increasingly demand high value added products, also which are environment- friendly.

It will allow to gain new and increasing market shares

The new approach of closing loop is also highly profitable

BIOMASSE E SCARTI
(vinacce e vinaccioli)

UTILIZZO
TRADIZIONALE

RIUTILIZZO GREEN O
ESTRAZIONI INNOVATIVI

ECONOMIA CIRCOLARE

VITICOLTURA CIRCOLARE

SMALTIMENTO DEGLI
SCARTI O
COMBUSTIONE

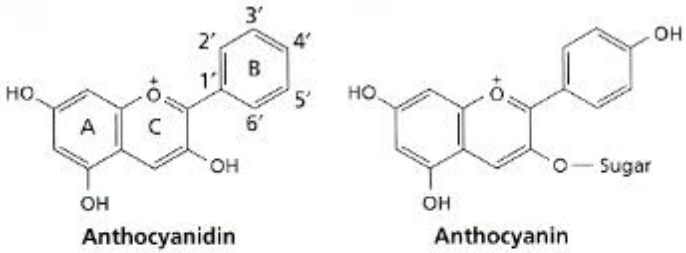
COMPOSTI NATURALI
ATTIVI ED ENERGIA

Optimization of optical methods for quality control of wine grapes to assess in the vineyard grape berry anthocyanins

Annalisa Romani

PHYTOLAB-DiSIA, Department of Pharmaceutical Sciences, University of Florence

IFAC CNR, Florence, ITALY



Anthocyanidin	Substituents	Color
Pelargonidin	4′— OH	Orange red
Cyanidin	3′— OH, 4′— OH	Purplish red
Delphinidin	3′— OH, 4′— OH, 5′— OH	Bluish purple
Peonidin	3′— OCH_3, 4′— OH	Rosy red
Petunidin	3′— OCH_3, 4′— OH, 5′— OCH_3	Purple

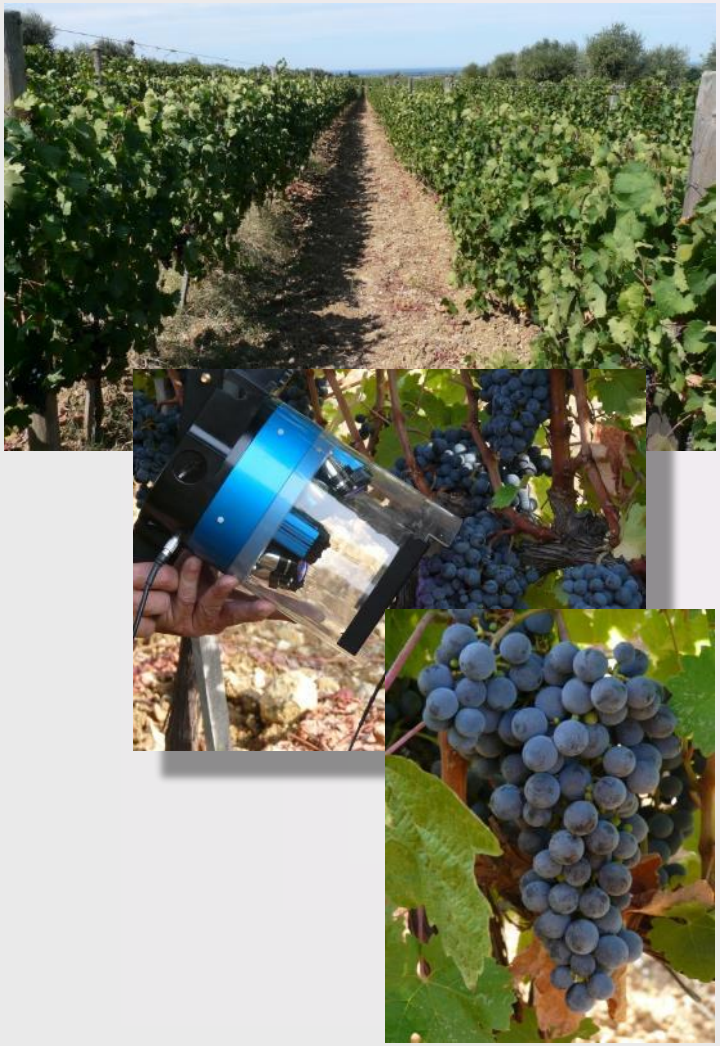
Technology and Innovation for a Sustainable Future: a Commodity Science Perspective

The evaluation of grape maturity, fundamental for the production of top-quality wines, can be possible:

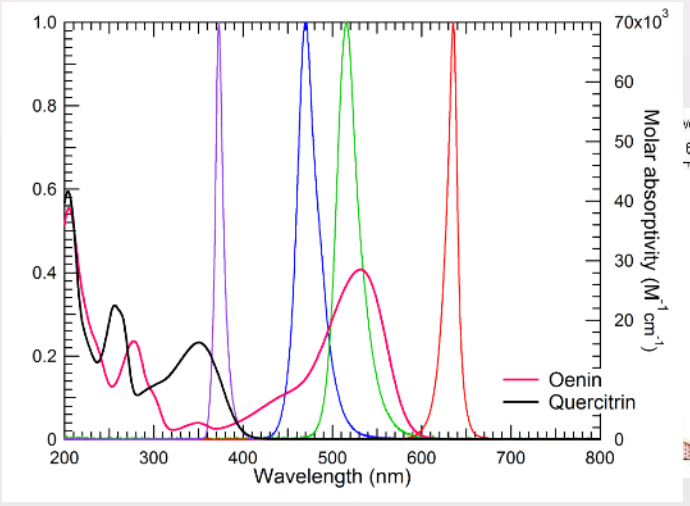
directly in the vineyard, using the portable optical sensor **Multiplex** (Force-A, France) by a non destructive analysis

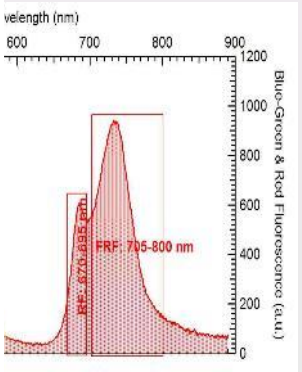
MT01 (Maselli Misure S.p.A.) in the laboratory with a screening and rapid method based on destructive diffuse reflectance spectrophotometry

Multiplex: the technique



This method is based on the screening of fruit chlorophyll fluorescence excitation by Anth and **allows the Anth contents of intact berry skin to be measured**.





Multiplex sensor

- Handheld (battery-operated)
- Rapid acquisition time for a single bunch sample (1 sec)
- Large detection area (8-cm diameter)
- LED sources pulsed and synchronized to detection
- No sample preparation.

Multiplex3®

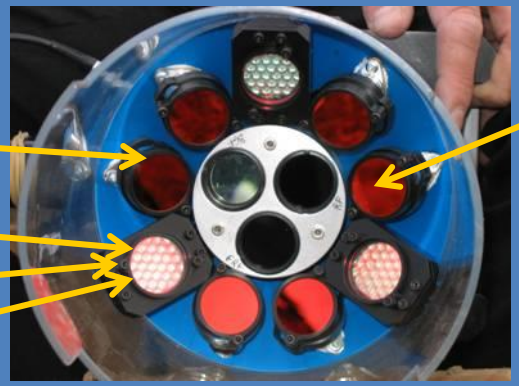
Excitation light emitting diode (LED)

UV

Blue

Green

Red



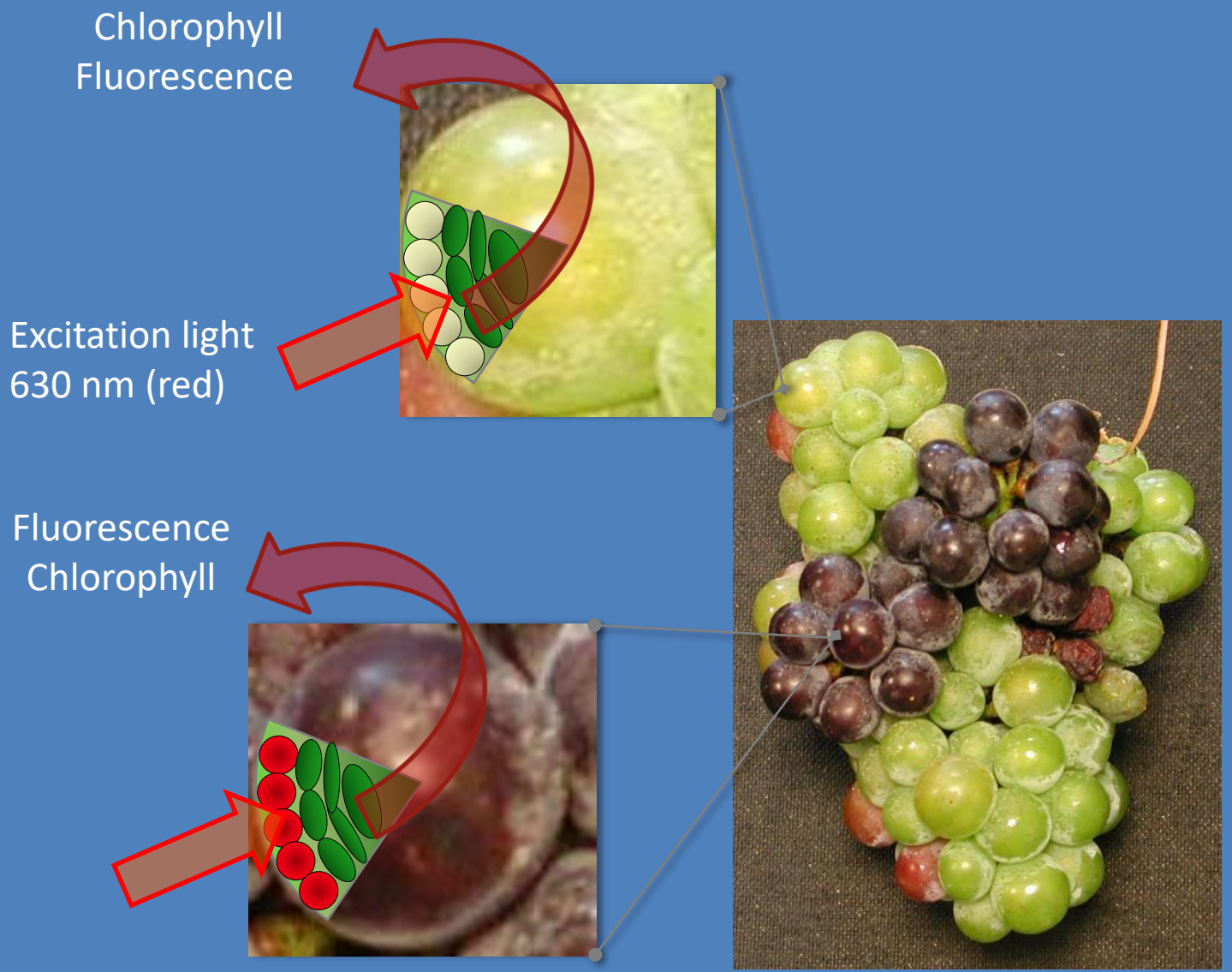
3 detection channels

Blue-green fluorescence (BGF)

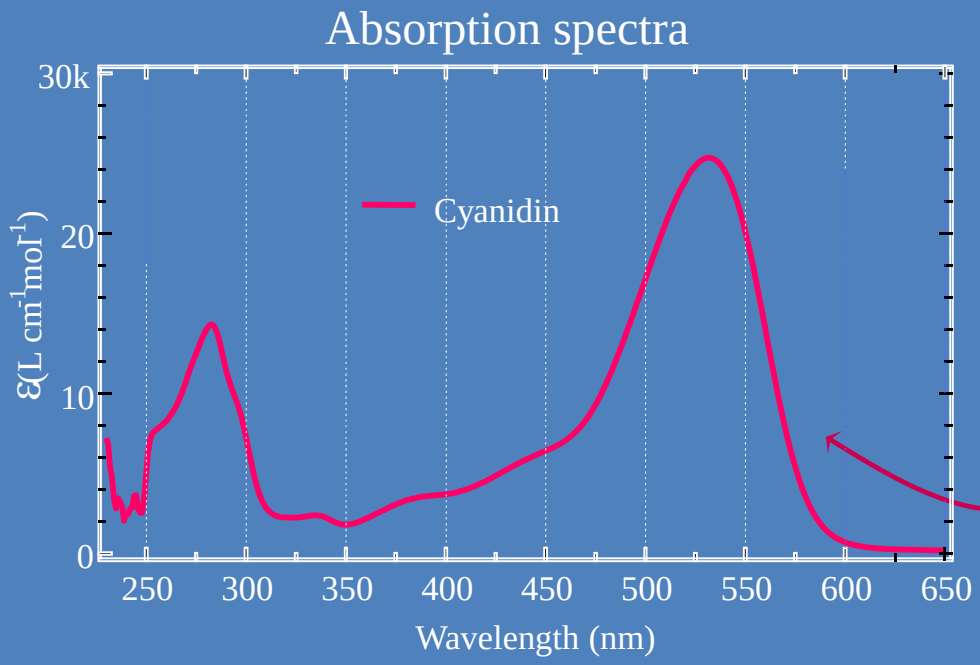
Red Fluorescence (RF)

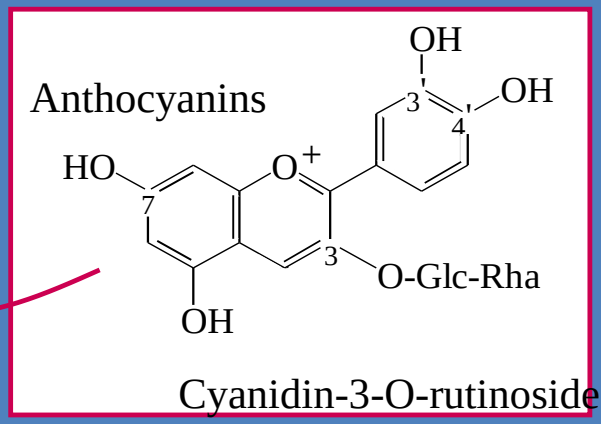
Far Red Fluorescence (FRF)

Anthocyanin accumulation during ripening



Anthocyanins Multiplex Index $ANTH = \log(FRF_{red} / FRF_{green})$





The definition of the Anth index given in such Equation, by comparing the Chl fluorescence intensity under G and R lights, respectively, represents a differential absorption measurement (Beer–Lambert's law) that is proportional to the Anth content.

HIGHER THE ANTH CONCENTRATION, LOWER THE CHL FLUORESCENCE SIGNAL.

Sampling in vineyard:

- Petit Verdot
- Syrah
- Merlot

CASALE DEL GIGLIO®
AZIENDA AGRICOLA

Veduta dell'azienda agricola Casale del Giglio alle Ferriere

Multiplex3®

- Cabernet Sauvignon
- Sangiovese
- Merlot
- Syrah

CASTELLO BANFI
MONTALCINO

Weekly measurements from veraison to harvest (2009-2011 seasons)

In field measurements

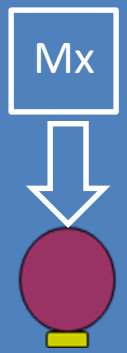
3 plots
2 rows per plot - 2 sides per row
25 grape bunches per side
100 measured bunches per cv. per plot

SENSOR CALIBRATION

3 X

For each day of measurements 3 bunches per variety were collected. Mx measurements *in situ* before harvest.

Measured again in the lab 3-4 hours after harvest.

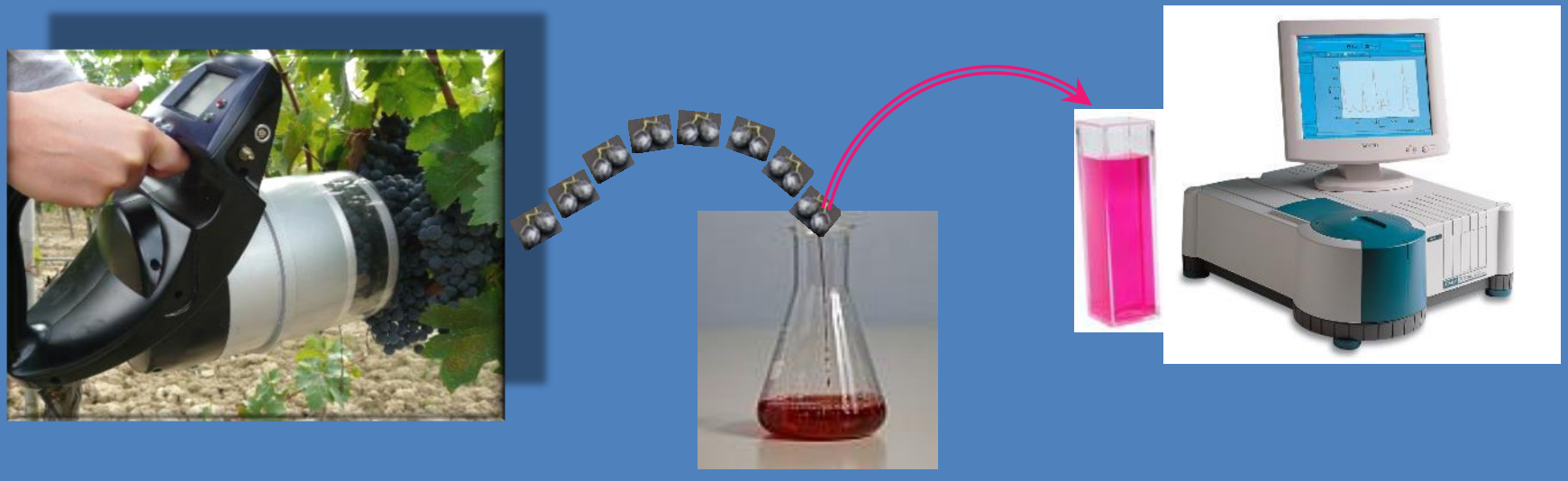


From the exposed side **a limited number of berries** was collected and measured by Mx

Frozen until hydroalcoholic extraction and spectrophotometric analysis of ANTH with a classical UV-Vis spectrophotometer Cary 50 (Varian).

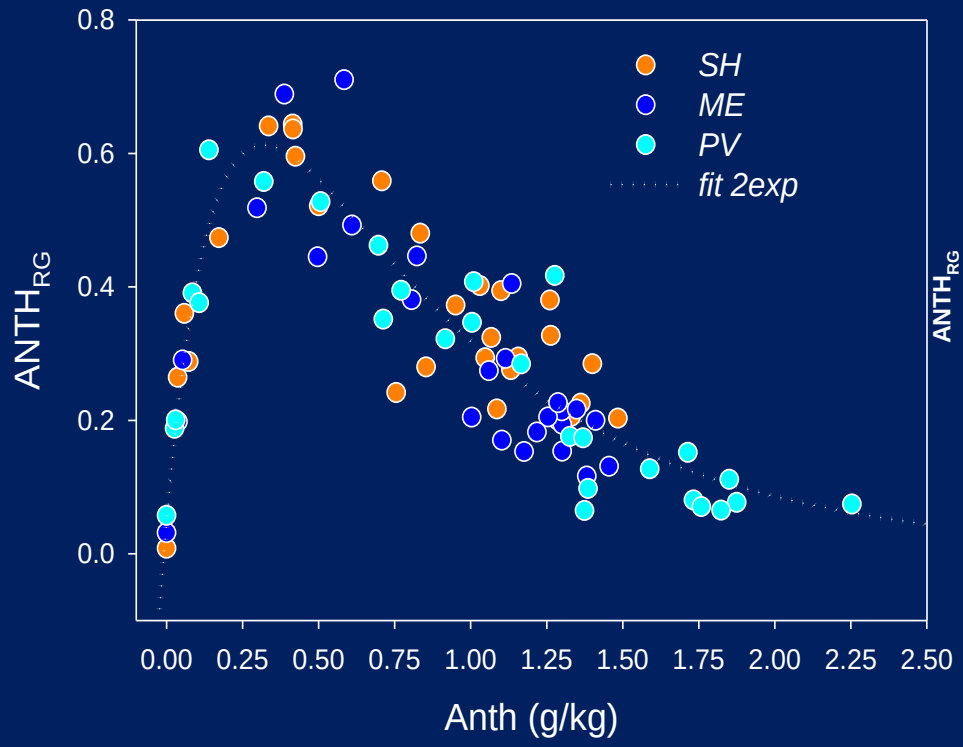
UV-Vis spectrophotometer Cary 50

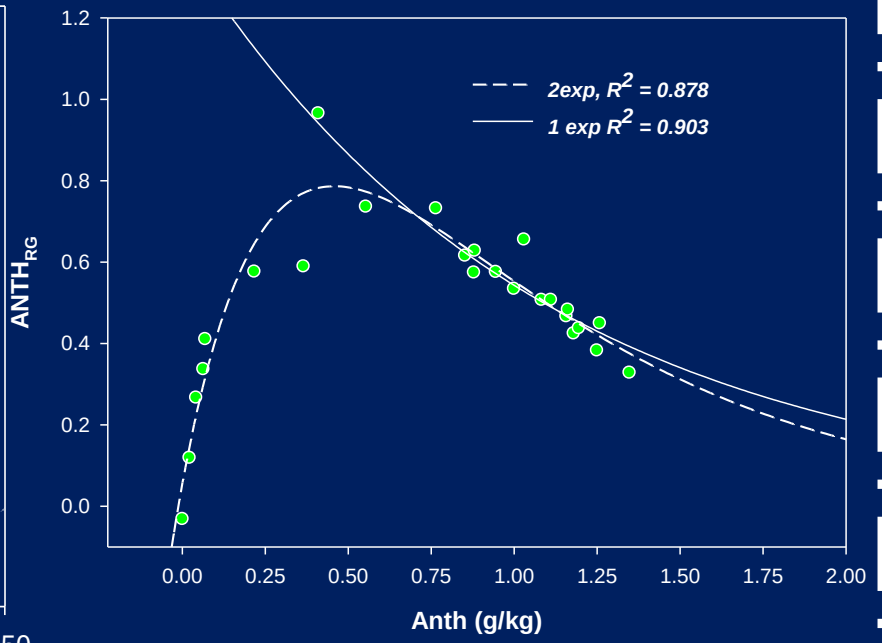
Data obtained from the destructive analyses have been used to calibrate the optical sensor. The calibration curves obtained for each cv have been inserted into the optical sensor.



CALIBRATION CURVES

Multiplex sensor and UV-VIS Spectrophotometric data

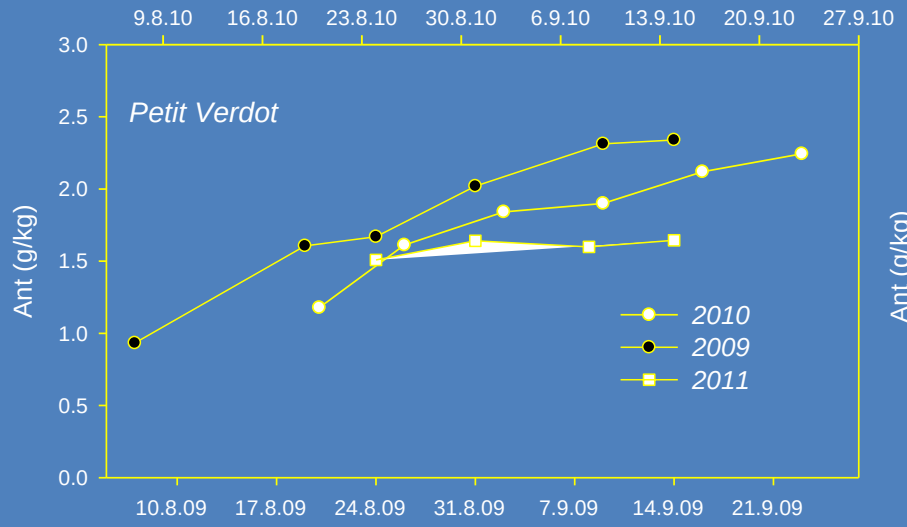


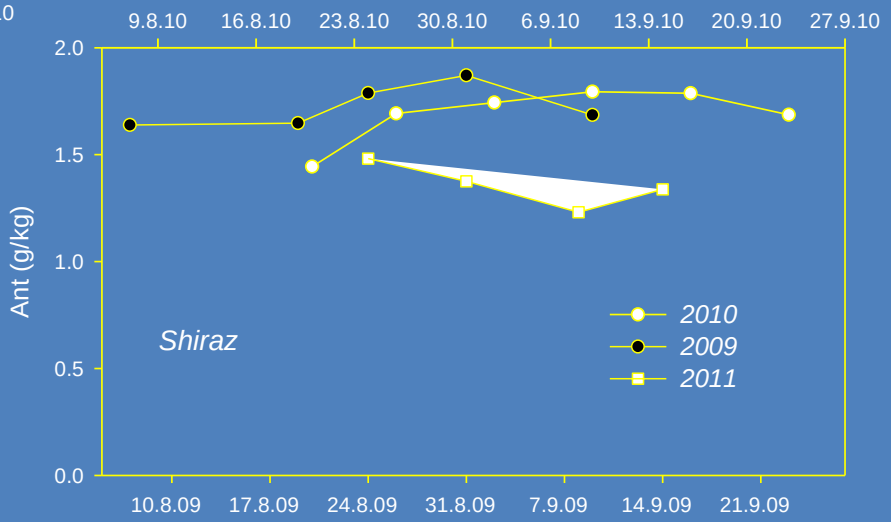


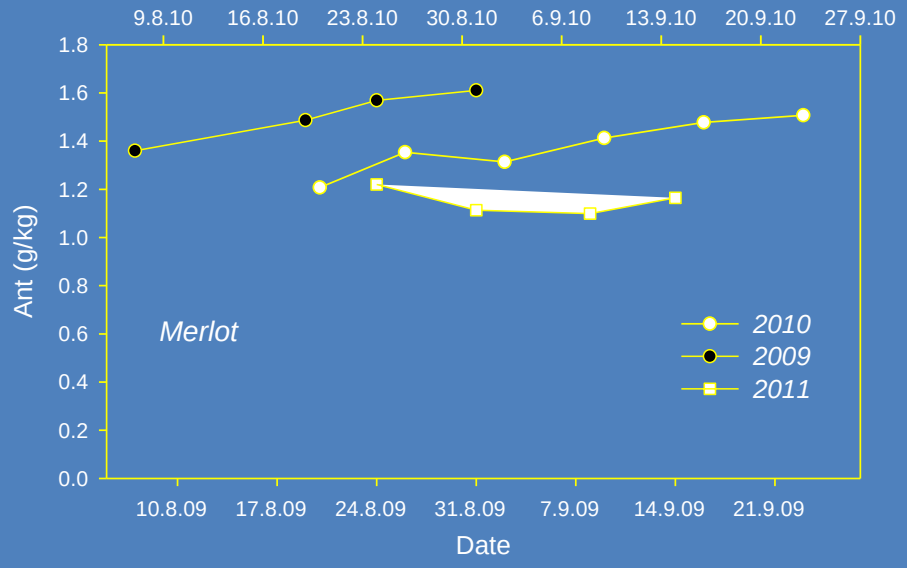
A total calibration curve for the all investigated cultivars.

A specific calibration curve for the clone SANGIOVESE GROSSO DI MONTALCINO

Time course of Anth accumulation data by Multiplex sensor







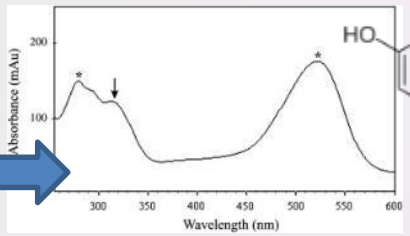
Evolution time of Anth accumulation in the same plots of the vineyard during the different seasons.

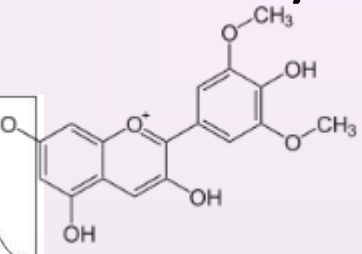
The time of accumulation of Anth under the same season was cultivar-dependent.

The Anth concentration expressed in g/kg of berries was deduced from the $ANTH_{RG}$ Mx index after spectrophotometric calibration.

Bunches first measured in field with the Multiplex...

... were then crushed and analyzed into the device ....





...finally the slurry was extracted by a hydroalcoholic solvent and analyzed by UV-Vis spectrophotometer to detect polyphenol content.

Diffuse Reflectance Spectrophotometer MT01

The MT01 allows to determine an important technological parameter: ANTH DISSOLUTION INDEX in the must.
In details, it uses various LED light sources of a specific wavelength detecting the optical density.

520 nm ⇨ **anthocyanin content**

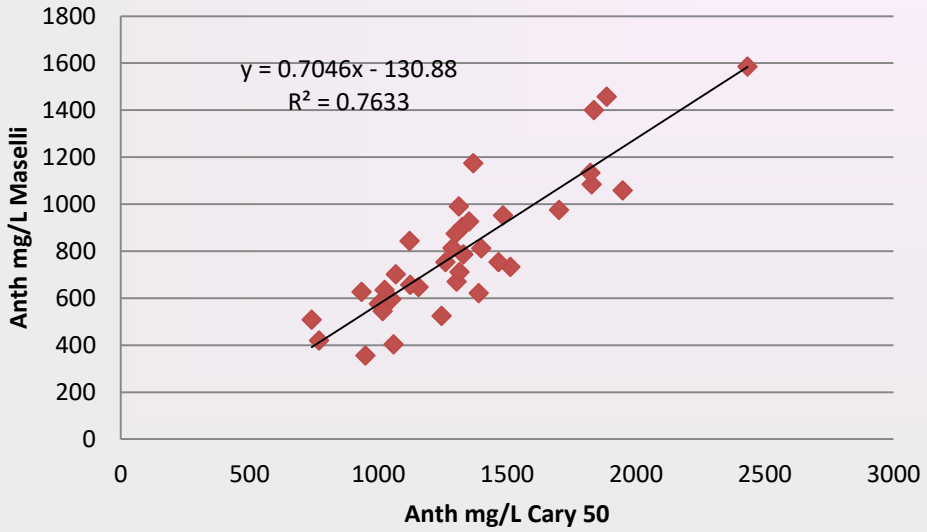
340 nm ⇨ **total polyphenols index**

280 nm ⇨ **tannin content**

The use of diffuse reflectance spectrophotometry is able to analyze in short times not filtered samples and not treated with chemical reagents, expensive and highly polluting.

MT01 (maselli misure PROCESS ANALYZERS)Calibration

For the calibration of the **MT01** device, one hundred berries were sampled at six dates for each cv. Weighed berries were crushed and the resulting slurry was then measured.



Validation of MT01 device for Anth determination by spectrophotometric analyses.

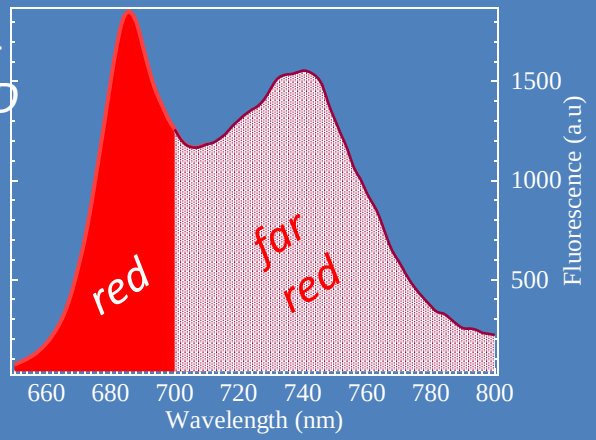
Multiparameter Optical Sensor

Multiplex3 ® FORCE-A

A single measurement give data about several compounds:

$$\text{ANTHOCYANINS ANT H Index} = \log \frac{\text{Fluorescence with red LED}}{\text{Fluorescence with green LED}}$$

$$\text{FLAVONOL FLAV Index} = \log \frac{\text{Fluorescence with red LED}}{\text{Fluorescence with UV LED}}$$

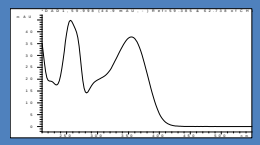


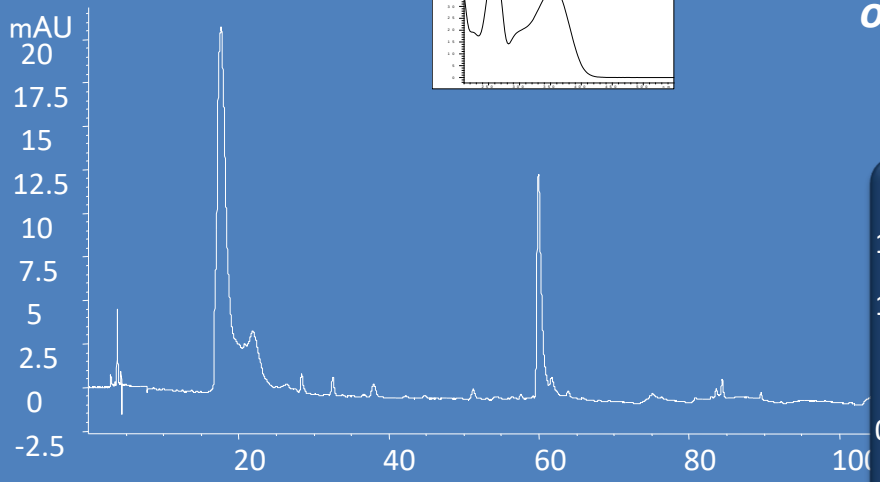
$$\text{CHLOROPHYLL CLO Index} = \frac{\text{Far red Fluorescence}}{\text{Red Fluorescence}} \text{ with red LED}$$

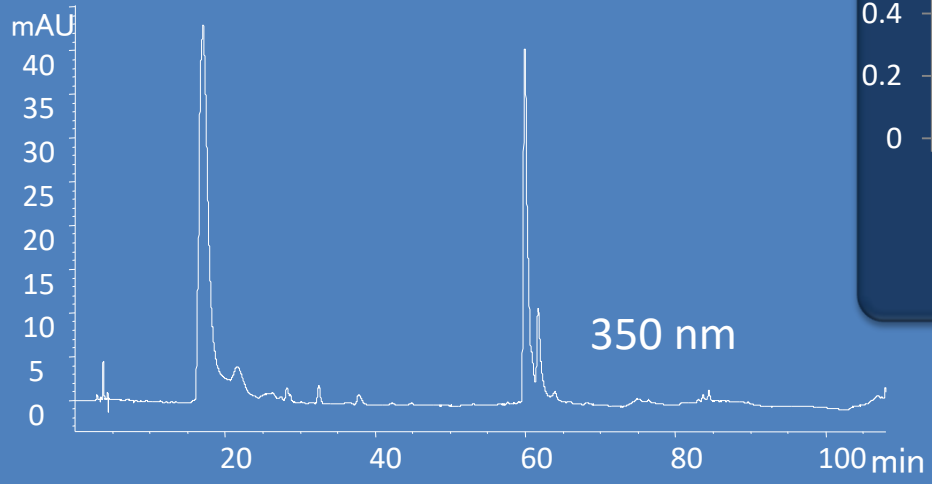
WHITE GRAPE VARIETIES

Flavonols

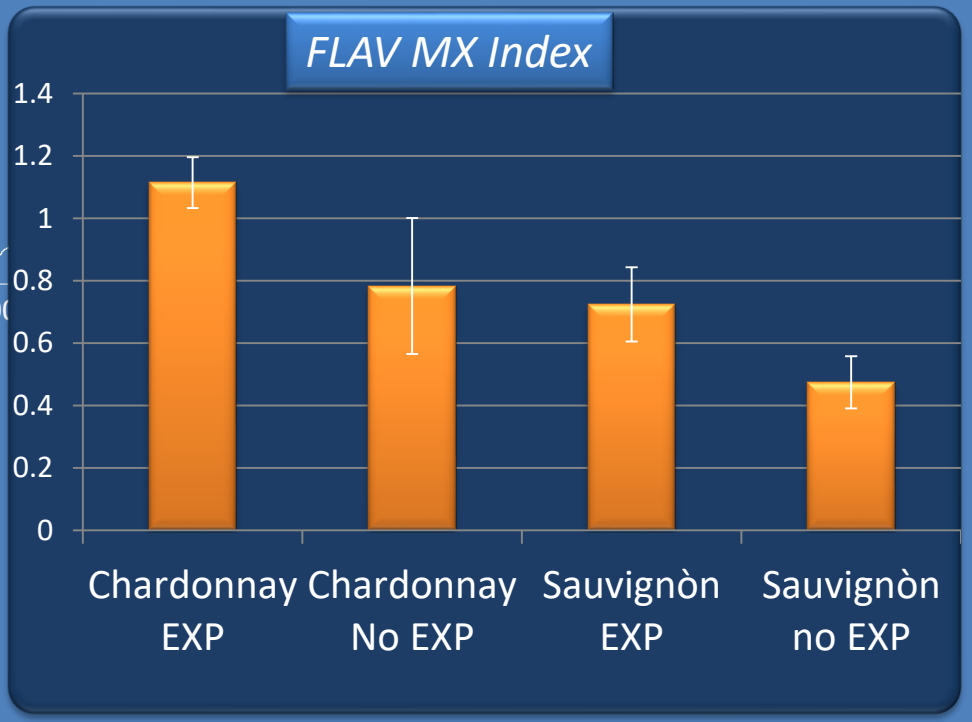
HPLC/DAD Analysis







Mutliplex (Mx) measurements on Chardonnay and Sauvignòn



Cluster sides exposed and not exposed to sun

The use of these optical methods is of wide interest, not only for the production of top-quality red wines, but in general for FRUIT QUALITY CONTROL.

The time-course evaluation of CHLOROPHYLL, ANTHOCYANIN and FLAVONOL concentrations in several vegetal matrices during fruit ripening, could useful to choose the best time of harvesting and fruit and other vegetables sorting (plums, kiwi, lettuce…) and, consequently, to evaluate the functional and organoleptic characteristics of the fruits and their processed products (olive oil, jam, juices).

MULTIPLEX

MT01

The optical methods (MULTIPLEX and MT01) have allowed to evaluate the influence of the sun exposition on the kiwifruit quality from the polyphenol content point of view.

CONCLUSION

Optical methods (Multiplex and MT01) can monitor the maturity trend of Anth accumulation for each specific red grape cv. and flavonol accumulation in white cvs.

Multiplex screening method can represent a new rapid and non-invasive tool for the assessment of phenolic maturity in red grape vineyards.

The calibration curves obtained by comparing the Mx index $ANTH_{RG}$ with the spectrophotometric destructive Anth analyses can be used to monitor Anth accumulation directly in the vineyard obtaining the Anth content in mg/g of grape.

MT01 allow to obtain quantitative data (anthocyanins, total polyphenol and tannins) in aqueous non filtered samples in short times and without the use of organic solvents. Method validation with a classical UV-VIS spectrophotometric analysis.

The wine under study is a Sangiovese 95% - Colorino 5% red wine (Chianti Rufina) fermented into stainless steel tanks, then divided into three aliquots and moved in the three different containers for the ageing process. The screening of the wine properties and metabolite patterns will last throughout the whole ageing period.

- **raw amphorae**
- **French oak wood at a medium toast level**
- **stainless steel**

- Examine the influence of different types of containers on sensory and organoleptic properties
- Evaluation of changes in aromatic composition with GC/GC/TOF
- HPLC/DAD/MS analysis of phenolic compounds

CAFFEIC/FLAVONOLS

Castello del Trebbio I° sampling

	Wood	*Steel*	*Amphora*
Kaempferolo glucoside (mg/L)	4.040	4.905	5.897

Castello del Trebbio II° sampling

	mg/L		
	Wood	*Steel*	*Amphora*
Acido caftarico	10.83	9.10	9.10
Acido cutarico	6.196	5.376	5.828
Acido fertarico	1.722	1.639	1.750
Der. Acido caffeico	0.900	0.930	0.920
Der. Caffeici	**19.65**	**17.04**	**17.60**
Miricetina glucoside	1.009	0.793	0.861
Quercetina glucuronide	5.399	4.801	5.292
Miricetina aglicone	0.857	0.788	0.835
Quercetina aglicone	2.717	3.074	6.397
Flavonoli	**9.98**	**9.46**	**13.39**

PROCYANIDINS/GALLIC ACID

Castello del Trebbio I° sampling

	Wood	*Steel*	*Amphora*
Proanthocyanidins expressed as catechin (mg/L)	190.9	240.7	279.2
Acido gallico (mg/L)	57.95	74.78	73.29

Castello del Trebbio II° sampling

mg/L	*Wood*	*Steel*	*Amphora*
Proanthocyanidins expressed as catechin (mg/L)	66.43	73.88	95.92

ANTHOCYANINS

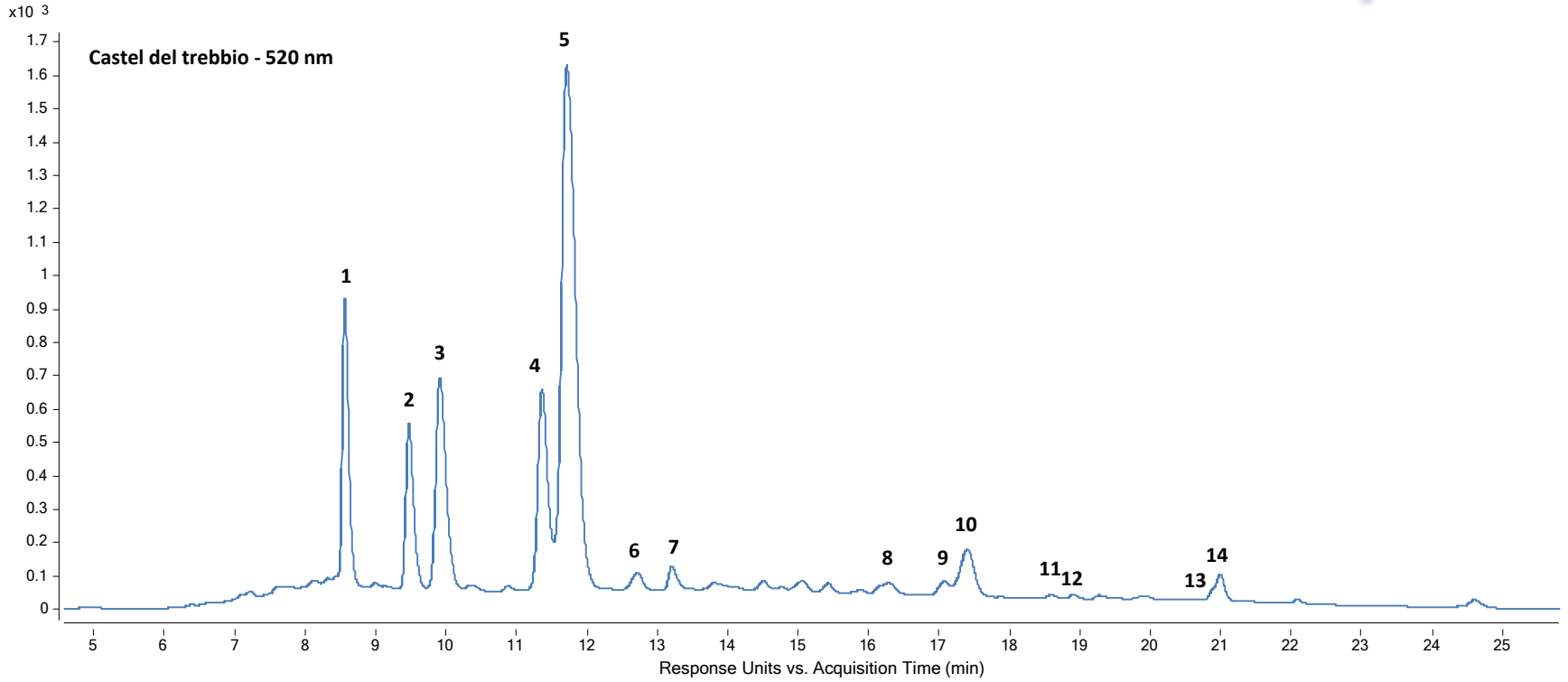
Castello del Trebbio I° sampling

	(mg/L)		
	Wood	*Steel*	*Amphora*
Delfinidina 3,5-diglucoside	0.000	0.000	0.000
Malvidina caffeoilglucoside	0.000	0.000	0.000
Delfinidina 3-glucoside	6.945	7.162	7.220
Cianidina 3-glucoside	3.921	4.057	3.116
Petunidina 3-glucoside	8.951	9.345	9.201
Peonidina 3-glucoside	5.723	7.003	6.389
Malvidina 3-glucoside	33.174	35.087	34.584
Delfinidina 3-acetil glucoside	0.689	1.064	0.744
Cianidina 3-acetil glucoside	0.666	0.752	0.611
antocianoside 1	0.000	0.000	0.000
antocianoside 2	0.595	0.855	0.676
Petunidina 3-acetil glucoside	1.697	1.611	1.530
Peonidina 3-acetil glucoside	0.654	0.759	0.736
Malvidina 3-acetil glucoside	3.431	3.777	3.677
Malvidina 3-cumaroil glucoside	1.714	1.753	1.266
antocianoside 3	1.223	1.134	0.963
Antocianosidi totali	**69.38**	**74.36**	**70.71**

Castello del Trebbio II° sampling

	(mg/L)		
	Wood	*Steel*	*Amphora*
Delfinidina 3,5-diglucoside	0.000	0.000	0.000
Malvidina caffeoilglucoside	0.000	0.000	0.000
Delfinidina 3-glucoside	7.378	7.165	7.950
Cianidina 3-glucoside	3.870	4.161	4.176
Petunidina 3-glucoside	9.106	9.718	10.054
Peonidina 3-glucoside	6.125	6.464	6.651
Malvidina 3-glucoside	34.433	37.540	37.042
Delfinidina 3-acetil glucoside	0.803	0.804	0.898
Cianidina 3-acetil glucoside	0.000	0.000	0.602
antocianoside 1	0.000	0.000	0.624
antocianoside 2	0.658	0.589	0.657
Petunidina 3-acetil glucoside	0.000	0.624	1.242
Peonidina 3-acetil glucoside	0.606	0.739	0.845
Malvidina 3-acetil glucoside	3.307	3.992	4.144
Malvidina 3-cumaroil glucoside	1.120	1.577	1.582
antocianoside 3	0.958	0.878	0.980
Antocianosidi totali	**68.36**	**74.25**	**77.45**

Anthocyanidins

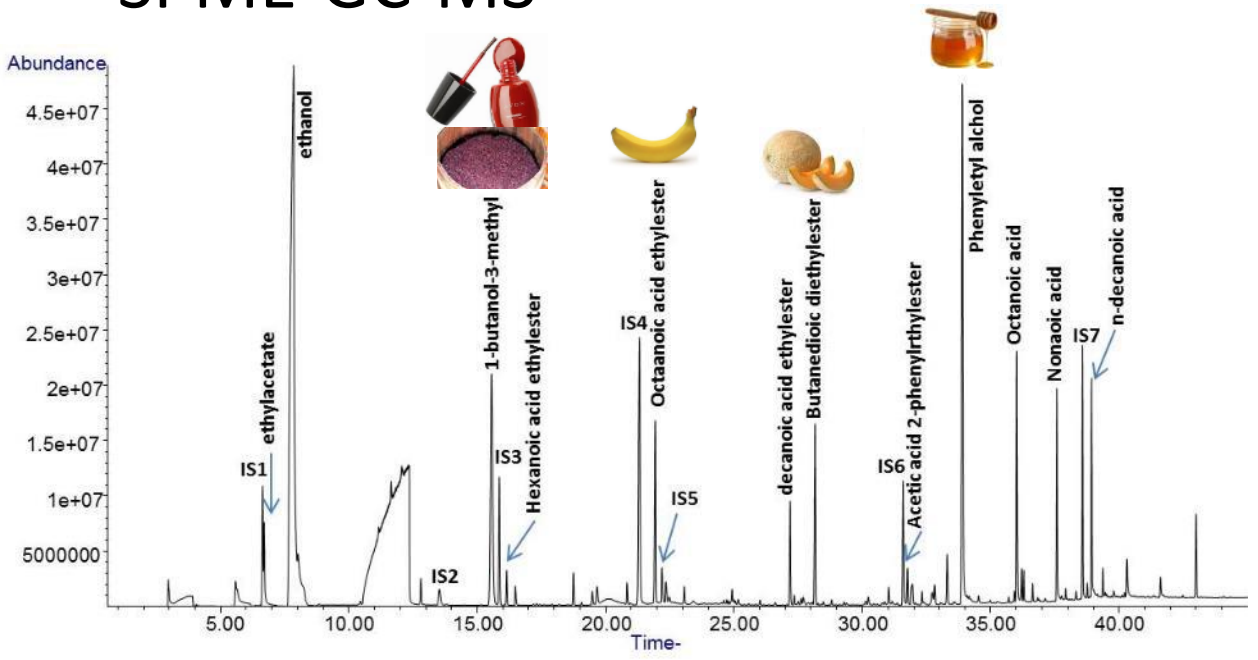


1. Delfinidina 3-glucoside
2. Cianidina 3-glucoside
3. Petunidina 3-glucoside
4. Peonidina 3-glucoside
5. Malvidina 3-glucoside
6. Delfinidina 3-acetil glucoside
7. Cianidina 3-acetil glucoside
8. Petunidina 3-acetil glucoside
9. Peonidina 3-acetil glucoside
10. Malvidina 3-acetil glucoside
11. Malvidina 3-caffeoil glucoside
12. Petunidina 3-cumaroil glucoside
13. Peonidina 3-cumaroil glucoside
14. Malvidina 3-cumaroil glucoside

SPME-GC-MS

The VOCs profile of red wines was determined by SPME (Solid-Phase MicroExtraction)-GC-MS. 1 ml of vine was placed in a 25 ml vial and then 4 ml of water and 2 g of NaCl were added. An Internal Standard (IS) in suitable amount was added to each sample. SPME Conditions: The profile of VOCs was determined by absorption of VOCs at 80° C (for 30-min) on a trivalent fiber Carboxen PDMS DVB 1 cm, followed by desorption at 280° C and, then, analysis by GC/MS. An Agilent 7890 a GC equipped with a 5975C MSD was used. The analyte separation was achieved with a column Agilent DB InnoWAX 50m, 0.20 µm id, 0.40 µm df. Chromatographic conditions were: initial temperature 40° C for 0,5 min, then 6° C min-1 up to 260° C. This temperature was maintained for1 min. A tentative compounds identification was performed by comparing Mass spectra of each peak with those reported in mass spectral databases.

***A. Romani, M. Campo, F. Ieri, L. Calamai. Volatile and antioxidant compounds profiles of red wine aged in different types of containers. 1st MS-WINE DAY. Bagno a Ripoli (FI), 16-17 Aprile 2015.**



Wine aroma perception deal with a large number of Volatile Compounds and the use of GC-MS can be useful for a correct evaluation of the aroma.

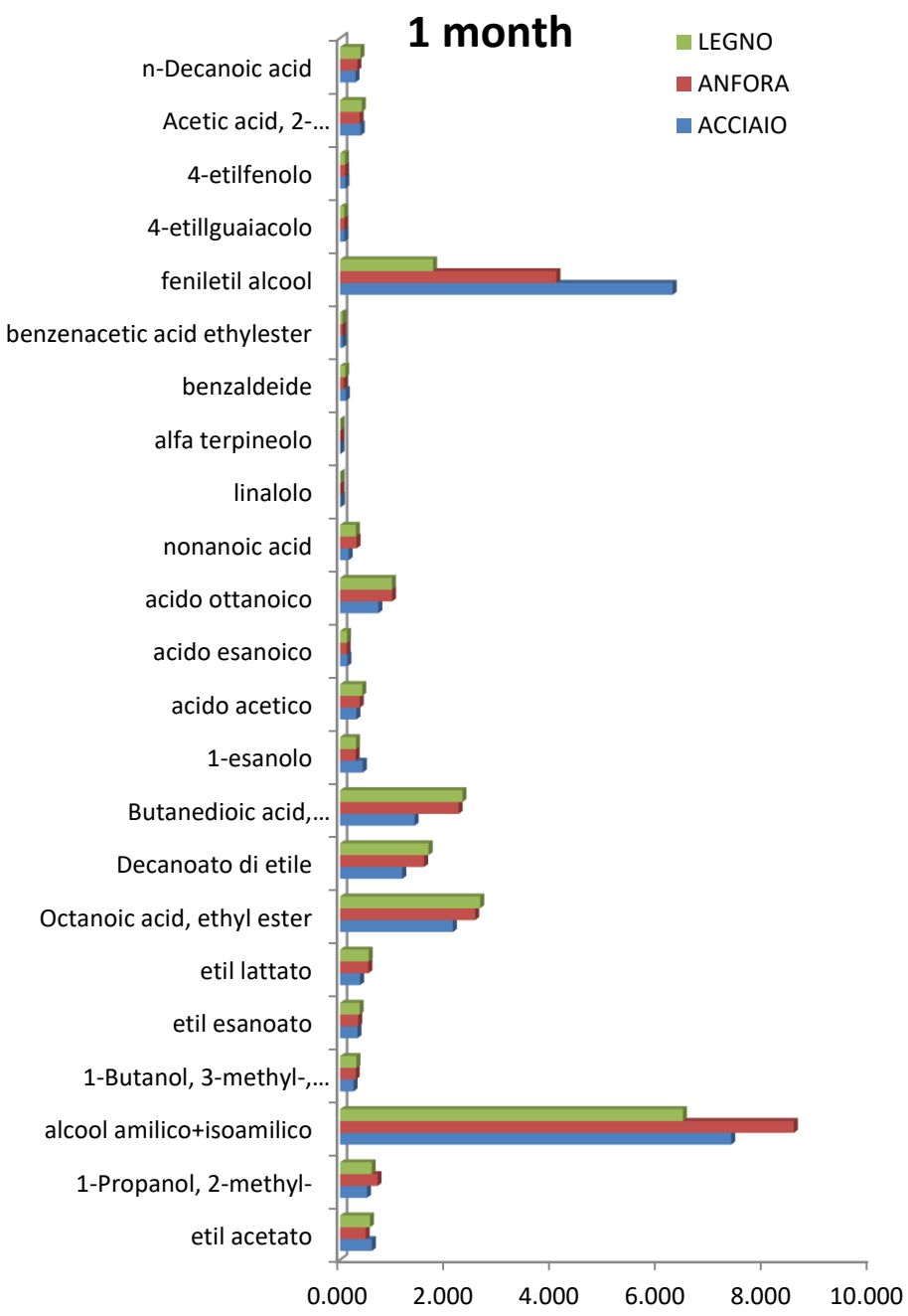
→ 2 and 3-methyl 1-butanol : “wine “nail polish”

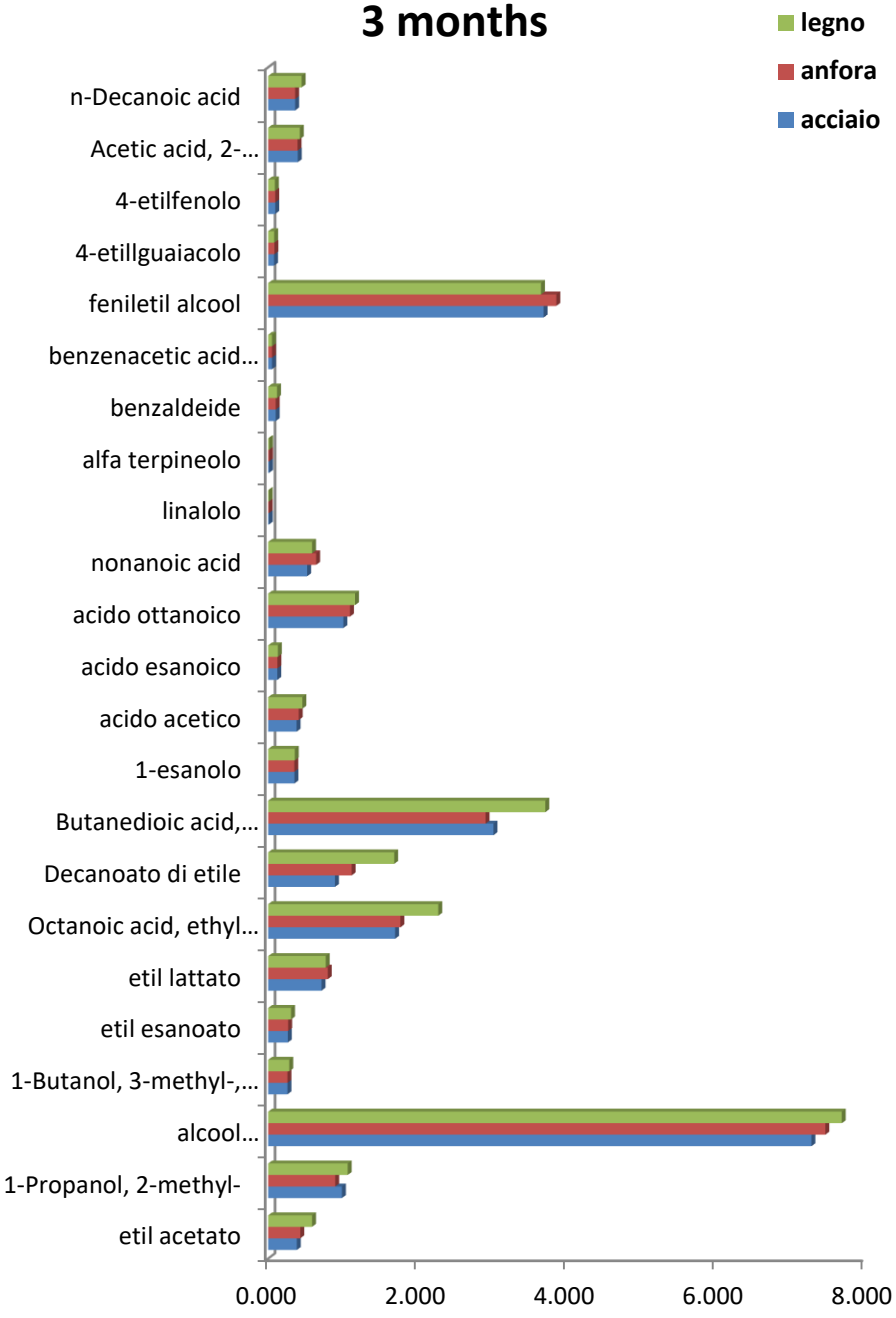
→ Diethyl succinate: “ripe melon” e “lavender”

→ 2-phenylethanol: “rose”, “talco” e “honey”

→ ethyl octanoate: “sweet” e “banana”

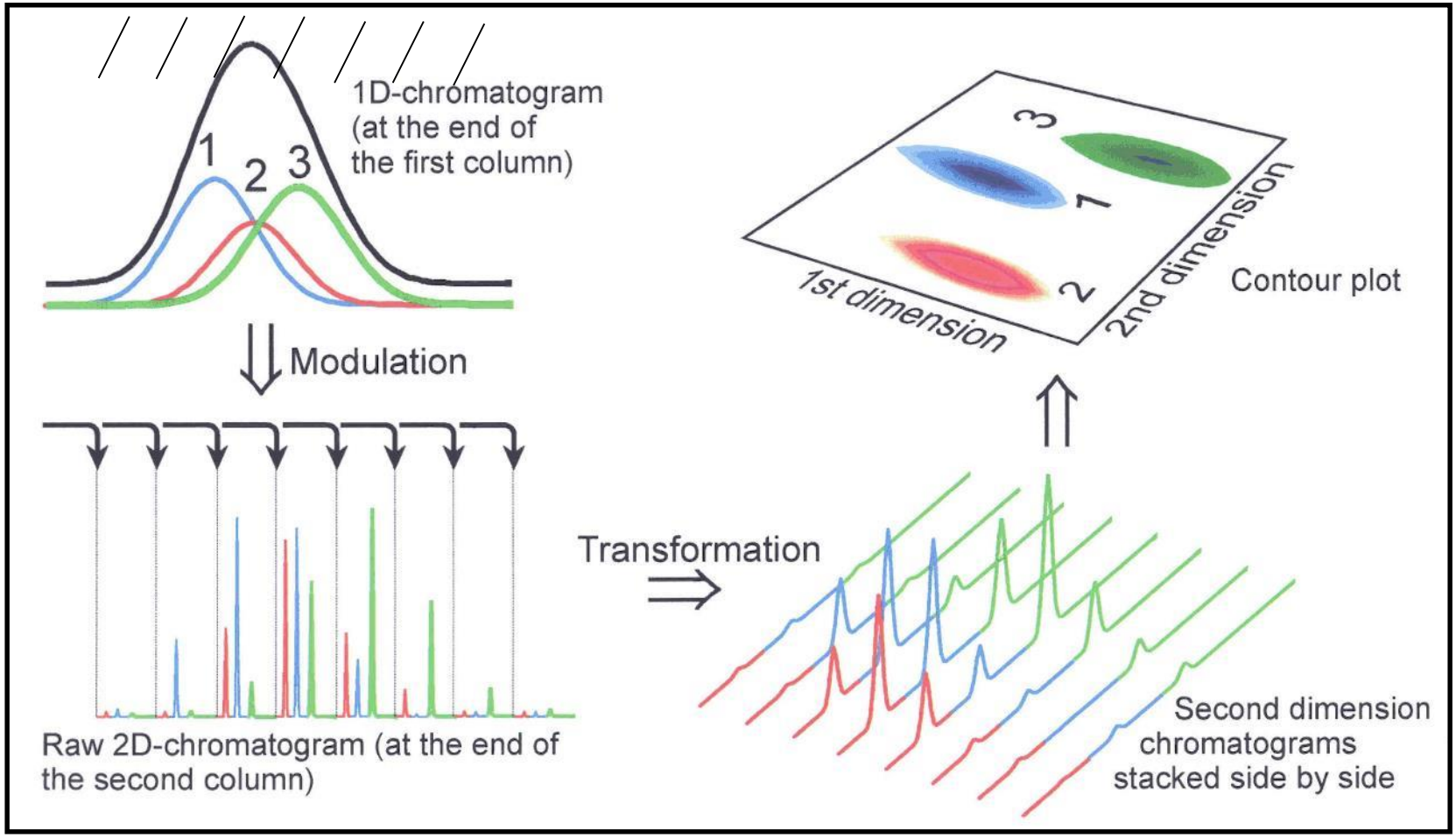
→ ethyl lactate: “strawberry ,red fruit, raspberry»


1 month
LEGNO
ANFORA
ACCIAIO
n-Decanoic acid
Acetic acid, 2-...
4-etilfenolo
4-etillguaiacolo
feniletil alcool
benzenacetic acid ethylester
benzaldeide
alfa terpineolo
linalolo
nonanoic acid
acido ottanoico
acido esanoico
acido acetico
1-esanolo
Butanedioic acid,...
Decanoato di etile
Octanoic acid, ethyl ester
etil lattato
etil esanoato
1-Butanol, 3-methyl-,...
alcool amilico+isoamilico
1-Propanol, 2-methyl-
etil acetato
0.000
2.000
4.000
6.000
8.000
10.000

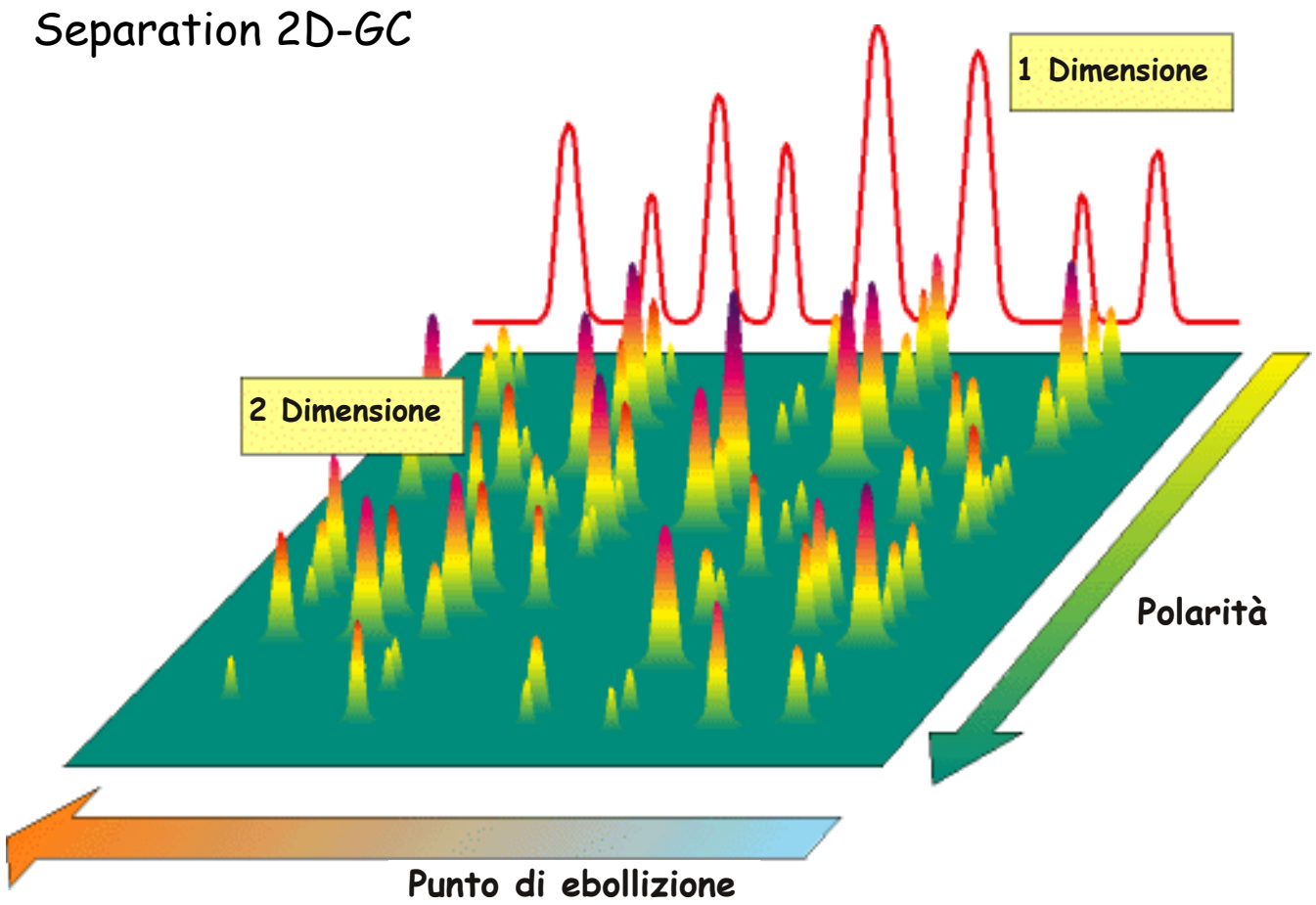

3 months
legno
anfora
acciaio
n-Decanoic acid
Acetic acid, 2-...
4-etilfenolo
4-etillguaiacolo
feniletil alcool
benzenacetic acid...
benzaldeide
alfa terpineolo
linalolo
nonanoic acid
acido ottanoico
acido esanoico
acido acetico
1-esanolo
Butanedioic acid,...
Decanoato di etile
Octanoic acid, ethyl...
etil lattato
etil esanoato
1-Butanol, 3-methyl-,...
alcool...
1-Propanol, 2-methyl-
etil acetato
0.000
2.000
4.000
6.000
8.000

GCMS 7890B, with GC 2D system and HP5 20m x 0.18 x 0.2 column in first dimension and BPX50 5m x 0.35 in second dimension. Spectrometer TOF-DS Markes, full spectra at 50Hz.

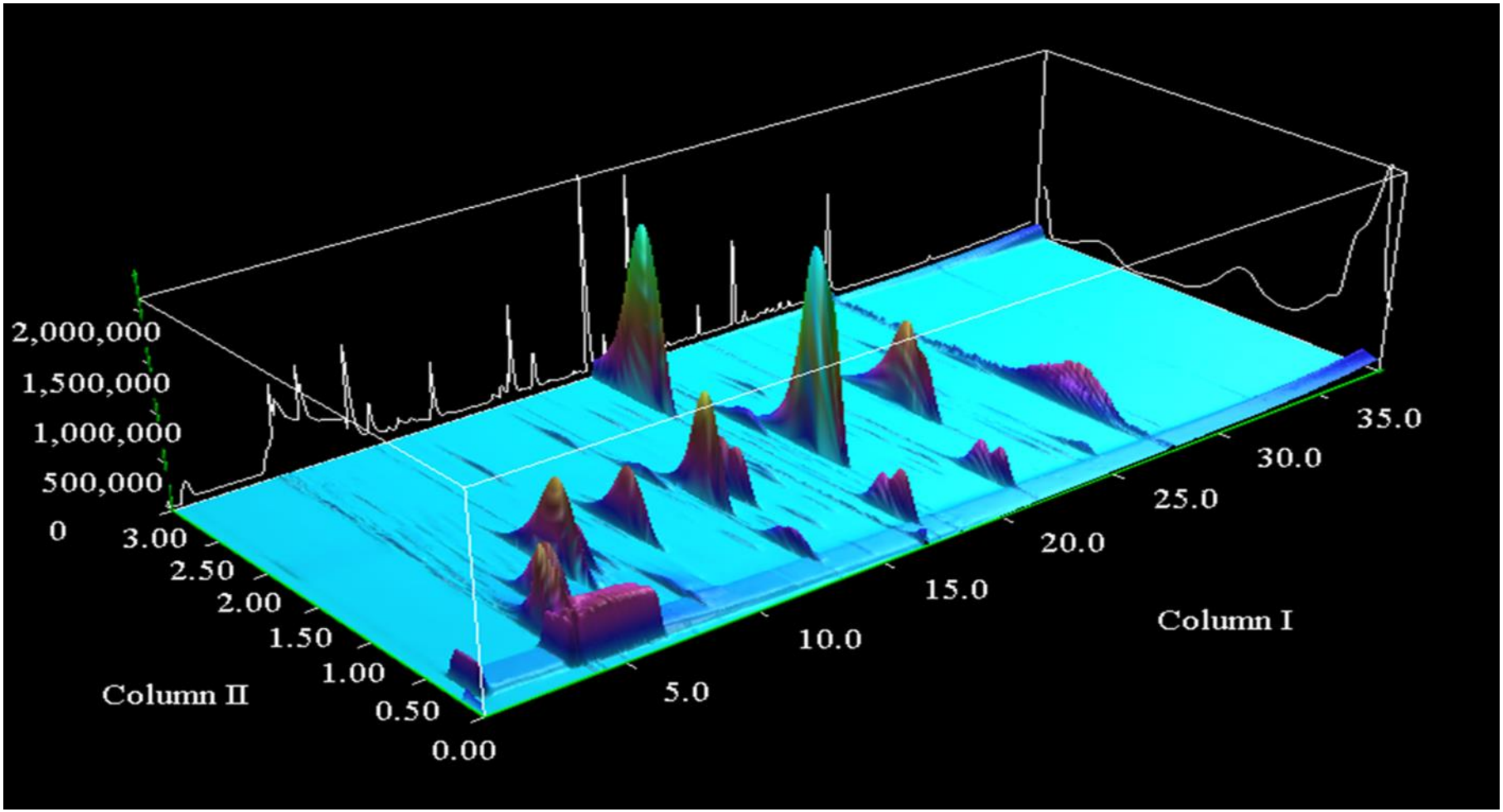
2D GC or GCxGC: blobs



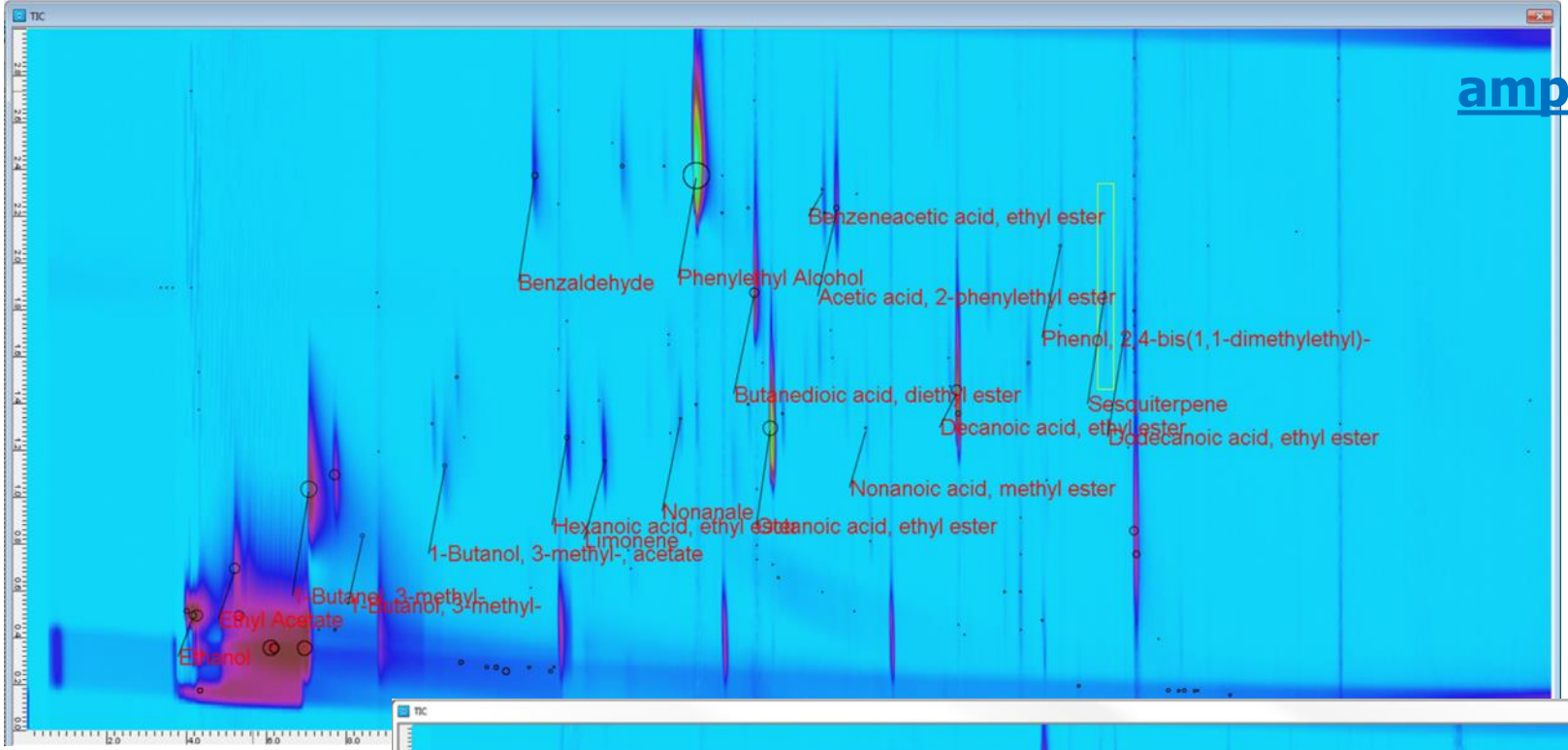
2D GC or GCxGC: Contour Plot



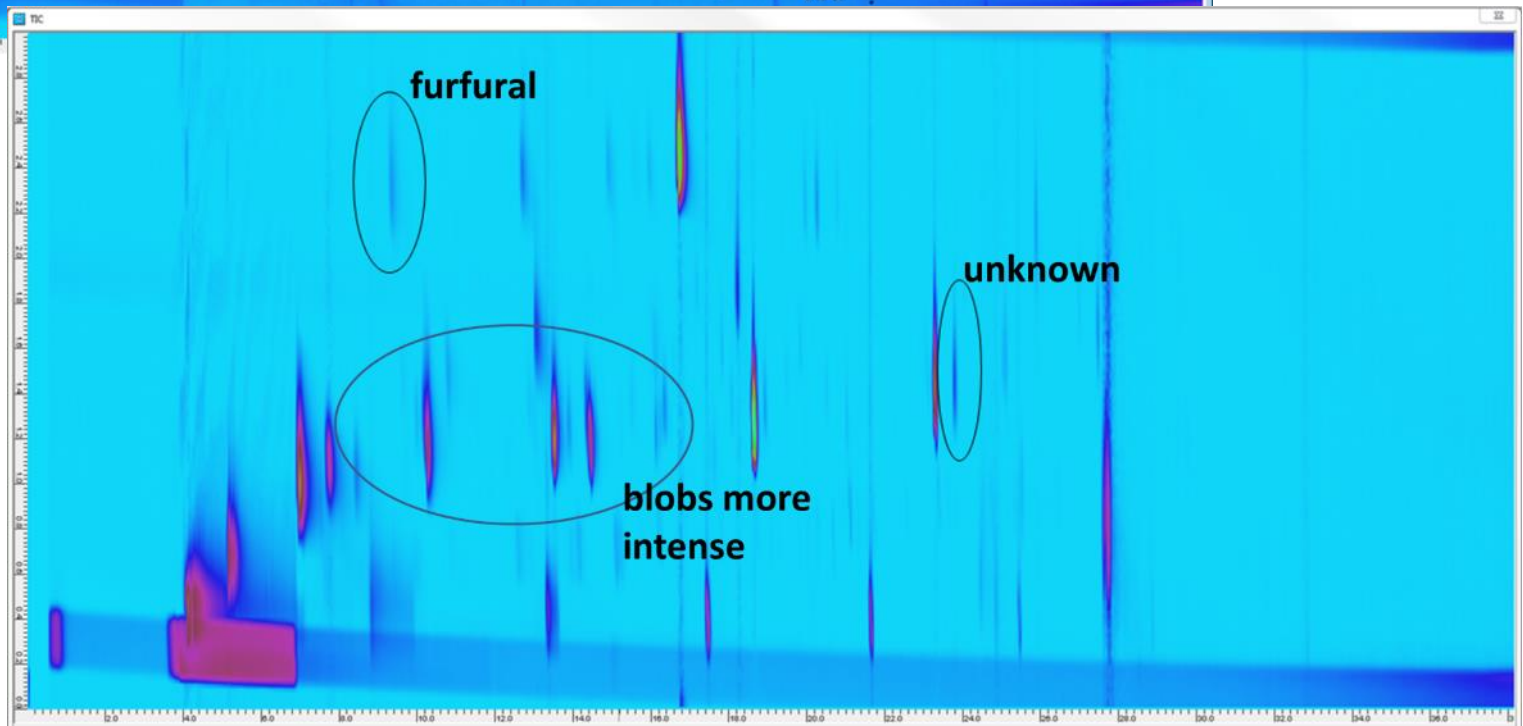
stainless steel (I)



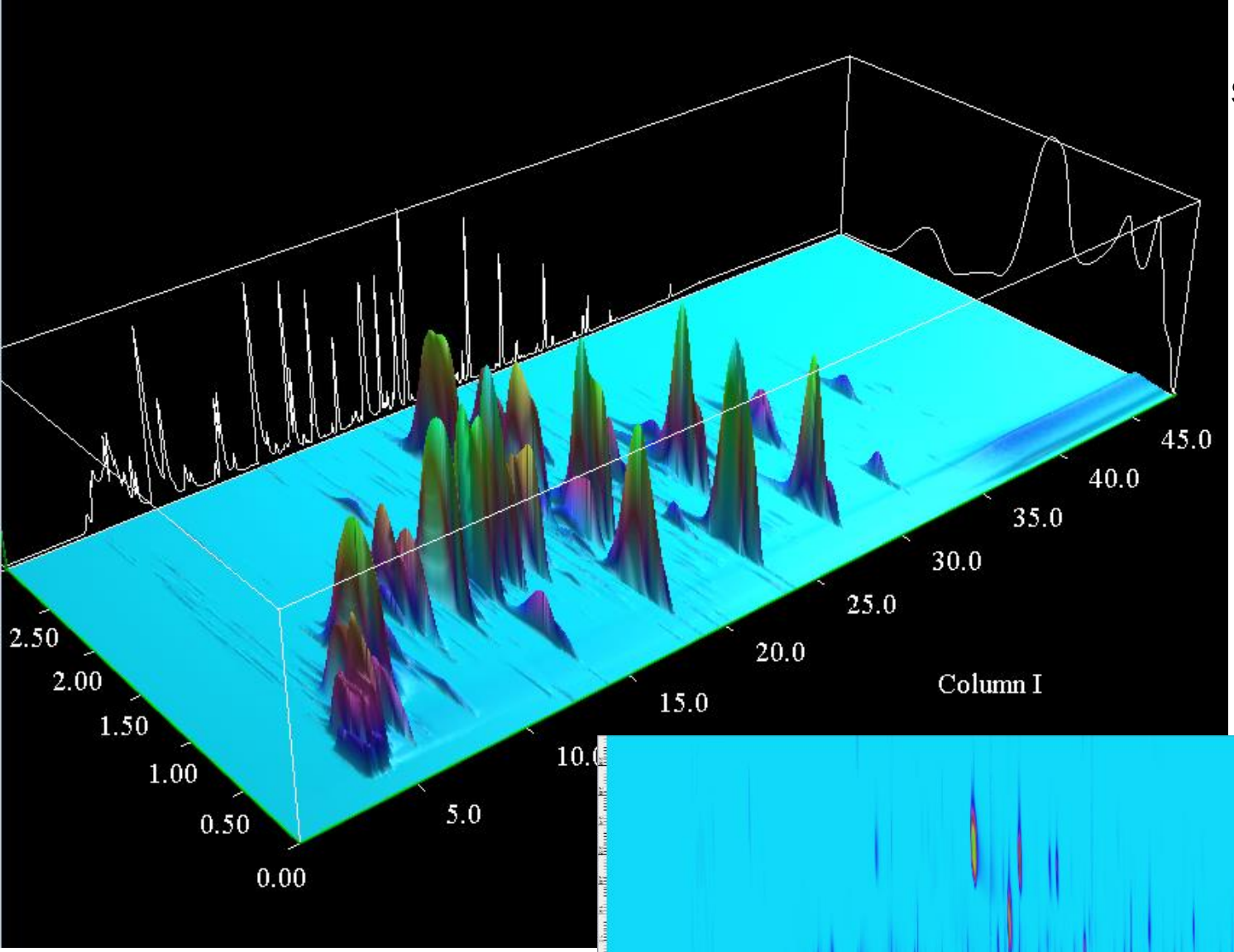
amphorae I

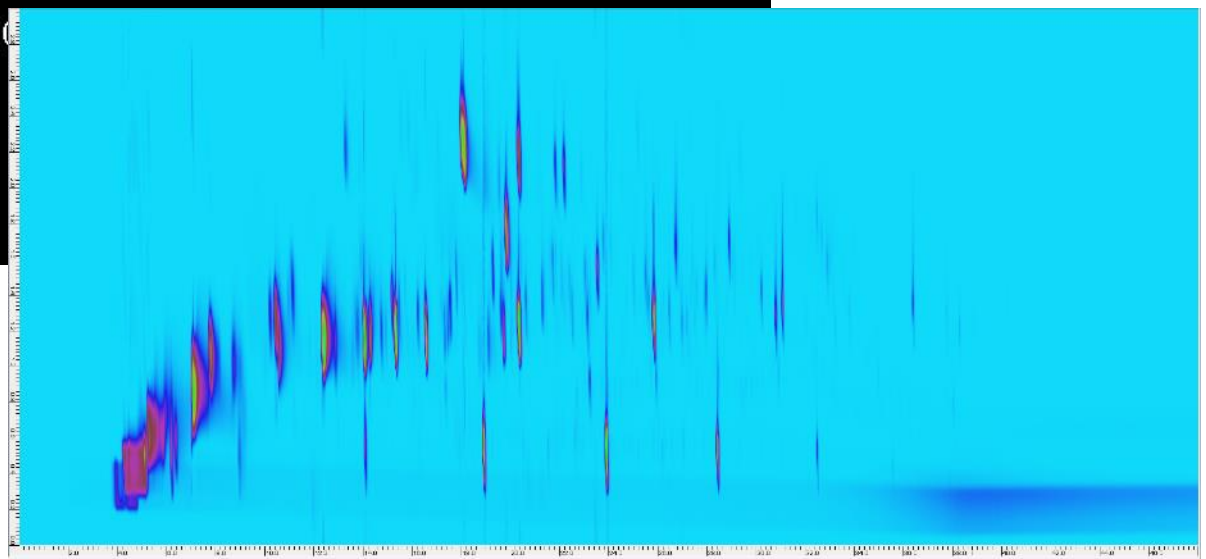


wood I

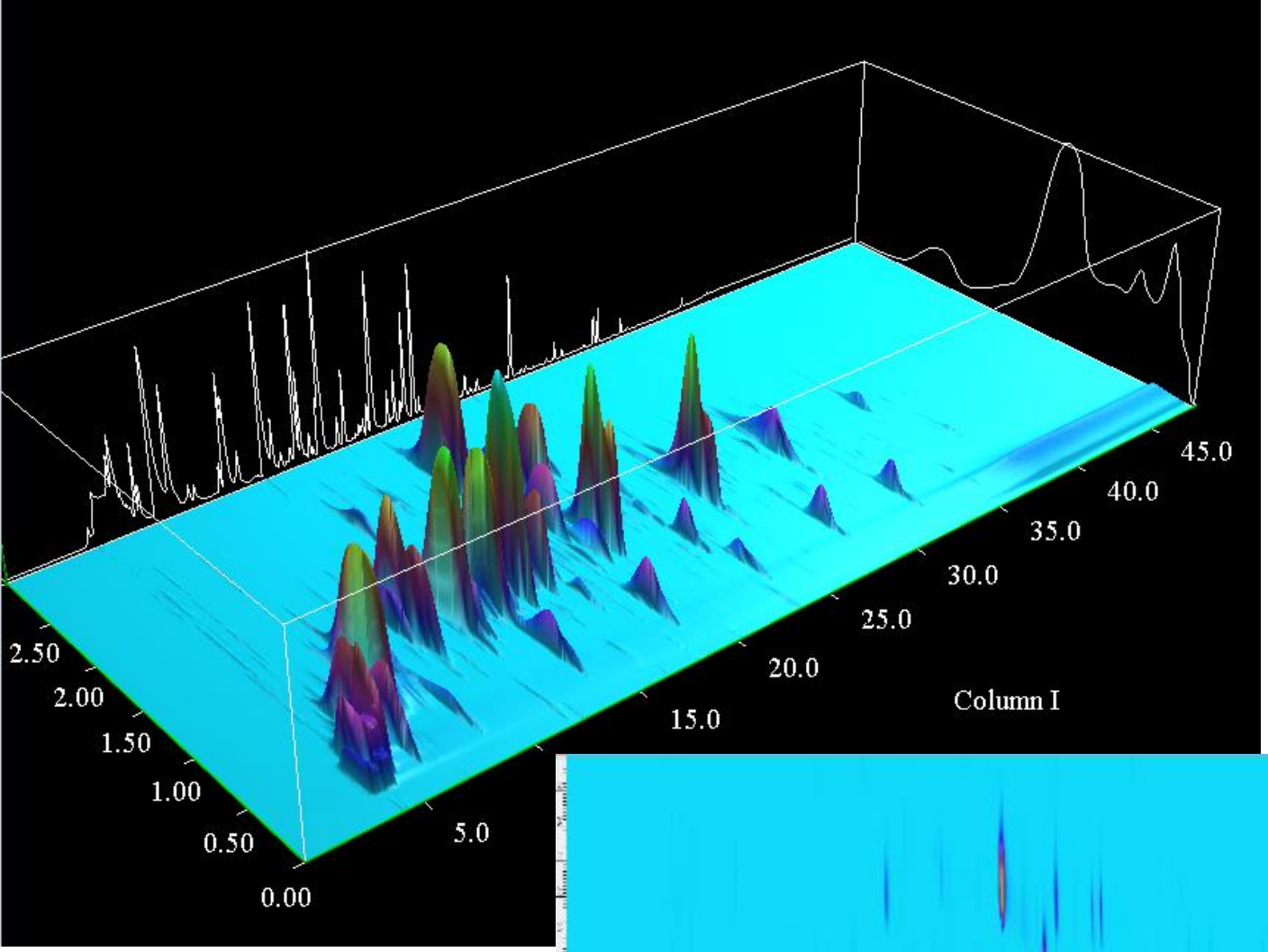


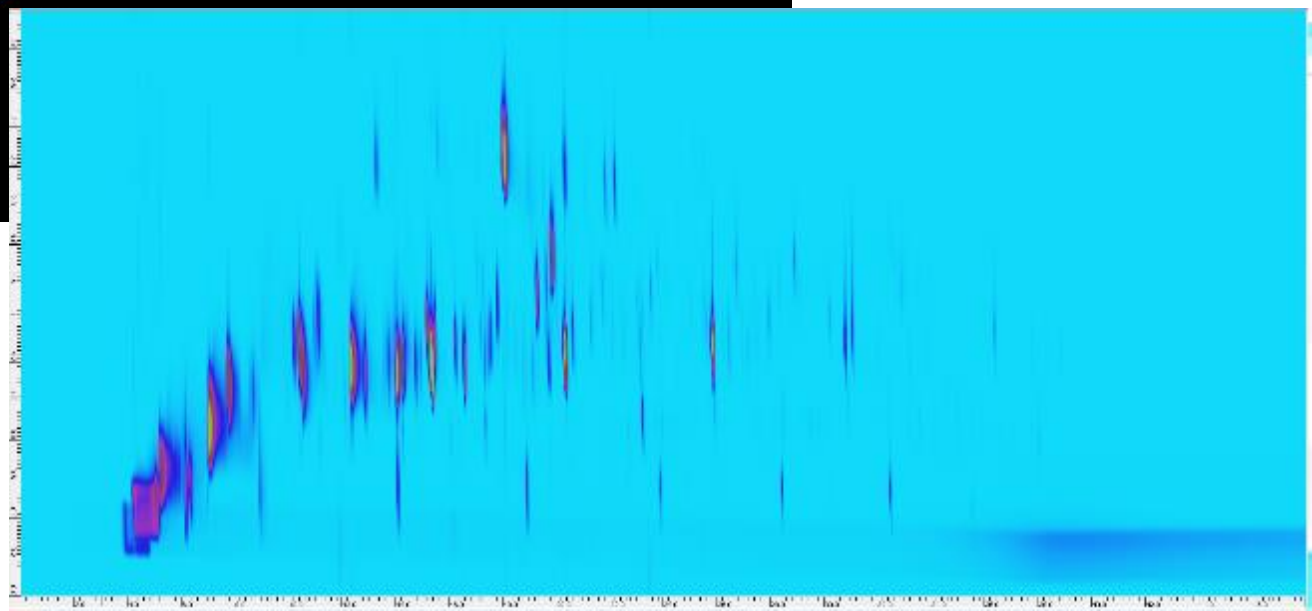
stainless steel II



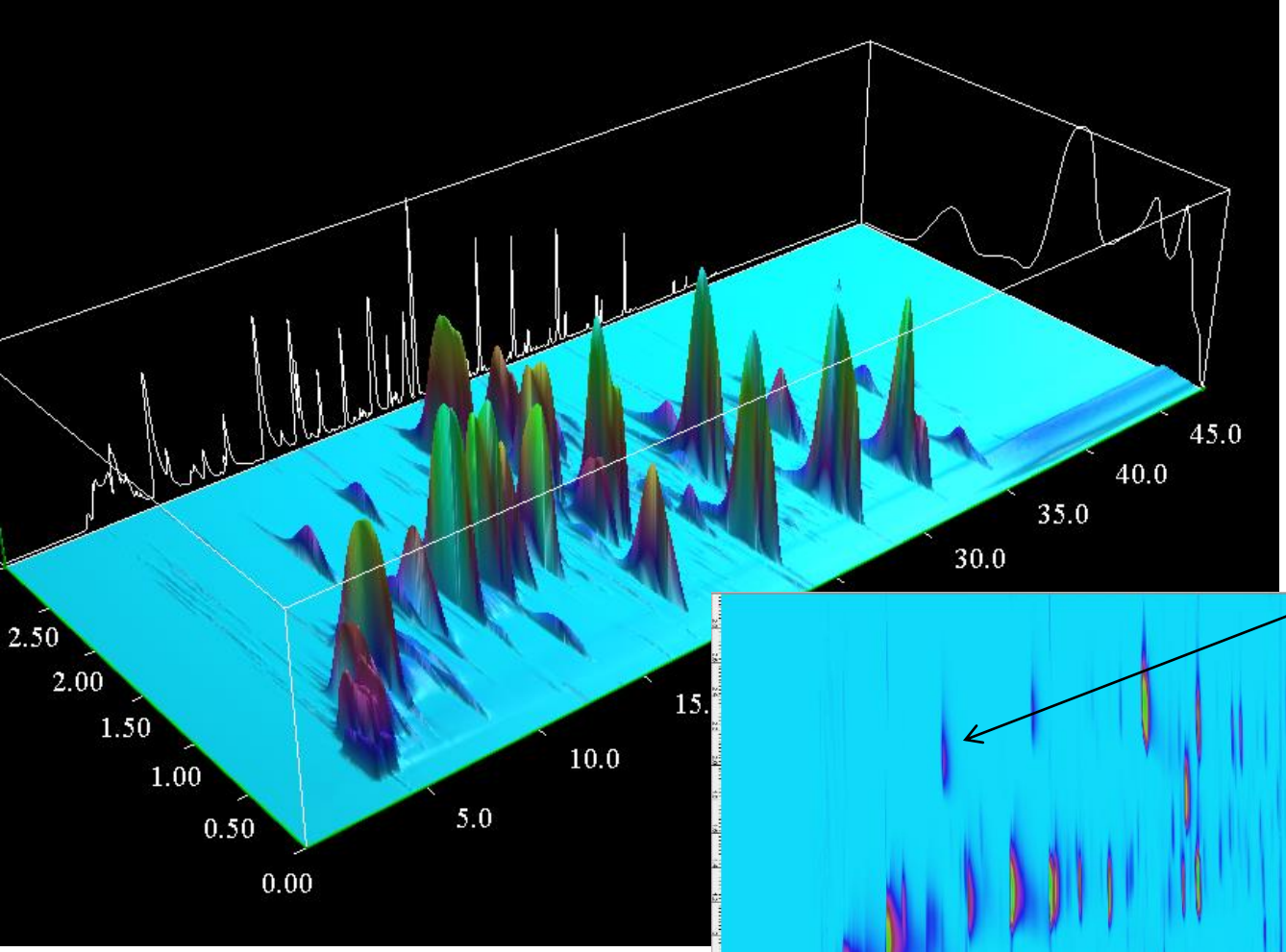


amphorae II

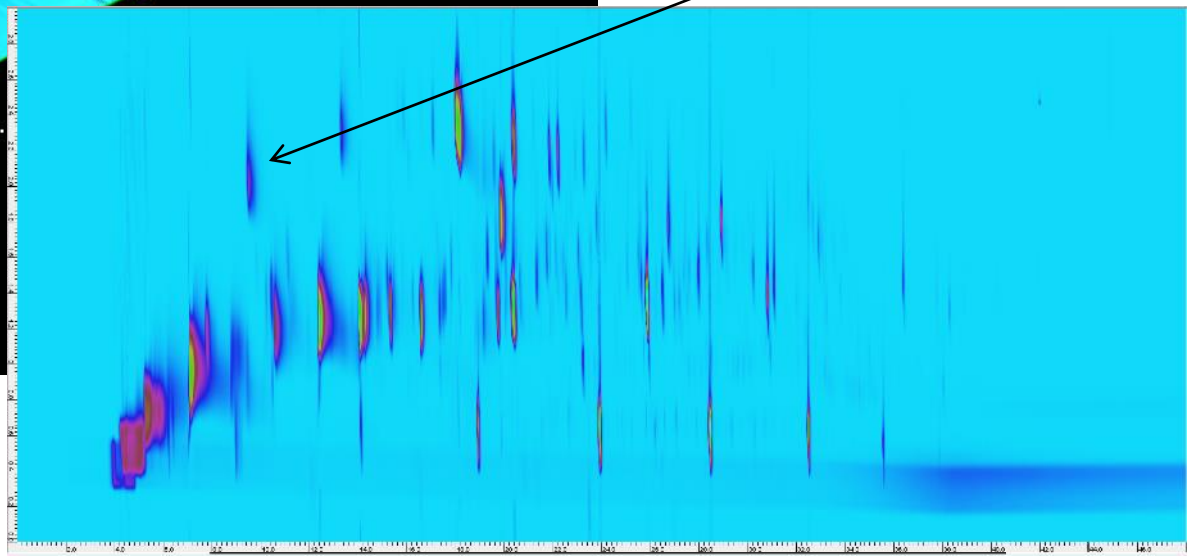




wood II



furfurale



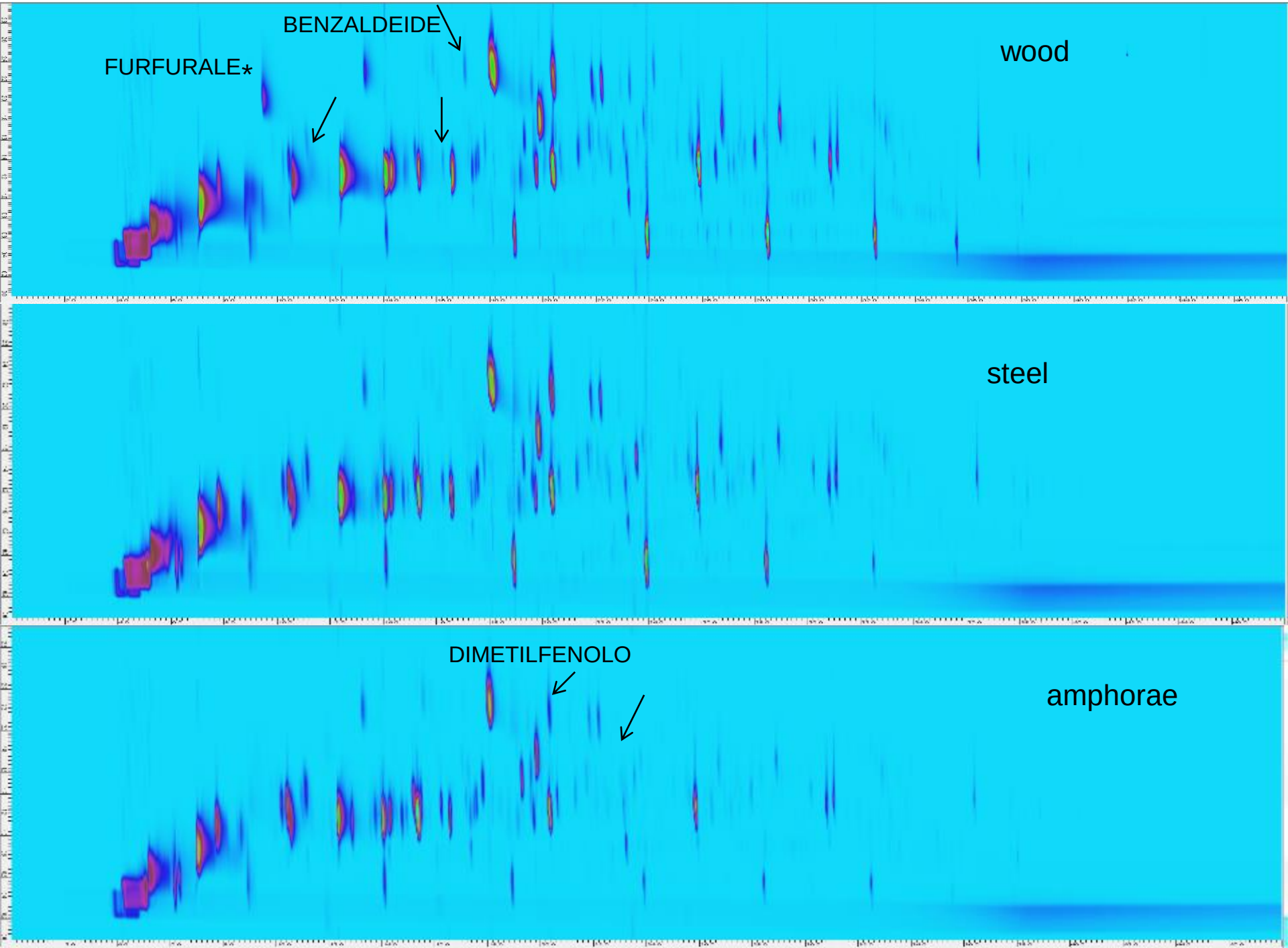
furfurale

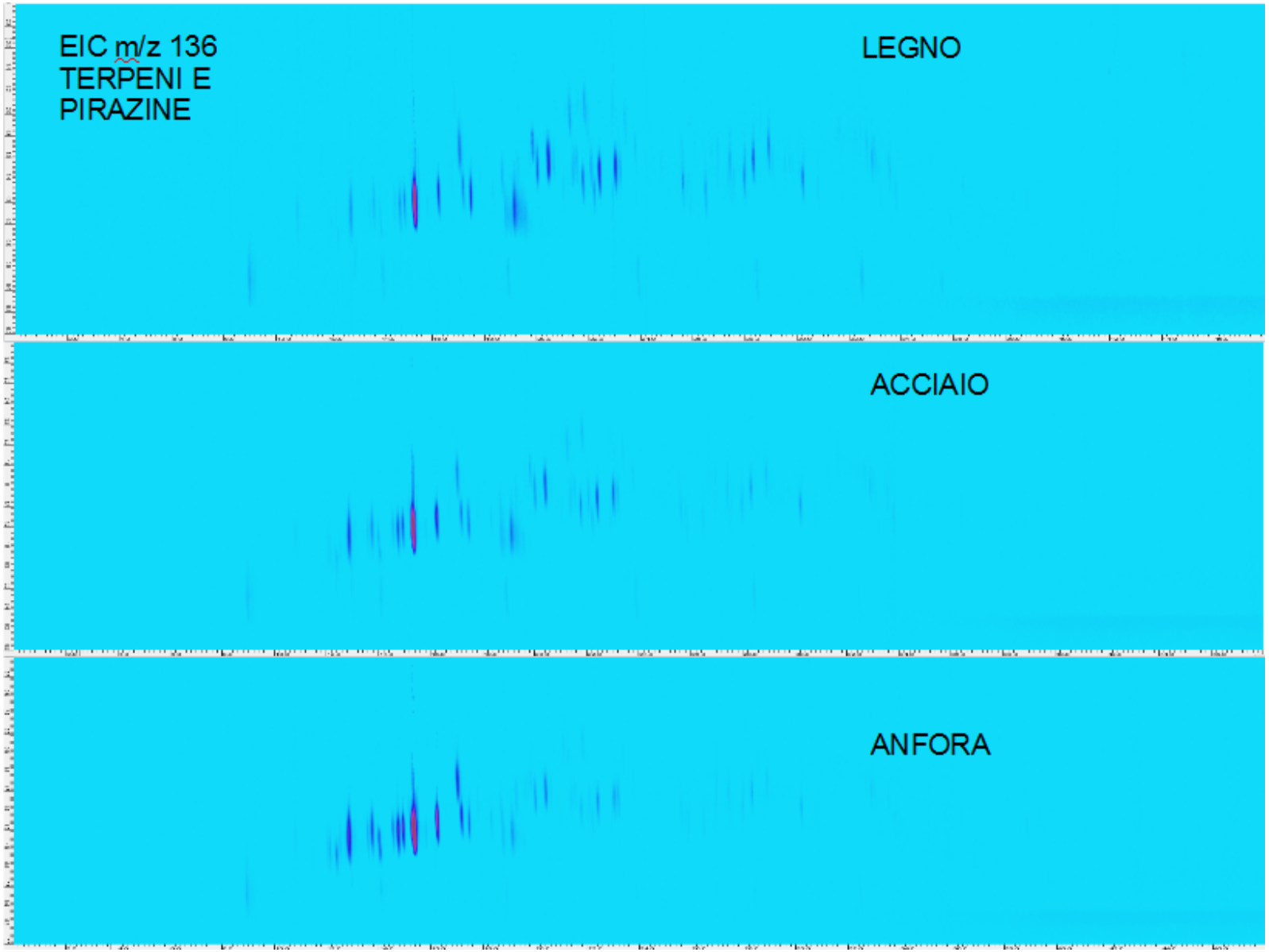
Odor Description:

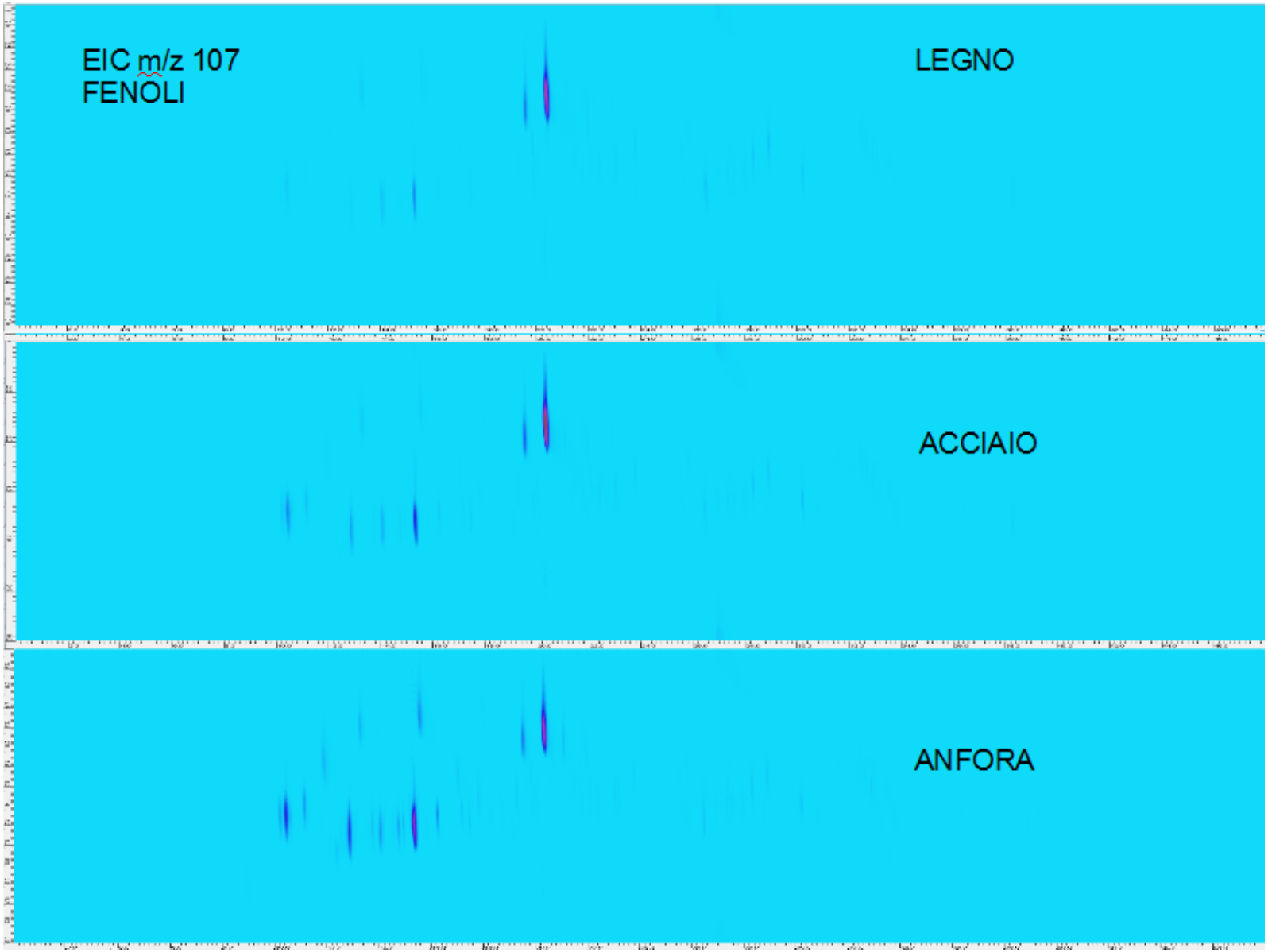
Sweet, brown, woody, bready, caramellic, with a slight phenolic nuance
Mosciano, Gerard P&F 18, No. 2, 38, (1993)

Taste Description:
at 30.00 ppm.

Brown, sweet, woody, bready, nutty, caramellic with a burnt astringent nuance
Mosciano, Gerard P&F 18, No. 2, 38, (1993)


BENZALDEIDE
FURFURALE*
wood
steel
DIMETILFENOLO
amphorae


EIC m/z 136
TERPENI E
PIRAZINE
LEGNO
ACCIAIO
ANFORA


EIC m/z 107
FENOLI
LEGNO
ACCIAIO
ANFORA

Samples :

a) Amedeo Sagrantino di Montefalco DOCG cultivar Sagrantino100% aged 10 years

b) Sagrantino di Montefalco versione Secco DOCG cultivar Sagrantino 100% 6 years

c) Sagrantino di Montefalco versione Passito DOCG cultivar Sagrantino100% 4 years

d) Montefalco Rosso DOC cultivar Sangiovese 65%; Sagrantino 10 % Merlot 20% Cabernet 5% aged 4 years

e) Montefalco Rosso versione Riserva DOC cultivar Sangiovese 60%; Sagrantino 15 % Merlot 20% Cabernet 5% aged 6 years

f) Benozzo IGT Umbria Rosso ricavato con 100% cultivar Sagrantino aged 3 years

g) Rossobastardo IGT Umbria cultivar Sangiovese 65%; Sagrantino 10 % Merlot 20% Cabernet 5% aged 3 years

The molecules identified in the grape and wine samples analyzed related to the class of hydroxycinnamic acids, flavonols, catechins and procyanidins are the following:

Flavonols
Quercetina 3-glucoside
Quercetina 3-glucuronide
Quercetina
Miricetina 3-glucoside
Miricetina
Kaemferolo 3-glucoside
Kaemferolo

Phenolic Acids
Acido gallico
Acido caffeico
Acido para cumarico
Acido ferulico acido vanillico
Acido *p*-OH-benzoico
Acido protocatechico
Acido siringico

hydroxycinnamic tartaric acids
Acido caffeoil tartarico
Acido *p*-coumaroil tartarico
Acido feruloil tartarico

Catechins and Procyanidins
(+) catechina
(-) epicatechina
Procianidina B3
Procianidina B1
Procianidina B6
Procianidina B2
Procianidina B7
Procianidina C1

Stilbenoids
Trans Resveratrolo glucoside
cis Resveratrolo glucoside
Trans Resveratrolo
cis Resveratrolo

JAM GRAPE SAGRANTINO
HPLC/DAD analysis polyphenolic compounds

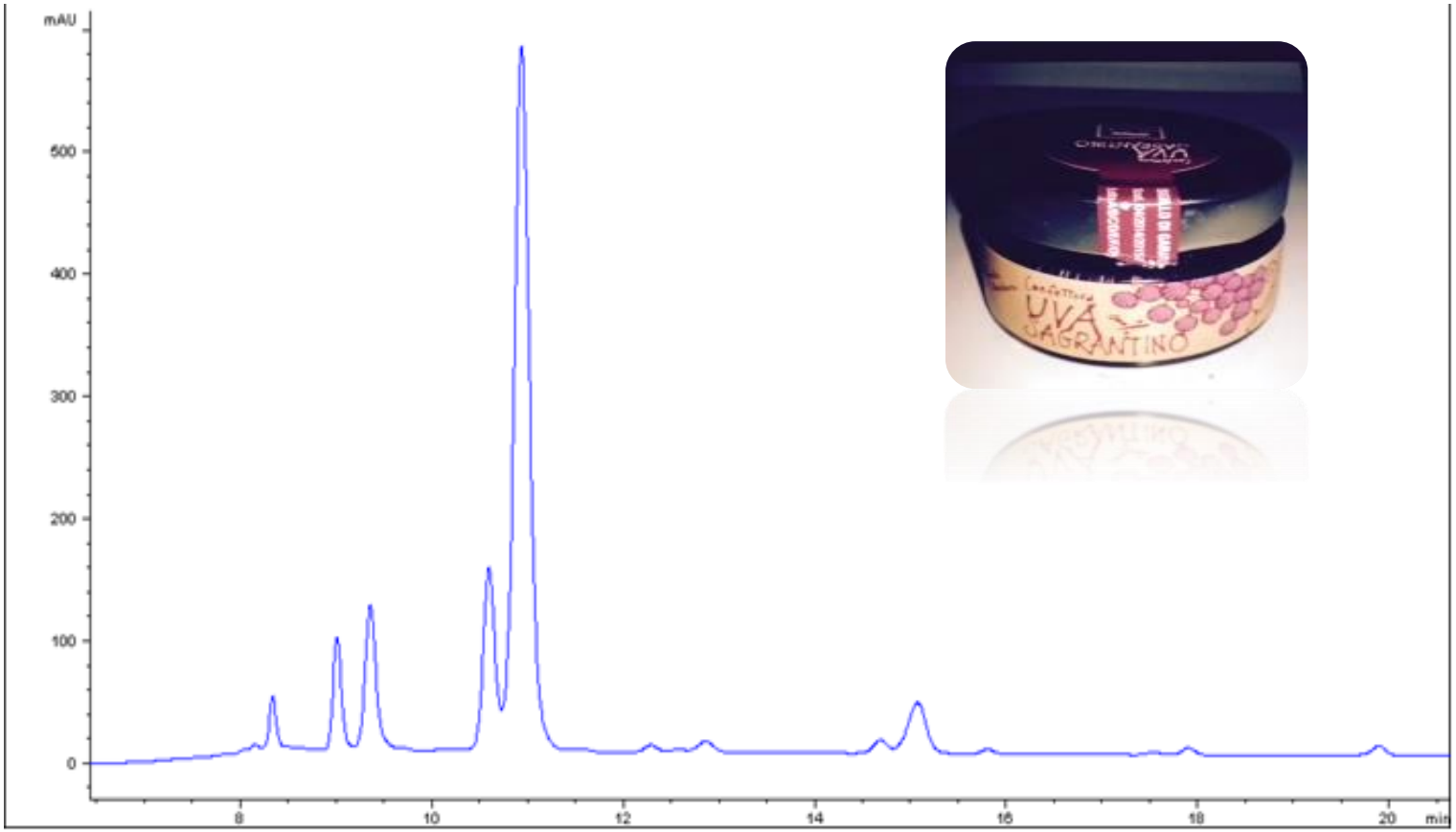
Sample preparation

3.0 g of jam were diluted in 5.0 mL of H2O acidified to pH 2.0 with HCOOH, then purified and extracted with ethyl acetate (AcOEt) and with acidified methanol at pH 1.7 for HCOOH (MeOH), by successive liquid-liquid distributions on inert solid support by Extrelut cartridge. The two fractions obtained were concentrated under reduced pressure until complete evaporation of the solvent, then diluted in 2.0 mL of H2O at pH 2.0 (AcOEt fraction) and with 5.0 mL of the same solvent (MeOH fraction). The obtained solutions were centrifuged to remove any insoluble compounds and analyzed.

Results

The flavonols are made mainly from quercetin rutinoside (rutin), while among hydroxycinnamic derivatives the most abundantare caffeoil-tartaric acid, p-cumaroil feruloil-tartaric acid and tartaric acid, as well as caffeic acid, p-coumaric and ferulic. There are also gallic acid and derivatives of catechin and epicatechin with the prevalence of proanthocyanidinic dimers and trimers. The table reports the qualitative and quantitative analysis for subclasses of polyphenolic derivatives in the jam sample.

	mg/g of jam
flavonols	0.114
hydroxycinnamic derivatives	0.389
Gallic acid	0.020
catechin and derivatives	0.039
anthocyanins	2.281
Total of polyphenols	2.842



In the figure the chromatographic profile, recorded at 520 nm, relative to the MeOH fraction of the jam is reported. All visible peaks are related to anthocyanosidic compounds. 3-O-glucosides of malvidin, peonidin, petunidin, cyanidin and delphinidin have been identified in accordance with the data available in the literature (*A. Baldi, A. Romani, N. Mulinacci, F.F. Vincieri, B. Casetta. Journal of Agricultural and Food Chemistry, 43, 1995, 2104-2109*), and the corresponding 3,5-O-diglucosides, the 3-O-acetylglucosides and 3-O-(6-O-p-cumaroyl) glucosides. The most abundant appear to be monoglucosides and in particular the malvidin 3-O-glucoside.

The table below, for comparison, shows the data obtained for three previously analyzed jams, apricot, peach and apple, sweetened with Stevia rebaudiana. Data are expressed in mg/g of jam.

Comaparison among Sagrantino jam and the others jam (nd-non detected)										
mg/g of jam	**sagrantino jam**	**apricot stevia**	**+ Peach stevia**	**+ apple stevia**	**+ Apricot Light GDO**	**Apricot Extra GDO**	**Red fruits Light GDO**	**Red fruits Extra GDO**	Strawberry **Light GDO**	Strawberry **Extra GDO**
Flavonols	**0,114**	**0,010**	**0,011**	**0,019**	**0,033**	**0,018**	**0,061**	**0,032**	**0,023**	**0,017**
hydroxycinnamic derivatives	**0,389**	**0,087**	**0,025**	**0,288**	**0,123**	**0,095**	**0,051**	**0,01**	**0,059**	**0,046**
Gallic acid	**0,020**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
catechin and derivatives	**0,039**	**0,129**	**0,250**	**0,150**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
anthocyanins	**2,281**	**nd**	**nd**	**nd**	**nd**	**nd**	**0.163**	**0.149**	**0.094**	**0.086**
Total of polyphenols	**2,842**	**0,226**	**0,286**	**0,457**	**0,155**	**0,113**	**0,275**	**0,192**	**0,176**	**0,149**

The Mediterranean Diet and the French paradox

Mediterranean Diet

In its general sense, the term refers to the dietary profile of the Italians in the early '50s, when Ancel Keys, American researcher, went in southern Italy realizing that mortality due to cardiovascular disease was extremely low.

The incidence of classic cardiovascular risk factors is not very different among the residents of the Mediterranean region and those of other Western countries. This suggests that other factors, many of which are still unidentified, can be favorably influenced by the components of the Mediterranean diet.

French paradox

At the end of the 80s, some scientists studied the correlation between mortality due to coronary heart disease and intake of animal fats in the diet.

Despite the high consumption of animal fat, the French did in fact have the lowest mortality rate from coronary heart disease. The researchers tried to give an answer to what today is still known as the French paradox.

Statistical observation of greater consumption of wine on French soil, led to the idea that this drink could offset the effects of high intake of animal fat. Since the negative effects of alcohol had already been widely documented and that the wine had proved more effective than other alcoholic beverages in reducing the incidence of these diseases, the second step was to assume that at the base of the French paradox not there was alcohol, but other substances present in wine, just as antioxidant molecules (anthocyanins, flavonoids, tannins, resveratrol)

GRAPE PHENOLS AND POTENTIAL HEALTH ACTIVITY

The last decade was characterized by a strong interest on grapes and wines phenolic in relation to the potential health benefits activities, in particular flavonoids and stilbenes.
Stilbene has recently been shown to be effective in stopping the carcinogenic process, both at the level of initiation, both of promotion and progression.
Among the flavonoids quercetin molecule is included and is known for its multiple biological activities including antioxidant capacity.
Among the stilbene we find resveratrol (trans-3, 4 ', 5-trihydroxystilbene), which is also a natural antioxidant polyphenols, present in many plants (including red grapes, berries, peanuts) and contained in the wine, particularly red wine.
Resveratrol and quercetin are considered natural bioactive molecules with phytotherapic and pharmacological potentialities that include addition to the aforementioned antioxidant activities, also has anti-inflammatory activity, anti-platelet, antiatherogenic, inhibitors of cell proliferation and immune modulating.
Are, in particular, be from 10 to 20 times more potent than vitamin E in protecting the oxidation of low density lipoproteins (LDL), unanimously recognized to be a powerful trigger mechanism of the arteriosclerotic process (1,2).

1, The relative antioxidant potencies of some polyphenols in grapes and wines. Baldi A., Romani A., Mulinacci N., Vincieri FF., Ghiselli A. In Wine nutritional and therapeutic benefits, edited by Ton R. Watkins, published by the american chemical society, 1997.

2, Guerrero RF, García-Parrilla MC, Puertas B, Cantos-Villar E. Wine, resveratrol and health: a review. Nat Prod Commun. 2009 May; 4(5):635-58.

French paradox

Polyphenols from Vitis vinifera: health properties

- Protective capillary
- Venotonic
- Astringent
- Antiviral
- Antiproliferative
- Antiradicalic: EC_{50} =5.75 µM

Lizarraga D. et al. (2007), FEBS J. ***274****, 4802-4811;*
Iwasawa A. et al. (2009), Biocontrol Science ***14****, 107-111;*
HO HY et al (2009), J. Agric. Food Chem. ***57****, 6140-6147;*
Stoclet J. et al. (2004), European Journal of Pharmacology ***500****, 299-313.*

DEVELOPMENT OF NEW PRODUCTSI "VINOTHERAPY"

- **Creation of a line of natural cosmetics that have as an essential basis the wine active ingredients (polyphenols), with name and brand recordable.**
- **This kind of brand extension is favored by those companies that give the greatest importance to the control of product quality, image and distribution.**
- **Ideal marketing tool that allows a company brand of going into sectors and market segments distant from the original business.**

Product containing red vine extract which help maintainig the well-being of the legs, all this according to the manufacturer, thanks also to the flavonoids in the extract of red vine leaves, which provide an aid to improve the microflow skin and oxygenation of the vessel walls.

estratto di mosto polvere	mg/g polvere
Procianidine	81.03
Flavonoli	32.65
Caffeici	45.98
Antociani	600.98
Polifenoli tot	**760.64**

phytotherapy

cosmetics

Natural well-being and ethical consumption

Novel Food and food supplements

BIOMASSE E SCARTI
(vinacce e vinaccioli)

UTILIZZO
TRADIZIONALE

RIUTILIZZO GREEN O
ESTRAZIONI INNOVATIVI

ECONOMIA CIRCOLARE

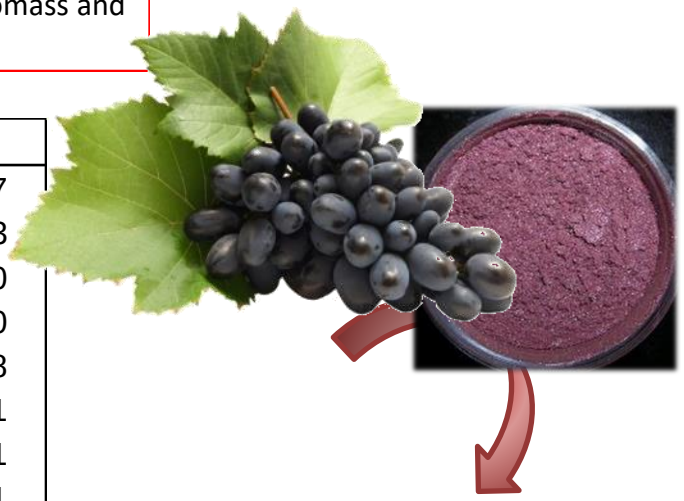
VITICOLTURA CIRCOLARE

SMALTIMENTO DEGLI
SCARTI O
COMBUSTIONE

COMPOSTI NATURALI
ATTIVI ED ENERGIA

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

Dried pomace	mg/g	mmol/Kg	%
Delphinidin-3-glucoside	0.040	0.086	0.7
Cyanidin-3-glucoside	0.079	0.175	1.3
Petunidin-3-glucoside	0.064	0.134	1.0
Peonidin-3-glucoside	0.265	0.530	4.0
Malvidin-3-glucoside	0.249	0.505	3.8
Cyanidin-3-acetyl glucoside	0.007	0.014	0.1
Malvidin-3-acetyl glucoside	0.078	0.145	1.1
Malvidin-3-caffeoyl glucoside	0.012	0.018	0.1
Petunidin-3-cumaroyl glucoside	0.016	0.025	0.2
Peonidin-3-cumaroyl glucoside	0.022	0.034	0.3
Malvidin-3-cumaroyl glucoside	0.098	0.152	1.2
Delphinidin aglycone	0.287	0.867	6.6
Total anthocyanins	**1.215**	**2.685**	**20.4**
Gallic acid	0.019	0.112	0.9
Catechin	0.030	0.102	0.8
Epicatechin	0.032	0.112	0.9
ECG dimers	0.891	1.010	7.7
catechin/epicatechin trimers digallated	10.661	9.112	69.4
Total tannins	**11.633**	**10.448**	**79.6**
Total polyphenols	**12.848**	**13.133**	**100.0**



Dried grape pomaces: HPLC/DAD/MS characterization

Quali-quantitative analysis of the hydroalcoholic extract of the dried pomace. The results are expressed as mg, mmol and % p/p of single compounds with respect to the sample weight.

Grape seed post-oil extraction residue

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

GRAPE SEED	mg/g	mmol/Kg
Gallic acid	0.042	0.246
Catechin dimer B3	1.687	2.919
Catechin	0.816	2.814
Catechin trimer	0.000	0.000
Catechin dimer B6	1.288	2.228
Catechin dimer B2	0.776	1.343
Epicatechin	0.578	1.993
Catechin trimer	0.487	0.562
ECG dimers	1.649	1.870
Catechin oligomers quantified as tetramers	26.25	22.74
ECG dimers	17.07	19.35
catechin/epicatechin trimers digallated	39.35	33.63
catechin/epicatechin trimers digallated	4.473	3.823
TOTAL	**94.46**	**93.52**

OIL EXTRACTION RESIDUE (hydroalcoholic extract)	mg/g	mmol/Kg
Gallic acid	0.036	0.209
Catechin dimer B3	1.513	2.618
Catechin	1.004	3.462
Catechin trimer	0.434	0.501
Catechin dimer B6	0.662	1.145
Catechin dimer B2	0.778	1.346
Epicatechin	0.882	3.041
Catechin trimer	0.402	0.464
ECG dimers	1.470	1.667
Catechin oligomers quantified as tetramers	17.05	14.77
ECG dimers	12.07	13.69
catechin/epicatechin trimers digallated	25.14	21.49
catechin/epicatechin trimers digallated	6.687	5.716
TOTAL	**68.13**	**70.12**

Grape seed post-oil extraction residue

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

GRAPE SEED	mg/g	mmol/Kg
Gallic acid	0.042	0.246
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Catechin trimer	0.000	0.000
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ECG dimers	17.07	19.35
catechin/epicatechin trimers digallated	39.35	33.63
catechin/epicatechin trimers digallated	4.473	3.823
TOTAL	**94.46**	**93.52**

OIL EXTRACTION RESIDUE (aqueous extract)	mg/g	mmol/Kg
Gallic acid	0.407	2.394
Catechin dimer B3	1.520	2.630
Catechin	0.644	2.221
Catechin trimer	0.544	0.629
Catechin dimer B6	0.766	1.325
Catechin dimer B2	0.963	1.666
Epicatechin	0.588	2.026
Catechin trimer	0.000	0.000
ECG dimers	1.057	1.199
Catechin oligomers quantified as tetramers	9.507	8.238
ECG dimers	5.494	6.229
catechin/epicatechin trimers digallated	13.47	11.52
catechin/epicatechin trimers digallated	8.919	7.623
TOTAL	**43.88**	**47.70**

Life

EVERGREEN

"EVERGREEN" - LIFE13 ENV/IT/000461

B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

Grape seed commercial extract

Grape seed commercial extract	mg/g	mmol/Kg
Gallic acid	0.004	0.024
Catechin dimer B3	2.217	3.836
Catechin	11.07	38.18
Catechin trimer	3.213	3.710
Catechin dimer B6	2.614	4.522
Catechin dimer B2	5.374	9.297
Epicatechin	13.62	46.96
Catechin trimer	3.706	4.280
Epicatechin gallate dimer	6.649	9.108
Epicatechin gallate	6.098	13.80
EC Oligomer calibrated as tetramers	54.88	47.55
Epicatechin gallate dimer	180.6	204.8
Trimers digallate	383.0	327.3
Trimers digallate	149.7	127.9
TOTAL	**822.7**	**841.3**

- Antioxidant capacity
- Antimicrobial activity

- Expensive
- Use of solvents during production process

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

New Formulations

GEL FORMULATION with grape seed post-oil extraction residue:

Release of condensed tannins into the ground to obtain antimicrobial and nematostatic/nematicidal effects

In a phase of characterization and optimization (laboratory + industrial scale)

The formulation may be enriched with other natural extracts.

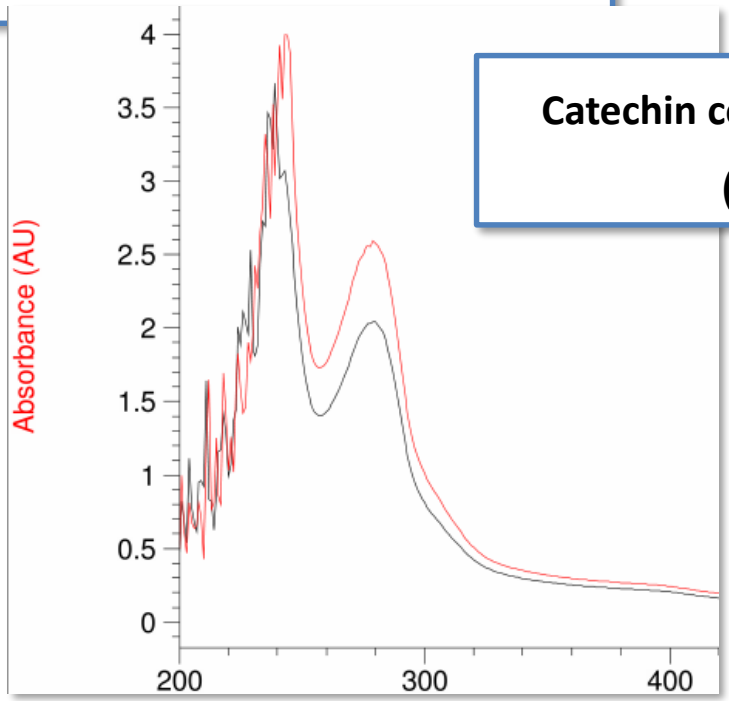
Dried grape skin from wine-making process

"EVERGREEN" - LIFE13 ENV/IT/000461

B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

New Formulations

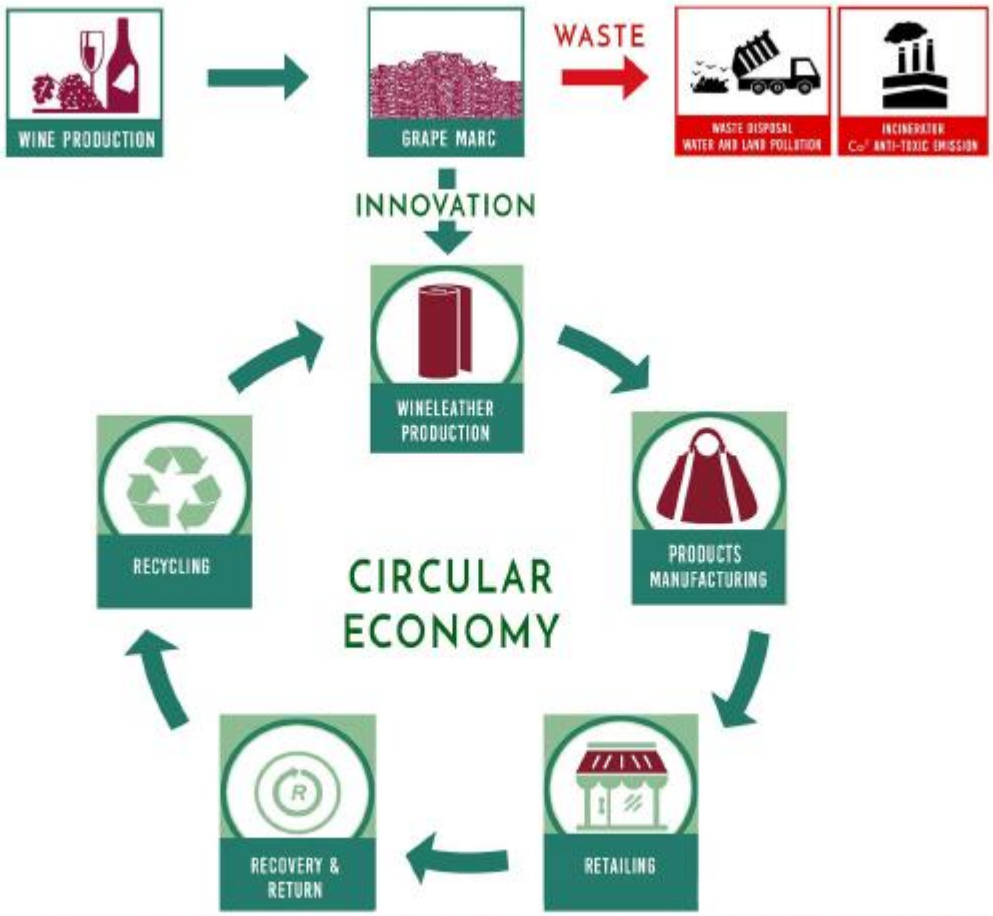
GEL FORMULATION with grape seed post-oil extraction residue:



Catechin content: 7.4 mg/g gel

(0.7% p/p)

"EVERGREEN" - LIFE13 ENV/IT/000461


WINE PRODUCTION
GRAPE MARC
WASTE
WASTE DISPOSAL
WATER AND LAND POLLUTION
INCINERATOR
Co^2 ANTI-TOXIC EMISSION
INNOVATION
WINELEATHER
PRODUCTION
PRODUCTS
MANUFACTURING
RETAILING
RECOVERY &
RETURN
RECYCLING
CIRCULAR
ECONOMY

PROCESSO PRODUTTIVO WINELEATHER

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WINELEATHER - UN NUOVO MADE IN ITALY

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Azienda che pone le sue basi su un concetto così semplice quanto antico: quello di estrarre colore da matrici vegetali.

PIGMENTO

Be_natural, la natura, prima di tutto.

Un ritorno all'applicazione di colorante naturale rispettando il principio della sostenibilità ambientale ed economica, così come in passato ma con modalità innovative, coniugando nuove tecnologie e professionalità.

PIGMENTO

Giallo *Yellow*

P (*Allium cepa* L.) Giallo Oro, Verde Brillante *Golden*

one *Brown*

Violette, Grigio Cenere *Violets, Ash Gray*

Applicazione su tessuti
Application on fabrics

Giallo Oro
Golden Yellow

Verde Brillante

Grigio Cenere

PIGMENTO

info@pigmentonaturale.com

Thank you for the attention

Annalisa Romani, Maria Francesca Belcaro

Phytolab (Pharmaceutical, Cosmetic, Food supplement Technology and Analysis) – DISIA

QuMAP – PIN Università degli Studi di Firenze

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PIN POLO UNIVERSITARIO CITTÀ DI PRATO

SERVIZI DIDATTICI E SCIENTIFICI PER L'UNIVERSITÀ DI FIRENZE