

## A New Process for the Management of Olive Oil Mill Waste Water and Recovery of Natural Antioxidants

APOSTOLIS AGALIAS,<sup>†</sup> PROKOPIOS MAGIATIS,<sup>†</sup>  
 ALEXIOS-LEANDROS SKALTSOUNIS,<sup>\*,†</sup> EMMANUEL MIKROS,<sup>†</sup>  
 ANTHONY TSARBOPOULOS,<sup>‡,§</sup> EVAGELOS GIKAS,<sup>§</sup> IOANNIS SPANOS,<sup>||</sup> AND  
 THRASYVOULOS MANIOS<sup>⊥</sup>

Laboratories of Pharmacognosy and Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis Zografou, 157 71 Athens, Greece, Department of Pharmacy, University of Patras, Panepistimiopolis Rio, 26504, Greece, GAIA Research Center, Bioanalytical Department, The Goulandris Natural History Museum, 13 Levidou Street, Kifissia, 145 62 Athens, Greece, Terra Nova Ltd., 39 Kaisareias Street, 115 27 Athens, Greece, and School of Agricultural Technology, Technological Education Institute of Crete, 71004 Heraklion, Greece

The high polyphenol content of the wastewater is the major environmental problem caused by the olive mills. A pilot scale system for the treatment of the olive oil mills wastewater was developed aiming at the recovery of high added value-contained polyphenols and the reduction of the environmental problems. The treatment system consists of three main successive sections: The first one includes successive filtration stages aiming at the gradual reduction of the wastewater suspended solids up to a limit of 25  $\mu\text{m}$ . The second section includes passing of the filtered wastewater through a series of adsorbent resins (XAD16 and XAD7HP) in order to achieve the deodorizing and decolorization of the wastewater and the removal/ recovery of the polyphenol and lactone content. The third section of the procedure includes the thermal evaporation and recovery of the organic solvents mixture, which has been used in the resin regeneration process, and finally the separation of the polyphenols and other organic substance contents using fast centrifuge partition chromatography. The final outcome of the whole procedure is (i) an odorless yellowish wastewater with a 99.99% reduced content in polyphenols and 98% reduced COD, (ii) an extract rich in polyphenols and lactones with high antioxidant activity and high added value, (iii) an extract containing the coloring substances of the olive fruit, and (iv) pure hydroxytyrosol.

**KEYWORDS:** Olive mill wastewater; hydroxytyrosol; polyphenols; resins; antioxidants

### INTRODUCTION

The Mediterranean basin is the main olive oil production area in the world. The annual worldwide olive oil production is estimated at about  $2 \times 10^6$  tons, with Spain, Italy, and Greece traditionally being the major producing countries, sharing nearly 80% of the global production. The olive tree is the main cultivation in Greece covering 7371 km<sup>2</sup>, and the major olive oil-producing area is the island of Crete. Over 400000 tons of olive oil are produced annually in Greece, accounting for over 80% of the total agricultural income of the local population (1). Currently, over 2200 olive oil mills are operating in Greece, the great majority of which are three-phase centrifugal mills

(2). From this type of mill, the main byproducts are (i) olive leaves, which in most cases are used by local farmers as animal feed; (ii) olive press cake, which is utilized by special oil-extracting factories for the production of a lower quality olive oil and a dry olive press cake used as fuel; and (iii) olive oil mill wastewater (OMWW), which is responsible for the largest environmental problem in the oil-producing areas (2).

Given that olive extraction by centrifugation typically generates about 5 m<sup>3</sup> of wastewaters per ton of produced olive oil, this would amount to more than  $2 \times 10^6$  m<sup>3</sup> of OMWW in Greece, currently disposed in evaporation tanks (2) during the oil-producing season, which usually lasts for about 100 days.

The treatment of OMWW is extremely difficult due to its large volume and the increased concentration of organic matter (BOD ranging between 15000 and 50000 mg/L and COD reaching values as high as about 220 g/L). It mainly consists of polysaccharides, sugars, polyphenols, polyalcohols, proteins,

\* To whom correspondence should be addressed. Tel: +30-210-7274598. Fax: +30-210-7274594. E-mail: skaltsounis@pharm.uoa.gr.

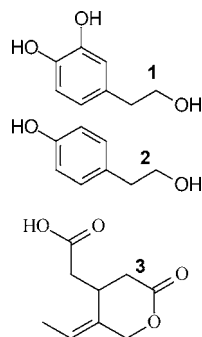
<sup>†</sup> University of Athens.

<sup>‡</sup> University of Patras.

<sup>§</sup> GAIA Research Center.

<sup>||</sup> Terra Nova Ltd..

<sup>⊥</sup> Technological Education Institute of Crete.



**Figure 1.** Structures of the main antioxidants isolated from OMWW. Key: 1, HT; 2, tyrosol; and 3, (5-ethylidene-2-oxo-tetrahydropyran-4-yl)-acetic acid.

organic acids, and oil (3). Moreover, OMWW contains considerable amounts of suspended solids that may reach up to 190 g/L (3).

In addition to the large volume and the increased concentration of organic matter, the major factor of the environmental problems imposed by the OMWW is the high concentration of polyphenols. These compounds present phytotoxicity (4, 5), toxicity against aquatic organisms (6), or suppression of soil microorganisms (7) and are difficult to decompose (8, 9). Although polyphenols can be considered an environmental problem, they are, on the other hand, a very important category of antioxidant phytochemicals that are useful for the pharmaceutical and cosmetic industry. It should be noted that the concentration of the polyphenolic compounds in olive oil ranges from 50 to 1000  $\mu\text{g/g}$  of oil depending on the olive variety and the extraction system and that this amount of antioxidants in the olive oil is only 1–2% of the available pool of antioxidants in the olive fruit (10). The rest is lost either in the wastewater (approximately 53%) or in the pomace (approximately 45%).

More than 30 biophenols and related compounds have been identified in OMWW, and the majority of them exhibit antioxidant activity and potential cardioprotective and cancer-preventing activities in humans as reviewed by Obied et al. (9). Among them, the most important constituents are hydroxytyrosol (HT), tyrosol, and the lactone (11) depicted in **Figure 1**. From a financial point of view, it should be noted that according to Obied et al. (9) the cost of 1 g of pure HT is about \$U.S. 1000–2000 for scientific/experimental purposes. Additionally, olive extracts with standardized concentrations in HT are already used in the cosmetic industry.

Up to today, a large variety of methods have been suggested for the treatment of OMWW, including composting (12), anaerobic digestion (13), aerobic treatment (14), mixing with municipal wastewater (15), direct land application (irrigation) (16), chemical oxidation in combination with biological treatment (17) or adsorption (3), and even the utilization of fungi species (18). However, all of the aforementioned methods aim at the decomposition/destruction of the contained polyphenols and not their exploitation.

In this context, we have developed a pilot scale system for the treatment of OMWW aiming at the recovery of high added value polyphenols contained in OMWW and a reduction of the environmental problems.

## MATERIALS AND METHODS

The experimental work of this paper was divided into three parts. (A) One regarding the adsorption study of pure HT, (B) one referring to a pilot scale process for the OMWW treatment, and (C) a final part regarding the methodology of pure HT isolation.

**Table 1.** Properties of the Tested Adsorption Resins

resin	XAD-4	XAD-7HP	XAD-16
copolymerisate	styrene–divinylbenzene	acrylate–divinylbenzene	styrene–divinylbenzene
specific surface ( $\text{m}^2\text{g}^{-1}$ )	750	450	750
porosity ( $\text{cm cm}^{-3}$ )	0.65–0.70	0.55	0.58–0.63
bulk density ( $\text{g cm}^{-3}$ )	0.62–0.63	0.62	0.61
particle size (mm)	0.3–1.2	0.3–1.2	0.3–1.2

**(A) Adsorption of HT on XAD4, XAD7HP, and XAD16.** Three resins, namely, XAD-4, XAD-7, and XAD-16 (Rohm and Hass Co.), were tested in the adsorption experiments. The properties of the adsorbents are presented in **Table 1**. Prior to use, all adsorbents were extracted with acetone for 8 h and then dried for 2 h under vacuum at 24 °C. HT used in all of the following adsorption experiments was isolated from OMWW as described in part C of the experimental section.

**Adsorption Experiments under Static Conditions.** A 0.100 g amount of each resin was introduced directly into a 250 mL conical flask, and resins were first “wetted” with 0.5 mL of methanol and then rinsed three times with deionized water before use. A 100 mL amount of an aqueous solution of HT was added into each flask. The initial HT concentrations ( $C_0$ ) of the solutions ranged from 200 to 1000 mg/L. The flasks were sealed and placed in a shaker. The batch equilibrium test run continued for over 24 h to ensure that an adsorption equilibrium had been reached, and the final concentrations of HT ( $C_s$ ) were determined. The adsorption capacity was calculated according to  $q_s = V_1(C_0 - C_s)/MW$ , where  $V_1$  is the volume of the solution (L),  $W$  is the weight of dry resin (g), and  $M$  is the molecular weight of HT.

**Adsorption Rate Experiments.** Under conditions essentially similar to those described in the above experiments, an aliquot of the solution was removed every 5 min and the concentration of the free HT was measured. After the first 10 min, more than 50% of the total final quantity had been adsorbed. After 60 min, the adsorbed quantity was more than 95% of the finally adsorbed quantity. This experiment was performed only for the finally selected XAD16 resin.

**Column Adsorption Experiments.** A 4.5 cm  $\times$  1.5 cm stainless steel column packed with XAD-4 or XAD-16 and connected with a Prep 100 pump was used. A 70.9 mmol/L HT solution was passed through the column at a flow rate of 6 BV/h, where BV is the total volume of resin bed. The temperature remained at 20 °C throughout all of the experimental procedures. Adsorption capacities of resins were evaluated in two ways: (i) breakthrough capacity, the total amount of adsorbed HT until the effluent concentration reached 0.05 mM; and (ii) the total capacity, the total amount of adsorbed HT until the eluate concentration reached the influent concentration. The adsorption capacities ( $q_c$ ) were calculated with the following equation:  $q_c = V_2 \times (C_1 - C_c)/V_3$ , where  $V_2$  is the volume of solution through column (L),  $C_1$  is the initial concentration of solution (mmol/L),  $C_c$  is the average concentration of the effluent, and  $V_3$  is the volume of polymeric adsorbent in column (mL).

**HT Monitoring.** In all of the above adsorption experiments, the concentration of HT was monitored by measuring the UV absorbance at 280 nm as recorded on a Shimadzu-160A spectrophotometer.

**(B) Process for the OMWW Treatment. OMWW Collection.** The wastewaters used in the pilot scale study were collected from a three-phase olive mill in Rouva municipality, Crete, Greece, in January 2004. The COD measured by the standard method (19) was 120 g/L, and total phenols measured by the Folin–Ciocalteu method (20) were 2.5 g/L. The wastewaters were stored in a tank at ambient temperature for 48 h before filtration.

**Filtration.** The first section of the OMWW treatment system included three successive filtration stages. The first filtration stage was performed using a stainless steel Waterscreen rotating screen. The length of the screen was 120 cm, its diameter was 60 cm, and its slots width was 250  $\mu\text{m}$ . The screen was self-cleaned (the suspended solids/sludge, which were accumulated at the screen’s surface, were removed by a stainless steel scrapper). The screen’s rotation speed ranged between 2 and 3 rpm. Wastewater was fed into the screen from its collection tank

through a 1 in. polyethylene pipe using a submerged Vortex pump. The screen feeding flow rate was 1.5 m<sup>3</sup>/h.

Consequently, the filtered wastewater was fed by means of a centrifugal pump to the second filtration stage, which consisted as previously of a rotating screen with 120  $\mu$ m slots width.

Then, during the third filtration stage, the wastewater was fed by a centrifugal pump (outflow pressure, 6.5 atm) into a Pall bagfilter with 25  $\mu$ m pore sizes. The bagfilter was placed in a stainless steel (304) device of 27 L internal space volume.

**Treatment with Resins.** The second section of the OMWW treatment procedure included passing the filtered wastewater through a series of adsorbent resins, and it consisted of two stages. The following described procedure refers to the treatment of 1.5 m<sup>3</sup> of filtered wastewater.

**First Stage.** This stage aimed at retaining the OMWW polyphenols content. Filtered wastewater was passed through a column of 180 cm height and 80 cm diameter filled with 275 L of XAD16 resin. Wastewater was fed into the resin using an 0.5 in. diameter PVC pipe using a Wilden diaphragmatic pump. The feeding flow rate was 375 L/h. The resin total feeding duration was 4 h.

Afterward, to regenerate the resin, the procedure was used as follows: (i) resin wash by countercurrent water flow under 3 bar of pressure for 15 min, in order to remove the suspended solids settled on the resin's surface (the washing process was performed until the water effluent was completely colorless); (ii) rearrangement of the resin's particles inside the column by cocurrent water flow for approximately 5 min; (iii) resin regeneration by cocurrent feeding of a 50/50 (v/v) ethanol/isopropanol mixture for 40 min and flow at a rate of 750 L/h; and (iv) resin washing by cocurrent water flow for approximately 30 min. After the completion of the above procedure, the resin was ready to be used again.

**Second Stage.** This stage aimed at retaining the organic substances, which produce the heavy dark color of the OMWW. Wastewater was refed, under the same operation conditions of the first stage, into a second column filled with 275 L of XAD7HP resin. After the completion of the second stage, to regenerate/reactivate the resin, the regeneration/reactivation procedure of the first stage was followed.

**Thermal Treatment—Solvent Recovery.** The third section of the OMWW treatment procedure aimed at the recovery of the organic solvents mixture, which has been used in section B of the process. Each one of the organic solvents mixtures that has been used in the first and second stage of section B was evaporated separately in a QVF thermal evaporation system consisting of a 200 L evaporation vessel. The organic solvent mixtures were thermally treated under 100 mbar vacuum at 45 °C. The condensation of the produced organic solvents vapors was facilitated using a coolant (glycol) at -7 °C. The evaporation rate was 50 L/h. The final semisolid residue obtained after the evaporation of the solvents used in the regeneration of XAD16 was 4.5 kg.

**(C) Purification of HT by Fast Centrifuge Partition Chromatography (FCPC).** The final step aimed at the purification of the polyphenols and other contained organic substances from section C using FCPC chromatography.

**Procedure A.** A 30 g portion of the section C residue was submitted to countercurrent chromatography, using a FCPC apparatus (Kromaton). The system of solvents used in this procedure was cyclohexane/EtOAc/MeOH/H<sub>2</sub>O: 4/6/4/6 (8 L). The capacity of the column was 1 L, the rapidity of the rotation was 900 rpm, and the flow rate was 18 mL/min. At first, the mobile phase was running in "ascending" mode through the stationary phase. When 6 L of the mobile phase had passed through the column and 60 fractions (100 mL) had been collected, the mode was changed to "descending" and 20 more fractions (100 mL) were collected. Finally, 5.8 g of HT (purity 90%) was isolated by the above-mentioned process. The purity of HT was established by high-performance liquid chromatography (HPLC), NMR, and MS spectrometry. Pure HT was obtained by preparative HPLC for comparison.

**Procedure B.** A 50 g portion of the section C residue was diluted in 1 L of acetone and then filtered. The acetone extract (25 g) was evaporated and subjected to reverse phase medium-pressure liquid chromatography with decreasing polarity mixtures of H<sub>2</sub>O—MeOH. The obtained water fraction was evaporated, giving 15 g of an oily residue, which was identified by NMR spectroscopy as a mixture of mainly

HT, tyrosol, and lactone (3:1:1). Then, the water fraction was subjected to chromatographic fractionation with a CH<sub>2</sub>Cl<sub>2</sub>/MeOH system of increasing polarity and gave tyrosol (21), lactone 3 (22), and HT (5) identified by NMR spectral analysis.

**HPLC Analysis of HT and Tyrosol in Wastewater.** The monitoring of the process through the two resins was accomplished by means of HPLC-UV detection. The methodology developed also incorporated a solid-phase extraction (SPE) procedure as the cleanup step.

A ThermoFinnigan HPLC system was used for the analysis comprised of a P4000 quaternary pump, a UV 2000 dual wavelength detector, a Rheodyne 7725i injector with an 100  $\mu$ L loop, and a Finnigan on line degasser. A C8 column (250 mm  $\times$  4.6 mm, 5  $\mu$ m) was used with a flow rate 1 mL/min. The gradient program applied to the analysis is presented in **Table 4**.

**Sample Pretreatment.** SPE Oasis HLB cartridge (60 mg/3 mL) was used. Each cartridge was equilibrated with 1 mL of MeOH and 1 mL of H<sub>2</sub>O, and 5 mL of sample was loaded. The cartridge was washed with H<sub>2</sub>O/MeCN (98/2) and 1 mL of *n*-hexane and eluted with 3 mL of Et<sub>2</sub>O. The solvent was removed under a gentle stream of nitrogen, and the residue was reconstituted using 200  $\mu$ L of MeCN/10% H<sub>2</sub>O.

## RESULTS AND DISCUSSION

Olive oil production, an agroindustrial activity of vital economic significance for many Mediterranean countries, is unfortunately associated with the generation of large quantities of liquid waste. The management, treatment, and safe disposal of those wastes imposes serious environmental concerns.

The main concept of the herein proposed methodology is that the polyphenols contained in the OMWW could be selectively adsorbed by a series of adsorption resins affording extracts or pure compounds useful for the pharmaceutical and cosmetic industry on one hand and producing a wastewater free of polyphenols and significantly reduced COD on the other hand.

HT was selected as a model for the adsorption studies because it is the main polyphenol in OMWW and furthermore possesses the most potent antioxidant activities.

**Resin Selection for HT Adsorption.** The resin selection is mainly based on its adsorption capacity for a selected compound. Equilibrium sorption studies under static conditions provide the capacity of the sorbent, which can be described by a sorption isotherm. Sorption equilibrium is established when the concentration of sorbate in the bulk solution is in dynamic balance with that in the interface. The equilibrium relationship between sorbent and sorbate at a given temperature defines an isotherm, which is characterized by certain constants whose values express the surface properties and the affinity of the sorbent (23).

The Freundlich isotherm is perhaps the most popular adsorption model for a single solute system, and it has been used in several studies to compare the adsorption of commercial resins (24). The mathematical form of this model is  $q_s = K_F C_s^{b_F}$  where  $q_s$  (concentration in the solid phase, mg/g) is the sorbate concentration inside the resin sited in equilibrium with  $C_s$  (concentration in the fluid phase, mg/L) and  $K_F$  (Freundlich adsorption constant, L/g) and  $b_F$  (Freundlich exponent, dimensionless) are temperature-dependent parameters for each adsorption system.  $K_F$  and  $b_F$  indicate the capacity and the intensity of the adsorption process, respectively. In particular,  $b_F$  represents the effect of residual concentration on adsorption capacity. This equation is usually applied in the linear form:  $\log q_s = \log K_F + b_F \log C_s$ . Plotting the equation on a log—log scale,  $K_F$  and  $b_F$  can be calculated from the intercept and the slope.

**Figure 2** shows the adsorption curves of HT calculated on the basis of Freundlich constants and experimental equilibrium data at 20 °C. Comparing different resins, usually the one with the highest amount of solute absorbed ( $q_s$ ) at a specified residual

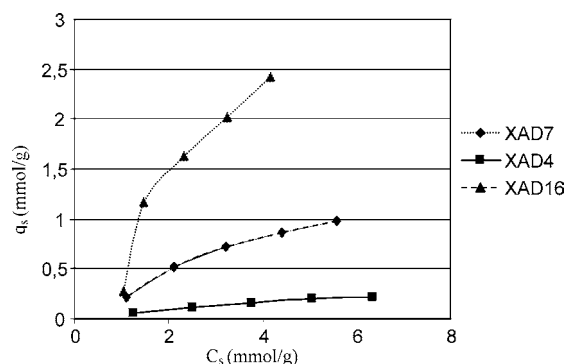


Figure 2. Adsorption curves of HT.

Table 2. Adsorption Characteristics of Tested Resins for HT under Static Conditions

adsorbent	regression equation	$K_F$	$n$	$R$
XAD-4	$y = 0.8705x - 1.3164$	0.048	1.15	0.9994
XAD-7HP	$y = 0.9048x - 0.638$	0.23	1.105	0.9618
XAD-16	$y = 0.689x - 0.0426$	0.41	1.45	0.9995

Table 3. Adsorption Capacities of Tested Resins for HT under Column Conditions

resin	breakthrough adsorption capacity (mmol/mL)	total capacity (mmol/mL)
XAD-4	0.354	0.899
XAD-16	0.425	1.37

Table 4. Gradient Program Applied to the HPLC Analysis of OMWW

time	MeCN	0.05 M ammonium acetate (pH 5)
0	10	90
10	30	70
17	35	65
19	10	90
20	10	90

concentration ( $C_s$ ) would be preferred for the particular application. Table 2 reports the regression equation of  $\log q_s$  vs  $\log C_s$  for Freundlich isotherms at 20 °C as well as the  $K_F$  and  $n = 1/b_F$  values for each resin.

Besides the adsorption study under static conditions, a second experiment concerning the column adsorption was also performed. The column adsorption capacity could be affected by the diameter of the column, the flow rate, and some other experimental factors. For comparison, all of these parameters were set as constant in the present study. At a relatively high flow rate of 6 BV/h and an elevated initial HT concentration of 70.9 mmol/L, the breakthrough adsorption capacities and the total capacities for XAD-4 and XAD-16 were calculated and are presented in Table 3.

On the basis of the above experiments, it was clear that both XAD-4 and XAD-16 are capable of successfully adsorbing HT but that XAD-16 possesses the highest breakthrough adsorption capacity and should be considered as the most preferred resin. XAD-7 is also highly efficient, but during the experiments with OMWW, it was observed that it presented a very high affinity to the contained colorants and consequently it could not be used for selective adsorption of HT.

**Process Application for OMWW.** Following the choice of the most suitable resin for the adsorption of HT in laboratory scale, we have developed a pilot scale treatment system

consisting of the three main successive individual wastewater treatment sections.

(A) The first section of the OMWW treatment system included three successive filtration stages as described in the Materials and Methods section that aimed at the gradual reduction of the wastewater suspended solids to 25  $\mu\text{m}$ . The removal of solids was necessary because their presence could block the pores between the resin beads. It should be noted that the sludge that was produced by the rotating screens (ca. 150 g/L) was transferred to a composting unit (data published elsewhere).

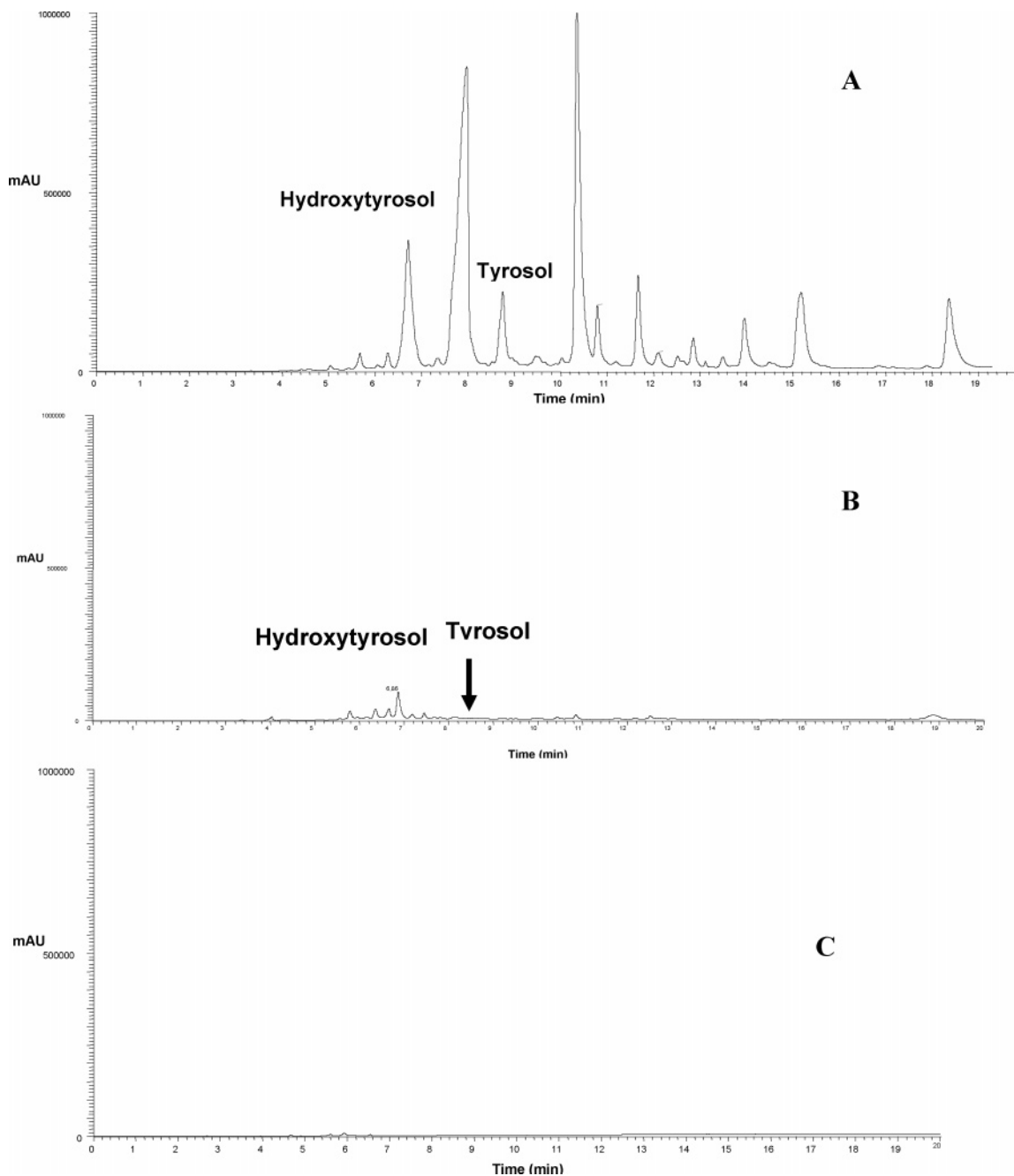
The bagfilter operation for the removal of the larger than 120  $\mu\text{m}$  suspended solids proved to be problematic due to formation of a sticky film onto the filter surface, which in a short time period led to the total blocking of the filter. The COD value after the three filtration steps was reduced from 120 to 52 g/L.

(B) The second section of the OMWW treatment procedure included the pass of the filtered wastewater through a series of columns filled with adsorbent resins. The OMWW/XAD bed volume ratio was roughly based on the breakthrough capacity for HT. First, the total phenols were expressed as their molecular equivalent in HT. Given that the total phenols of OMWW were 2.5 g/L, the molecular equivalent was 16 mmol HT/L of OMWW. With a breakthrough capacity of 0.425 mmol/mL, it was found that for the treatment of 1 L of OMWW the minimum bed volume of XAD16 should be 37 mL. Several trials showed that this quantity was not efficient for the removal of HT because several other nonpolyphenolic constituents of OMWW (e.g., lactones) could also be adsorbed by the resin, in competition for the adsorption sites. The final selection of the OMWW/XAD bed volume ratio was based on the reduction of the total phenol value of the eluting wastewater and it was optimized at 5.5. The first column contained XAD16 in order to achieve the selective retention of the polyphenols and secoiridoids content. The second column contained XAD7HP and was proved efficient in the complete deodorizing and decolorization of the wastewater; in addition, it was used to accomplish the removal of polyphenols not captured by the first resin.

As can be seen in the HPLC chromatograms (Figure 3), all of the amount of HT in OMWW was successfully adsorbed. As it can be seen from the results, 87% of HT and 100% of tyrosol remain bound on the first resin and the remaining 13% bound on the second one. Therefore, 100% from HT and tyrosol can be recovered from the combination of the two resins.

After passing from the aforementioned treatment stages, the OMWW becomes an odorless, light yellow colored liquid. The total phenol measurement showed a value of 0.25 mg/L corresponding only to the 0.01% of the initial value. The COD value of the final wastewater was significantly reduced from 120 to 2.1 g/L, but the achieved value still remains over the acceptable limit for disposal in natural receivers. However, the absence of polyphenols from the final wastewater permits the use of biological treatment with other already proposed methodologies (17).

(C) The third section of the procedure aimed at the thermal evaporation and recovery of the organic solvents mixture. The residue produced after the evaporation of the organic solvents mixture, which has been used for the regeneration of the resin of the first column, is an odorless viscous liquid with a yellow-brown color. It contains the major percentage of the polyphenol and lactone quantity that originally existed in the OMWW. It is noteworthy that the optimum time for the OMWW treatment is between 2 and 4 days after its production because after the fourth day the obtained extract has an "acid" odor.



**Figure 3.** HPLC chromatogram (UV 280 nm) of (A) untreated OMWW, (B) wastewater after first resin XAD16, and (C) wastewater after second resin XAD7HP.

The residue produced after the evaporation of the organic solvents mixture, coming from the second column, is a solid in powder form with dark brown-red color. This residue contains almost the total of the coloring substances quantity that originally existed in the OMWW. These substances are responsible for the heavy dark color of the wastewater.

Each one of the organic solvents mixtures, which is recovered in the third section of the wastewater treatment system, is reused in the respective stage of the second treatment section where it was initially used.

**Isolation of Pure HT.** The final step of the developed methodology deals with the isolation of pure HT from the extract eluting from the XAD16 resin. Several attempts employing normal or reversed phase medium-pressure liquid chromatography proved not very successful due to the complexity of the process, the increased solvent consumption, and the overall time

needed. The final solution was found in the application of FCPC chromatography. The developed FCPC methodology allowed the isolation of 90% pure HT in an 1 h step directly from the extract obtained from the XAD16 resin effluent, without any pretreatment. It should be noted that the methodology described in the experimental section was applied for only 30 g of extract affording 5.8 g of HT and it can be easily and reliably scaled up. In this case, treatment of 1 m<sup>3</sup> of OMWW could lead to 0.58 kg of HT.

The final outcome of the herein described methodology is (i) an odorless yellowish wastewater with a 99.99% reduced content in polyphenols and 98% reduced COD, (ii) an extract rich in polyphenols and lactones with high antioxidant activity and high added value, (iii) an extract containing the coloring substances of the olive fruit, and (iv) pure HT. The proposed process could be considered as a new approach to the manage-

ment of OMWW combining environmental protection and production of raw materials useful in the pharmaceutical and cosmetic's industry.

#### LITERATURE CITED

- (1) Migdalas, A.; Baourakis, G.; Kalogeras, N.; Meriem, H. B. Sector modeling for the prediction and evaluation of Cretan olive oil. *Eur. J. Oper. Res.* **2004**, *152*, 454–464.
- (2) Manios, T. The composting potential of different organic solid wastes: Experience from the island of Crete. *Environ. Int.* **2004**, *29*, 1079–1089.
- (3) Zouari, N. Decolorization of olive oil mill effluent by physical and chemical treatment prior to anaerobic digestion. *J. Chem. Technol. Biotechnol.* **1998**, *73*, 297–303.
- (4) Aliotta, G.; Fiorentino, A.; Oliva, A.; Temussi, F. Olive oil mill wastewater: Isolation of polyphenols and their phytotoxicity in vitro. *Allelopathy J.* **2002**, *9*, 9–17.
- (5) Capasso, R.; Cristinzio, G.; Evidente, A.; Scognamiglio, F. Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters. *Phytochemistry* **1992**, *31*, 4125–4128.
- (6) Fiorentino, A.; Gentili, A.; Isidori, M.; Monaco, P.; Nardelli, A.; Parrella, A.; Temussi, F. Environmental effects caused by olive mill wastewaters: Toxicity comparison of low-molecular-weight phenol components. *J. Agric. Food Chem.* **2003**, *51*, 1005–1009.
- (7) Kotsou, M.; Mari, I.; Lasaridi, K.; Chatzipavlidis, I.; Balis, C.; Kyriacou, A. The effect of olive oil mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*. *Appl. Soil Ecol.* **2004**, *26*, 113–121.
- (8) Davies, L. C.; Vilhena, A. M.; Novais, J. M.; Martins-Dias, S. Olive mill wastewater characteristics: Modelling and statistical analysis. *Grasas Aceites* **2004**, *55*, 233–241.
- (9) Obied, H. K.; Allen, M. S.; Bedgood, D. R.; Prenzler, P. D.; Robards, K.; Stockmann, R. Bioactivity and analysis of biophenols recovered from olive mill waste. *J. Agric. Food Chem.* **2005**, *53*, 823–837.
- (10) Rodis, P. S.; Karathanos, V. T.; Mantzavinou, A. Partitioning of olive oil antioxidants between oil and water phases. *J. Agric. Food Chem.* **2002**, *50*, 596–601.
- (11) Nousis, L.; Doulias, P. T.; Aligiannis, N.; Bazios, D.; Agalias, A.; Galaris, D.; Mitakou, S. DNA protecting and genotoxic effects of olive oil related components in cells exposed to hydrogen peroxide. *Free Radical Res.* **2005**, *39*, 787–795.
- (12) Papadimitriou, E. K.; Chatjipavlidis, I.; Balis, C. Application of composting to olive mill wastewater treatment. *Environ. Technol.* **1997**, *18*, 101–107.
- (13) Sabbah, I.; Yazbak, A.; Haj, J.; Saliba, A.; Basheer, S. Biomass selection for optimal anaerobic treatment of olive mill wastewater. *Environ. Technol.* **2005**, *26*, 47–54.
- (14) Hoyos, S. E. G.; Nieto, L. M.; Rubio, F. C.; Cormenzana, A. R. Kinetics of aerobic treatment of olive-mill wastewater (OMW) with *Aspergillus terreus*. *Process Biochem.* **2002**, *37*, 1169–1176.
- (15) Croce, F.; Poulson, S.; Hendricks, D. Combined treatment of olive mill effluent and municipal waste-water in a small tourist community. *Water Sci. Technol.* **1994**, *29*, 105–110.
- (16) Lopez, R.; Martinez-Bordiu, A.; de Lome, E. D.; Cabrera, F.; Sanchez, M. C. Soil properties after application of olive oil mill wastewater. *Fresenius Environ. Bull.* **1996**, *5*, 49–54.
- (17) Fiorentino, A.; Gentili, A.; Isidori, M.; Lavorgna, M.; Parrella, A.; Temussi, F. Olive oil mill wastewater treatment using a chemical and biological approach. *J. Agric. Food Chem.* **2004**, *52*, 5151–5154.
- (18) Aggelis, G.; Iconomou, D.; Christou, M.; Bokas, D.; Kotzailias, S.; Christou, G.; Tsagou, V.; Papanikolaou, S. Phenolic removal in a model olive oil mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process. *Water Res.* **2003**, *37*, 3897–3904.
- (19) Open Reflux Method, 5220B. *Standard Methods for Examination of Water and Wastewaters*, 18th ed.; American Public Health Association: Washington, DC, 1992.
- (20) Waterman, P. G.; Mole, S. *Analysis of Phenolic Plant Metabolites*, 1st ed.; Blackwell Scientific Publications: Oxford, United Kingdom, 1994.
- (21) Ida, Y.; Satoh, Y.; Ohtsuka, M.; Nagasao, M.; Shoji, J. Phenolic constituents of phellodendron amurense bark. *Phytochemistry* **1993**, *35*, 209–215.
- (22) Gil, M.; Haidour, A.; Ramos, J. L. Two glutaric acid derivatives from olives. *Phytochemistry* **1998**, *49*, 1311–1315.
- (23) Scordino, M.; Di Mauro, A.; Passerini, A.; Maccarone, E. Adsorption of flavonoids on resins: Cyanidin 3-glucoside. *J. Agric. Food Chem.* **2004**, *52*, 1965–1972.
- (24) Li, A.; Zhang, Q.; Chen, J.; Fei, Z.; Long, C.; Li, W. Adsorption of phenolic compounds on Amberlite XAD-4 and its acetylated derivative MX-4. *React. Funct. Polym.* **2001**, *49*, 225–233.

---

Received for review October 26, 2006. Revised manuscript received February 1, 2007. Accepted February 5, 2007. The present work was financed by the EU LIFE-Environment program MINOS. The MINOS project was ranked among the TOP-20 LIFE programs in EU for 2005.

JF063091D