沙漠研究 **22**-1, 61 -64 (2012)
Journal of Arid Land Studies

"ICAL 1 / DT X"
－Refereed Paper－

Valorization of Olive Processing By-Products: Characterization, Investigation of Chemico-Biological Activities and Identification of Active Compounds

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Abstract: The valorization of food processing by-products, wastes and effluents is a challenging opportunity for the sustainable and competitive development of several relevant industrial sectors. One of the best examples of a biorefinery applied to plant processing wastes is that of the olive oil sector by-products including leaves and olive mill wastewaters (OMW). Management of OMW, in particular, has received much attention over recent years including all possible strategies, i.e. treatment, disposal, recovery and reuse. Besides being a serious environmental problem, OMW can also represent a precious resource of potentially valuable molecules. This later approach appears to be the new frontier in olive processing by-products valorization.

The present paper will review the scientific works done in our laboratory concerning the valorization of olive processing by-products mainly OMW and olive leaves through the recovery of valuable molecules. Analytical chemical techniques such as solvent extractions and characterization of extracts in respect with their phenolic content were performed. Different methods were also used to investigate the activities of different extracts and fractions. Therefore, the antioxidant assays using DPPH (1,1'-diphenyl-2-picrylhydrazyl), beta-carotene, ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) and bioassays including anti-tumoral activity, and in vivo activities in animal models were carried out. Besides, analytical chromatography and spectroscopic techniques were used by our group either for a deep characterization of active fractions or for the identification of new molecules.

Key Words: Antioxidants, Bioactivities, Olive by-products, Polyphenols, Valorization

1. Introduction

Olea europaea L. is a typical tree widely cultivated for oil production in the Mediterranean area. Tunisian olive plantation counts about 60 million trees covering more than 1.600.000 ha. The sector of olive oil accounts for around 47% of agro-alimentary exports and 5.5% of total exports of the country. Olive leaves and olive mill wastewater, resulting from the water use during oil extraction, as the two main olive processing by-products, have attracted considerable attention as valuable resources of bioactive molecules.

OMW and olive leaves are considered as an inexpensive source of highly valuable molecules. These molecules mainly belong to the family of polyphenols. The most studied olive polyphenols are oleuropein, a secoiridoid group member, and its hydrolysis derivative; hydroxytyrosol (2 (3,4-dihydroxyphenyl) ethanol) (Obied *et al.*2005).

In respect with this topic, our research team has been implicated, during the last few years, into a local and a Mediterranean strategy in order to establish practical methods devoted to a rational management of olive processing wastes in particular OMW and olive leaves. Here we are reviewing the majority of our work in relation with the development of a sustainable valorization process of the aforementioned by-products.

2. Olive Mill Wastewater as a Source of Valuable Molecules

It is well admitted that OMW represents a great challenge as an environment-harming factor in olive oil producing countries. Chemically, OMW is characterized by a high organic load thus limiting its biological treatment. Phenolic compounds were found as major contributors to the toxicity and the antibacterial activity of OMW (Sayadi *et al.*, 2000). However, these phenolic compounds are endowed with several biological activities such as antioxidant properties (Visioli *et al.*, 1999).

OMW has attracted a considerable attention as a source of natural polyphenols. Indeed 98% of olive fruit biophenols are lost in OMW during oil extraction (Rodis *et al.*, 2002). **Table 1** illustrates the major phenolic monomers retrieved in OMW with their spectroscopic data determined using gas chromatography coupled with mass spectrometry.

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Table 1. Mass spectrometry data of the Major Phenolic Monomers. Identified in the crude OMW.

Compounds	mass spectrometry Data (m/z)	Concentration in OMW (mg/l)
Hydroxytyrosol	370; 267; 193; 179.	1433.4
Tyrosol	282; 267; 193; 179; 163; 149; 103.	851
3,4-dihydroxyphenyl acetic acid	384; 369; 311; 296; 281; 252; 179; 164; 147	87.9
p-hydroxyphenyl acetic acid	296; 281; 252; 179; 164; 147; 133.	274
Caffeic acid	396; 381; 307; 293; 249; 233; 219; 191.	321
p-coumaric acid	308; 293; 249; 219; 203; 179; 175; 139; 115	298
Ferulic acid	338; 297; 282; 267 253; 223; 207; 193; 179; 165; 141; 126; 89	95

Owing to the increased preference for natural food ingredients, which are generally believed to be safer and less subject to hazards compared to synthetic additives, our research team has developed a convenient method aiming the recovery of valuable molecules from OMW as natural agro-industrial waste. Particular emphasis was made to recover hydroxytyrosol. To achieve this goal, a continuous counter-current liquid-liquid extraction unit was set. This unit allowed extracting more than 85% of the hydroxytyrosol originally present in the OMW (Allouche *et al.*, 2004).

3. Olive Leaves a Raw Material for the Recovery of Bioactive compounds

The work done in relation with the valorization of olive leaves focused at first on the identification and characterisation of major polyphenols in particular oleuropein, flavonoids and hydroxytyrosol. The most important fact is that olive leaves were found to contain higher amounts of oleuropein reaching nearly 6.5 g per 100 g fresh olive leaves (Bouaziz and Sayadi, 2005). **Figure 1** shows an HPLC UV-spectrum of olive leaves extract where oleuropein is the major phenolic compound.

In addition to OMW, olive leaves could also be a natural source of hydroxytyrosol due to their richness in oleuropein. Indeed, oleuropein is an ester of hydroxytyrosol and the glucosylated form of the elenolic acid (**Fig. 2**). The hydrolysis of an ester molecule yields a molecule of hydroxytyrosol. Hence, olive leaves represent a raw material for the preparation

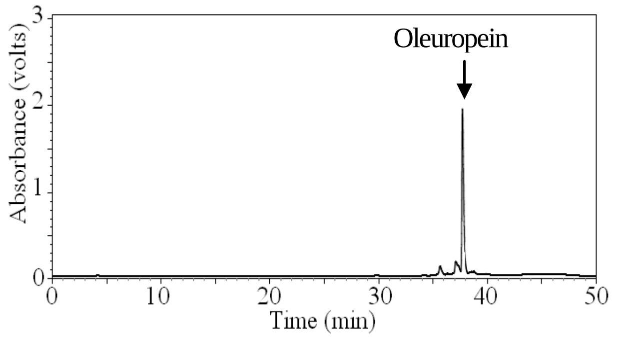

Fig. 1. HPLC chromatogram of an olive leaf extract. Detection was performed at 280 nm.

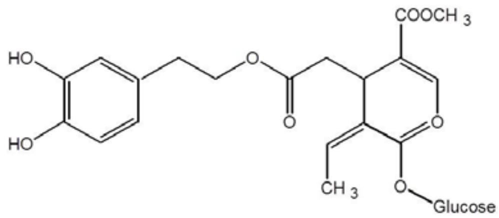

Fig.2. Oleuropein chemical structure.

of either hydroxytyrosol rich extract or even highly purified fractions. Both chemical (acid) and enzymatic hydrolysis was applied to oleuropein rich extracts. So far, hydroxytyrosol was the major compound of the hydrolyzates (Bouaziz and Sayadi, 2005; Bouaziz *et al.*, 2006).

4. Use of Olive Leaves and OMW Compounds for the Stabilisation of Edible Oils

Olive and husk refined oils are gaining increasing importance in food industry. However, these oils are highly susceptible to oxidation. The loss of nutritional quality and the production of undesirable off-flavours are the principal markers of lipid peroxidation in oils. To overcome this problem, compounds recovered from OMW were used as substituents to synthetic food additives (BHT and BHA).

Through the evaluation of the antiradical and antioxidant activities of the OMW extract and pure compounds identified in this extract, our team showed that hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid had the highest radical-scavenging effects on 1,1-diphenyl-2-picrylhydrazyl radical and the highest antioxidant activities using the b-carotene linoleate assay (**Table 2**) (Fki *et al.*, 2005). When incorporated in oil matrix, OMW extract and pure phenolic compounds resulted in an interesting protecting effect of refined olive and husk oils against induced lipid peroxidation.

Similarly olive leaves extract (oleuropein rich extract) and olive leaves hydrolyzate extract (hydroxytyrosol rich extract) were studied in oil matrix using olive and husk refined oils (Bouaziz *et al.*, 2008). Authors concluded to a great

Table 2. Characterization of OMW polyphenols and evaluation of their antioxidant activities.

	IC50 (µg/ml)	bleaching (h)
OMW extract	1.2	>236
Hydroxytyrosol	0.57	>236
Tyrosol	10.9	122
3,4-Dihydroxyphenyl acetic acid	0.64	>236
p-Hydroxyphenyl acetic acid	12.2	188
Caffeic acid	0.87	>236
p-Coumaric acid	9.5	148
Ferulic acid	1.21	>236
BHA	0.91	>236
BHT	0.89	>236

stabilizing effect of refined oils with both olive leaves and hydrolyzate extracts.

5. *In vivo* Bioactivities of Olive Compounds

5.1. Anticholesterolemic effects of olive compounds in rat model

Atherosclerosis is the principal cause of pathogenesis of the myocardial and cerebral infractions thus being the leading cause of death worldwide. Hypercholesterolemia resulting from modified lipid metabolism is being the inducing factor of atherosclerosis. Therefore it would be of interest to find anti-hypercholesterolemic molecules or preparations. Our team has investigated the possible implication of olive compounds in the reduction of hypercholesterolemia in rat models. OMW extract and hydroxytyrosol were tested (Fki *et al.*, 2007; Jemai *et al.*, 2008). These authors have showed that phenolic rich extract from OMW and hydroxytyrosol recovered from olive leaves or OMW had very pronounced hypocholesterolemic effects. Indeed these treatments were able to reduce both total circulating cholesterol, low density lipoprotein-cholesterol (LDL-C) and to increase the level of high density lipoprotein-cholesterol (HDL-C) in serum. In addition olive polyphenols favorably modified the antioxidant system within liver, heart and kidneys. Such later activities were suggested to be the origin of the hypocholesterolemic activity of olive phenolic compounds.

5.2. Hydroxytyrosol and Oleuropein could have anti-diabetic activities

Diabetes has been shown to implicate diverse and complicated mechanisms correlated with an overproduction of free radicals and many other disorders exerting deleterious effects on organs acting in the glycemia regulation. Due to their antioxidant activities, olive polyphenols were suggested to have anti-diabetic potential. For this reason hydroxytyrosol and oleuropein were recovered from olive olives and were investigated *in vivo* in rat model for their potential to prevent alloxan induced diabetes (Jemai *et al.*, 2009). Authors have focused on the analysis of the glucidic and oxidative stress parameters (**Table 3)**. Olive leaves compounds significantly reestablished blood circulating glucose and hepatic glycogen in animals treated with alloxan. This phenomenon was coupled with the restoration of the total cholesterol in serum and the thiobarbituric acid reducing substances in the liver.

Table 3. Antidiabetic and antioxidant activities of polyphenols recovered from olive leaves. PG (plasma glucose); HG (hepatic glycogen); TC (total cholesterol); TBARS (ThiobarbituricAcid-Reactive Substances).

Treatment	PG (µg/dl)	HG (mg/g)	TC (mg/dl)	TBARS(Mmol/ 100mg)
Control	1.05	10.6	98	12
Diabetic control	2.9	6	168	17.2
Oleuropein	1.5	16	105	13.1
Hydroxytyrosol	1.55	14.6	107	12.8

Table 4. Effect of olive leaves extract treatment on cell cycle distribution in human breast cancer cells MCF-7; OLE: olive leaves extract.

	Cell cycle distribution		
Treatment	G0/GA	S	G2/M
Control	39.9	33.3	26.1
OLE 2000µg/ml	46.9	27.6	24.9
OLE 2400µg/ml	53	24	22.4
OLE 2800µg/ml	53.1	23.1	23.2

6. Bioactivities of Olive Leaves Extract in Cancer Cells Model

Recently we have investigated the activities of hydroxytyrosol rich extract from olive leaves against the proliferation of cultured cancer cells. Breast cancer cells MCF-7 were used as cancer model. Anti-proliferation assays using MTT and neutral were performed (Bouallagui *et al.* 2011).

The studied extract showed a cell growth inhibiting effect through the arrest of the division cell cycle at G0/G1 phase (**Table 4**). Such growth arrest was the consequence of the reduction of the cyclinD1 level. At the up-stream level, cyclinD1 protein level decrease was the result of decreasing level of the peptidyl prolyl cis-trans isomerase Pin1 which is implicated in many physiological processes.

7. Conclusion

The bioactivities exerted by olive polyphenols highlighted once again the olive tree by-products, including both olive leaves and OMW, as a natural source of antioxidants which could have advantageous applications to prevent oxidative stress related disorders. Thus, processes aiming the recovery of antioxidant polyphenols from olive by-products will be a promising alternative for the valorization of such agro-industrial wastes.

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