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Isolation of Purified High Added Value Products from Olive Mill Wastewater Streams through the Implementation of Membrane Technology and Cooling Crystallization Process

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Olive Mill Wastewater (OMW) and olive leaves are the main by-product associated with olive oil production. OMW is a by-product characterized by the very high concentration of organics and phenolic compounds rendering its biodegradability very difficult. On the other hand, due to the antioxidant properties of phenolic compounds, their recovery and application into different scientific fields, including food, pharmaceuticals, etc, may ameliorate significantly health issues.
The current study aimed at the development of an integrated treatment process for the exploitation of OMW to zero waste emission. The proposed method for the more efficient treatment of OMW includes the implementation of membrane technology for the isolation and fractionation of the high added value by-products contained in the concentrate streams of nanofiltration and reverse osmosis. The final reverse osmosis permeate subtracted from the organic loading may be used for reuse in the OMW premises, while the reverse osmosis concentrate, enriched in the phenolic fraction, may be further treated for the investigation of the selective recovery of purified phenolic compounds by cooling crystallization.
The recovery of phenolic compounds from OMW streams, was investigated from synthetic OMW prepared according to literature data survey. Glucose and tyrosol were used as model compounds for sugars and for the phenolic fraction respectively. The implementation of membrane filtration employed for the fractionation of monosaccharides and polyphenols in the concentrate streams of nanofiltration and/or reverse osmosis. Finally, the phenolic compounds in the concentrate were obtained by cooling crystallization.

1. Introduction

Olive tree cultivation is of profound importance, especially for the Mediterranean countries where 95 % of the total olive oil and edible olives crops are produced (Paraskeva et al., 2007). The beneficial properties and constituents, present in olive oil, have classified it as an essential component for a healthy nutrition. Among the compounds that have rendered olive oil an important food product, are polyphenols, the most important among them being tyrosol, oleuropein, caffeic acid, vanillic acid and hydroxytyrosol. Olive oil extraction systems from olive mills is associated with the co-production of large quantities of aqueous streams known as Olive Mill Wastewater (OMW). OMW is characterized by very high organic loading and phenolic content that is highly phytotoxic. The phenolic fraction is responsible for the inhibition of the development of microorganisms responsible for the biodegradation of OMW (Iakovides et al., 2014). Moreover, because of the fact that the produced by-product is estimated to be 1.1-1.5 times higher than the corresponding weight of milled olives, the efficient management of OMW is of paramount importance for environmental control issues in all countries involved in olive oil production (Arvaniti et al., 2012). A sustainable methodology for OMW may be applied involving the recovery of phenolic compounds present in OMW streams. This class of compounds has been known for their antioxidant properties which have been demonstrated in the potential treatment of a number of pathologic situations.

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Hydroxytyrosol has been reported to have cardioprotective (Visioli et al., 1999) and antiatherogenic activity (Léger et al., 2000) whereas tyrosol the second main phenolic compound present in OMW, is believed to have anti-inflammatory (Marrugat et al., 2004) and neuroprotective effect (Vauzour et al., 2010). A number of treatment methods are currently employed for the management of OMW but it seems that there is no economically feasible solution (Zagklis et al., 2013).

The present work focuses on the development of an OMW treatment process based on the application of different physicochemical methods. As a first step, the implementation of membrane technology is proposed for the fractionation of phenolic compounds in the concentrate streams of Nano-Filtration (NF) and Reverse Osmosis (RO). The final permeate from the RO is appropriate for soil irrigation as it follows the Greek Environmental Quality Standards for wastewater disposal (Israilides et al., 2006) while the possibility of extraction of high added value compounds from the NF and RO concentrates by cooling crystallization was investigated (Kontos et al., 2014).

2. Material and methods

2.1 Chemical reagents-Analysis

Synthetic crystalline Tyrosol and Glucose monohydrate were obtained from Sigma-Aldrich (98 % purity) and Carlo Erba Reagents respectively. The quantitative determination of tyrosol was done with the Folin-Ciocalteu method (Waterman and Mole, 1994) involving spectrophotometric analysis at 760 nm. Carbohydrates were measured spectrophotometrically using L-tryptophan reagent and glucose as standard at 525 nm (Josefsson, 1983).

2.2 Membrane filtration

The membrane characteristics and the process followed for the fractionation of the phenolic compounds in the UF, NF and RO retentate have been recently described in details elsewhere (Zagklis and Paraskeva, 2013). The modules were supplied by HAR SpA, Milan, Italy and experiments took place under cross flow filtration in batch operation.

2.3 Cooling crystallization experiments

The experimental set-up used for the experiments was described in detail elsewhere (Kontos et al., 2015). The apparatus consisted of a cooled cylindrical surface (ø=25 mm, L= 120 mm) where crystallization took place, and a water jacketed double walled Pyrex® glass reactor (inner diameter 6.5 cm, active volume 250 mL). Both the cooled surface temperature, T_{cold}(5 ^{o}C), and the temperature T_{hot} (usually 70 ^{o}C) of the reactor walls, was kept constant through the circulation of hot and cold water supplied from two different heat exchangers.

In a typical crystallization experiment, the tyrosol and sugar containing solution was added in the reactor at temperature T_{hot}. Next, a cooled surface at temperature T_{cold} was introduced in the solution. The large temperature gradient applied, between the solution and the cooled surface, was the driving force for the initiation of crystallization selectively on the cooled surface. At the end of the cooling cycle, the cylinder was carefully removed from the reactor and the total amount of the crystals formed were measured spectrophotometrically as already described.

3. Experimental results

3.1 Isolation of phenolic content

Membrane technology was, initially, employed for the fractionation and isolation of phenolic compounds from OMW streams. Specifically, OMW was used as a feed stream processing through UF, NF and RO membranes. As shown recently (Zagklis and Paraskeva, 2013), it was found that most of the phenolic compounds (mainly complex phenolics, in a percentage around ca. 62%, associated with large particles) remained in the UF retentate. The NF step has a beneficial effect for the separation of the free complex polyphenols (around ca. 28%) in the NF retentate. Finally, the free simple phenolic compounds (around ca. 9%) were successfully isolated in the RO concentration through the application of high operational Trans-Membrane Pressure values. Similar results were also reported in the work of (Ochando-Pulido et al., 2015) demonstrating that the isolation of the low molecular weight compounds (simple phenolic compounds and carbohydrates) can be separated from the waste in the concentrate of the RO step. In order to investigate the possibility of further isolation of the phenolic compounds from the carbohydrates, the adsorption/desorption on specific resins was applied. Through this treatment, a sufficient amount of carbohydrates was removed and the phenols were isolated by vacuum distillation. The final results are summarized in Table 1.

Table 1: Phenolic and carbohydrate content in OMW, RO concentrate and distillation residue

	Raw OMW	RO concentrate	Distillation residue
Phenols gL^{-1}	2.64	2.09	377.5
Carbohydrates gL^{-1}	12.34	14.96	293.92

As may be seen from Table 1, the total phenolic content was substantially increased in the distillation concentrate. Specifically, the phenolics were estimated around 378 gL^{-1} whereas their initial concentration was 2.64 gL^{-1}. The final results were very promising, posing the challenge of investigating the possibility of extracting phenols in crystalline form through cooling crystallization. Despite the fact that a sufficient increase in the phenolic content was achieved, the cooling crystallization process was not applied to the distillation residue because of its high viscosity, rendering crystallization very difficult. As a result, the investigation was focused on the crystallization of high added value constituents from the RO concentrate. The schematic diagram of the process and the apparatus used is shown in Figure 1.

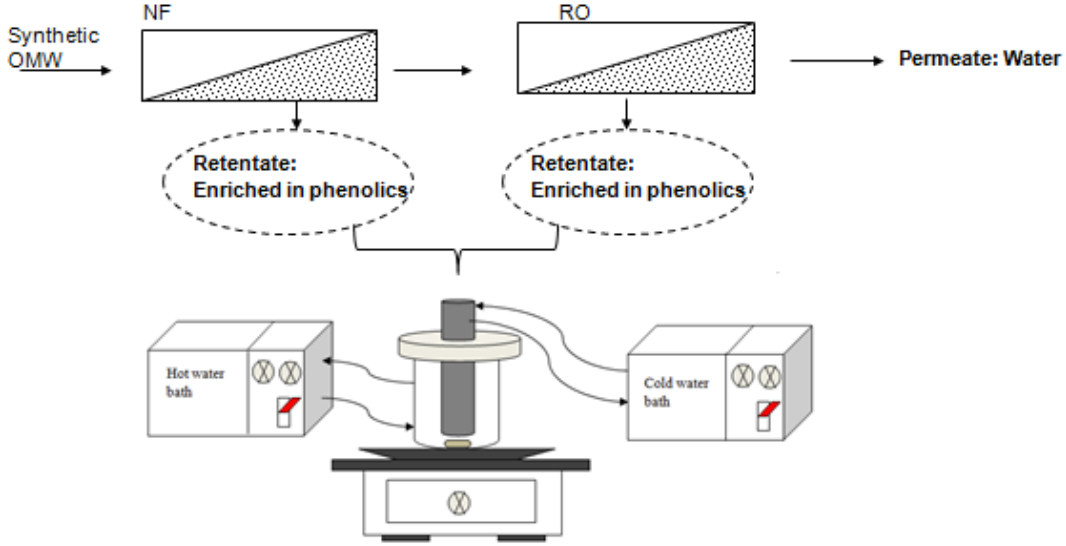

Figure 1: Schematic diagram of the process and Apparatus of membrane filtration process and experimental setup of cooling crystallization

As may be seen from Figure 1, a combination of two physicochemical methods is suggested for the treatment of OMW. Specifically, membrane filtration is implemented for the fractionation of low molecular weight compounds on the concentrate step of NF and RO whereas the final permeate of the RO was pure water suitable for irrigation or for reuse in OMW premises. Finally, the NF and RO concentrates, substantially enriched in phenolic content, were used for the application of cooling crystallization for the investigation of tyrosol's selective recovery.

3.2 Crystallization of tyrosol and monohydrate glucose

The recovery of phenolic compounds from OMW was investigated through preliminary experiments in which tyrosol and monohydrate glucose were used as model compounds. As a first step of the current study, the effect of supersaturation of both components to the recovered solid was examined. The solubility data of the monohydrate glucose in water were taken from the work of (Young, 1957), whereas for tyrosol the solubility values reported in the literature (Queimada et al., 2009) were used. The solubility data for the two model components used in the present study are shown in Figure 2.

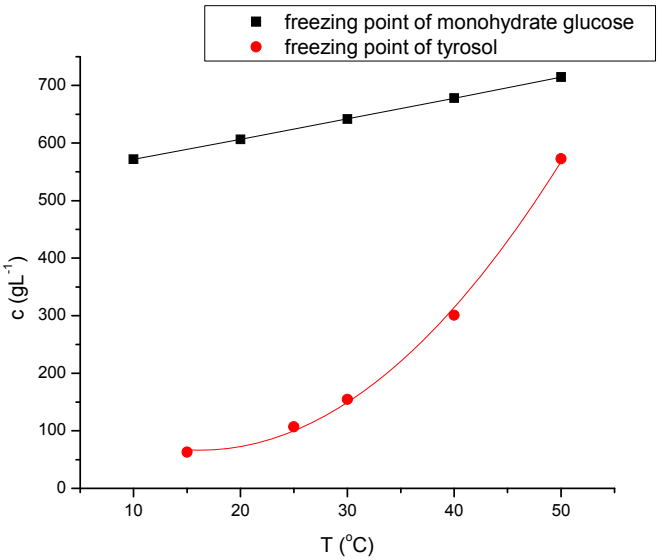

Figure 2: Solubility of glucose monohydrate and tyrosol in water as a function of temperature; (■) Glucose monohydrate, (●) Tyrosol.

Concerning tyrosol, the initial concentration in the aqueous solution was 65 gL^{-1}. The anticipating melting point for tyrosol was 15 ^{o}C. For the case of mono-carbohydrates their initial concentration varied in order to examine their effect on the solid obtained by crystallization. More specifically, 30, 65 and 600 gL^{-1} of glucose was added separately in the presence of tyrosol and past the dissolution of both components in the reactor, the cooled surface was immersed. Past up to 2.5 h from the onset of crystallization the cooled surface was removed from the solution and the formed crystals were smoothly distributed around the cylinder as shown in Figure 3.

Figure 3: Evolution of crystallization of tyrosol and glucose on a cooled surface

In Figure 3, the progress of crystal growth of tyrosol in the presence of glucose is presented. The applied temperature gradient, T_{hot}-T_{cold}, resulted to a sufficiently high concentration difference, the driving force for the initiation of nucleation and crystal growth on the cold surface. The concentration levels selected in the present work followed findings that in the concentrate of the RO, the ratio value of phenols over the corresponding value of the sugars was ca. 1:1 (Zagklis et al., 2015).

3.3 Effect of carbohydrate concentration on the total phenolic recovery

In the following set of experiments, mixtures of tyrosol (initial concentration 65 gL^{-1}) and glucose (at different initial concentration values) were added in the reactor and experiments were done in order to examine the possibility of tyrosol's crystallization in the presence of carbohydrates. These experiments were carried out under optimum constant temperature values (T_{hot}=70 ^{o}C, T_{cold}=5 ^{o}C) in a way that a substantially high

temperature gradient was applied. The dependence of the recovered solid as a function of the different concentration values of glucose is shown in Figure 4.

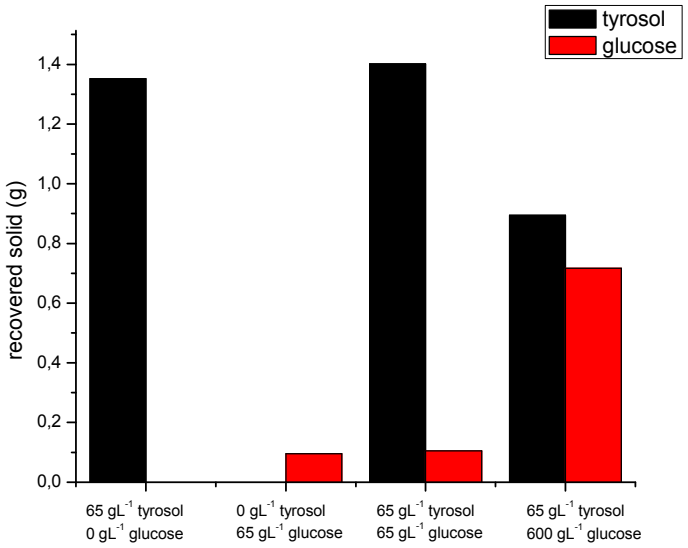

Figure 4: Recovery of tyrosol and glucose deposited on the cooled surface for different concentration values of glucose

As may be seen from Figure 4, the first experiment took place in the presence of tyrosol with initial concentration of 65 gL^{-1} (16.25 g tyrosol in 250 mL water) without glucose. The recovered tyrosol measured was found to be ca. 1.4 g. The same experiment was repeated for the case of a solution containing 65 gL^{-1} of glucose only. Due to the fact that the initial glucose concentration was much lower than the corresponding solubility value, the final recovery was very low (around 0.1g). The low recovery was attributed to the bulk solution retained on the cooling cylinder.

Additional experiments were done in the presence of both components. In the first case, low initial concentration of glucose was selected, so that it was insignificant with respect to its effect on the phenol melting point T_{cold}. At this condition, cooling crystallization was tested for the recovery of tyrosol selectively on the cooled surface. For this reason, past the end of crystallization, sweating of the crystallized solid was applied for the removal of the entrapped impurities (Chianese et al., 2002) (in this study monohydrate glucose was considered as impurity) that takes place mainly at the early stages of crystallization (Parisi and Chianese, 2001). It was estimated that increase of the initial concentration of glucose up to 65 gL^{-1} did not affect the crystallization of tyrosol and the respective recovery by cooling crystallization.

Higher glucose concentrations (600 gL^{-1}) resulted in a slight decrease of the recovery of tyrosol by cooling crystallization as may be seen in Figure 4. The experiments in the present work showed that the separation of phenolic compounds from carbohydrates is feasible provided that a suitable supersaturation is developed. Finally, it should be noted that the combination of membrane filtration and cooling crystallization may be a suggested as the core processes of a novel methodology for the more efficient treatment of OMW and for the recovery of high added value products.

4. Conclusions

The main objective of the present work was the investigation of phenolic compounds recovery from OMW. For this reason, membrane technology can be used as a first treatment step for the fractionation and separation of low molecular weight compounds. Most of the simple phenolic compounds and mono-carbohydrates can be concentrated using the NF and RO process. Further separation of phenolic compounds from carbohydrates by cooling crystallization applied in the concentrate streams, may be achieved. Model experiments, involving tyrosol and glucose monohydrate showed that this separation is possible. Tyrosol was successfully crystallized on a cooled surface by the application of high temperature gradients. Nevertheless, the achieved recovery of tyrosol was rather low (8.6 %), a fact attributed to the relatively low supersaturation levels applied.

The results of the present work contribute to the development of a novel method for the exploitation of OMW aiming to zero waste emissions.

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