Multifunctional platform for the production of antioxidants and energy from olive-mill industry

•*Annalisa Romani, *Lisa Banelli, •*Arianna Scardigli

*University of Florence, Department of Pharmaceutical Sciences, Via Ugo Schiff 6, 50019, Florence, Italy.
•MerQuRIS , Commodity Sciences and Quality of Natural Resources Laboratory, Via delle Pandette 9, 50127, Florence, Italy; PIN – QuMAP, Quality of Goods and Product Reliability Laboratory, Piazza Giovanni Ciardi 25, 59100, Prato, Italy.
Corresponding author: annalisa.romani@unifi.it

In the field of activities aimed at the exploitation and innovation of the olive oil sector, has been designed and optimized an industrial closed cycle platform for the recovery of antioxidants and the production of biogas from Olive oil waste waters. Polyphenols are phytotoxic for the environment and inhibit the fermentation processes for the production of biogas but, at the same time, the biological activity promotes the recovery as chemicals with high added value.

Preliminary pre-oxidation, extraction of polyphenols with membrane technology (patent ENEA), bio-digestion process to obtain biogas (as a fuel for cogeneration) and fertilizer. The recovery of polyphenol compounds, the biogas productivity, the continuous process and the interaction between high technology and environmental and economic sustainability, making this platform highly innovative compared other plants already existed (Spain and Greece).

Moreover in the pomaces stored in the absence of air, are activated bacterial processes that lead to obtain fractions enriched in hydroxytyrosol.

Hydroxytyrosol is a powerful antioxidant and cardioprotective (Covas M.I. *et al*, 2006; Visioli F. *et al*, 1998), it's the compound with greater functional activity present in extra virgin olive oil. It retards the oxidation of LDL (Visioli F. and Galli C., 1998). Hydroxytyrosol reduces the gene expression of iNOS and COX-2 in cell lines, preventing the activation of the transcription factors NF-KB, STAT-1α and IRF-1 (Maiuri MC *et al,* 2005). According to some authors it inhibits platelet aggregation in vitro (Petroni A. *et al*, 1995).

The anaerobic biodigestion for the production of biogas, fertilizer and cogeneration with previous aerobic phase (PCT/IT/2009/000246) was performed on two-step reactor. The first step is characterized by biochemical reactions that lead to the reduction of molecular masses by enzymatic hydrolysis of complex substances (cellulosic, polymeric and organic compounds with high molecular weight) and acidophilic reactions with formation of simple substances; this stage is characterized by formation of low molecular weight organic acids (acetic, succinic, propionic,

etc..). The second step is defined as methanogenic because it generates a microbial flora that uses organic acids with low MW formed in the previous stage, to form biogas.

The polyphenols from *Olea europaea* L. matrices (olive oil by-products, leaves, stems and olive pulps), are known for the highly antioxidant properties and protective biological and biomedical effects (Covas M.I., 2007; Brunelleschi S. *et al*, 2007; Franconi, F. *et al, 2006*). The chemical characterization and the quantitative evaluation of these minor polar compounds could be useful to obtain active principles with high bioavailability in cosmetic and functional food products. The main constituent of olive leaf is oleuropein, which could be broken down to elenolic acid, a powerful anti-bacterial molecule, and hydroxytyrosol which has an antioxidant activity three/four times higher than grape extracts. Animal studies have proven the effectiveness of olive leaf extract to lower blood pressure, and this effect seems to be mainly ascribed to oleuropein. An innovative process of separation has been used by physical technologies (EP2338501(A1)) defined as BAT (Best Available Technology) and recognized from EPA (Environmental Protection Agency).

Through the new sustainable extractive technologies by membrane separation four PHENOLEA fractions with different biophenol concentrations have been produced from Phenofarm srl, and the HPLC/DAD/MS quali-quantitative analyses have been performed for each sample.

In particular, PHENOLEA OH-TYR is an extract obtained from olive oil by-products which contains hydroxytyrosol and derivatives more than 90%; PHENOLEA LEAVES-S is an extract obtained from dried olive leaves, containing hydroxytyrosol and derivatives more than 50%, secoiridoids as oleuropein 20-35%, hydroxycinnamic derivatives as verbascoside more than 10%, flavonoids, luteolin and apigenin 7-*O*-glucosides, 2-7%, and, finally, trace amounts of lignans; PHENOLEA LEAVES-F obtained from olive green leaves, containing hydroxytyrosol and derivatives more than 20%, secoiridoids 60-70%, hydroxycinnamic derivatives as verbascoside more than 3%, flavonoids, luteolin and apigenin 7-*O*-glucosides, more than 2%, and lignans about 5%.

Finally, PHENOLEA RED is an extract obtained from red olive pulps, containing besides the usual *Olea* polyphenol classes also anthocyanosides, in particular anthocyanosides evaluated as cyanidin 3-*O*-rutinoside 15-20%, secoiridoids as oleuropein 60-70 %, flavonoids, luteolin and apigenin 7-*O*-glucosides 10-15%, and, finally, hydroxycinnamic derivatives as verbascoside 3-7%.

In Table 3 the quantitative data are reported for each polyphenolic class present in PHENOLEA extracts and in two powders obtained spray-drying the respective olive leaf extracts (S and F)

	PHENOLEA S mg/L	PHENOLEA F mg/L	PHENOLEA OH-TYR mg/L	PHENOLEA RED mg/L	PHENOLEA S SPRAY DRIED mg/g	PHENOLEA F SPRAY DRIED mg/g
Hydroxytyrosol der.	4970.28	5566.87	28846.90	6783.22	15.30	23.57
Secoiridoid der.	5320.60	34536.40	0	0	33.33	89.99
Elenolic acid der.	1729.83	5201.12	2248.80	1458.75	12.46	17.81
Hydroxycinnamic der.	1041.90	1237.20	424.16	996.90	4.45	6.14
Flavonoids	705.98	579.57	0	72.56	3.83	5.53
Coumarins	73.60	149.80	0	0	0.22	0.30
Lignans	traces	2355.94	0	0	4.34	7.92
TOTAL POLYPHENOLS	**13842.19**	**49626.90**	**31519.86**	**9311.43**	**73.93**	**151.26**

Table 3. HPLC/DAD quantitative analyses of the different *Olea* fractions and spray dried.

The quantitative evaluation of anthocyanosides as 0.047 g/L has been performed by spectrophotometric method at 520 nm, using Keracianin (Cyanidin 3-*O*-rutinoside), the main anthocyanoside present in red olive extracts, as standard.

The Phenolea fractions antiradical activities, evaluated using a DPPH solution ($3.16\ 10^{-4}$ mM), show the following EC_{50} values: 0.006757 mM for Phenolea F, 0.00544 mM for Phenolea S, and 0.012195 mM in the case of Phenolea OH-TYR.

Individual extracts and their combination allow specific applications due to the previously reported biological activities, and synergies of use for multiple and new formulations in different application fields. Associations with natural extracts or molecules derived from other plant species are under study.

REFERENCES

- Brunelleschi S. *et al* (2007) Pharmacol Res., 56:542-549.
- Covas M.I., (2007) Pharmacol. Res., 55: 175-186.
- EP2338501(A1)"Process for producing concentrated and refined actives from tissues and byproducts of Olea europaea with membrane technologies", Pizzichini, D.; Romani A. and Phenofarm, 20.06.2011
- Franconi, F. *et al* (2006) J. Agric. Food Chem., 54(8): 3121-3125.
- Maiuri MC. *et al* (2005) Naunyn-Schmiedeberg's Archives of Pharmacology, Springer, pp. 457-465.
- PCT/IT2009000246, filing date 05/06/09. RACE s.r.l. "Integrated process for recovery of a polyphenol fraction and anaerobic digestion of olive mill wastes".
- Petroni A. *et al* (1995) Thrombosis Research, Apr. 15, 78(2), pp. 151-60.
- Visioli F. and Galli C. (1998) *J. Agric. Food Chem.*, 46 (10), pp. 4292–4296.
- Visioli F. *et al* (1998) Biochemical and Biophysical Research Communications, Elsevier.