EU project LIFE13 ENV IT 000461 / “EVERGREEN”

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

Monitoring visit - May 13, 2016

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TIMETABLE

Action		2014				2015				2016			
Action number	Name of the Action	I	II	III	IV	I	II	III	IV	I	II	III	IV
A. Preparatory actions:													
B. Implementation actions:													
B.1	Demonstration of the performances of traditional pesticides for the control of bacterial and nematode diseases of plants important for the EU												
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												
B.3	Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and of their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale												
B.4	Demonstration of the biological activity of the high quality polyphenolic extracts recovered from not edible biomass and waste, agaist plant pathogenic bacteria and nematodes, *in planta*												
B.5	Demonstration of kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from not edible biomass and waste												
B.6	Demonstration of the null toxicity profile of the high quality polyphenolic bioactive molecules recovered from not edible biomass and waste, on model organisms and microorganisms												
B.7	Demonstration of the *in vivo* performances of the high quality polyphenolic bioactive preparations, recovered from not edible biomass and waste, at pilot scale level in field screenings												

TIMETABLE

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Action number	**Name of the Action**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
A. Preparatory actions:													
B. Implementation actions:													
C. Monitoring the impact of the project actions:													
C.1	Monitoring on the environmental impact of copper compounds and nematicides for the cropdefence against phytopathogenic bacteria and nematodes												
C.2	Monitoring of the absence of side effects for the high quality standardised polyphenolic preparations on common targets on any living organism at labotatory level												
C.3	Monitoring of the absence of a direct selection operated by the polyphenolic preparations towards the emergence of bacteria resistant to the polyphenolic molecules themselves, at laboratory levels												
C.4	Monitoring the short term environmental benefits from the use of the high quality standardised polyphenolic preparations in plant disease control at pilot scale level in field screenings												
C.5	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												
C.6	Monitoring the absence of a selection on the polyphenolic preparations on the selection of copper and antibiotic resistant bacteria, on plant and in soil, from laboratory to in field screenings												
C.7	Monitoring of technical-socio-economic assessment of the project												

ACTION B2: 2015 Jan – 2015 Dec

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: 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

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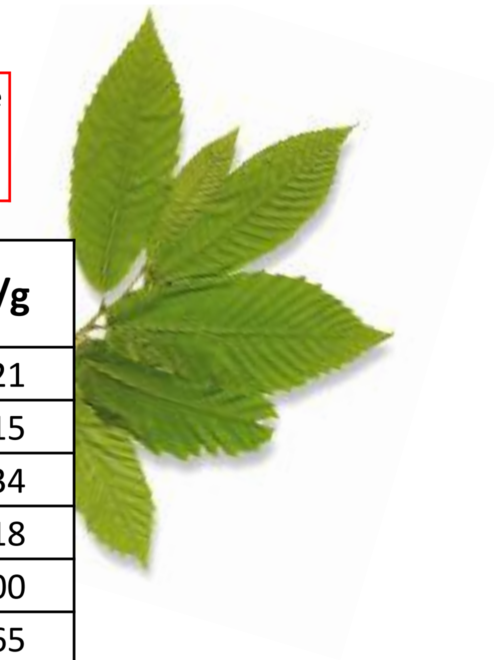

Sweet Chestnut leaf aqueous extract	mg/mL ext	mg/g
Monogalloyl glucose I	0.046	1.821
Gallic acid	0.045	1.815
Monogalloyl glucose II	0.008	0.334
Gallagic derivatives*	0.023	0.918
Ellagitannins with ellagic acid units**	0.000	0.000
Ellagitannins with HHDP- or NHTP- units**	0.022	0.865
Ellagic acid	0.025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

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 olive leaves and paste

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**

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**, *Submitted*.

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**

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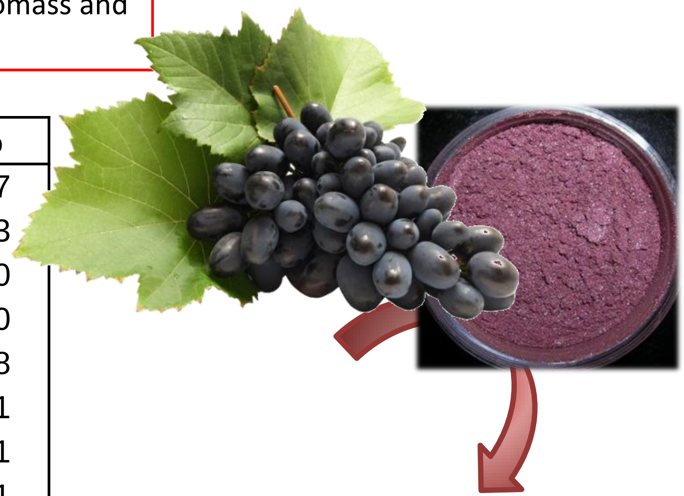
B2	Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

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**

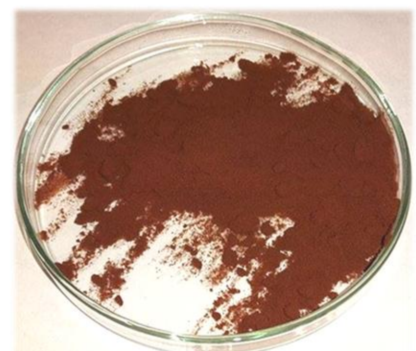
B2 Demonstration of the qualitative and quantitative yields of the extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste, at laboratory scale.

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



Grape seed post-oil extraction residue

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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**

ACTION B3: 2015 Jan – 2015 Dec

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

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.

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

Olea fractions stability

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.

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

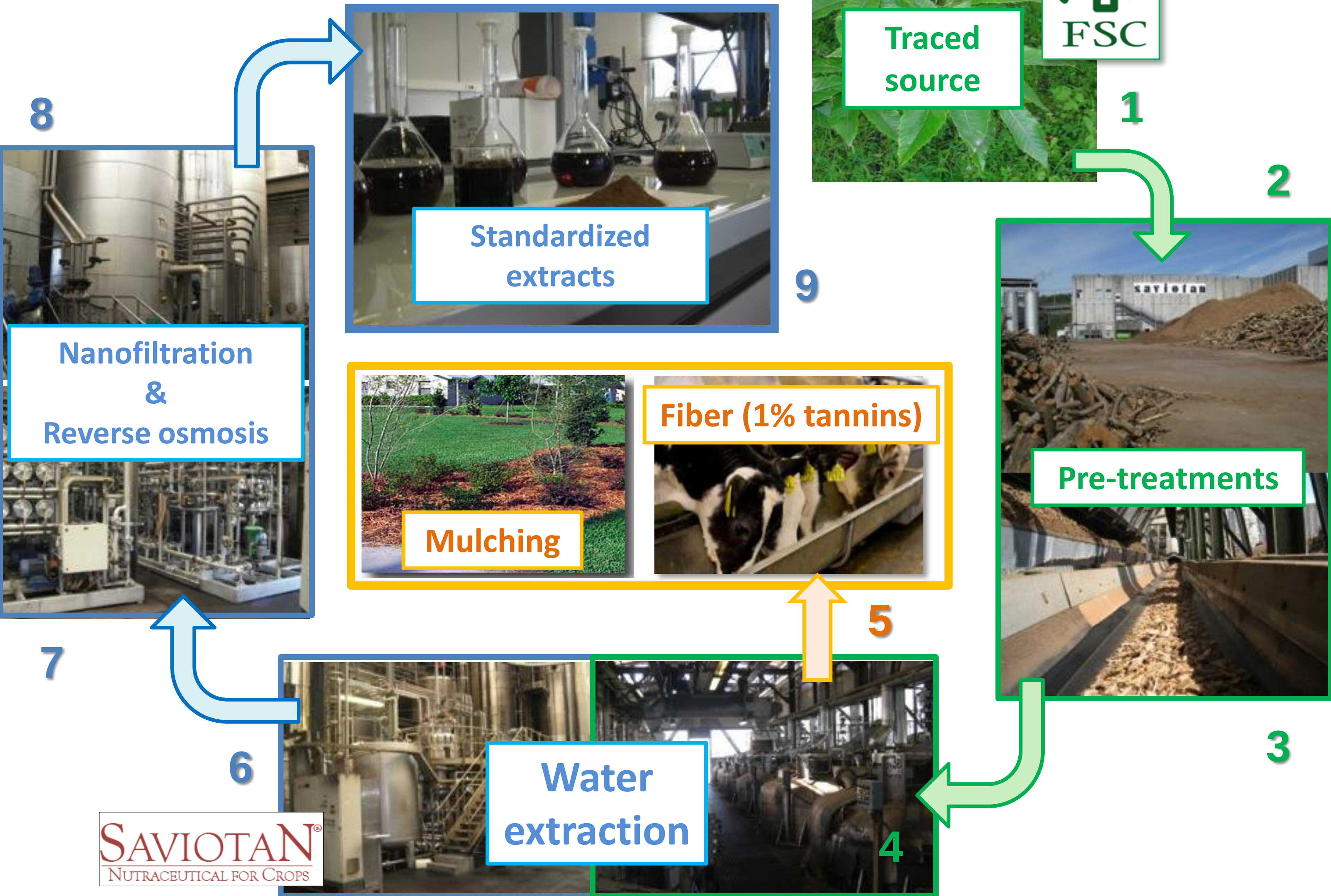
- Folin-Ciocalteu
- DPPH·
- ORAC (commercial fractions)

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

ACTION B5: 2015 Jul – 2016 Mar

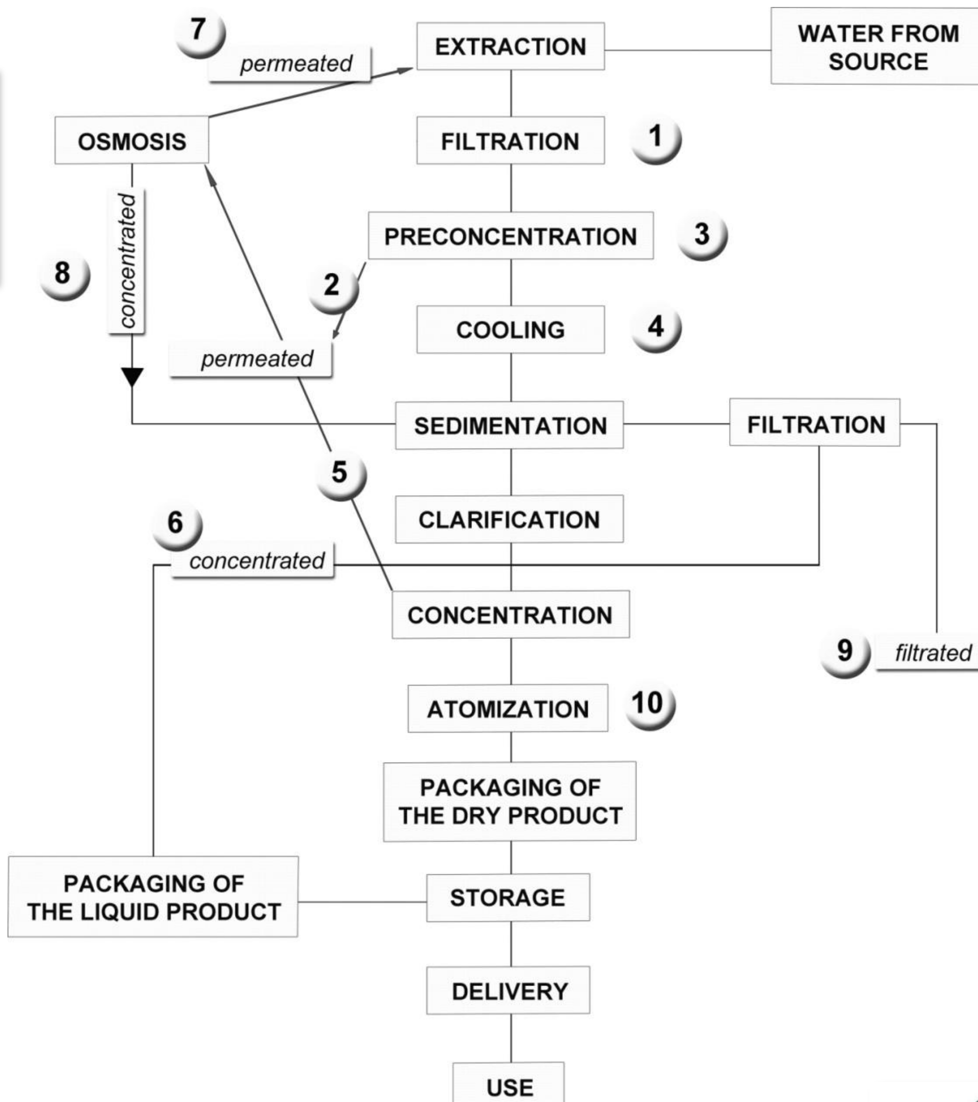
Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not edible biomass and waste

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



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



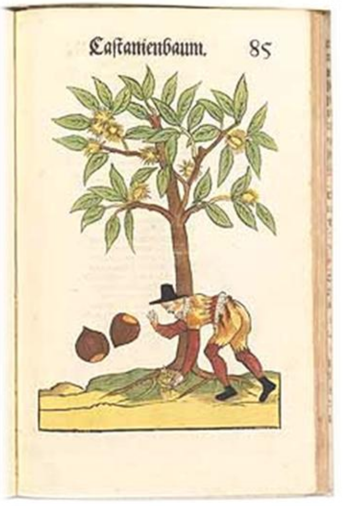
B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

SWEET CHESTNUT

Quali-quantitative analysis HPLC/DAD/ESI-MS Tannic subclasses: all Kilo-scale process fractions

Quantitative analysis by subclasses (%)

	GA/TOT	GA/GTs	(C+V)/TOT	(C+V)/ETs	GTs/TOT	ETs/TOT	TOT*
1	42.6	74.3	27.3	63.9	57.3	42.7	**37.9**
2	74.4	86.4	10.6	75.8	86.1	13.9	**15.6**
3	24.3	69.4	40.5	62.3	35.0	65.0	**82.0**
4	38.3	79.0	33.1	64.1	48.4	51.6	**75.3**
5	62.2	83.6	17.1	66.4	74.3	25.7	**43.2**
6	6.0	17.9	33.8	50.7	33.3	66.7	**121**
7	100.0	100.0	0.0	-	100.0	0.0	**2.68**
8	63.0	84.0	15.1	60.1	74.9	25.1	**138**
9	31.8	59.6	31.1	66.7	53.3	46.7	**102**
10	21.4	48.4	24.4	43.9	44.4	55.6	**372**



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

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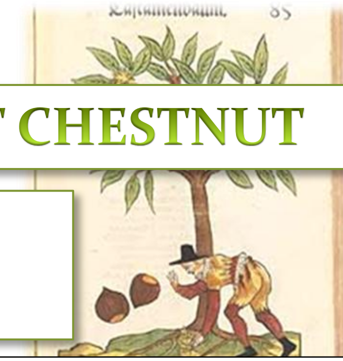
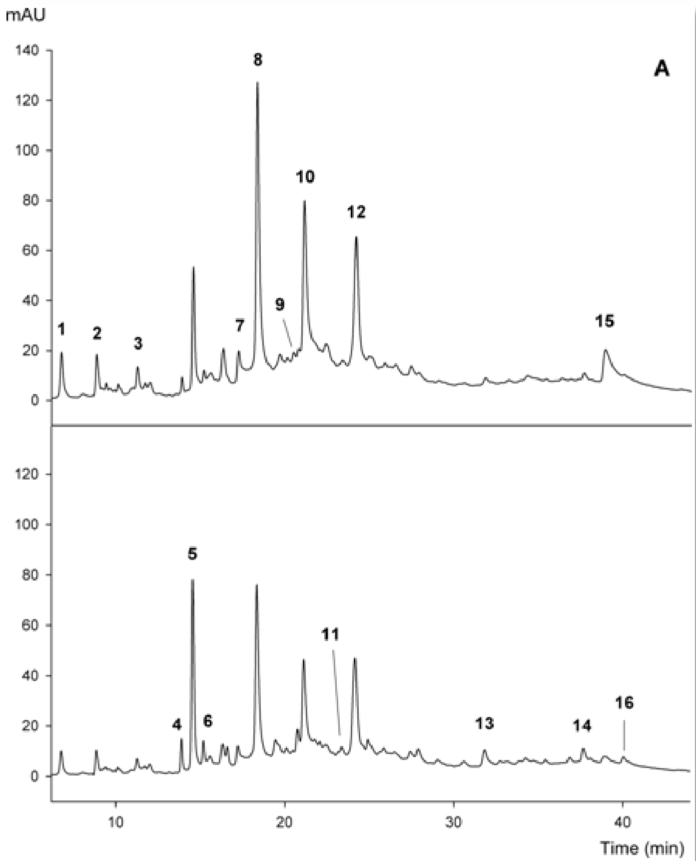


SWEET CHESTNUT

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.

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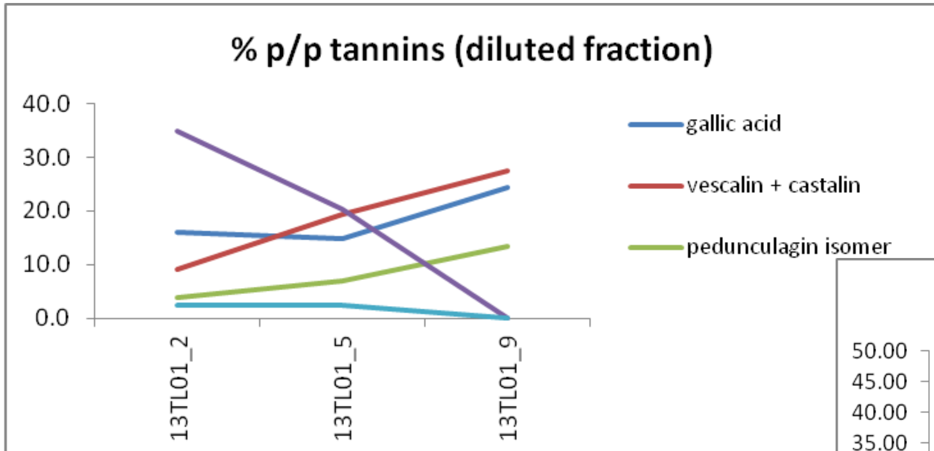
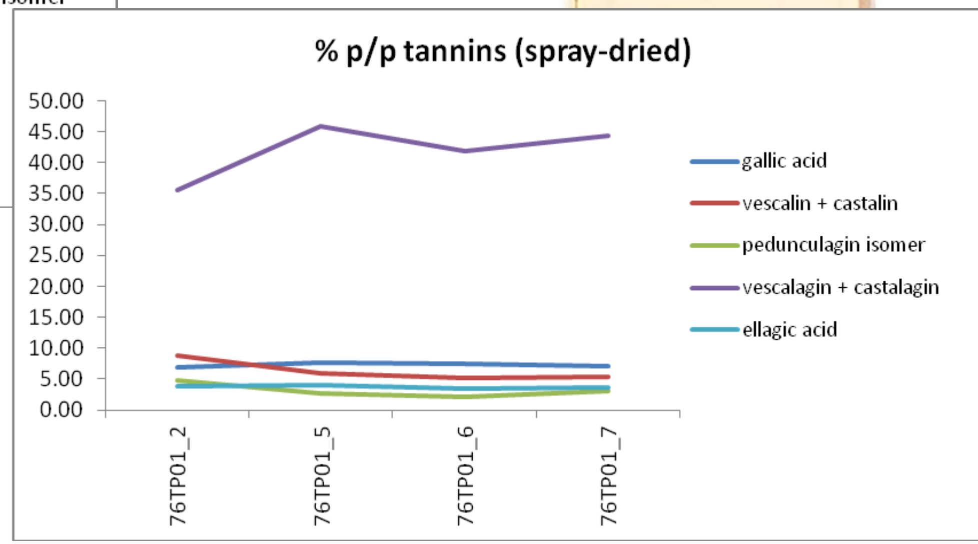
B5 Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not-edible biomass and waste.

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

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.

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.

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. *XXV*th International Conference of Polyphenols, *Polyphenols Communcations 2010.* **2010**, 2, T4. 75.

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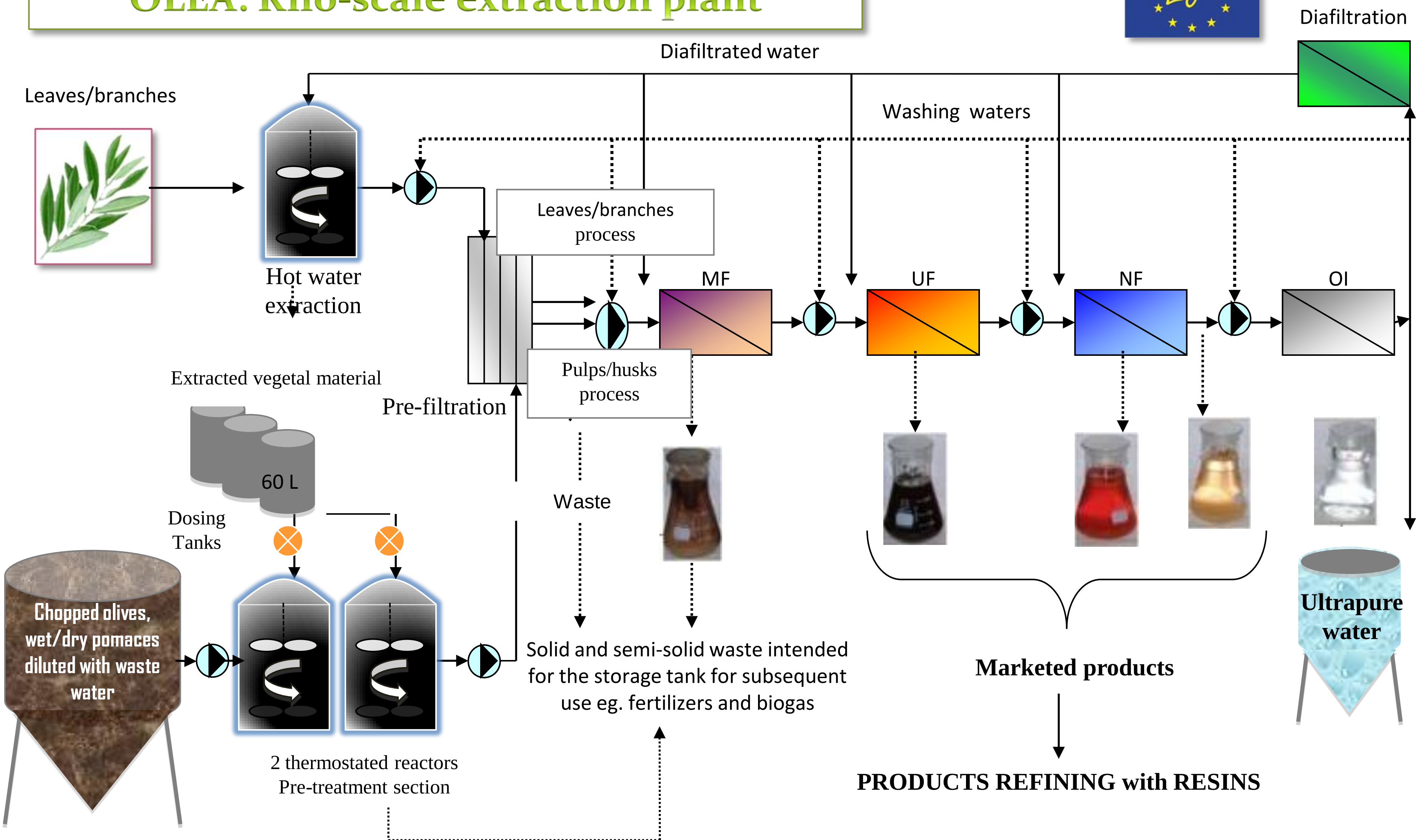
OLEA: Kilo-scale extraction plant



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**, *Submitted*.

EVERGREEN

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

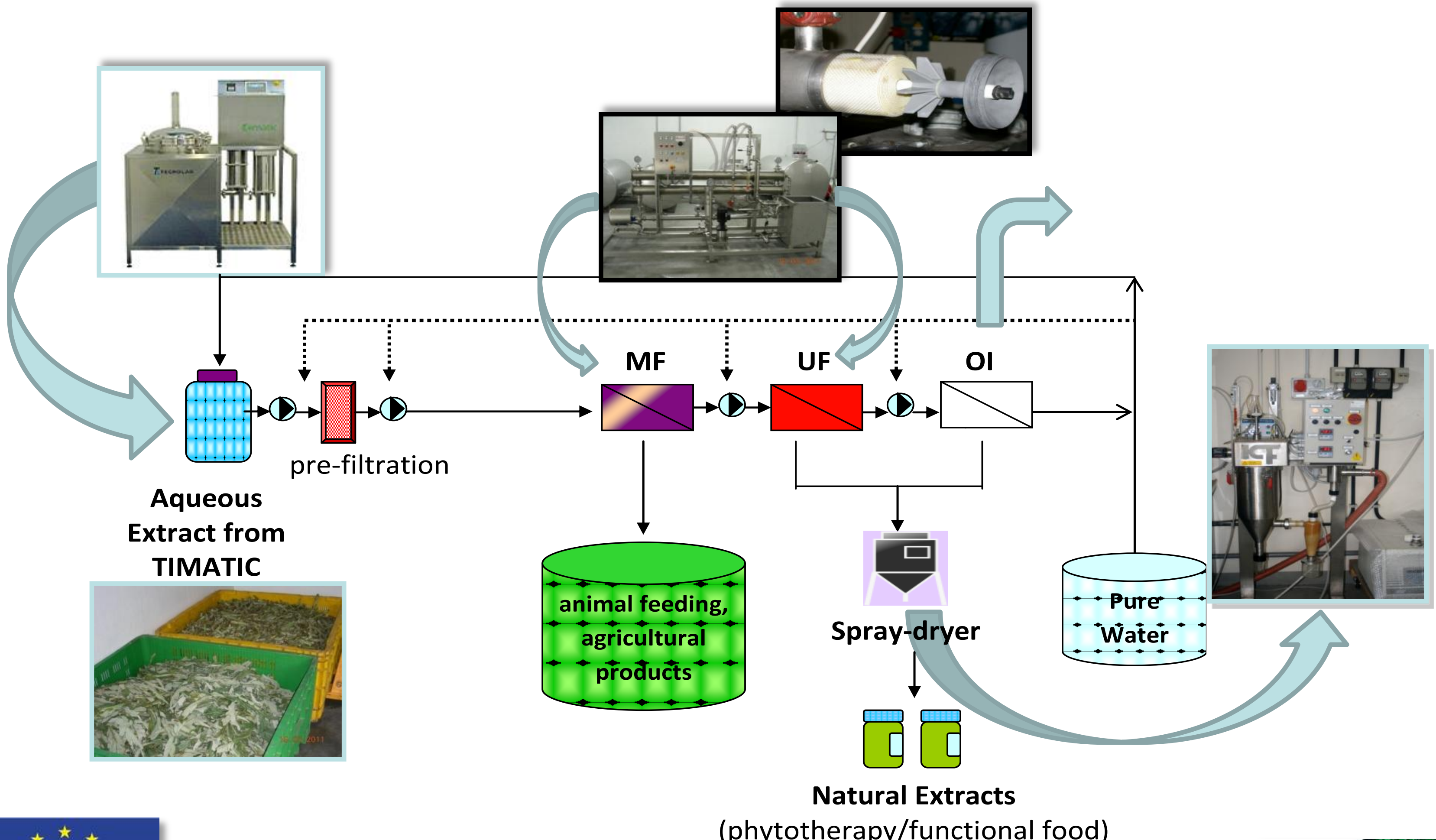
Soft Extract Olea OH-Tyr	Concentrated fraction (mg/g)
Hydroxytyrosol derivatives	279.89 ± 18.24
Secoiridoid der.	nd
Elenolic acid der.	0.51 ± 0.04
Hydroxycinnamic derivatives	7.83 ± 0.25
Flavonoids	nd
Verbascoside	1.69 ± 0.17
Lignans	nd
Total Polyphenols	**289.93 ± 18.70**

Olea fractions: chemical stability

Total polyphenols content (mg/g)	Phenolea FF	Phenolea FS	Phenolea OH-Tyr
T0	248.07	60.75	303.14
T12	240.23	54.50	276.70

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.



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**, *Submitted*.

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

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)

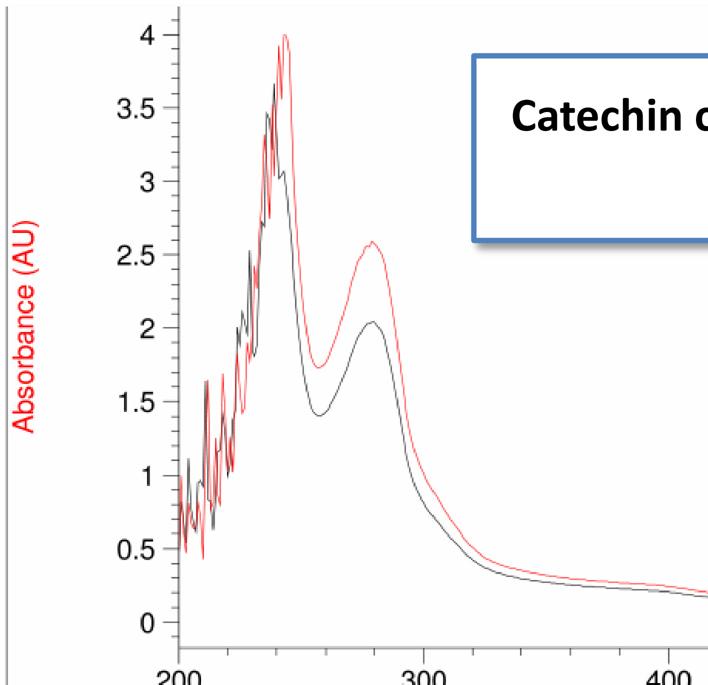
The formulation may be enriched with other natural extracts.

Dried grape skin from wine-making process

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)

Stability of the liquid formulations: accelerated aging at 40°C

- **Formulation #1: TC/O conc (concentrated solution)**. Sweet Chestnut liquid fraction (20%)/Olea liquid fraction 3.22% polyphenols (10%) [to dilute to 100 for use].
- **Formulation #2: TC/O dil (diluted solution)**. Sweet Chestnut liquid fraction (2%)/Olea liquid fraction 3.22% polyphenols (1%) [to dilute to 10 for use].

	mg/mL GAE									
	T0	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Form1	154.56	149.64	156.67	151.87	154.71	153.26	155.60	154.12	152.32	148.59
Form2	10.286	11.276	11.545	11.162	11.160	11.580	11.161	11.151	11.285	11.520

Folin-Ciocalteu assay results for the SC/OE formulations as such (**T0**) and during accelerated aging at 40°C (**T1-T9**). The results are expressed as mg/mL GAE (Gallic Acid Equivalents). Measures: T0 nov 27, 2015; T1 dec 4, 2015; T2 dec 11, 2015; T3 dec 18, 2015; T4 dec 23, 2015; T5 jan 7, 2016; T6 jan 14, 2016; T7 feb 15, 2016; T8 mar 15, 2016; T9 apr 15, 2016.

Romani A., Campo M. Scardigli A., Biancalani C., Cerboneschi M., Tegli S. Acts of the XXVIII International Conference on Polyphenols, Vienna 11-15 jul 2016