Treatment of two-phase olive mill wastewater and recovery of phenolic compounds

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ABSTRACT

Purpose:

The semi-solid wastes (pomace or alperujo) produced in the two phase olive oil extraction process contain extremely high organic load and phenolic substances that makes their treatment processing imperative before there safe dispose to the environment. Efficient treatment of such kind of wastes should be sought to reduce the hazardous effects to the environment and restrict the unsustainable disposal of waste to aqueous receptors or farmlands. On the other hand if the phenolic compounds are isolated and being properly purified, they could be exploited commercially offering an extra motive for farmers for the effective treatment of agro-industrial wastes.

Method: The separation and recovery of phenolic compounds from the olive mill solid wastes (OMSW) is attempted through a combination of extraction and membrane filtration processes. In the first stage, the extraction process with mixtures of water and ethanol was optimized by testing extraction parameters (e.g. optimal amount of solvents, solvent's content in ethanol, time, temperature etc.) in series of experiments in laboratory scale. Next, extraction was conducted in a larger volume with 6 kg of OMSW and the treatment was continued in a pilot membrane filtration system, consisted of Ultrafiltration (UF), Nanofiltration (NF) and reverse osmosis (RO) membranes.

Results: Recovery of phenols (expressed in Total Phenolic Content, TPC) was maximized in a solution where 40g of OMSW were suspended in 100 mL solvent (50% ethanol- 50 % water), after 1 hour stirring at 100 rpm, at room temperature 25ºC. The extracted solution was fed to a combined scheme of UF, NF and RO membranes, where all fat, lipids and solids were removed while the phenolic compounds were concentrated in the retentate streams of NF and/or RO. TPC at the concentrate stream of RO was 225 mg/L whereas at the final effluent was lower than 10 mg/lt. The COD value at the final effluent (permeate stream of RO) COD was much lower (~ 280 mg/L) than in the feed stream (>32,000 mg/L) suggesting use of the effluent as water suitable for irrigation.

Conclusions: The experimental results demonstrated the possibility of recovery of large quantities of phenolic substances from the two-phase olive mill wastewater whereas the final permeate was almost clean water.

Keywords: Phenols, extraction, membrane filtration, two phase pomace, olive mill solid waste

Introduction

The main products from olive trees are table olives and olive oil, which are essential components for a healthy nutrition and are related to the good Mediterranean dietary. Among the compounds that make olive oil important ingredient in human's diet are the contained polyphenol compounds, which show antioxidant activity. The most important phenols present in olive oil are tyrosol, oleuropein, caffeic acid, vanillic acid and hydroxytyrosol [1, 2].

Olive oil extraction processes have changed during the centuries aiming to the improvement of olive oil production, in terms of both quality and quantity. During the production of oil, large amount of waste is produced which is phytotoxic due to its high content in polyphenols [3]. Nowadays, the most used types of olive oil mills are the two- and the three-phase decanter systems. The two-phase system was introduced in the early 90s and it was characterized as ecological because it produces fewer amounts of liquid wastes compared to the three-phase system [4] where warm water is used to enhance the extraction of olive oil from the pulp of olive fruits. Moreover, it is less complicated, more reliable and less expensive than the three-phase one, with significantly lower energy and water consumptions. The two-phase systems are already widely used in Spain, whereas their use is always increasing in other Mediterranean countries such as in Italy, Greece and Portugal. During the olive oil production using the two phase system, a semi-solid waste (65% moisture) is produced which is called olive mill solid waste (OMSW) or "two-phase pomace" or "alperujo" [5]. On the other hand, the treatment of the two-phase system wastes presents great difficulties caused by the moisture and carbohydrate concentrations that characterize this type of wastes [6]. As in the case of the wastewater produced from three phase systems (Olive Mill Wastewater, OMW), the OMSW needs to be treated properly due to its high concentration in solid, fat, lipids, carbohydrates and polyphenols. OMSW treatment, with parallel recovery of specific byproducts of high added value, such as polyphenols, is of high interest for all Mediterranean countries. The classic aerobic or anaerobic methods for the treatment of heavy organic wastewaters are limited by the heavy organic load in terms of fat, lipids and phenolic compounds [7-9] which prohibit microbial growth. Thus, other physicochemical methods are currently being involved for the treatment of agroindustrial wastewaters [10]. Membrane filtration is among the widely used physicochemical method for the treatment of olive mill wastewaters (OMW), as it is published by various research teams [3, 11-17]. In Paraskeva et al, 2007a [12] OMW produced by the three phase system was treated using a pilot scale set-up consisting of UF, NF and RO membranes, whereas several operational parameters were investigated for the optimum operation of membranes. A pre-treatment step of OMW filtering was performed in order to reduce membrane fouling. The final permeate (~70%) of the initial volume was found to meet the EU and national characteristics, in order to be discharged at the environment without environmental risk or to be used at irrigation systems for water economy. The concentrates obtained from the NF modules of the pilot scale membrane modules were tested as ecological herbicides in series of experiments with different herbs and along with the possibility of water reuse, the cost for the OMW treatment could be balanced [18]. The membrane module set-up was combined also with other techniques such as the anaerobic digestion of the OMW using a periodic anaerobic baffled reactor where the decrease of hydraulic retention time enhanced the membrane

system retention of COD [19]. Based on recent literature survey, the most effective methods for OMWW purification are membrane filtration, coagulation/flocculation, anaerobic digestion and Fenton oxidation [10]. Ultrafiltration membrane system, solar photo Fenton oxidation, UF and NF module membrane system were compared for their OMW treatment efficiency while cost and SWOT analysis showed that the use of a photo Fenton oxidation method combined with a needed pre-treatment step as well as membrane system filtration are of the most efficient methods [20]. Membrane filtration was also used for the isolation of organic compounds of high added value from agro-industrial solid wastes and wastewaters coming from olive oil mills and winey which do not accomplish the criteria for commercial use [21]. To this direction, several techniques or the combination of techniques has been developed for the simultaneous purification of OMW and the recovery of high added value by-products such as the use of UF, NF and RO membrane modules, use of resins and cooling crystallization [22, 23]. In the case of OMW high phenolic content was recovered using the UF, NF and RO membrane module followed by rotary evaporation [23, 24, 25].

In the present study, the already tested methods for the treatment of three phase olive mill waste are used for the treatment of two-phase semi-solid waste (pomace) and are completed by an extra step for the extraction of organics and phenolics from the matrix of solids. The separation and recovery of the phenolic compounds by the extraction method was examined in series of laboratory experiments. In the beginning, a parametric investigation aiming at the enhanced phenolic recovery through the extraction experiments was performed. The parameters that evaluated for the optimum conditions for the extraction were: the type of solvent used during the extraction, the addition of small quantities of HCl solution during the extraction, the extraction temperature, the repeatability of extraction, the stirring rate and the time of extraction. At a second stage, the semi-solid waste was treated and the phenolic content was isolated using a pilot scale of membranes with larger volumes of extracted solution (120 L). The first membrane in the proposed scheme consisted of an Ultrafiltration (UF) module, where all suspended solids, fat, lipids and high molecular organic molecules were removed. The second membrane was Nanofiltration (NF) module, where most of the organics and the phenolic content were isolated. The third module of reverse osmosis (RO) membrane was used for the tertiary treatment of the permeate stream of NF membrane. The final permeate effluent was almost clean water with limited concentrations of phenolics and organics (in COD values).

Materials and methods

Olive mill wastewater

The OMW was collected from two different olive mills in Patras (region Achaia, Greece). Both olive mills are using two-phase extraction systems. The first sample was collected during November and the second one on December 2016. The kind of used olives for oil extraction was "Koroneiki" (Olea Europea) which is the most commonly cultivated olive tree variety at the region. The wastewater, after the collection, was separated into smaller vessels and stored at -20 ^{o}C to prevent any alteration of its characteristics. The first OMW sample was used for the extraction experiments while the second one was used for the experimental series at the pilot scale.

Extraction experiments

The extraction laboratory experiments were carried out using a jar test apparatus (Flocculator "FLOC-6" RAYPA). Six beakers of 600 mL were employed and the extraction took place under mechanical stirring. Next, the extracted solution was separated from the solid matrix using Whatman glass microfiber filters of 1.6 μm pore diameter. In order to obtain the optimum conditions for TPC recovery, a parametric study was performed. Firstly, the optimum type of solvent for extraction was investigated. Water and mixtures of water and organic solvent (ethanol with purity of 95%) were tested at various concentrations: pure water, 25% ethanol in water, 50% ethanol in water, 75% ethanol in water and 100% ethanol. For the first extraction experiments, 20 gr of semi-solid waste was suspended in solution of 100mL of solvent, at 25 ^{o}C, under stirring (100 rpm, 1 hour). Next, all experimental series were performed using two types of solvents: i) pure water and ii) mixture of 50% ethanol and 50 % of distilled water (hereinafter 50% E-50% W). The parametric study included investigation of: the optimum solvent; the optimum quantity of semi-solid waste that should be used in each experiment; the optimum dose of HCl solution (1N), that might enhanced hydrolysis of the organics in simple compounds; the temperature; the maximum recovered quantities of phenols (iteration of the extraction with the same solids); the stirring rate; the duration of the extraction time.

Membrane modules

A pilot scale of membranes [12, 18] was used in cross-flow mode and a batch operation was employed. UF module was ceramic (Zirconia), of 0.24 m^2 filtration area and 100 nm pore size. The NF module was spiral wound (advanced polyamide) with MWCO of 800 Dalton whereas the RO membrane was designed to reject the 98% rejection of monovalent ions. All membrane modules and the pilot plant units were supplied by HAR SpA, Milan, Italy.

The feed tanks of UF and NF units were of 180 L volume and 100 L respectively. During cross flow filtration the concentrate stream was recycled in the corresponding feed tank and the permeate stream was collected in a separate tank until the end. At the end of each experiment, the membranes were cleaned using a NaOH (1N) solution for 30 min and finally using water until neutral values of pH. In the case of the polymeric membrane, bisulfite solution was circulated for 20 min for sterilization.

Total Phenolic Content Measurement Total phenolic Content (TPC) was measured using Folin-Ciocalteu method, as described in [26]. This method detects the hydroxyphenyl groups which are present in a solution. The method is based on the ability of phenolic compounds to reduce phosphomolybdic acid and phosphotungstic acid compounds contained in the Folin-Ciocalteu reagent. The reaction forms a blue chromophore which is consisted of a complex whose maximum absorption depends on the alkaline solution and the TPC. From the absorbance value of the compounds in a photometer at 760 nm, the concentration of TPC in gallic acid equivalents is determined. Since the reductive effect of the phenolic compounds is promoted in an alkaline environment, sodium carbonate solution (Na_2CO_3) is added during the process. TPC concentration is calculated from a standard concentration versus absorbance curve for a particular phenolic compound. The standard curve is

constructed using Gallic acid solutions of known concentrations and the concentration of the phenolic of each sample is expressed in Gallic acid equivalents.

Total Carbohydrates Measurement

The determination of carbohydrates was based on the method described by Josefsson (1983) and summarized in [26] along with all recipes needed for the physicochemical characterization of all streams. The method involves reaction of carbohydrates with L-tryptophan in the presence of borate and concentrated sulfuric acid for 20 minutes in a boiling water bath and photometry at 525 nm. The principle of the method is based on the formation of coffee-violet (for hexoses) or brown-green complexes (for pentoses) between the polyaromatic reagent compounds and the sugar molecules. This method is also suitable for the calculation of complex carbohydrates, such as cellulose, since concentrated sulfuric acid can break down the glycosidic bonds of the polymer.

The carbohydrate concentration is calculated either using a standard optical absorption curve, based on the concentration of standard glucose solutions, or by direct measurement of the concentration using the appropriate colourimeter. For the first method, in particular, 5 glucose solutions corresponding to a concentration range of 5 to 90 mg / L are prepared. The concentration of the sugars in each sample is expressed in glucose equivalents. Tryptophan has been shown to produce derivatives of about the same absorption intensity with different monosaccharides.

Results and Discussion

In the first part of the present work a parametric investigation was performed in order to find the optimum conditions for the extraction of the maximum quantity of TPC (Total Phenolic Content) from the two-phase olive mill semi solid waste (OMSW or 'pomace'). In the beginning, the effect of the type of the solvent used during the extraction was investigated. Pure water or ethanol or mixtures of ethanol and water at different percentages were tested. The maximum possible extraction of phenols from the semi-solid pomace was obtained in a series of experiments with different percentages of solid and solvent. (gr pomaces/ lt of solvent). The addition of HCl solution during the extraction was tested also in order to study the effect of possible further hydrolysis of organics and of the phenolic compounds. Another important parameter that was tested was the temperature during the extraction, which was varied in the range of (10-60)^{o}C. Other tested parameters were the rate of stirring and the extraction time. The extraction of phenolics from a certain amount of semi solid samples was repeated in some experiments with fresh solvent in order to find the maximum available quantity of phenolic content that can be extracted from the semi-solid waste.

Effect of the solvent

Extraction experiments were performed using water and ethanol (95% purity) as solvents or ethanol-water mixtures only, because these are the only safe solvents accepted readily by the food industry. Pure ethanol purchased from the market was used as organic solvent whereas water was deionized. 20 gr of two phase pomace was used for the extraction using 100mL of solvent for 1 hour at 25 ^{o}C, while the stirring rate was kept at 100 rpm. Five different percentages of the two solvents were used a) pure deionized water, b) 25% ethanol in water, c) 50% ethanol in water (50% E-50% W), d) 75% ethanol in water and e) 100% ethanol. The TPC and carbohydrates concentrations obtained after the extraction experiments are depicted in Figure 1. Both TPC and carbohydrates concentration values increased as the fraction of ethanol in water increases until 50% ethanol in water. For higher concentration values of ethanol in water TPC and carbohydrates concentration values decreased. Thus, the optimum solvent type was chosen as the mixture 50% of ethanol in water. Figure 1 also shows that all carbohydrates values were much higher than the values of phenols, as it happened in all fruits.

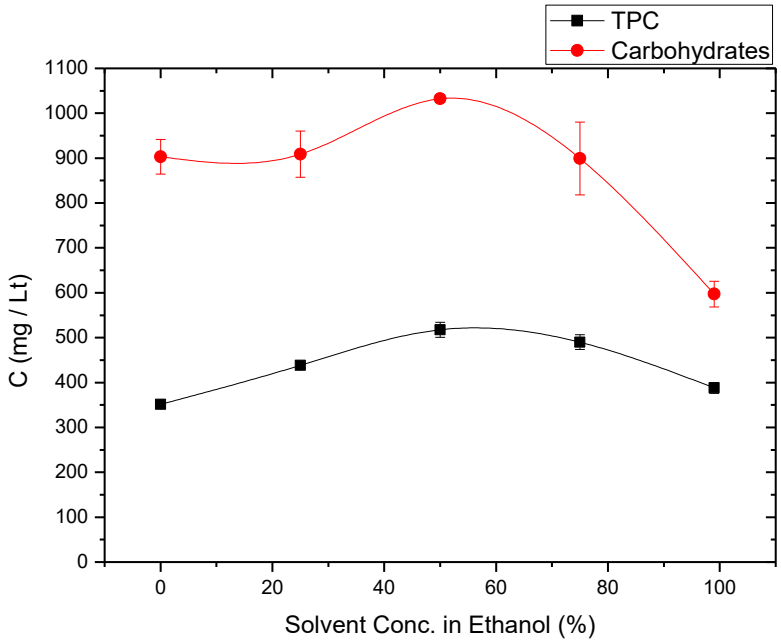

Figure 1: TPC and carbohydrates concentrations obtained after the extraction experiments using solvent of varying concentration in ethanol (95% purity).

Maximum quantity of solids per solvent

At this stage the quantity of semi-solid waste that should be used in order to recover the maximum TPC quantity was investigated. The experiments were performed at 25 ^{o}C and 100 rpm of stirring rate, using 100mL of two different solvents with: a) pure water and b) mixture of 50% ethanol in water. The obtained experimental data are summarized in Figure 2, where it is shown that both carbohydrate and phenol concentrations are increased as the amount of used semi-solid material is increased for both solvents, as expected (solid lines). Figure 2 confirms the remark obtained in Figure 1, that the use of the mixed solvent (50% E-50% W) enhanced the extraction of organics. However, what is important in this case, is the extracted quantity of organics per gram of solid wastes. Dot lines which are referred to

the right axis of Figure 2, suggest that the most effective extraction was obtained when only 20 or even 40 gr of solid was diluted in the volume of 100 ml of the solvent. It seems that at high values of the used solid wastes, the extracted solution is saturated with organics and there is a possibility of phenols or carbohydrates to remain in the mass of the solid waste. From the present investigation the concentration which seems to be optimum for recovering of significant amounts of TPC, and relatively less amount of carbohydrates is the 20 gr of waste per 100mL of solvent. However, the value of 40gr was chosen in order more phenolic content or carbohydrates to be accumulated in the solution that will be further treated with membranes and consequently to isolate as much as possible phenolic compounds.

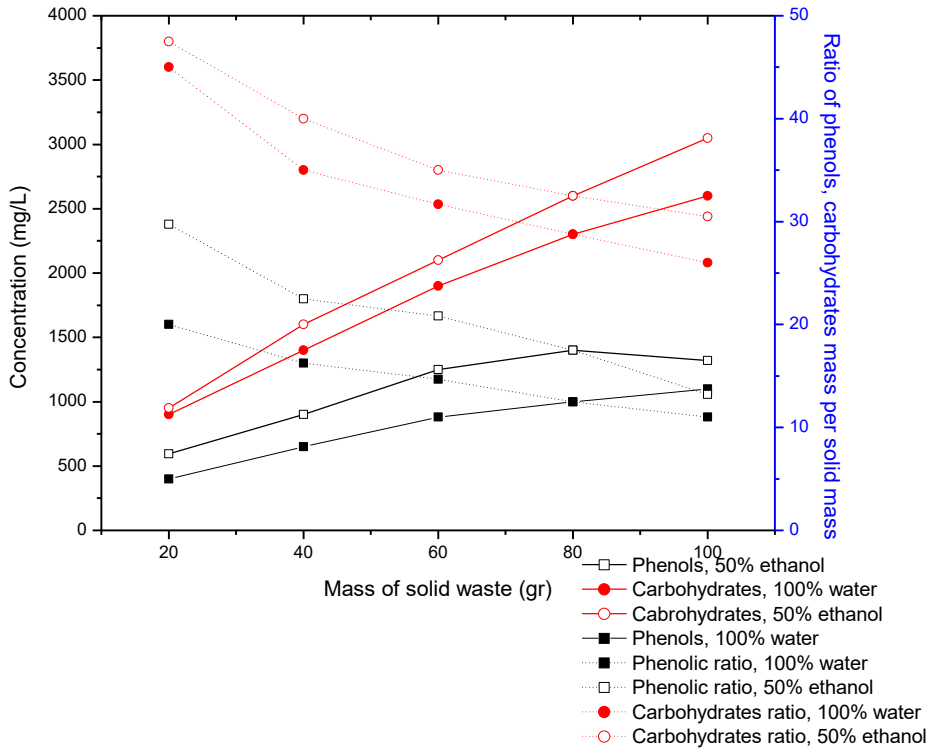

Figure 2. Concentration of TPC and carbohydrates as a function of the mass of solid waste (solid lines), and Ratio of TPC and carbohydrates mass per mass of solid (dot lines).

Effect of of HCl

Figure 3 shows the effect of HCl which has been reported in the literature as a reagent that might help hydrolysis of organics and aiming to improve the recovery of phenolics [5]. In the present series of experiments, 40 gr of solid olive oil mill waste were used in two different solvents of 100mL for each extraction set following the result obtained in Figure 2. The extractions were performed using water and mixture of 50% E-50% W as solvents. Figure 3 shows that TPC concentration values for both solvents slightly decreased upon the addition of HCl. On the contrary, the obtained carbohydrates concentration values, found to be increased with the addition of HCl. Figure 3 suggests that HCl should

be avoided because it does not help the enhancement of the recovery of the phenolic compounds, which are the interesting compounds with high added value and is the main target of the present work.

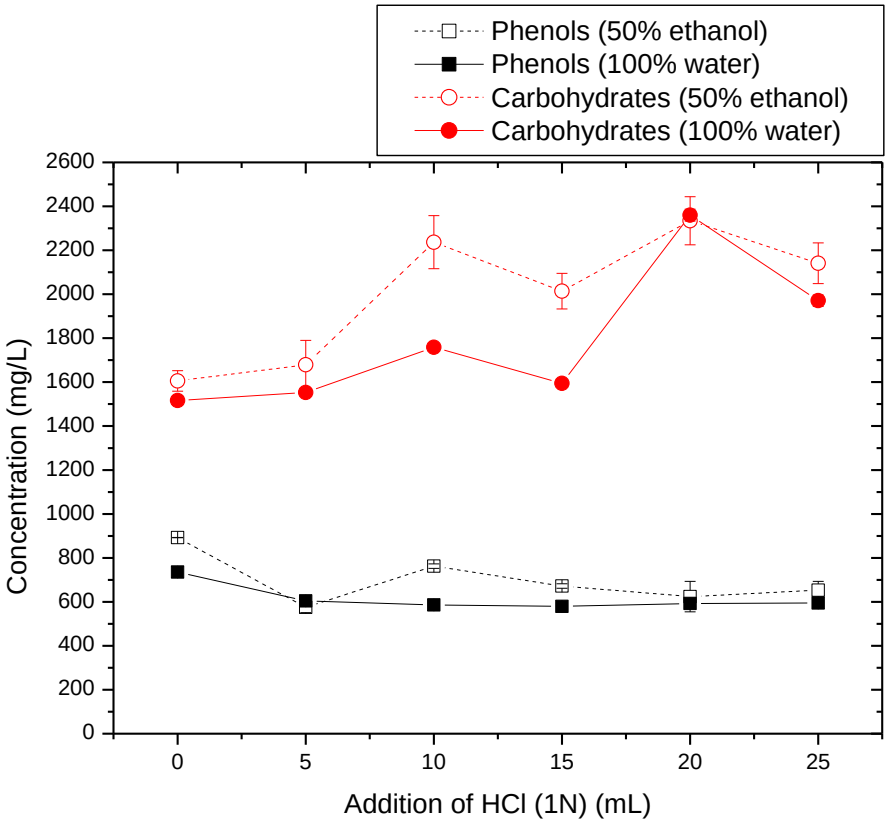

Figure 3: TPC and carbohydrates concentrations as a function of added HCl (1N).

Temperature effect

Figure 4 shows the effect of temperature on the extraction of phenolics and carbohydrates, in a series of experiments performed with 40 gr of solid waste suspended in 100 ml of two different solvents. Again, here the solvents were pure water for the first set of experiments and a mixture of 50% E-50% W, for the second set. The stirring was held at a rate of 100rpm for 1 hour. The obtained results show that there is a significant increase in TPC values when the temperature was increased up to 60 ^{o}C when the second solvent was used (50% E-50% W). The extraction of TPC with pure water as solvent gave much lower values of TPC for all tested temperature values.

On the contrary the values for the concentration of carbohydrates were found to be higher when the extraction was performed with pure water as solvent. Thus, Figure 4 suggests that one shall choose mixtures of alcohol and water (50% E-50% W), rather than pure water because more phenolics and lower amount of carbohydrates are extracted at the same time. Figure 4 also shows that the increase of

temperature at values more than 50ºC resulted in the extraction of more carbohydrates, in both scenaria with solvents, while in the case of phenolics, the effect is significant only for the mixture of ethanol and water. For the case of extraction of phenolics with pure water Figure 4 shows that there is a decrease initially in TPC values in the range of 10-20 ºC while above 20 ºC a slight increase in TPC values was observed. Comparing TPC and carbohydrates recovery it seems that the optimum temperature range is between 30 and 50ºC. However, since the TPC concentration values are sufficient for temperature values above 25ºC which was adopted as optimum for practical reasons and for energy economy.

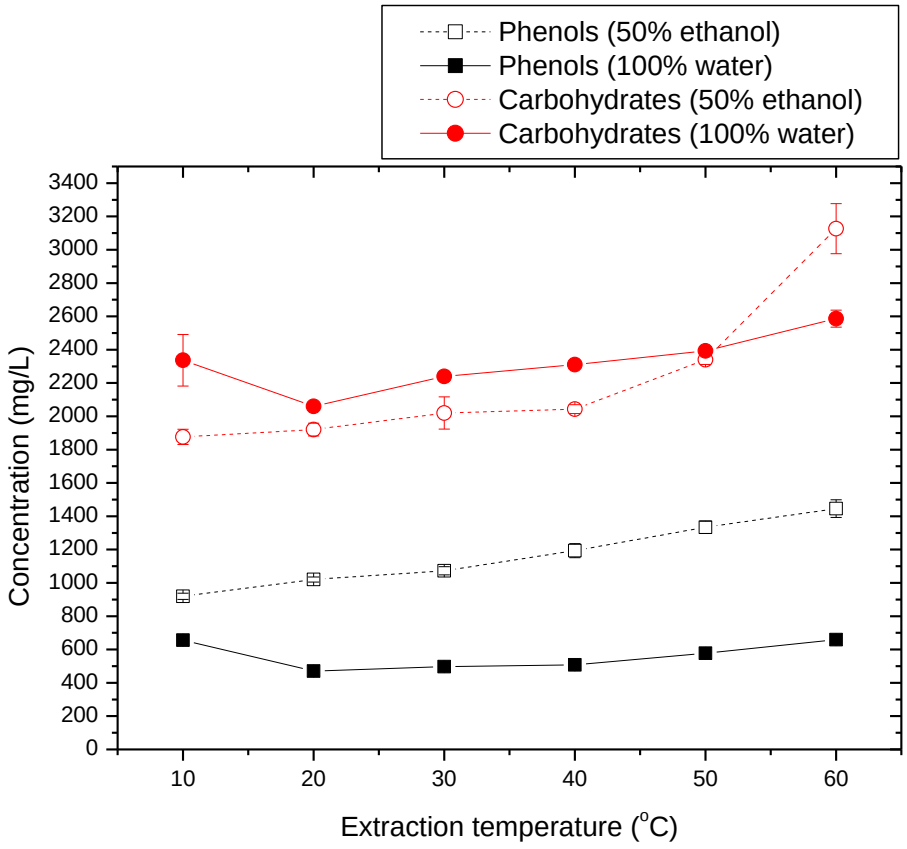

Figure 4: TPC and carbohydrates concentrations as a function of the extraction temperature.

Optimization of the maximum recovery of phenolics from the solid waste

At this stage three successive extraction experiments were performed using the same quantity of 40 gr of solid waste and fresh solvent each time. The experiments were conducted at 25ºC, under stirring (100rpm) for 1 hour for both tested solvents (pure water and 50% E- 50% W). Figure 6 shows the obtained values for TPC and carbohydrates as function of extraction repeatability. As Figure 6 shows, the second and the third trial for recovery of phenolics and carbohydrates from the same solid waste were resulted in much reduced recovery rates. It is clear, that after the third cycle all material available for extraction was removed from the solid matrix. Again here, the mixed solvent (50% E- 50% W)

gave the best results in terms of the best recovery of phenolics and in terms of the less recovery of the carbohydrates, which is in accordance with the target of the present work. Thus, it is concluded that TPC and carbohydrates recovery are high enough from the first extraction and no significant increase is obtained from the second and third extraction.

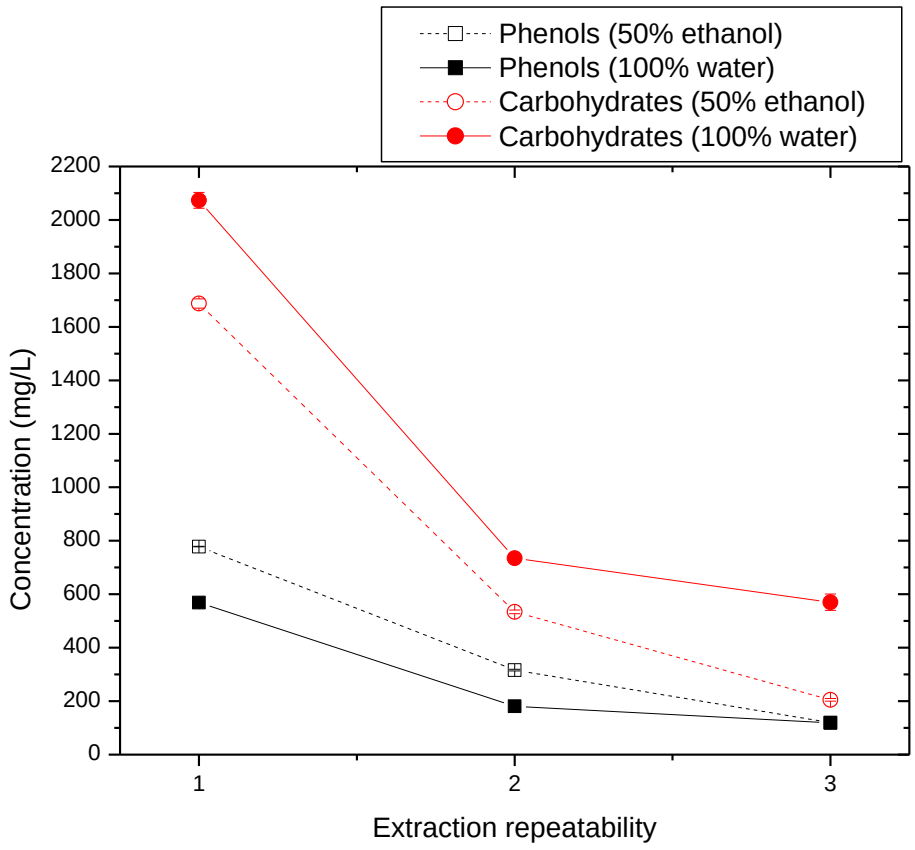

Figure 6: TPC and Carbohydrates concentration values versus repeatability with respect to the solid residue.

Effect of stirring rate

At this stage, the effect of stirring rate on the extraction of phenols was investigated. Again 40 gr of solid waste was used for the extraction of organics in a volume of 100mL of two different solvents. Again water and 50% E- 50% W solvents were used, for 1 hour, at 25 ºC but the stirring was varied at rates of 0, 50, 100, 150 and 250 rpm. The obtained TPC and carbohydrates concentrations are depicted in Figure 7. Concerning the TPC concentration values (solid and dot black curves), an increase is observed up to 50 rpm for both solvents. By further increasing the stirring rate up to 150 rpm, no significant change in the phenolic load was observed. A slight extra increase is obtained at 200 rpm in the case of when pure water was used as solvent. Carbohydrates concentration values obtained during extractions with water solvent increased with increasing stirring rate. When the used solvent is 50% E-50% W, carbohydrates concentration values were increased up to 100 rpm, then a slight decrease was

obtained at 150 rpm which was followed by an increase at 200 rpm. Comparing TPC and carbohydrates concentration values, the optimal stirring rate is found at 100 rpm since at this rate TPC recovery was maximized.

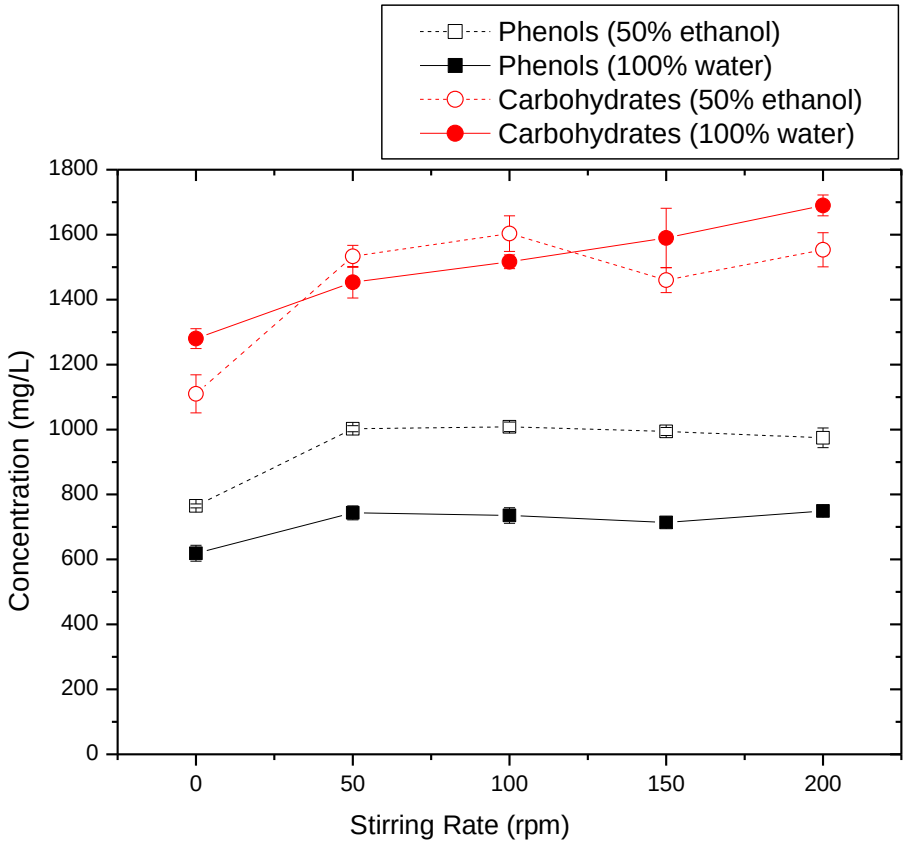

Figure 7: TPC and carbohydrates concentration values versus stirring rate.

Effect of the extraction time

At this stage the time for the total duration of the extraction was investigated. As in all experiments described above, 40 gr of olive oil mill waste were extracted using each time 100mL of two different solvents, at 25 ^{o}C and 100 rpm of stirring rate. TPC and carbohydrates concentrations for the different types of solvents and for increasing extraction time are depicted in Figure 8. As Figure 8 shows there is no significant change in the TPC concentrations with increasing the extraction time, for both solvents after 60 minutes. With regard to carbohydrates concentration (red curves), the concentrations remain constant for the first hour of extraction for both solvents. For longer times the carbohydrates recovery appeared to reduce when the solvent was pure water, whereas it increased significantly when the solvent used consisted of 50% E- 50% W. Thus, it is concluded that the optimum extraction time is 1

hour (60 min) since TPC concentration kept constant after 1 hour and at the same time the recovery of carbohydrates was not too high.

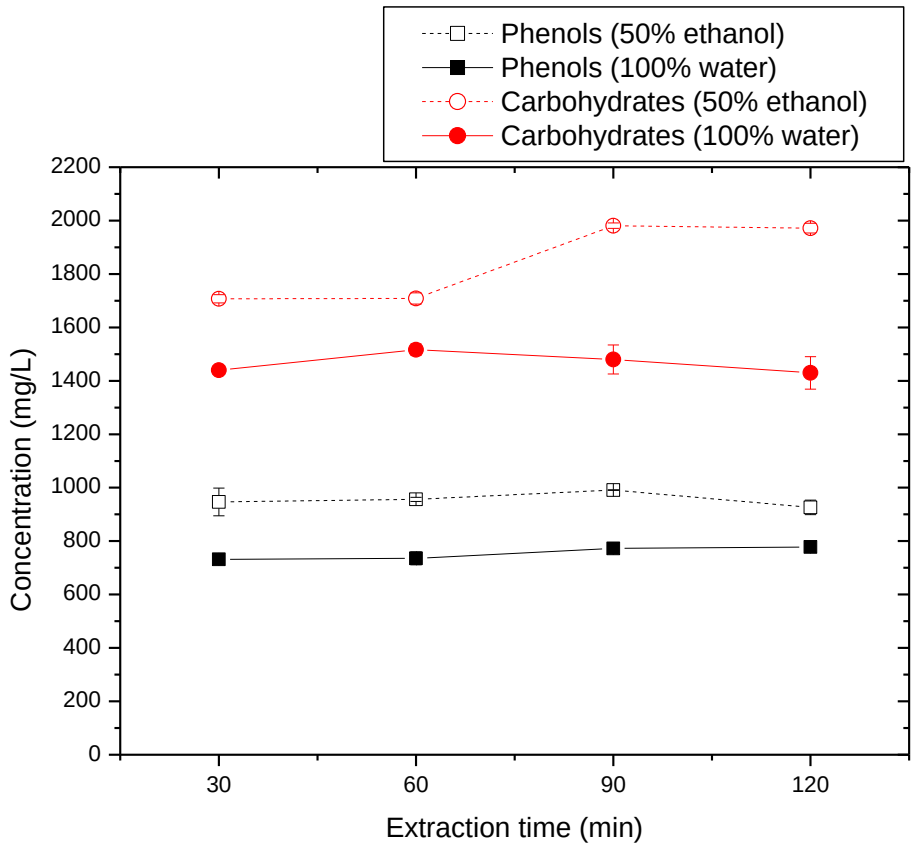

Figure 8: TPC and carbohydrates concentrations as function of the duration of extraction.

The optimal conditions for the present experimental analysis is referred to the maximum recovery of phenolic substances and if possible to the minimum recovery of carbohydrates. From the above analysis of all experimental data, for both solvents (pure water and 50% E- 50% W), the optimum TPC extracted values were achieved using 40 gr of two-phase olive oil mill waste, 100mL of solvent, without the addition of HCl, at room temperature 25°C, after 1 hour stirring at 100 rpm.

The difference between the two types of solvents lies in the recovery of the phenolic substances which in the case of 50% ethanol in water is higher (Table 1). When 50% of ethanol in water is used as solvent, the maximum recovery in phenols is about 1g (mean value 970 mg) TPC per L of two-phase mill waste extract, whereas when pure water is used as solvent, a significantly lower amount of TPC (~ 660 mg TPC/L of waste) is obtained. This difference is attributed to the higher solubility of polyphenols in organic solvents than in water. Although the parametric investigation concerns mainly the higher TPC recovery, it is important to investigate also the optimum recovery of total carbohydrates. As Table 1 shows the obtained carbohydrates concentration values are similar for both

solvents, thus the choice of solvent used in the extraction process does not affect the recovery of total carbohydrates.

Table 1: TPC and carbohydrates concentration values (mg/L) in the extracted solution

	Maximum TPC concentration (mg/L)		**Maximum Carbohydrate concentration (mg/L)**	
Solvent Type **Conditions**	**100% H_2O**	**50 % ethanol (95%), 50 % H_2O**	**100% H_2O**	**50 % ethanol (95%), 50 % H_2O**
Temperature, 20ºC	471.0	1020.3	2060.0	1373.3
Rate, 100 rpm	735.5	1008.3	1516.7	1603.3
Duration, 60 min	735.5	956.3	1516.7	1708.3
HCl, 0 mL	678.0	892.5	1436.0	1605.3
Mean Concentration	**655.0**	**969.3**	**1632.3**	**1572.6**

Pilot scale experiments

Pilot scale experiments were performed using the pilot plant of UF, NF and RO membranes described in details in Methods and Materials section. 6 kg of solid waste were used to extract a solution of 120 L based on most of the parameters obtained from the batch extraction process described above. Although the optimum ratio was found to be 1:5 in the laboratory experiments, a smaller ratio (1:20 ratio) was chosen in order to avoid membranes fouling. Additionally, although the solvent 50% E- 50% W was found as optimum, pure water was chosen for the extraction of organics from the OWSM in pilot scale experiments in order to reduce the experimental costs. The extracted solution was sieved carefully with stainless steel sieves with different size of pores, the smallest being the one with pore openings of 125 μm. The sieved solution free of large suspended particles was introduced into the membrane scheme (Figure 9). The volume balances in each membrane modules is shown also in Figure 9. The permeate stream from UF was used as feed for the NF module while the permeate stream of NF was fed to RO module. What is important is the final treated volume (~80 L) which was almost free of organics and this stream can be considered as appropriate for irrigation or recycling in the premises of the olive mill factory. As for the concentrates, small particles (with size less than 125 μm), fat, lipids and large molecules with high molecular size were isolated in the concentrate stream of Ultrafiltration (20 L).

Other organics with intermediate sizes are removed in the concentrate stream of NF (10 L) and all remaining organics were retained in the RO concentrate stream (11 L)

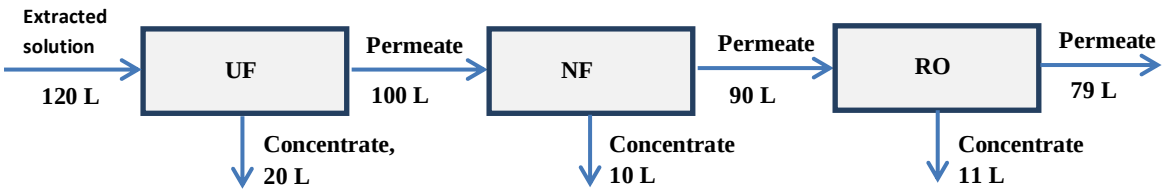

Figure 9: Schematic diagram of the feed, permeate and concentrate streams during the pilot scale experiment.

TPC and carbohydrates concentration as well as COD values were measured at all stages of the experiment at all fractions of the concentrate and permeate streams (Figures 10-12). According to the experimental data obtained for the TPC (Figure 10) a considerable amount of TPC is recovered at the concentrate stream of the UF reaching ~550mg/L and at the concentrate stream of NF membrane where 652mg/L were retained. In the concentrate stream of RO 225 mg/L of phenolics were isolate and we assume that this fraction contains simple phenols with low molecular weight size. The corresponding values for the permeate stream for the three modules were 203, 37 and 7 mg/L for UF, NF and RO streams, respectively. TPC concentration at the final effluent (permeate after the RO membrane is less than 10 mg/L, showing that the polyphenols of the olive oil mill waste were almost completely recovered in the concentrate streams. Thus, the final stream can be disposed for irrigation purposes of cultivated fields or fruit trees. All selected streams are treated further to remove most of the solvent in order phenolic samples with high purity to be isolated. This is the issue of the current work.

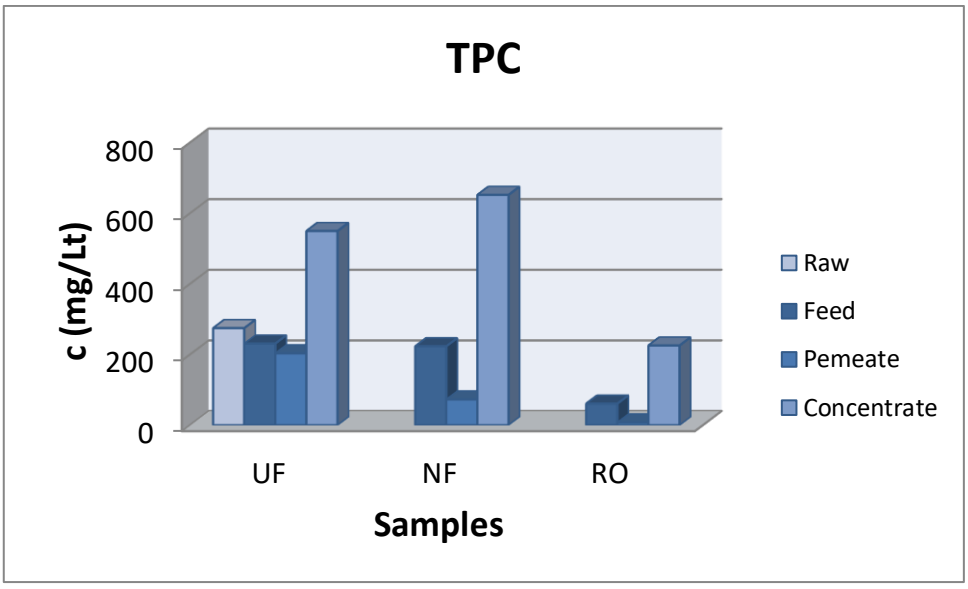

Figure 10: TPC concentration at the raw, feed, permeate and concentrate streams at the UF, NF, RO membranes.

Carbohydrate recovery showed a similar behavior as the recovery of phenols when the extracted sample was filtered, in the proposed scheme of UF, NF and RO modules. Carbohydrates concentration values (Figure 11) show a decrease at the concentrate stream after NF and RO modules, as expected, since polysaccharides are large molecules and are retained in the concentrate stream of UF. The carbohydrates concentration at the concentrate streams of the UF module was ~4200mg/L, at the NF module was ~3200 mg/L and at the RO module was 2500 mg/L. The final effluent (permeate stream of RO) has only a small concentration of carbohydrates (~146 mg/L), where at the intermediate steps of the UF and NF permeate stream values were 1780 mg/L and 787 mg/L, respectively. The concentration of carbohydrates at the final effluent at 146 mg/L was found to be more that the accepted limits for free disposal to aqueous receptors, however this small value of 146 mg/L do not prohibit the safe disposal of the final effluent for irrigation purposes.

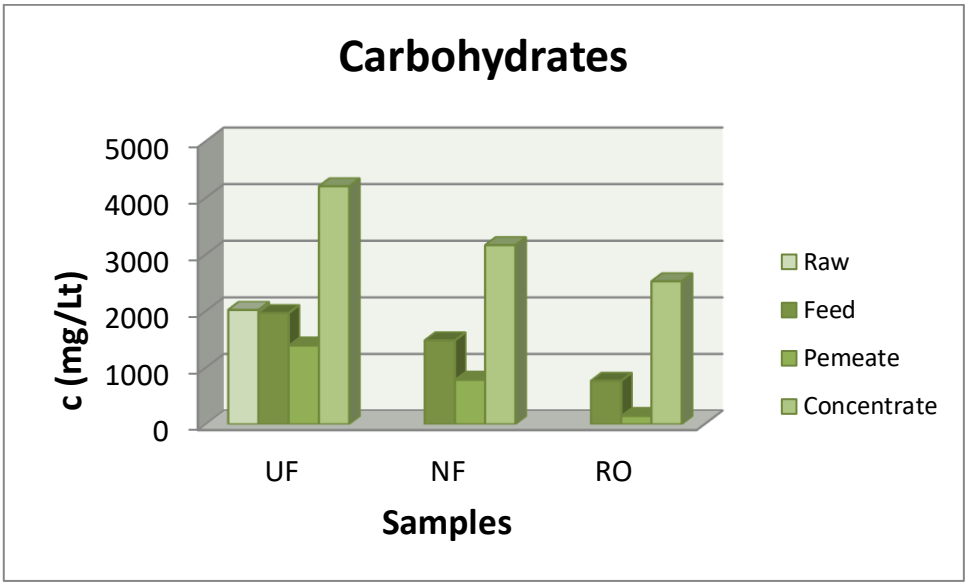

Figure 11: Carbohydrates concentration at the raw, feed, permeate and concentrate streams at the UF, NF, RO membranes.

Figure 12 shows the corresponding COD values obtained in the concentrate and permeate streams of the membrane scheme. The highest COD concentration was obtained after the implementation of UF membrane at its concentrate stream exceeded the 32000 mg/L. The concentrate stream of NF contained 16,000 mg/L organics expressed as COD values and at the concentrate step of RO the retained organics had a concentration of 12,000 mg/L. The COD at permeate stream of UF was up to 7360 mg/L, at NF was 6885 mg/L and after the RO membrane was 284mg/L. This final value for COD is too low in comparison with the values at the feed of the membrane module showing that the final permeate is almost clean.

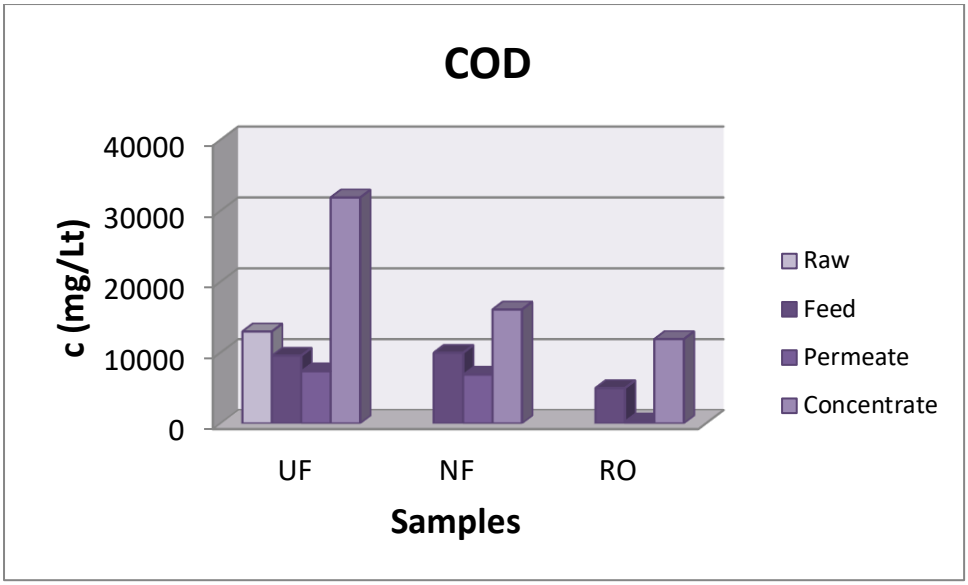

Figure 12: COD at the raw, feed, permeate and concentrate streams at the UF, NF, RO membranes.

Conclusions

Series of extraction experiments were performed in order to investigate the optimal conditions for the extraction of the maximum amount of phenolic compounds and if possible the minimum extraction of carbohydrates. Two different types of solvents were examined (pure water and) in series of experiments where the effect of different parameters was investigated, i.e. maximum solid to solvent ration, the addition of HCl, the temperature during the extraction (10-60 ^{o}C), the extraction repeatability, the rate of stirring (rpm) and the extraction time. When the solvent of 50% E- 50% W was used, TPC and carbohydrates concentrations were found to increase in all tests. Extraction repeatability on the same waste using new solvent each time was found to give small increase in TPC recovery. Increasing the stirring rate increases the carbohydrates concentration but in the case of TPC recovery, it is almost stabilized after 100 rpm. The optimal TPC recovery was achieved for both solvents after the extraction of 40g of OWSM, without the addition of HCl, at room temperature 25^{o}C, after 1 hour stirring at 100 rpm.

Finally, a pilot scale membrane set-up consisting of UF, NF and RO membranes was used for the recovery of TPC and carbohydrates. The TPC and carbohydrates concentration as well as the COD values were measured at the raw olive oil mill waste, at the filtered feed stream of the UF and at all permeate and concentrate streams after each membrane module. The final effluent in the permeate stream after the RO membrane was found almost clean from polyphenols, whereas only low concentration of carbohydrates and COD were detected. TPC at the concentrate stream of RO was 225 mg/L of phenolics whereas at the permeate was lower than 10mg/L. Carbohydrates concentration at the final effluent was found was 146 mg/L and COD was almost ~ 284mg/L. These concentration values give the evidence that it is possible to clean the two phase olive mill solid waste (OMSW-pomace) with

simultaneous recovery of high amounts phenolic substances. The high recovery of polyphenols is of high interest due to their high added value whereas at the same time the clean final permeate can be reused in other applications.

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