Adsorption of phenolics isolated from agricultural byproducts on solid sorbents.

M. P. Kodjapashis[1,2], A.D. Zentelis[2,3], A. S. Stefanopoulos[1,2], G. A. Velissaris[1,2], V. K. Zarkada[1,2], D. P. Zagklis[2], V. Sygouni[1,2], C.A. Paraskeva[1,2,*]

[1]Foundation for Research and Technology, Hellas, Institute of Chemical Engineering Sciences, FORTH/ICE-HT, GR-26504 Patras, Greece
[2] Department of Chemical Engineering, University of Patras, GR-26504 Patras, Greece
[3] Department of Pharmacy, University of Patras, GR-26504 Patras, Greece

Presenting author email: sygouni@upatras.gr

ABSTRACT

Agricultural by-products contain substantial amounts of phenolic compounds, which have special antioxidant properties and contribute to human health. In this work, a method to recover high concentrations of phenolic substances from a typical type of agricultural by-product, such as olive leaves, was investigated. Phenolic substances were isolated by extraction using various environmentally friendly solvents such as water, ethyl alcohol and water-alcohol mixtures. Then the extracts were fractionated on a membrane array, Ultrafiltration-Nanofiltration-Reverse Osmosis. The NF concentrate fraction, which contained the low molecular weight organics (simple phenols, carbohydrates) was further treated by a sorption/desorption process in a cylindrical column packed with XAD16N resin. The aim of the present experimental study was to optimize the isolation and enrichment of phenolic compounds through their selective adsorption on resin. In the first series of experiments, standard compounds such as gallic acid (phenolic compound) and glucose (carbohydrates) were tested to find the optimal sorption and desorption times of the phenol and carbohydrate families and the sorption parameters were estimated using mathematical models. Next, experiments were performed with the NF fraction. The results showed that XAD16N resin selectively adsorbed phenolic compounds over polysaccharides. Afterwards, the hydrophilic adsorbed small size polysaccharides were removed by desorption with water, while immediately afterwards an alcohol/water mixture was introduced, and the desorbed mixture mainly consisted of phenolic substances. To identify the distinct types of phenolics, a thorough liquid chromatography study was performed on the sorption and desorption samples collected at the outlet of the column, and the sorption-desorption versus time plots were found to agree.

Keywords: phenolics, adsorption, desorption, agricultural, membranes, extraction.

INTRODUCTION

Waste elimination and the sustainable use of resources maximizing the value of products and materials through their entire lifecycle, are included in a circular model of "reduce-reuse-recycle". The biorefinery approach focuses on the valorization of biomass and waste streams into a wide range of products such as biofuels, biochemicals, and biomaterials [1]. The concept is the creation of a closed-loop system characterized by the waste elimination and by-products exploitation. Biorefinery technologies integrated with existing processes may reduce greenhouse gas emissions, create new economic opportunities, and improve resource efficiency. The idea of applying biorefinery technologies in agriculture by-products is a sector under substantial increase the last decades.

Agricultural by-products contain substantial amounts of toxic phenolic compounds and fatty substances, and for this reason their uncontrolled disposal in the environment is prohibited by law. The presence of phenols limits the growth and activity of the microbial population that contributes to the biodegradation of an organic waste. On the other hand, it has been proven that phenolic compounds have special antioxidant and disinfectant properties, which in turn contribute to health and strengthening the human immune system and are already used as additives in the food industry, cosmetics, or pharmaceuticals [2].

Phenolic compounds are found for example in biomass residues, including *Olea europaea L.* byproducts. Olive leaves contain significant amounts of phenolics, including secoiridoids, flavonoids, and phenolic acids [3,4]. Oleuropein, the most abundant and well-studied secoiridoid found in olive leaves, exhibits a wide range of biological activities, including antioxidant, anti-inflammatory, and anti-cancer effects [5,6]. Hydroxytyrosol and tyrosol found in olive leaves, are known for their strong antioxidant and anti-inflammatory properties their health benefits such as reduction of risk of cardiovascular and neurodegenerative disease, etc. [7]. The phenolic content of olive leaves varies depending on factors such as cultivar, harvest time, and extraction methods. The optimization of the extraction and isolation methods is crucial for the characteristics of the by-products and their commercial applications.

For the separation of phenolic compounds from complex mixtures, a widely used method is their adsorption on resins. Polymeric resins contain functional groups with high affinity towards phenolic compounds [8]. The process includes the injection of the mixture through a bed packed with resin under controlled experimental conditions (temperature, pH, and flow rate). The process allows the selective retention of phenolic compounds by the resin, while other components of the mixture are collected at the outlet. At a second stage, the retained phenolic compounds may be recovered from the resin using appropriate solvents (i.e., methanol, ethanol), and they may further be purified using appropriate techniques (i.e., chromatography or crystallization) [9].

Commercial resins such as amberlite XAD4, XAD7HP, and XAD16 were found effective for the adsorption of phenolics of small molecular weight whereas XAD16 was also used for the separation of phenols from olive mill wastewaters [10,11], but the absence of pretreatment resulted to a low-purity phenolic product. Other resins that have been used for the same purpose include the ion-exchange IRA958 Cl, Optipore SD-2, FPX66, and XAD761 [12] after a microfiltration pretreatment for the removal of suspended solids. The resin XAD16 was efficient when phenolic adsorption was evaluated between neutral (XAD16) and ionic resins (IRA958 Cl, IRA 67) [13].

This work investigates the recovery of phenolic substances in high concentrations from agricultural by-products, such as olive leaves, which are collected as waste during the extraction of olive fruits in olive mills. Phenolic substances initially were isolated by solid-liquid extraction using various environmentally friendly solvents such as water, ethyl alcohol and mixtures of water and ethyl alcohol in various volume ratios. The extracts were then fractionated on a series of membrane arrays such as Ultrafiltration, Nanofiltration and Reverse Osmosis [9, 10, 14]. Each membrane unit provided a filtrate stream and a concentrate stream. For the case under consideration, the fractions of the concentrate and permeate streams of NF and the concentrate stream of RO, where organic compounds with low molecular weight were isolated, are of interest. These compounds are mainly, simple phenols and small size carbohydrate compounds. Unfortunately, the presence of carbohydrates in the fractions of the membranes makes difficult the isolation of phenolic substances at high concentrations (>60%). To achieve this goal, the use of special resins is involved which are selective in the adsorption of phenols and are much less selective in the adsorption of sugars [15-16]. Thus, the aim of the present study was to find a low-cost process for the isolation and enrichment of the phenolic compounds via the selective adsorption on the XAD16N resin [9, 10, 14-16].

Previous studies have shown that XAD16N can successfully separate phenolics [9, 10, 14, 17]. For the more careful study of the sorption and desorption of phenolics on resins, 2 series of experiments were carried out. In the first series, experiments were carried out with standard solutions of phenols and sugars. More specifically, gallic acid was used as a representative of the family of phenolic substances and simple glucose as a standard for sugars. The experiments have been performed by injection through packed beds of pure solutions of gallic acid, pure sugars (only glucose) and one mixture of organics that was obtained in the NF concentrate stream. The experiments in packed beds were performed keeping the mass of resin constant for various flow rates of the injected extract.

The experimental results were then fitted using appropriate mathematical models and showed a particularly good agreement with the experimental data and the system adsorption parameters were evaluated [17]. In the second series of experiments, membrane fractions of two by-product extracts such as olive leaves were used. The results showed that the resin XAD16-N selectively adsorbed phenolic compounds over carbohydrates.

The desorption of the sorbed substances is then carried out in two stages. In the first stage, desorption of hydrophilic sugars was achieved with the injection of water and then a mixture of alcohol and water (1:1 v/v) was introduced to recover the phenolic substances. To identify the several types of phenolic substances, a thorough liquid chromatography study (HPLC) was performed on the samples taken at the outlet of the bed during the sorption and the desorption processes.

The liquid chromatography experimental data agree with the plots of sorption and desorption versus time and gave information on the separation time of the main compounds of olive leaf extracts which are hydroxytyrosol, tyrosol, L-tryptophan, caffeic acid, p-coumaric acid and oleuropein.

MATERIALS AND METHODS

Experiments in packed beds

A cylindrical packed bed of 30 cm in height, internal diameter equal to 2.97 cm and total volume of 208 mL was used in the present experimental study. Two syringe pumps were used for the injection of the extract into the bed at constant total flow rate of 300 ml/hr. Samples were selected at the outlet of the bed for the measurement of total phenolic content and carbohydrates (Figure 1). The used packing material of the bed was resin AmberLite™ XAD™16N Polymeric Adsorbent (Sigma Aldrich). It is a non-ionic, hydrophobic, cross-linked polymer with excellent adsorptive properties due to its macroporous structure and high specific surface area. This material can be used to adsorb hydrophobic molecules from polar solvents and volatile organic compounds. The bed was completely packed using 146 g of resin. The resin's density ranged between 1.015 -1.025 g/mL and the porosity of the bed was estimated ~ 35 % [1, 3].

Figure 1: Experimental apparatus for the adsorption/desorption processes at constant flowrate of 300 ml/hr in a 30-cm height column packed with XAD -16N resin (non-ionic, cross-linked polymer**)**

The adsorption of the solution with the mixture of organics tested (concentrate fraction in NF membrane) lasted for 9 h to ensure that full saturation of the resin bed has been completed. In the desorption process, a first step with water exclusion was applied for 3 h to remove the trapped hydrophilic carbohydrate compounds followed by a second desorption step with a mixture (1:1 v/v, water/ethanol) for 11 h. In both the adsorption and desorption processes the flowrate was constant at 300 mL/h. The discretization of phenolics into distinct types of phenols was further analyzed via chromatographic methods (HPLC measurements). The differences in times in the chromatographic diagrams are attributed to the fact that the sample contains a mixture of phenolic compounds with different adsorption capacity. The desorption process with water, lasted 3 hours due to the high concentration of carbohydrates. As far as the desorption with the mixture is concerned, it took a long time since, as it was shown in the chromatograph (HPLC), different phenolic substances came out with each of them having its own adsorption/ desorption capacity.

As for adsorption in standard experiments with a constant flow rate of 300 mL/h, it lasted 6 hours for gallic acid, glucose, and their mixtures. Desorption with pure water (at the same flow rate) lasted 90 minutes and desorption with an ethanol-water mixture lasted about 100 minutes. As for the lower flow rate at 200 mL/h, adsorption lasted longer since less phenolic substances entered the same volume. Thus, in this experiment it took 10 hours to complete the sorption

experiment. So, the desorption also took longer due to having to remove solvent trapped in the void volume with a lower flow rate and thus the desorption took about 200 minutes for both water and ethanol-water mixture desorption. For the high flow at 400 ml/hr, the experiment lasted less. Thus, for adsorption the time for carrying out the experiment was 5 hours and for desorption with water it was about 80 minutes and about the same for desorption with a water-alcohol mixture (80 minutes).

Measurements

Total Phenolic Content

Total phenols were measured by the Folin-Ciocalteu method, which detects the total hydroxyl phenolic groups in a solution due to the ability of phenolic compounds to reduce compounds of phosphomolybdic acid and phosphotungstic acid contained in the Folin-Ciocalteu reagent [18]. Specifically, the Folin-Ciocalteu reagent is reduced to a mixture of blue oxides of tungsten and molybdenum. During the application of the method, sodium carbonate solution (Na_2CO_3) is added, because the reducing action of phenolic compounds is achieved in an alkaline environment. The blue color formed shows maximum absorption at about 760 nm and the intensity of the color is proportional to the concentration of phenolic compounds. From the standard curve of concentration versus absorbance for a particular phenolic compound, the concentration of phenolics is calculated. The construction of the standard curve was done using gallic acid and the value of the phenolic concentration of each sample is expressed in equivalents of gallic acid. A UV-Vis spectrophotometer (Shimadzu UV-1601) was used to measure the absorbance at 760 nm. Gallic acid 97.5-102.5% (titration) (Sigma Aldrich), Na_2CO_3 – anhydrous ≥ 99.5% (Sigma Aldrich) which was prepared to 200 g/L solution in the laboratory and Folin & Ciocalteu's reagent 2 N (Sigma Aldrich) were the reagents used for the identification of total phenolic compounds.

Total carbohydrates concentration

Carbohydrates were measured with the L-tryptophan reagent. The principle of the method is based on the formation of brown-violet or brown-green complexes between the polyaromatic compounds of the reagent and the sugar molecules. The method involves reaction of carbohydrates with L-tryptophan in the presence of boric and concentrated sulfuric acid for 20 minutes in a boiling water bath. The absorption was measured with UV-Vis spectrophotometer (Shimadzu UV-1601) at 525 nm [19] The calculation of the concentration of carbohydrates is done using a standard curve of optical absorption, as a function of the concentration of glucose standard solutions. For the method specifically, 5 glucose solutions are prepared that correspond to a range of concentrations between 5 and 90 mg/L. The sugar concentration value of each sample is expressed in glucose equivalents. Tryptophan has also been shown to give derivatives of approximately the same absorption intensity with different monosaccharides. The following reagents were used: D-glucose anhydrous (Merck), L-Trypthopan ≥ 98% (Sigma Aldrich), H_2SO_4 96% (Penta Bioscience Products), and Boric acid ≥ 99.5% (Sigma).

HPLC measurements

For the qualitative analysis of phenolic compounds and amino acids, the initial sample, the output fractions during adsorption as well as those of desorption, the reverse phase high-performance chromatography technique (Reverse Phase – HPLC) was applied. All samples before their analysis were initially centrifuged at 4000 rpm (Hettich EBA 20) and filtered using UptiDisc syringe filters 0.2 μm Nylon. A Waters 2695 Alliance HPLC instrument (Waters Inc., Milford, CT, USA) and a C-18 column (Phenomenex Prodigy 5u ODS3, 100 A, 250 x 4.6 mm) coupled with a C- pre-column were used for the separation of the compounds. 18 (Phenomenex ODS, 4 x 3.0 mm guard column), while for their detection Dual wavelength Detector (Waters

2487) set at 280nm and 360nm. As the mobile phase, the solvents chosen were 0.1% Trifluroacetic acid (Carlo Erba Reagents, RS-Pour LC-MS) in water (Honeywell, HPLC grade) which constituted the solvent (A) while acetonitrile (Honeywell, HPLC) was used as the solvent (B) grade). For the effective separation of the compounds, a gradient elution was followed with a flow of 0.8 ml/min which is described based on the solvent (B) as follows: 0-5 min 10%, 5-7 min 17%, 7-14 min 17%, 14-17 min 22%, 17-28 min 22%, 28-32 min 26%, 32-42 min 26%, 42-45 min 90% B, 45-50 min 90%, 50-55 min 10%, 55-60 min 10%. Regarding the injection volume and the column temperature, the values 10μL and 25oC were chosen, respectively.

Ten standard phenolic compounds were selected for the identification of the substances at the different steps of adsorption. The standard compounds were selected having in mind the main phenolic compounds found by many researchers working with the by-products of olive oil extraction process. The following compounds were used: Gallic acid monohydrate (Sigma-Aldrich,≥98%), 2-(4-Hydroxyphenyl)ethanol (Aldrich, 98%), 2-(3,4-Dihydroxyphenyl)ethanol (Sigma-Aldrich, ≥98%), p-Coumaric acid (Sigma, ≥98%), Caffeic acid (Sigma, ≥98%), trans-Ferulic acid (Aldrich, 99%), L-Tryptophan (PanReac AppliChem, 98,5-101,0%), Oleuropein (Sigma, ≥98%), (+)-Catechin hydrate (Sigma, ≥98%), (-)-Epicatechin (Sigma, ≥98%).

RESULTS AND DISCUSSION

A. Experiments with pure compounds in packed beds

a. Adsorption and desorption of glucose

Experimental data obtained during the glucose adsorption experiments in resin packed beds, are summarized in Fig. 1. In Fig. 1a, the dimensionless concentration of glucose at the outflow of the bed versus time, for various initial glucose concentrations at constant flow rate, is depicted. The increase rate of the concentration is similar for all initial glucose concentrations, thus, the initial glucose concentration in this range of values does not significantly affect the adsorption process. In Fig. 1b. the dimensionless concentration of glucose versus time is depicted for various values of flow rate, and at initial glucose concentration equal to C_{in}=1 g/L. It can be observed that the required time for the ratio C_{out}/C_{in} to reach the unity decreased for increasing flow rate. In Fig. 1a, the rate of glucose concentration changes versus time during the glucose adsorption process at constant flow rate equal to 300 mL/h and at various initial concentration values is summarized. The observed peaks of the curves denote the termination of the adsorption process. The peaks of the curves coincided independently of the initial concentration and the rate of concentration increase depended on the initial glucose concentration.

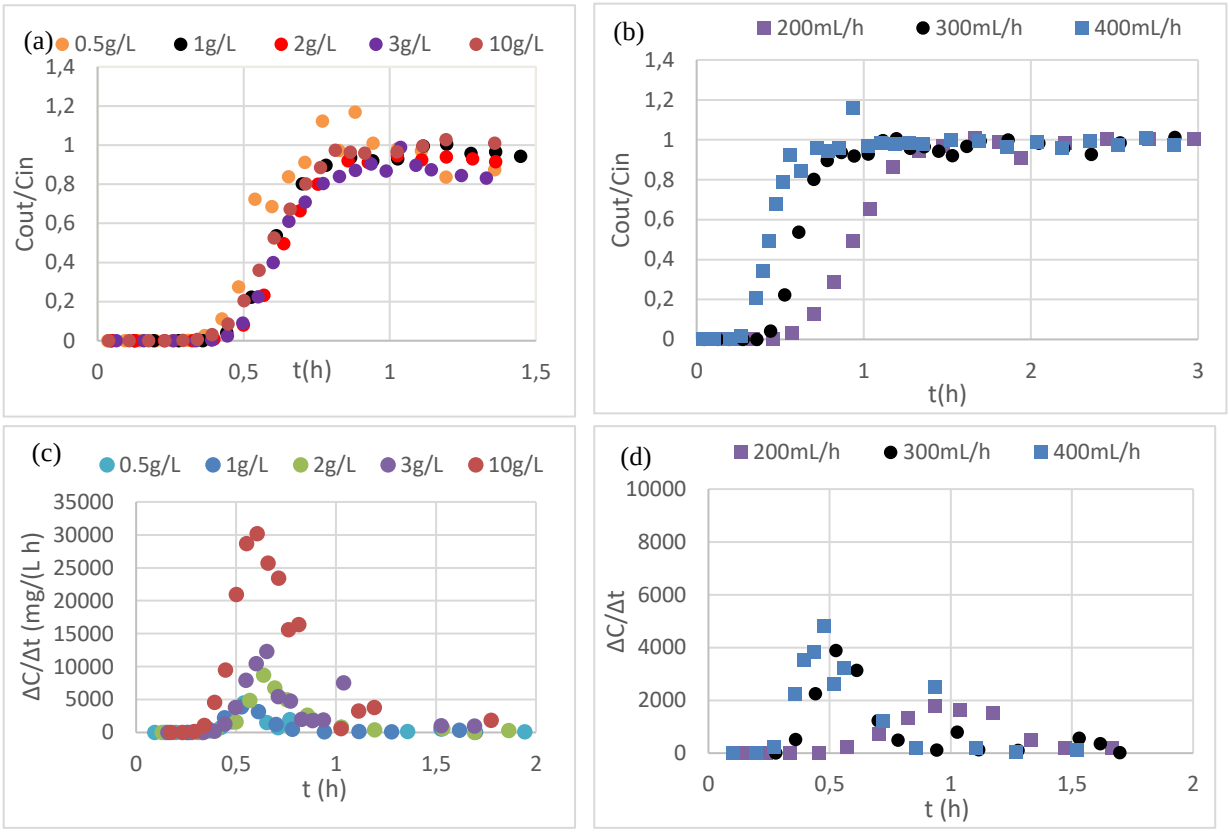


Fig. 1 (a) Dimensionless glucose concentration at the bed's outflow during the adsorption of glucose at constant inflow rate q=300 mL/h and different initial glucose concentrations, C_{in}=0.5 g/L, 1 g/L, 2 g/L, 3 g/L, and 10 g/L. (b). Dimensionless glucose concentration at the outlet of the bed during the glucose adsorption process at initial flow glucose concentration C_{in}=1 g/L and at different inflow rates q=200 mL/h, 300 mL/h, and 400 mL/h. (c). Rate of glucose concentration change versus time during the adsorption of glucose at constant flow rate equal q=300 mL/h and at initial glucose concentrations C_{in}= 0.5 g/L, 1 g/L, 2 g/L, 3 g/L, and 10 g/L. (d). Rate of glucose concentration change versus time for the adsorption process at initial glucose concentration C_{in}=1 g/L and different inflow rates q=200 mL/h, 300 mL/h, and 400 mL/h.

The experimental data obtained in the series of desorption experiments are summarized in Fig. 2. Desorption experiments were performed in the saturated columns presented above, during the adsorption process, for four different initial glucose concentration (Figure 1a) and three different flowrates (Figure 1b). The dimensionless glucose concentration at the outflow of the bed, C_{out}/C_{in}, versus time, for constant flow rate equal to 300 mL/h and for various initial glucose concentrations did not present significant differences (Fig. 2a). In all cases, the required time for the dimensionless concentration, C_{out}/C_{in}, coincided, thus, for the investigated concentration range, the glucose was eluted in the same time interval, independently of the adsorbed glucose quantity. In Fig. 2b, the dimensionless concentration C_{out}/C_{in} versus time for the same initial glucose concentration C_{in}=1 g/L and for various flow rates are summarized. It can be observed that the required time for glucose elution depended on the flow rate. In all cases, the required time for the adsorption process was equal to the required time for the desorption process.

In Fig. 2c, the rate of glucose concentration changes versus time for constant initial glucose concentration, C_{in}=1 g/L, and at various flow rates is depicted. It can be observed that the peaks follow a parabolic model. The rate of concentration change depended on the initial

concentration and the inflow rate. In Fig. 2c, a comparison of the rate of the glucose concentration change is done for the glucose elution at different initial concentrations. As previously, the peaks of the curves took place at the same time, thus, the rate of concentration change depended on the concentration, but it did not affect the required time for the elution of glucose. In Fig 2d, a comparison of the rate of concentration changes versus time, for constant initial glucose concentration equal to C_{in}=1 g/L and for different inflow rates is presented. The peaks of the curves did not take place at the same time and more specifically the peaks appeared at shorter times corresponded to the higher inflow rates, thus the elution was ended earlier for higher inflow rates. The amount of the adsorbed glucose on the resin grain surfaces can be calculated by evaluation of the area above of the curves in Figure 1a and 1b (~ 1.3 – 13 mg gallic acid /g of resin) or after the fitting of the data with the kinetic model, the Thomas model (1-11 mg gallic acid/g of resin).

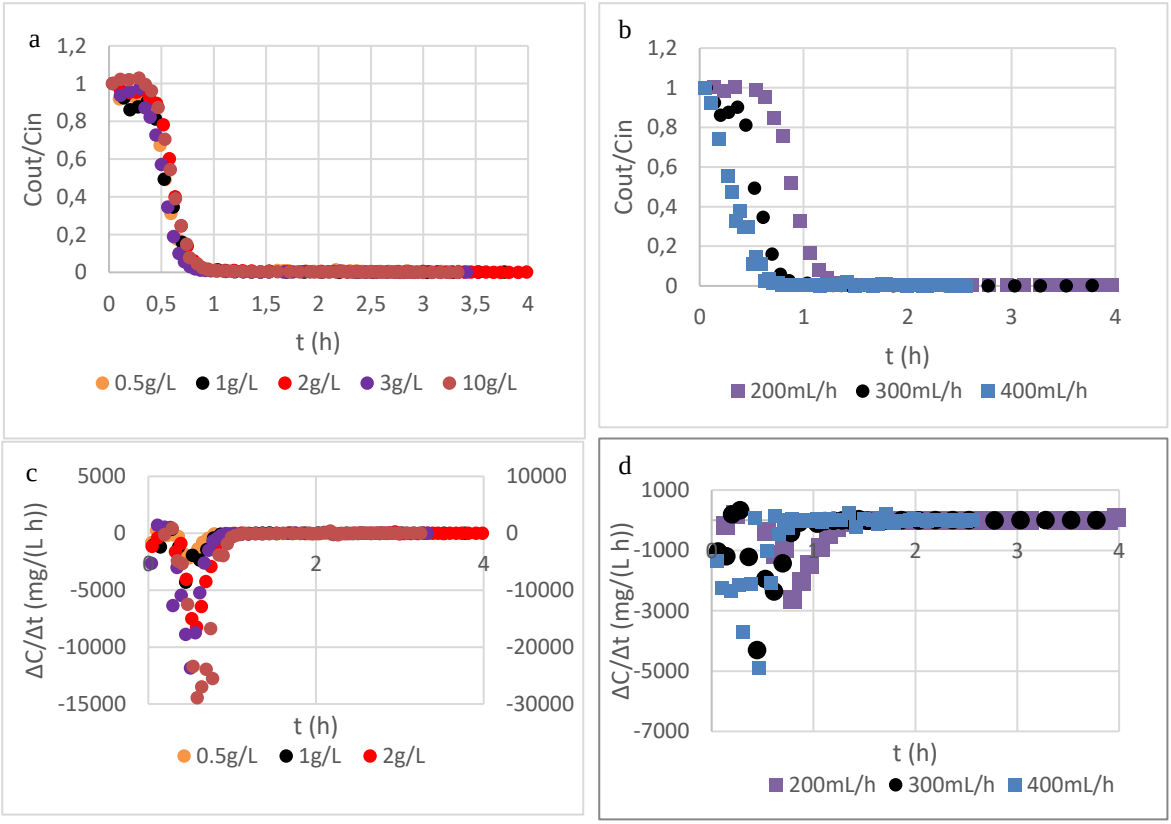


Fig. 2(a) Dimensionless glucose concentration at the outlet of bed, during the desorption process, at constant inflow rate 300 mL/h, in the 4 resins beds that were saturated with initial glucose concentrations C_{in}= 0.5 g/L, 1 g/L, 2 g/L, 3 g/L and 10 g/L (b). Dimensionless concentration during the desorption process at different inflow rates q_{in}=200 mL/h, 300 mL/hr and 400 mL/h in the 3 resins beds that were saturated with initial glucose concentrations C_{in}= 1 g/L (c). Rate of concentration change versus time during the desorption process described in Fig 2(a), (d). Rate of concentration change versus time during the desorption process described in Fig. 2(b)

The modeling of the adsorption experimental data on the grains of the resin was done with the aid of the Thomas Model [17]

$$\frac{C_t}{C_{in}} = \frac{1}{1+\exp\left(k_{Th}q_e\frac{x}{v}-k_{Th}C_{in}t\right)} \qquad (1)$$

where C_t corresponds to the concentration of phenols in the bulk at the effluent of the adsorption column at time t, C_{in} corresponds to the input concentration of phenols, k_{TH} is the Thomas rate parameter (mL min^{-1} mg^{-1}), x is the amount of adsorption resin in the column (g), and v is the flow rate (mL min^{-1}).

The solver add-on in Microsoft Excel was used for the optimization of model parameters in all cases by minimizing the residual error between experimental and-model predicted values. Those parameters for the five experiments are summarized in Tables 1 and 2.

Table 1. Thomas kinetic parameters, mass of adsorbed and eluted glucose at constant flow rate equal to 300 mL/h and at different concentration values.

C_{in} (mg/L)	K_{th} x 10^3 (L mg^{-1} h^{-1})	Total adsorbed glucose (mg)	Total desorbed glucose (mg)
0.5	25.303	104.302	93.223
1	17.389	176.6884	177.27
2	7.524	392.1676	418.745
3	6.006	614.286	472.605
10	1.933	1659.497	1655.705

Table 1 shows the values of K_{th} parameter values for the five adsorption experiments performed and presented in Figure 1(a) for the five different initial glucose concentration (C_{in}). It is observed that the value for the K_{th} parameter decreases as the C_{in} increases. Table 1 also shows total adsorbed glucose on the resin grains in adsorption experiments for five initial concentrations of glucose (0,5, 1, 2, 3, 10 mg/L) presented in Figure 1(a) and for duration of the experiment up to 1.5 h. The adsorbed material was evaluated after integration of the area above of the five curves in Figure 1a (multiplied by the absolute values of initial glucose concentrations and the flowrate), since the area below of those curves denotes the mass of glucose that penetrates the column and selected at the outlet of the resin bed. The total desorbed mass of glucose is presented in the last column of Table 1, where the eluted mass is calculated after the integration of the area below the curves in Figure 2a and multiplied by the initial concentration and the flowrate (300 mL/h). It is interesting to notice that the difference between total adsorbed and desorbed glucose is small which suggests that in desorption experiments of multiple mixtures of phenols and carbohydrates it is obligatory to apply first desorption with pure water to ensure the removal of the highest percentage of the hydrophilic carbohydrates.

Table 2. Thomas kinetic model parameters: mass of adsorbed and eluted glucose at constant initial flow rate equal to 1 g/L and different inlet flow rates.

Q_{in} (mL/h)	K_{th} x 10^3 (L mg^{-1} h^{-1})	Total adsorbed glucose (mg)	Total desorbed glucose (mg).
200	0.0889	193.529	163.413
300	0.0174	176.689	177.27
400	0.0190	160.367	132.68

Table 2 shows the values of K_{th} parameter values for the three adsorption experiments performed and presented in Figure 1(b) for different flow rates and for constant glucose concentration (C_{in}=1 mg/L)). It is observed that the value, for the K_{th} parameter decreases as the flowrate increases. Table 2 also shows total adsorbed glucose on the resin grains in adsorption experiments for the same experiments presented in Figure 1(b) and for duration of the experiment up to 3 h. The adsorbed material was evaluated after integration of the area above of the three curves in Figure 1b (multiplied by the absolute value of initial glucose concentrations and the flowrate). The total desorbed mass of glucose is presented in the last column of Table 2, where the eluted mass is calculated after the integration of the area below the curves in Figure 2a and multiplied by the initial concentration and the corresponding

flowrate (300 mL/h). It is interesting again here in the observation that the difference between total adsorbed and desorbed glucose is small which suggests all carbohydrates were removed from the resin bed.

b. Gallic acid adsorption and desorption experiments

Figure 3a summarizes the experimental data of the dimensionless gallic acid concentration at the outlet of the bed during the adsorption process on resin XAD-16N at constant flow rate equal to 300 mL/h versus time, and for different initial gallic acid concentrations 0.5 g/L, 1 g/L και 2 g/L. The required time for the saturation of resin bed is smaller in the case at 2 g/L (4 h), whereas for the case of 0.5 g/L the saturation was obtained after 5.5 h. The intermediate case of the adsorption experiment with 1 g/L of gallic acid reached the equilibrium in 4.75 h. After 5 h the resin bed was completely saturated, and this time is five times greater than in the case of glucose adsorption. The rate of the gallic acid concentration change increased for increasing gallic acid initial concentration as Figure 3c shows. In Figure 3b, the dimensionless gallic acid concentration data at the outflow of the bed during the adsorption experiment at initial gallic acid concentration equal to 1 g/L versus time and at different flow rates of 200 mL/h, 300 mL/h and 400 mL/h are presented. For the same initial gallic acid concentration (C_{in}=1 g/L), the required time for the completion of the adsorption decreases when the flow rate decreases, and the rate of concentration change increased with increasing flow rate (Figure 3d). The total quantity of the adsorbed gallic acid was not affected by the flow rate.

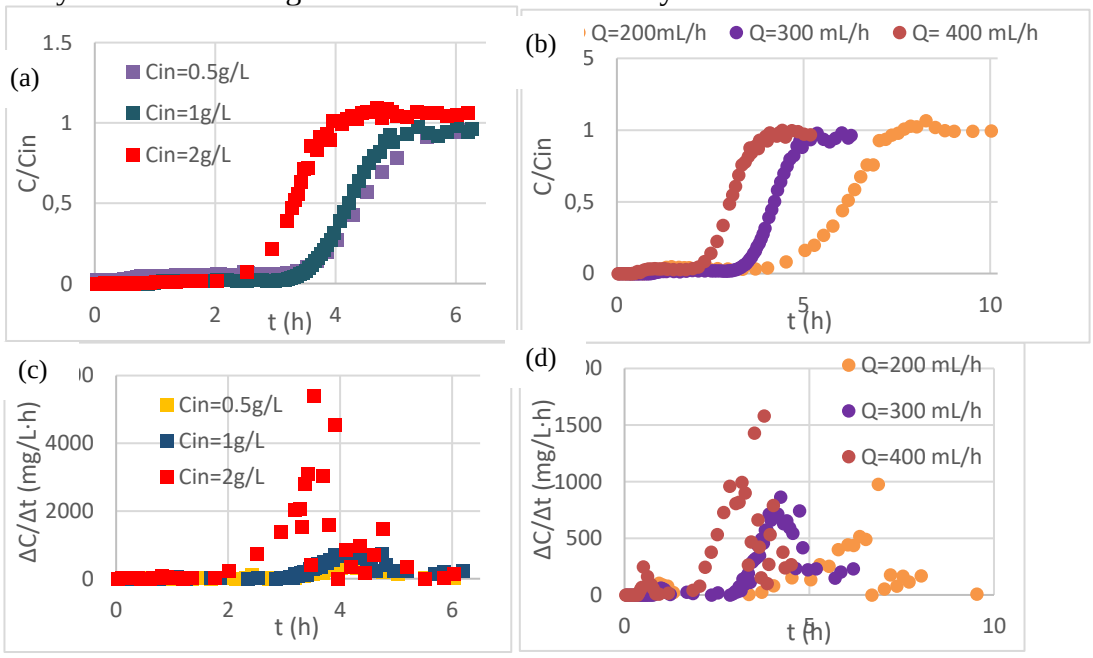


Figure 3. (a) Normalized concentration of gallic acid versus time and 3(c) Rate of gallic acid change versus time during the adsorption experiment for different gallic acid concentrations C_{in}= 0.5 g/L, 1 g/L, 2 g/L, at inflow rates equal to 300 mL/h 3(b). Dimensionless gallic acid concentration versus time at the outflow of the bed and 3(d) rate of concentration change, during the adsorption experiment different flowrates of 200 mL/h, 300 mL/h, 400 mL/h, and constant gallic acid concentration, C_{in}= 1 g/L.

The saturated beds with gallic acid for different initial values of C_{in} (Figure 3a) and for different flowrates (Figure 3b) were further treated to recover the trapped phenolics and other organics. As the experiments with glucose showed, one shall apply first a step of desorption with pure water and then apply a second step with a mixture of water and ethanol (1:1 v/v) to release all remained trapped compounds. It is expected that the eluted solution in the first step to be rich in phenolic compounds. The experiments described in Figure 4a, shows values of gallic acid at the outlet of the resin bed after injection in the inlet of the bed of pure water for 2 h followed

by injection of the mixture of ethanol-water (1:1 v/v). The experiments were those described in Figure 3a, where adsorption was performed at a constant flow rate (300 mL/h) and at different values of the initial gallic acid concentration. Figure 4a shows that only a small quantity of gallic acid leaves the resin bed during the injection with pure water. It is interesting to note that in the next 30-45 almost all gallic acid ejected and can be selected as a pure solution of phenolics. The desorption was continued for one more hour but no extra phenolics were recovered. Thus, the experiment with the standard of the phenolic compound, i.e., the gallic acid suggests that it can be separated from the carbohydrates with which it co-exists in fruit and vegetable by-products. The rate of gallic acid concentration change at the outflow of the experiment, was increased with increasing initial gallic acid concentration, while the elution time was not affected (Figure 4c). Figure 4b shows the case of desorption of phenolics being adsorbed in the resin bed under the conditions described in Figure 3b. Figure 4b shows that here the time needed to remove trapped phenols differ and depends on the value of the flowrate. As expected during the case of flowrate 400 mL/h, the appearance of phenolics starts at 1.7 h, at flowrate 300 mL/h (as above) phenolics appear after two hours while if the flowrate decreases to 200 mL/h, the appearance of gallic acid was observed after 4 h. The rate of gallic acid concentration change was increased with increasing the solution's inflow rate. The elution time was not affected by the flow rate and the elution process was finalized earlier for the higher flow rate (Figure 4d).

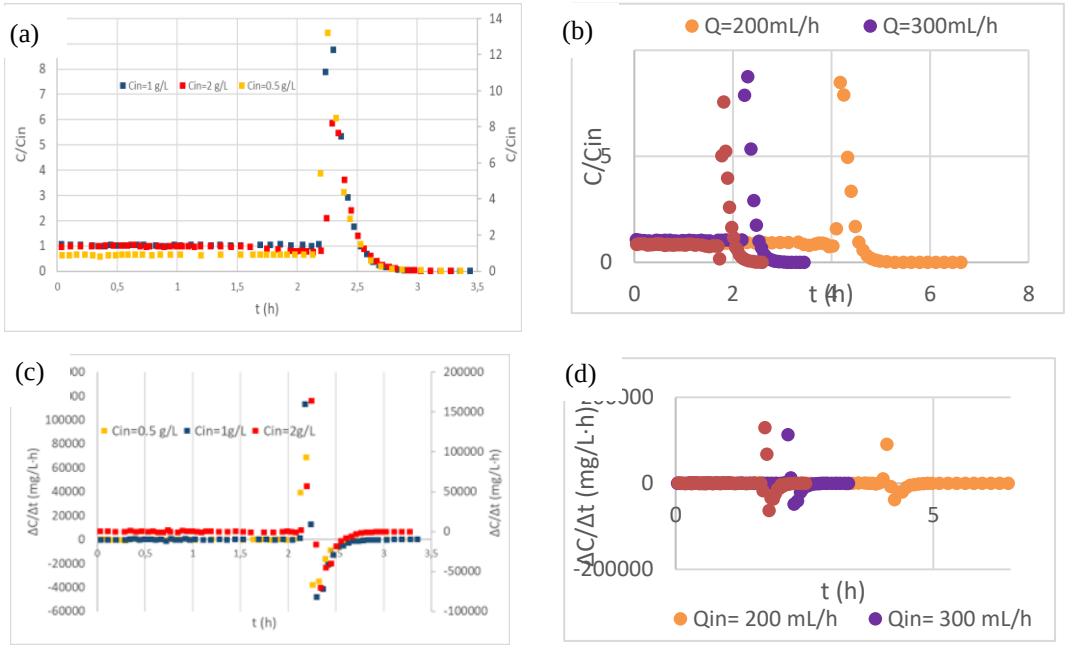


Figure 4. (a). Dimensionless gallic acid concentration at the outflow of the bed (note that the curve for C_{in}= 0.5 g/L corresponds to the secondary y-axis) and (c). Rate of concentration change versus time, both during the elution experiment at constant inflow rate equal to 300 mL/h at initial concentrations equal to 0.5 g/L, 1 g/L, 2 g/L. Note that the curve for C_{in}=2 g/L corresponds to secondary y-axis. (b). Dimensionless gallic acid concentration and (d). rate of concentration change, both at initial concentration equal to Qin=1g/L and flow rates equal to 200 mL/h, 300 mL/h, 400 mL/h.

Using the experimental data of Figures 3a & 3b and 4a & 4b, information on the equation of the Thomas kinetic model for the adsorption of gallic acid (Table 3a and 3b) and on the total

phenolics that have been adsorbed on the resin grains within the packed bed (Tables 4a and 4b) were obtained.

Table 3a: Calculated Thomas kinetic constant for gallic acid for experiments with different initial concentrations of gallic acid

Flow rate (mL/h)	Initial Concentration (g/L)	k_{th}
300	0.5	0.00363
300	1	0.00351
300	2	0.00163

Table 3b: Calculated Thomas kinetic constant for experiments with different flow rates

Flow rate (mL/h)	Initial Concentration (g/L)	k_{th}
200	1	0.00201
300	1	0.00351
400	1	0.002985

Tables 3a & 3b show the values of k_{th}, Thomas rate parameter (mL min^{-1} mg^{-1}) for different initial concentrations glucose (constant flow rate) and for different flow rates (constant initial glucose concentration. The values for k_{th} vary in the range of 0.0015 to 0.0035 mL min^{-1} mg^{-1} and an average value for k_{th} =0.0025 mL min^{-1} mg^{-1} could potentially be used in the case where more than one type of simple phenolics compound is present in the solution.

Table 4a: Adsorbed gallic acid on the resin grains for experiments with different feed concentrations

Flow Rate(mL/h)	Initial feed concentration (g/L)	mg of adsorbed gallic acid	mg gallic acid/g of resin
300	0.5	518.58	3.55
300	1	749.82	5.13
300	2	1582.59	10.84

Table 4b: Adsorbed gallic acid on the resin grains for experiments with different flowrates

Flow Rate(mL/h)	Initial feed concentration (g/L)	mg of adsorbed gallic acid	mg gallic acid/g of resin
200	1	762,60	4,65
300	1	749,82	5,13
400	1	958,89	6,57

The evaluation of the amount of the adsorbed gallic acid on the resin grains within the bed was made after the integration of the area above the curves in Figure 3a and 3b, data in Table 4a and Table 4b, respectively. The areas below the curves 3a and 3b can give information on the eluted concentration of gallic acid. Tables 4a & 4b show that the selected resin XAD 16N, has an adsorbing capacity of 4-10 mg of gallic acid per gram of resin material.

B. Adsorption Experiments in packed resin beds with the NF membrane concentrate fraction of olive leaf extract.

Based on the information with the pure compounds, the gallic acid as a representative of the phenolic family and the glucose as the representative of the family of carbohydrates, adsorption, and desorption experiments with a fraction of the membrane filtration array scheme were designed and performed.

Adsorption

In the present work, the fraction of NF concentrate stream was selected as a test sample for the investigation of adsorption and desorption capacity of the selected XAD 16N resin material using an agroindustrial liquid byproducts (extract of olive leaves). The membrane fraction characteristics in UF, NF and RO and the extract's antioxidant properties were discussed in authors group previous works [6,16]. Here, the idea is to repeat the work with the pure compounds (gallic acid and glucose) with a rich in phenols membrane fraction. The chosen solution found to contain 4500 mg/L of total phenolics. The same resin bed as in the previous experiments was used at a flowrate of 300 mL/h in both, adsorption, and desorption experiments. Samples were collected in both the adsorption and desorption steps.

Fig. 5 shows the evolution of phenolic concentration at the outlet of the 30 cm height resin bed as a function of time. The initial value of the phenolics in the tested NF extract was close to 4600 mg/L and the solution was injected into the bed at constant rate, Q= 300 mL/h. As Fig. 5 shows during the 1st hour upon the initiation of the adsorption experiment, no phenolics were found in the outlet stream. Gradually and between 1-5 h, the concentration of phenolics reached at a constant value of C_{out}=3000 mg/L.

It seems that after 5 h of the duration of experiment, efflux of phenolic compounds continues to occur up to the 10 h that the sorption process lasted, but at an extremely low rate and never reached the feed concentration ($C_{\_phenols\_init}$= 4600 mg/L). A draft calculation of area above the curve, it is estimated that approximately 7400 mg of phenolics were adsorbed on the resin grains.

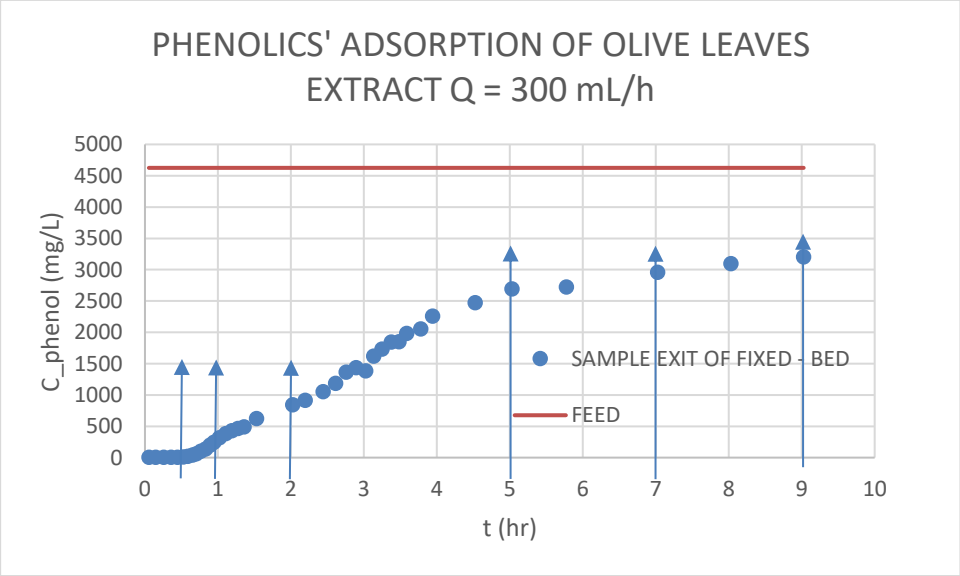


Figure 5. Concentration of gallic acid at the outlet of the resin bed versus time.

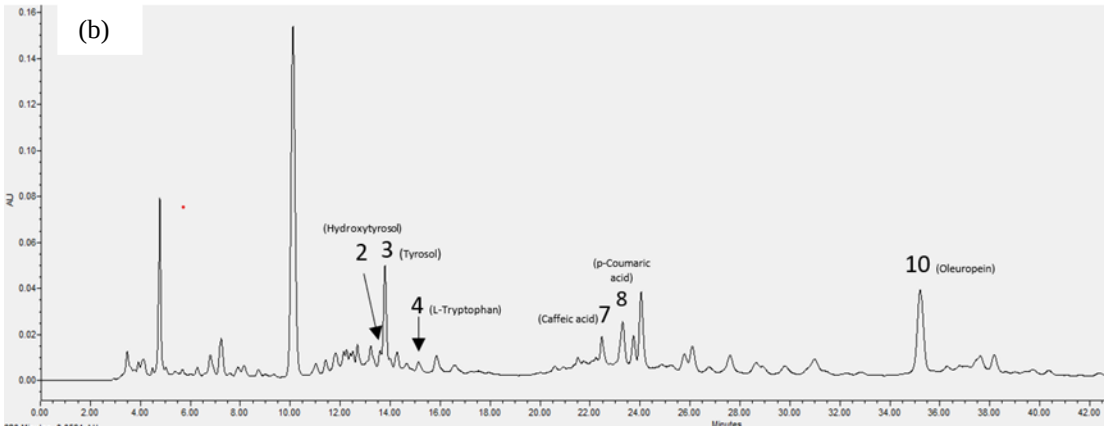
(Q=300 mL/h, NF extract with total phenolics in the inlet, C_{in}, = 4600 mg/L). Arrows at time ½, 1, 2, 5, 7 and 9 hrs denote the time that samples were collected and analyzed for their content in phenolic compounds in HPLC unit)

According to the experiments with gallic acid (Figures 3a, b, c, and d), a complete saturation of the resin should be expected after 3, maximum 5 h from the initiation of the experiments. Figure 5 shows the antagonism between the phenolic compounds and other organic compounds that cover the active adsorption sites on the external grain surface of the resins and even after 9 h and with a high initial C_{in} (4600 mg/L). Thus, the next step was to investigate the composition of the samples taken from all stages of the adsorption (and later of desorption). Thus, chromatography study in an HPLC device was engaged to record the composition of the solutions at various times of the process.

HPLC analysis- results and discussion

Before the chromatography investigation of the compounds included in the feed and samples selected in the outlet of the resin we have chosen to identify 10 compounds that are usually exists in fruit oils olive leaves, olive liquid and semiliquid wastes. The ten compounds were: Gallic acid monohydrate, 2-(4-Hydroxyphenyl)ethanol, 2-(3,4-Dihydroxyphenyl)ethanol, p-Coumaric acid, Caffeic acid, trans-Ferulic acid, L-Tryptophan, Oleuropein, (+)-Catechin hydrate, (-)-Epicatechin. A 10% ACN (HPLC) in water (HPLC) solvent was used for the preparation of the standard solution, as the elution program started with this percentage and was then filtered using a 0.2 μm filter. The elution was carried out under the same conditions as the sample, i.e., injection volume 10 μL, temperature 25 ^{o}C, flow 0.8 ml/min and wavelength 280nm and 360nm.

The first diagrams obtained in the HPLC analysis are the diagram for the standards and the feed solution (NF concentrate fraction), Figures 6a and 6b, respectively. As Figure 6(a) shows there is a clear distinction among the curves in the chromatograph for the ten standard phenolics compounds and one can easily identify the phenolic compounds are included in the feed solution. The time in which each of the ten standard compounds is eluded from the chromatography column is given in Table 5. The main phenols of interest, gallic acid, hydroxytyrosol (plus tyrosol) and oleuropein can be identified clearly in Figure 6b (feed solution). Also signals for the presence of caffeic and p-coumaric acids appeared in the Chromatographic diagram of Figure 6(b).



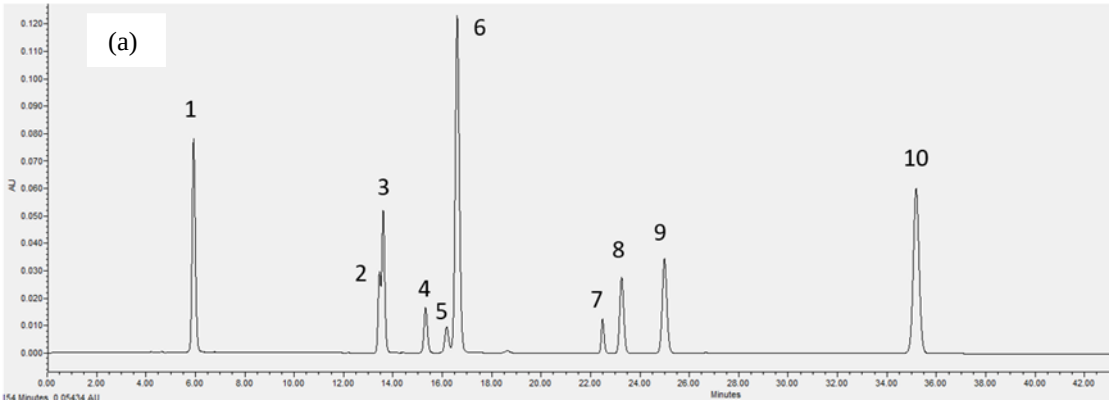


Figure 6. (a) HPLC analysis for the ten standard compounds 1,2,…10 and determination of retention time, in Table 5 and (b) HPLC analysis for the phenolic compounds in the feed solution

Table 5.The ten standard (prototype) compounds as shown in the chromatographic diagram Figure 6a and the corresponding retention time for each compound.

a/a	Compound	Retention Time (RT) at 280nm, (min)
1	Gallic acid monohydrate	5.9
2	Hydroxytyrosol	13.4
3	Tyrosol	13.6
4	L-Tryptophan*	15.3
5	(+)-Catechin hydrate	16.1
6	(-)-Epicatechin	16.6
7	Caffeic acid	22.5
8	p-Coumaric acid	23.2
9	trans-Ferulic acid	25.0
10	Oleuropein	35.2

*L-tryptophan is an amino-acid; however, it has aromatic sidechains in its chemical composition which can be identified at 280 nm

According to the Figure 5 (adsorption vs time), for the first hr, phenolics were not detected at the outlet of the resin bed and there was a gradual increase up to five hours and thereinafter the amount of phenolics in the exit was almost constant even at 9 h. It is interesting to investigate the mechanisms of adsorption during this period. Figure 7 (a), (b), (c) and (d) shows the composition of phenolics in the outlet of the bed. Figure 7a corresponds to the feed solution (t=0), Figure 7 (b) to the outlet solution after 30 min, Figure 7(c) to the solution excluded after 1 h and finally the ejected solution in the outlet after 2 h (Figure 7d).

Comparing Figure 7a (data from the feed solution) with the excluded solution after 30 min (Figure 7b) it is observed that only few compounds were detected after half an hour at 280nm. As it is clearly shown in Figure 7(b) the presence of compounds that absorb at 280 nm is negligible, the only peaks that appear are within the first 6 min as well as two small ones which are eluted with RT 11.5 and 12.4 min. Nevertheless, due to the exceedingly small absorption (maximum peak does not even reach 0.002 AU) one can say that it is free from phenolic compounds. Note also, that the sample was not diluted. Figure 7(c) shows the phenolics that were detected after one hours from the initiation of the experiment. In this sample, organic compounds begin to be detected which are eluted within the first 15 min. Considering that quite polar compounds are eluted in this region and that the feed during adsorption consists mainly of water, one may conclude that the adsorption capacity of the resin for these compounds has

begun to decrease due to diffusion phenomena between resin and solvent stream, while for the less polar ones it remains completely active because of two reasons: a) the column is not yet saturated b) the forces developed between characteristic groups of the resin and the compounds are stronger than the ability of the solvent to displace them (diffusion phenomena do not appear). One may notice in Figure 7c that at 14.9 min that the peak of L-tryptophan is also observed for first time. At 12.9 min a peak like that of hydroxytyrosol and tyrosol appears, but because the elution time of these compounds is at 13.4 and 13.6 min (for the standards) this conclusion is not so safe (there is too long a shift, when the shift of L-tryptophan is a few seconds. As the sorption process continues, increasingly polar compounds are eluted from the column, as it can be seen from the absorbance of sample in Figure 7c, which ranges from 0.01 – 0.085 (AU absorbance units), it increases to the range 0.01-0.22 AU (Figure 7d, 2 h absorption). In addition, the large peak at 10min also begins to appear as well as two peaks at 23.4 and 24.9 min (Figure 7d). By comparing this sample, the feed, and the standards we can see that the peak at 23.4 min corresponds to p-Coumaric acid (present in the feed).

Figures 8a, b, c, and d, show chromatographic diagrams from the samples collected during adsorption process after 5 h from the initiation of the experiments. As Figure 5 shows all these samples are in the area where the curve of adsorption tends to asymptotic curve, that is the point from which hereinafter no more phenolics are adsorbed on the resin grains, because of the saturation of their adsorptive sites with other organic compounds. Figure 8a, shows again the feed chromatographic diagram for reasons of direct comparison with the rest of the graphs of the composition of the samples at the exit in phenolic substances. Figure 8b, presents the composition of phenolics at the outlet of the resin bed after 5 h of adsorption process.

Despite the high dilution of the sample that has taken place, the absorption compared to the previous ones (in Figures 7 b, c, and d) is high. Characteristic is the intensity of the peak at 10 min (from 0.04 AU without dilution it has gone to 0.14 AU with dilution). What was mentioned earlier also applies here, with the difference that the compounds hydroxytyrosol and tyrosol are now detected (so they are eluted) even in low concentrations. Nevertheless, oleuropein, caffeic acid, compounds no less polar and significant amounts of p-coumaric acid are still retained in the resin.

The chromatogram (Figure 8c) shows sample at the output of the bed at 7 h during the adsorption step. It has undergone the same dilution as sample in 5 h and the absorbance shows the same magnitude (high) of levels. What is interesting in this diagram is the fact that now there is a large elution of hydroxytyrosol and tyrosol (especially for tyrosol, it increases from 0.025 to 0.06 AU). Gradually, more peaks begin to appear after 16 min, however their relative absorption intensity is exceptionally low compared to the other compounds eluted in the first 16 min. Figure 8d (9 h).

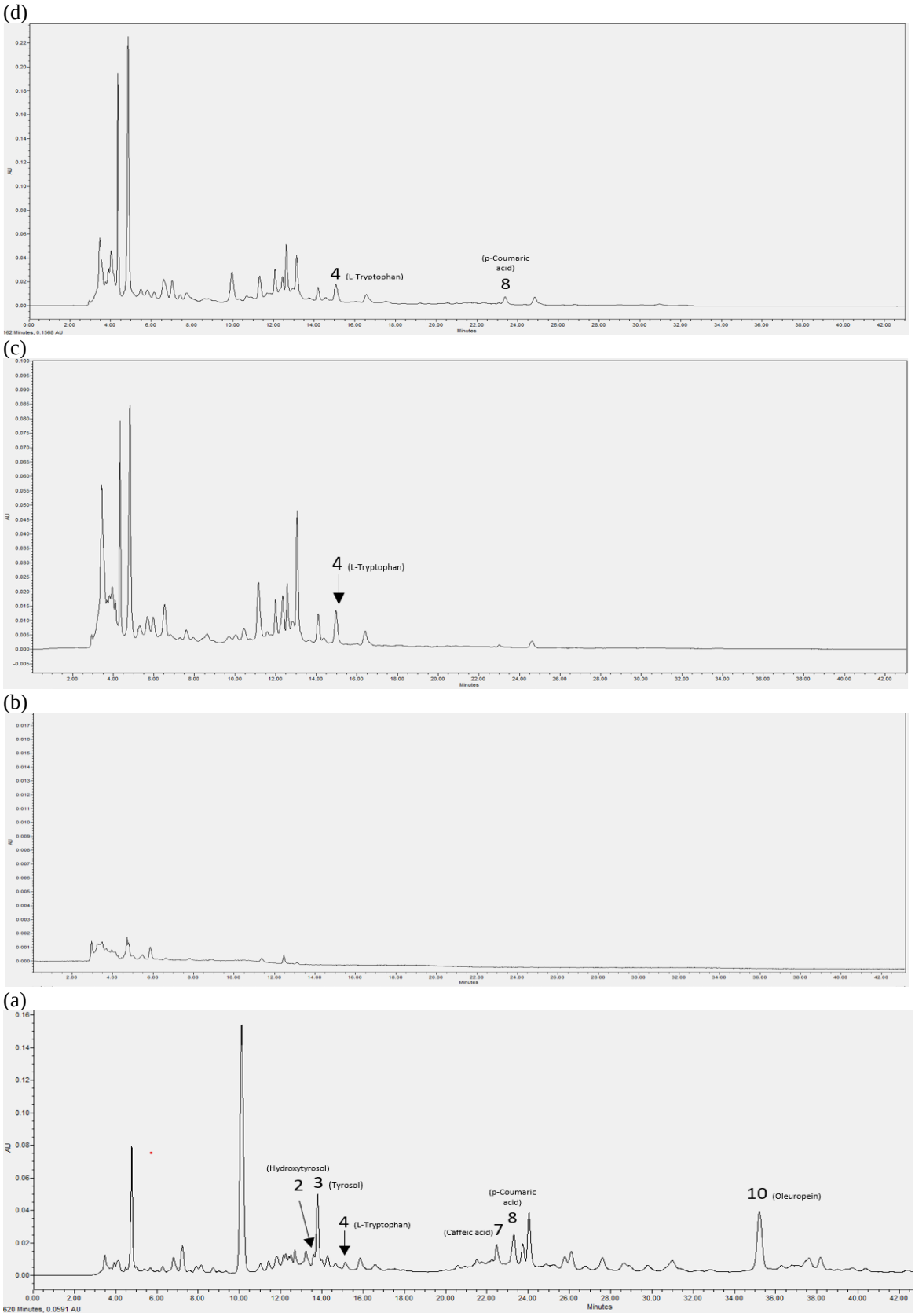
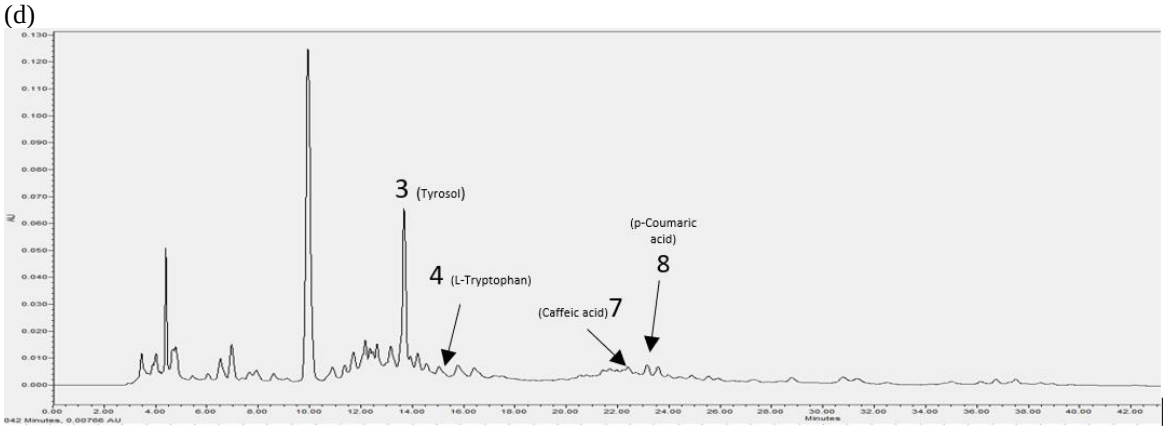
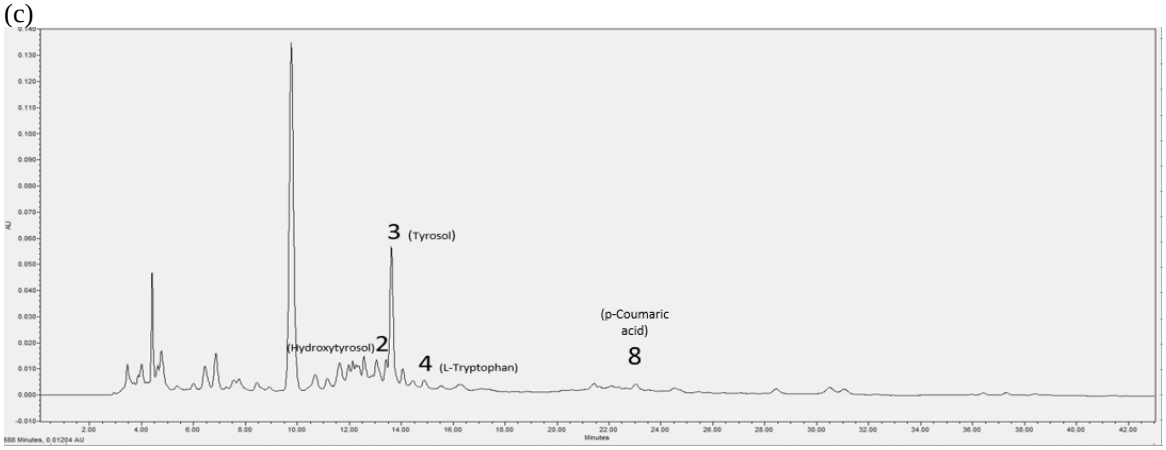


Figure 7. HPLC analysis for the (a) Feed sample and for the collected samples at the outlet of resin bed at (b) ½ h (c) 1 h and (d) 2 h.

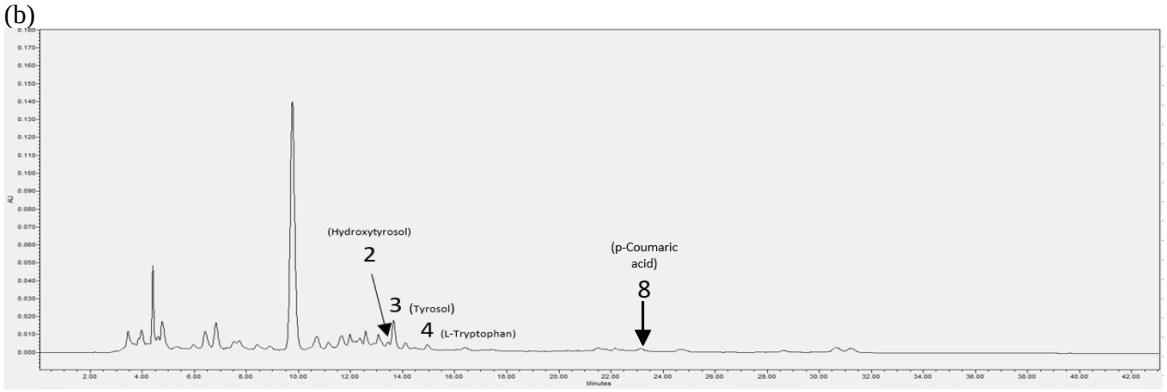
(d)



(c)



(b)



(a)

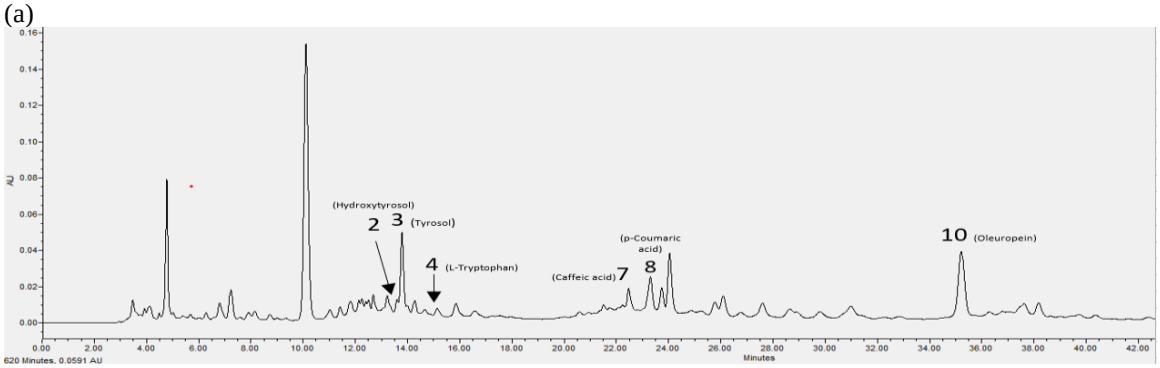


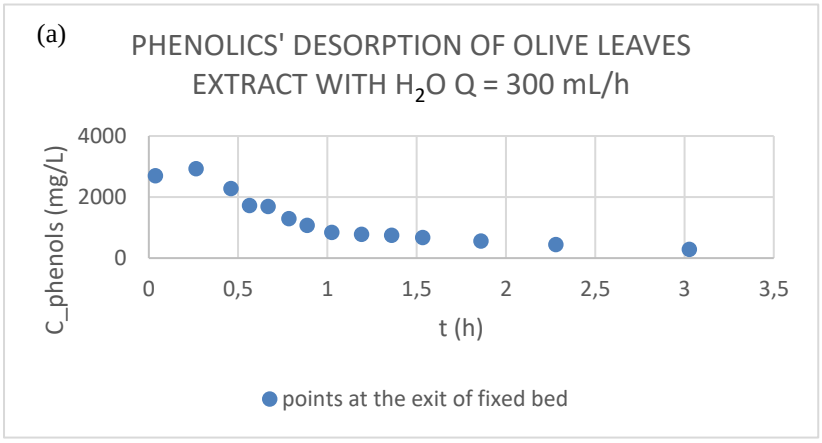
Figure 8. HPLC analysis for the (a) Feed sample and for the collected samples at the outlet of resin bed at (b) 5 h (c) 7 h and (d) 9 h

C. Desorption Experiments in packed resin beds with the NF membrane concentrate fraction of olive leaf extract.

Desorption

Fig. 9a and b shows the evolution of phenolic concentration at the outlet of the resin bed as a function of time during the process of desorption with water and next with mixture of water and ethanol (50%- 50%). Figure 9a shows that during the desorption process with water extremely low amounts of phenolic compounds are eluted from the resin bed. Most of phenolics are eluted with water only during the 1st hour of desorption. A draft calculation of the area below the curve shows that only 1100 mg of the trapped phenolics are being eluted from the grains of the resin during the first step which is a small part of the totally absorbed material in adsorption process (7800 mg). Thus, it is clear that the first step with the water did not remove most of the phenolics but on the contrary, water still help in the removal more carbohydrates.

Figure 9b, shows the evolution of phenolic concentration at the outlet of the resin bed as a function of time during the process of desorption with the mixture of water and ethanol (1:1 v/v). Figure 9 b shows that the mixture of water and ethanol is highly effective in the elution of the adsorbed phenolics and in a period of 2 hours all trapped phenolics are eluted. Again, a draft calculation of the area below the curve shows that during the second step an amount of 6.7 g of phenolics can be selected in 11 h (Figure 9b). What is especially important is to observe from Figure 9b that if a sample is collected during 0.5-1.25 hr will collect samples with extremely high concentration of phenolics (> 20.000 mg/L), which was the main target of the present work. The amount of phenolics that eluted in the period 0.5 hr -1.6 is ~ 6 g of the total 6.7 g that eluted during the entire duration of the desorption (11 hr).



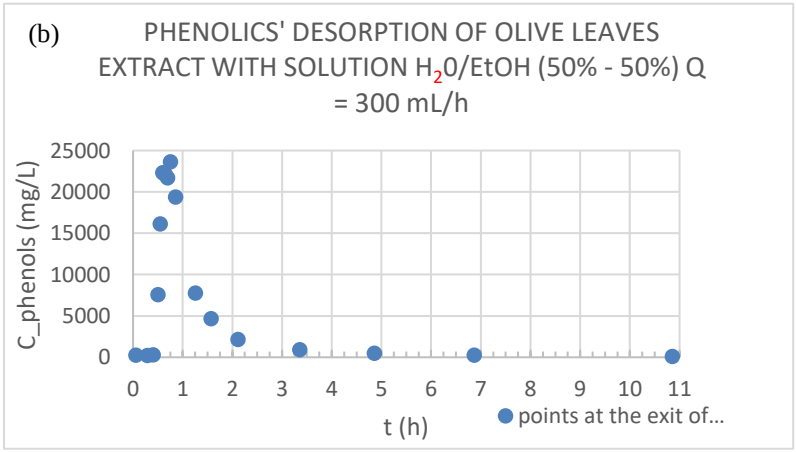


Figure 9. a. Concentration of phenolic compounds during the first stage of desorption with pure water, b. Concentration of phenolic compounds during the second stage of desorption with a mixture of ethanol and water (1:1, v/v).

Figure 10 shows the chromatographic analysis obtained in the sample during the desorption process with pure water after 3 hours from the initiation of the desorption process. As Figure 10 shows only compounds shown in peaks 2 and 3 that correspond to hydroxytyrosol and tyrosol. The size of peaks 2 and 3, are quite high but this is because no dilution was applied to the sample received at time, t=3 h. Also, the profile of curves of peaks is as of that of the adsorption at 5 – 9 hours (see chromatograms 8 b, c, and d) in the adsorption with corresponding hours at 5, 7 and 9). Before 10 min the number of peaks is noticeably lower compared to those of adsorption, possibly because they have been removed within these 3 hours and/or the column had retained exceedingly small amount of these compounds. After 15 min a small number of peaks also appears, which leads us to the conclusion that the less polar compounds such as caffeic acid, p-coumaric acid, oleuropein etc. remain inside the column and are not eluted during desorption with water.

All other phenolics still being trapped within the resin bed, connected with that grain resin surface higher interactions that of carbohydrates which were released very readily as the desorption with water was presented above (see also, Figure 2a & 2b).

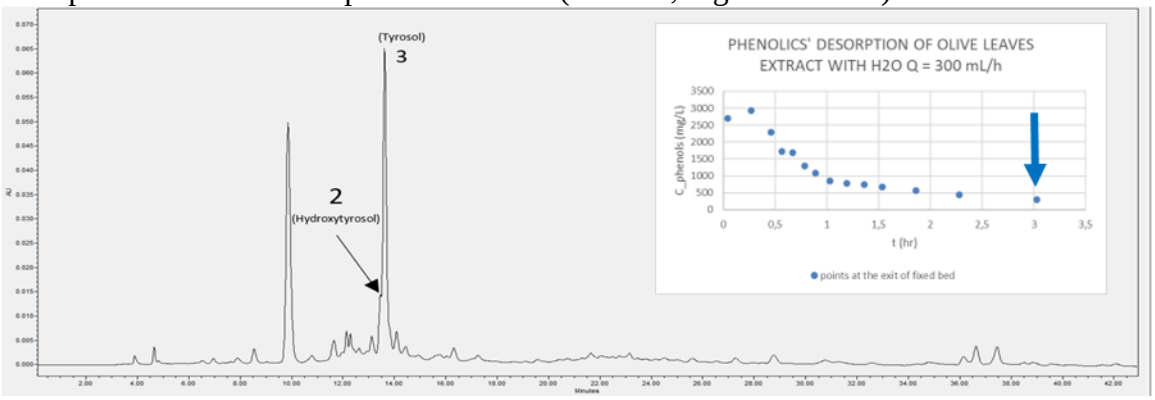


Figure 10. HPLC analysis for the samples collected the outlet of resin bed at the first step of the desorption process with only water after 3 hours from the initiation of the desorption process.

Figures 11(a, b, and c) shows the chromatographic diagrams of samples selected in the second step of desorption where the solvent now is a mixture of water and ethanol (1:1 v/v). The results show the composition of the samples collected at the first 17 min (Figure 11 a), 35 min (Figure 11b) and 41 min (Figure 11c) As Figure 11d shows these samples correspond to samples received at the peak of the desorption and most of phenolics are eluted from the resin bed.

As Figure 11a shows the desorption fraction after 17 min and essentially the first minutes of desorption shows qualitatively the same profile as that of desorption with water after 3 hours (see chromatogram in Figure 10). An explanation for this could be that in the time interval of 17 min the compounds have not had time to elute from the resin bed. Probably the desorption mechanisms inside the column have started and compounds are re-entrained in the stream towards the exit of the column but have not yet had time to be eluted.

Chromatographic diagrams Figures 11 b and d (samples after second desorption step after 35 ~~min~~ and 41 min respectively) are the most important result of the whole work, as they demonstrate the column mainly retains less polar compounds and that these are eluted in large concentrations at the end of the process. The presence of fewer peaks ~~g~~ at 280nm combined with the higher concentration of phenolics measures with Folin-Ciocalteu assay show that few compounds give larger concentrations of phenolic compounds as well as fewer peaks and simultaneously with higher intensity in terms of absorbance units, which means cleaner samples. More specifically, an increased elution of the compounds caffeic acid, p-coumaric acid and especially oleuropein was observed, tyrosol is located to a lesser extent while in terms of locating Hydroxytyrosol it was not possible too be detected (due to its small content in this fraction).

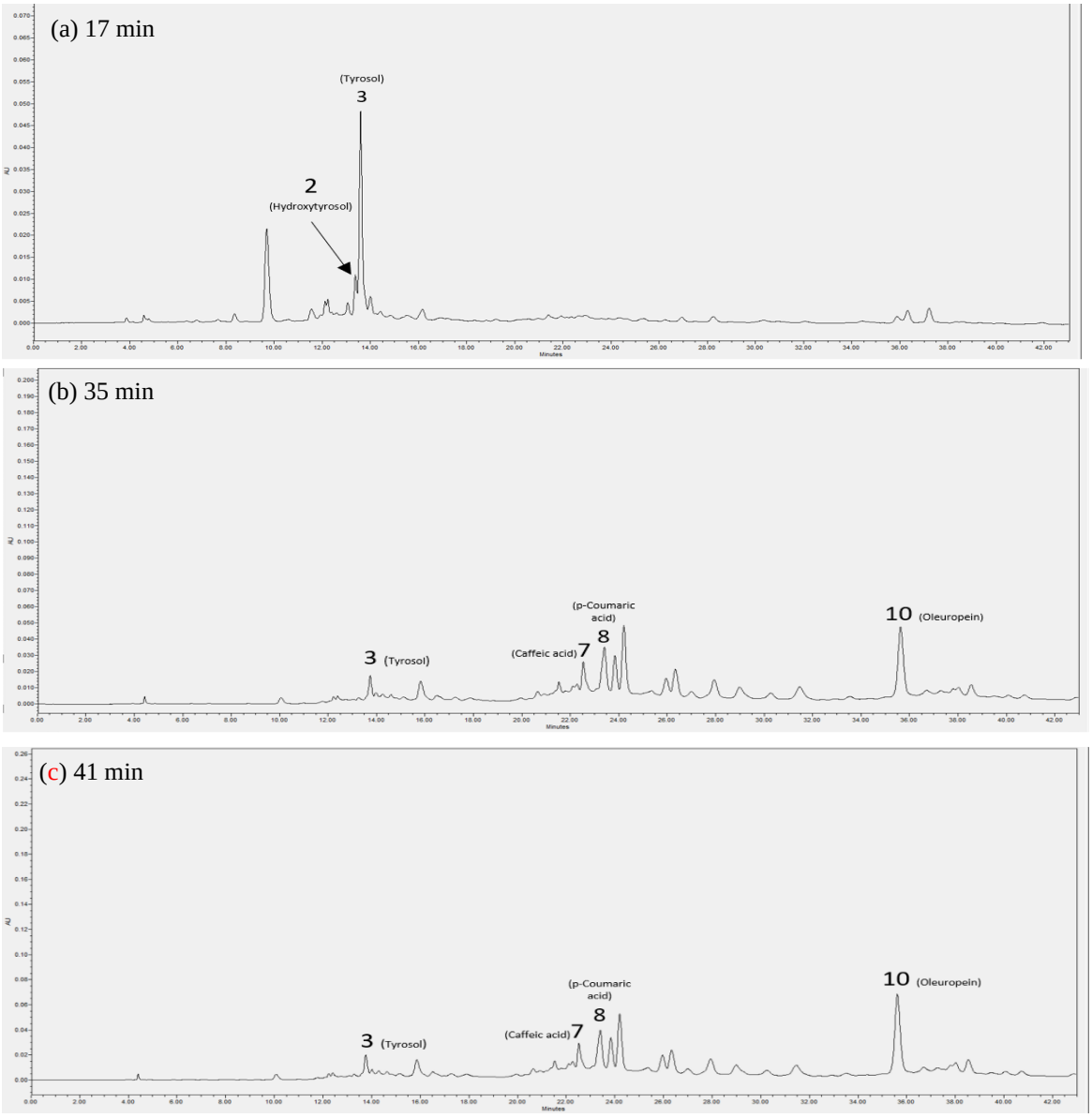


Figure 11 HPLC analysis for the samples collected samples at the outlet of resin bed at the second stage of the desorption process, a) at 17 min, b) at 35 min and c) at 41 min and Figure 11 d: Corresponding concentration of phenolic compounds during the second stage of desorption and sampling points.

Figures 12(a, b, c, and d) shows the chromatographic diagrams of samples selected in the second step of desorption where the solvent now is a mixture of water and ethanol (1:1 v/v) at different desorption times (a) 2 h, (b) 5 h and (c) 7 h. Figure 12 (d) was given to help the reader to understand the time intervals that the samples were taken.

After 2 hours of desorption with the water-ethanol mixture (1:1 v/v), the column has started to empty of compounds that it had retained during adsorption, as shown in the chromatogram of Figure 12a where the chromatographic diagram of the fraction is presented desorption, after 2 hours of elution. Few compounds are detected with the difference that the compound that dominates now is only that of oleuropein while all the others have decreased to a significant extent.

As the desorption process continues with the ethanol-water mixture beyond 2 hours, fewer and smaller peaks appear. Indicatives are the chromatograms of the desorption at 5 hours (chromatogram in Figure 12b) and at 7 hours (chromatogram in Figure 12c), in which, despite the non-dilution of the samples, no peaks appear, apart from oleuropein which was detected as a minor peak.

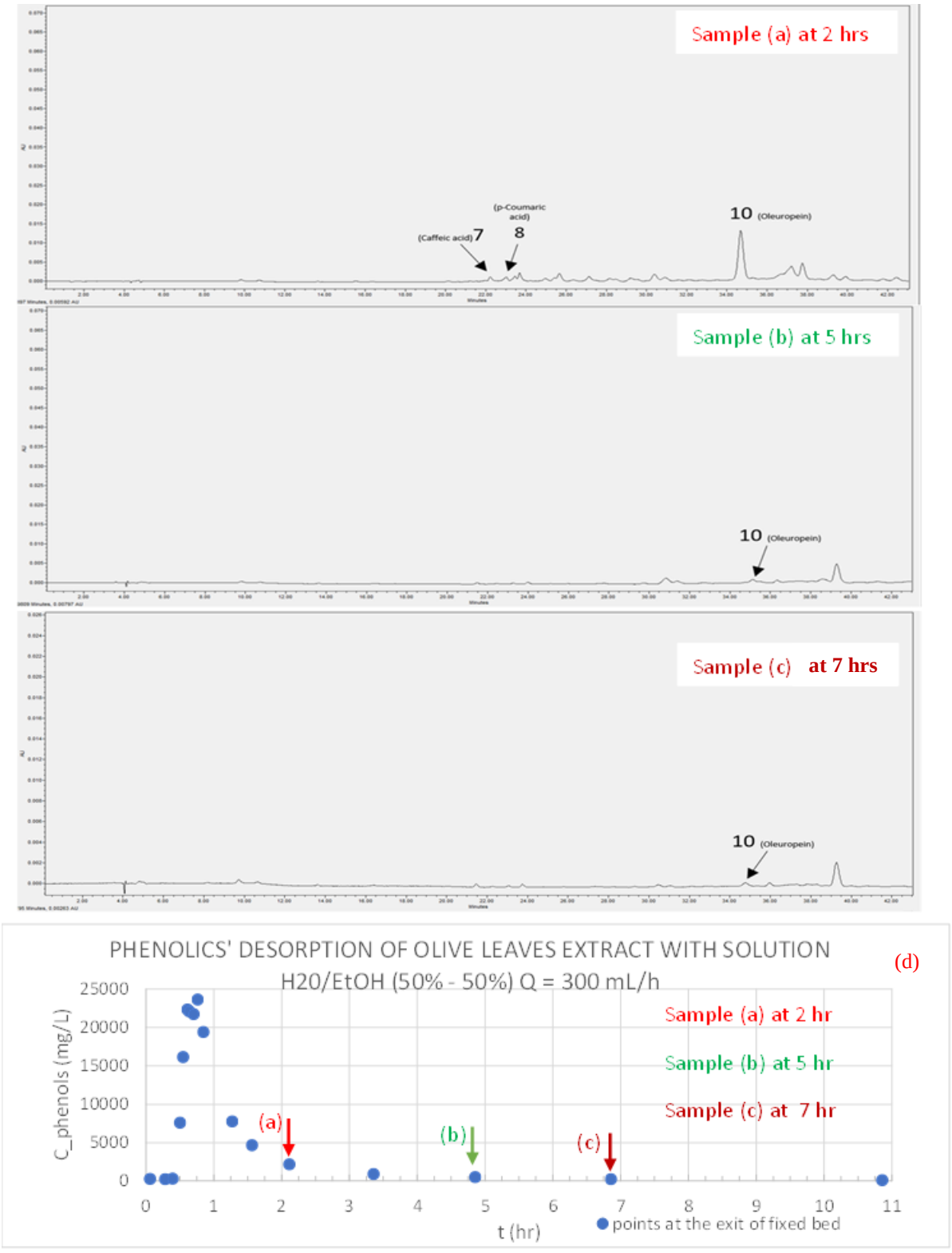


Figure 12. HPLC analysis for the samples collected samples at the outlet of resin bed at the later stages of the desorption process at (a) 2 h, (b) 5 h, and (c) 7 h, and Figure 12 (d) Corresponding concentration of phenolic compounds during the second stage of desorption and sampling points.

CONCLUSIONS

The analysis of sorption and desorption experiments of standard solutions, gallic acid (as a phenolic compound) and glucose (as a carbohydrate), showed that the times for saturation (sorption) and removal of organic compounds (desorption) of the column were much shorter than when tested a concentrate obtained from the original olive leaves extract sample in the NF membrane retention fraction. More specifically, in the experiments where the incoming sample contained only glucose, it was observed that the resin bed was saturated in 1h from in all cases tested (3 different initial concentrations, 1, 2 and 3 g/L, and 3 different flow rates 200, 300 and 400 mL/h). Also, the duration of desorption with water lasted only 1 h and the column was completely cleaned of the strongly hydrophilic glucose. This indicates that physical adsorption on the surface of the granules with loose bonds that break relatively easily during desorption.

In contrast to glucose, the sorption and desorption of pure gallic acid solution lasted longer until complete saturation of the column (sorption) or remove all adsorbed gallic acid, for the three different concentrations tested (0.5, 1 ,0, 2 g/L) for all three different flow rates (200, 300 and 400 mL/h). The duration of absorption in the above experiments lasted about 4-6 h compared to glucose absorption which was completed in one hour. The experiment highlights the fact that the sorption mechanisms of phenolics are more complex and include chemical sorption with stronger bonds without excluding simple physical sorption. This fact is also reinforced by the observation during the desorption, where when pure water was initially passed through the bed, the adsorbed phenolic substance remained attached to the resin grains and only when the desorption was done with a mixture of ethanol and water, the relatively less hydrophilic molecule of gallic acid.

In both, the gallic acid and glucose sorption experiments, the experimental data were fitted with the sorption model (Thomas) and the kinetic parameter, kth, and the amount of sorbed substance were calculated. Both the Thomas model and the integration of the relevant curves in the sorption diagrams showed that the average sorption of gallic acid and glucose is about 7 mg of sorbed substance per g of resin.

Of particular interest is the investigation of the sorption and desorption phenomena of raw sample i.e., the extract from agricultural by-products. In the present work, a sample of olive leaf extract was used which was further fractionated by filtration on an array of membranes in series, Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO). In the present work, the retention fraction of NF was selected, which was rich in phenolic compounds (~ 4500 mg/L). The first major observation during the sorption of this sample in the resin bed was that, despite using the same bed (height 30 cm, resin mass 146 g) and flow rate (300 mL/hr), the bed never reached saturation (in terms of phenolics) apparently due to the intense competition between the different components contained in the mixture. The mixture contained hydrophilic compounds such as carbohydrates and hydrophobic compounds such as phenolic compounds. Because both hydrophilic and hydrophobic compounds were not of the same type, they show different adsorption capacity on the surface of the resin grains and this was particularly reflected in the extensive study done by analyzing the samples of various times, with the liquid chromatography method. Desorption took place in two steps. In the first step, clean water was pumped into the bed to ensure the removal of all hydrophilic compounds i.e., the carbohydrates were eluted. In the second step, a mixture of alcohol and water was injected into the bed and thus the release of the hydrophobic compounds (phenolic compounds) started. At the end of the desorption process, it was observed both in the plots of phenolic concentration with time and

in the chromatograms that all phenolic compounds have been removed from the resin. Interestingly, desorption with a water-alcohol mixture (1:1, v/v/) removed almost all of the adsorbed phenolic substances. During the desorption process, within 2 h c.a. 7500 mg of phenols could be obtained in a volume of 600 mL, corresponding to a concentration of 12.5 g/L.

The chromatographic analysis showed that the sample of the NF fraction contained hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, and of course oleuropein which was the main phenolic compound contained in olive leaves. During the sorption, it could be seen in the chromatograms, that up to 2 h of operation of the process, all the phenolic compounds examined, remained trapped inside the bed (due to adsorption) and only a little p-coumaric acid appeared at the outlet. At longer time (5-9 h) the of tyrosol and hydroxytyrosol appeared at the outlet. Towards the end of this cycle, caffeic acid and p-coumaric acid appeared (in insignificant amounts). Oleuropein which was the main phenolic compound did not appear in the outlet samples and this was very encouraging for a pre-planned plan to isolate and enrich samples of oleuropein, which is a substance with high added value. The desorption experiments were done in two steps, firstly using pure water and then with a water-ethanol mixture. At the end of the first step of desorption, tyrosol and hydroxytyrosol were observed at the outlet, at the end of the process. Then the desorption continued with a water-ethanol mixture, and it was observed that in the initial stages of the desorption (17 min) the molecules of hydroxytyrosol and tyrosol continued to be eluted. As the process progressed, the presence of alcohol enhanced the release of other phenolic compounds, such as caffeic acid and coumaric acid, and at longer times, oleuropein also was appeared. At much longer times (5 and 7 hr) all the remaining compounds (caffeic and p-coumaric acid and oleuropein) were also released in exceedingly small quantities. Thus, it is obvious that a design of sorption and desorption experiments is possible to be done to isolate the main phenolics at high purities and to scale up at pilot and industrial scale.

AKNOWLEDGEMENT
We acknowledge the support of this work by the Project “PPP_Phenolics” (code 03828), which is implemented under the Action “2nd Call for H.F.R.I. Research Projects to support Faculty Members and Researchers” funded by Hellenic Foundation for Research and Innovation.

REFERENCES

1. Awasthi, M.K., Sindhu, R., Sirohi, R., Kumar, V., Ahluwalia, V., Binod, P., Juneja, A., Kumar, D., Yan, B., Sarsaiya, S.: Agricultural waste biorefinery development towards circular bioeconomy. Renew. Sustain. Energy Rev. (2022). https://doi.org/10.1016/j.rser.2022.112122
2. Olszowy, M.: What is responsible for antioxidant properties of polyphenolic compounds from plants? Plant Physiol. Biochem. (2019). https://doi.org/10.1016/j.plaphy.2019.09.039
3. Pereira, A.P., Ferreira, I.C.F.R., Marcelino, F., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A., Pereira, J.A.: Phenolic compounds, and antimicrobial activity of olive (Olea europaea L. Cv. Cobrançosa) leaves. Molecules. 12, 1153–1162 (2007).
4. Ghomari, O., Sounni, F., Massaoudi, Y., Ghanam, J., Drissi Kaitouni, L.B., Merzouki, M., Benlemlih, M.: Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. Biotechnol. Reports (2019), https://doi.org/10.1016/j.btre.2019.e00347
5. Butt, M.S., Tariq, U., Iahtisham-Ul-Haq, Naz, A., Rizwan, M.: Neuroprotective effects of oleuropein: Recent developments and contemporary research. J. Food Biochem. (2021) https://doi.org/10.1111/jfbc.13967
6. Papageorgiou, C.S. Lyri, P., Xintaropoulou, I., Diamantopoulos, I., Zagklis, D.P., Paraskeva, C.A.: High-Yield Production of a Rich-in-Hydroxytyrosol Extract from Olive (Olea europaea) Leaves. Antioxidants (2022). https://doi.org/10.3390/antiox11061042
7 Ercelik, M., Tekin, C., Tezcan, G., Ak Aksoy, S., Bekar, A., Kocaeli, H., Taskapilioglu, M.O., Eser, P., Tunca, B.: Olea europaea Leaf Phenolics Oleuropein, Hydroxytyrosol, Tyrosol, and Rutin Induce Apoptosis and Additionally Affect Temozolomide against Glioblastoma: In Particular, Oleuropein Inhibits Spheroid Growth by Attenuating Stem-like Cell Phenotype. Life 2023, 13.
8. Issabayeva, G., Hang, S.Y., Wong, M.C., Aroua, M.K.: A review on the adsorption of phenols from wastewater onto diverse groups of adsorbents. Rev. Chem. Eng. (2018). doi:10.1515/revce-2017-0007
9 Zagklis, D.P., Paraskeva, C.A.: Isolation of organic compounds with high added values from agro-industrial solid wastes. J. Environ. Manage. (2018). doi:10.1016/j.jenvman.2017.04.083
10. Zagklis, D.P., Vavouraki, A.I., Kornaros, M.E., Paraskeva, C.A.: Purification of olive mill wastewater phenols through membrane filtration and resin adsorption/desorption. J. Hazard. Mater. (2015). doi:10.1016/j.jhazmat.2014.11.038
11. Frascari, D., Bacca, A.E.M., Zama, F., Bertin, L., Fava, F., Pinelli, D. Olive mill wastewater valorisation through phenolic compounds adsorption in a continuous flow column. Chem. Eng. J. (2016), https://doi.org/10.1016/j.cej.2015.07.048
12. Frascari, D., Rubertelli, G., Arous, F.; Ragini, A., Bresciani, L., Arzu, A.; Pinelli, D.: Valorisation of olive mill wastewater by phenolic compounds adsorption: development and application of a procedure for adsorbent selection. Chem. Eng. J., 360, 124–138 (2019).
13. Pinelli, D., Molina Bacca, A.E., Kaushik, A., Basu, S., Nocentini, M., Bertin, L., Frascari, D.: Batch and continuous flow adsorption of phenolic compounds from olive mill wastewater: a comparison between nonionic and ion exchange resins. Int. J. Chem. Eng. 2016, (2016).
14. Zagklis, D.P, Paraskeva, C.A.: Membrane filtration of agro-industrial wastewaters and isolation of organic compounds with high added values. Water Sci. and Technol. (2013).

doi:10.2166/wst.2013.683

15. Zagklis, D.P., Paraskeva, C.A.: Preliminary Design of Phenols Purification Plant. J of Chem. Technol. and Biotechnol. (2020). DOI 10.1002/jctb.5930
16. Zagklis, D.P., Papageorgiou, C.S., Paraskeva, C.A.: Technoeconomic Analysis of the Recovery of Phenols from Olive Mill Wastewater through Membrane Filtration and Resin Adsorption/Desorption, Sustainability 13 (4), 2376, (2021).
17. Kodjapashis, M., Zentelis, A.D., Zagklis, D.P., Sygouni, V., Paraskeva, C.A.: Resin adsorption of phenolic compounds from olive leaf and coffee residue extracts: Batch and packed column adsorption experimental investigation and mathematical modeling, accepted, Separations (2023), https://doi.org/10.3390/xxxxx
18. Waterman, P.G. and Mole, S. (1994) Analysis of Phenolic Plant Metabolites. Blackwell Scientific Publications, Oxford, 73-99.:).
19. Josefsson, B. (1983). “Rapid spectrophotometric determination of total carbohydrates,” in Methods of Seawater Analysis, eds K. Grasshoff, M. Ehrhardt, and K. Kremling (Verlag Chemie GmbH), 340–342.).