Research highlights

- Phenolic compounds were recovered, concentrated and purified from artichoke wastewaters
- Artichoke wastewaters were clarified by UF with tubular ceramic membranes
- The UF permeate was concentrated by NF with a spiral-wound polymeric membrane
- Macroporous resins were tested to produce purified phenolics from the NF retentate
- Samples were analysed for total antioxidant activity, sugars and phenolic compounds
Purification of artichoke polyphenols by using membrane filtration and polymeric resins

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Abstract

The present study aimed at evaluating the potential of an integrated process based on the use of membrane technology and adsorbent resins for the recovery, concentration and purification of phenolic compounds from artichoke wastewaters. In particular, artichoke wastewaters coming from the blanching step were pre-treated by ultrafiltration (UF) in order to remove suspended solids and macromolecular compounds. The UF permeate was submitted to a nanofiltration (NF) process producing a concentrated fraction enriched in phenolic and sugar compounds. Three different macroporous resins were tested through adsorption/desorption methods to produce purified phenolic fractions with high antioxidant activity. Samples produced in UF, NF and adsorption-desorption tests were assayed for phenolic composition (chlorogenic acid and apigenin 7-O-glucoside), sugar composition (fructose, glucose and sucrose) and antioxidant activity. Among the three different tested resins, the S 7968 offered the best performance in terms of adsorption/desorption ratio for chlorogenic acid, with a total adsorption/desorption yield (TADY) of 63.39%; for the apigenin 7-O-glucoside the S 7968 and the S 2328 resins showed a TADY in the range 68.31-78.45%.

Keywords: Artichoke wastewaters; Ultrafiltration; Nanofiltration; Macroporous resins; Phenolic compounds.
1. Introduction

The artichoke (*Cynara scolymus* L.) is an ancient herbaceous perennial plant typically cultivated in the Mediterranean area with the main producers being Italy and Spain, where its commercial production contributes substantially to the agro-economy [1].

The results of different clinical investigations have widely demonstrated the health-protective potential of artichoke extracts in terms of hepatoprotective, anticarcinogenic, antibacterial, anti-HIV and hypocholesterolemic activity [2,3]. These properties are linked to their special composition which includes high levels of phenolic compounds and inulin. In particular, mono- and di-isomers of caffeoylquinic acids (chlorogenic acid and cynarin) and flavonoid O-glycosides (luteolin and apigenin derivatives) have been identified as the main responsible compounds for the biological properties of artichoke extracts and their marked antioxidant activity [4-8].

The artichoke-based industry generates huge amounts of agricultural waste (up to 60% of the harvested product) consisting mainly of the leaves, stems and the external parts of the flower which are not suitable for human consumption. Blanching waters represent additional residues of the canning artichoke industry.

The management of artichoke processing wastes is a serious environmental issue due to their perishable character. The common disposal of artichoke byproducts is as organic mass, animal feedstuff [9], ensilage [10], fiber and fuel production [11].

There is a considerable interest in preventive medicine and in the food industry in the development of natural antioxidants from botanic sources. Therefore, research efforts have been intensified to discover and utilize methods for the extraction, separation and purification of these compounds from artichoke by-products. The recovery of polyphenols is nowadays conducted in distinct steps following the so-called "5-Stages Universal Recovery Processing" [12]. Feasible protocols based on the use of methanol
and water extractions to obtain phenolic-rich extracts [13] and inulin [14] from
artichoke agroindustrial wastes have been proposed. Separation methods for the
enrichment of phenolic compounds from plant-based materials, including liquid-liquid
extraction, ultrasound-assisted extraction, heat treatment, enzyme-assisted extraction,
supercritical fluid extraction and chromatography have been recently reviewed by
Azmir et al. [15]. Unfortunately, most of these methodologies cause the degradation of
the targeted compounds due to high temperature and long extraction times as in solvent
extractions, or pose some health-related risks due to the unawareness of safety criteria
during irradiation. The requirement of costly and high purity solvents with low selective
e extractions are additional drawbacks for large scale productions.
Membrane processes offer several advantages (low temperature, absence of phase
transition and low energy consumption) when compared with conventional technologies
for concentrating and/or fractionating bioactive phenolic compounds from different
vegetable sources. In particular, pressure-driven membrane technologies, such as
ultrafiltration (UF) and nanofiltration (NF), have been widely investigated for the
recovery and concentration of bioactive compounds from natural products and by-
products of their industrial transformation [16,17]. Successful applications include the
concentration by NF of biologically active compounds from mate (*Ilex paraguariensis*)
[18], *Sideritis* ssp. L. (an endemic plant of the Balkan Peninsula) [19] and coffee
extracts [20], the fractionation of proanthocyanidins from winery extracts [21], the
recovery of phenolic compounds from bergamot juice [22] and orange press liquor [23].
The integration of UF and NF units have been also proposed for the production of soy-
protein hydrolysate with high antioxidant capacity [24], for the concentration of
anthocyanin extracts from aronia fruits (black chokeberry) [25] and for the enrichment
of polyphenolic compounds relatively to other compounds such as carbohydrates in ethanolic extracts of *Eucalyptus globulus* bark [26].

The combination of membrane operations with other conventional separation technologies (i.e. adsorption, precipitation, crystallization) offers new and interesting perspectives in order to increase the selectivity of the process [27]. For example, the combination of adsorption/desorption with UF and UF-NF coupled processes, have been applied to isolate total polyphenols and caffeic acid from *Green tea leaves* [28] and to purify phenolics compounds in distilled grape pomace press liquors in order to increase the antioxidant capacity of the final products [29].

In a previous work the combination of two different NF membranes was proposed in order to obtain two enriched fractions containing phenolic compounds and sugars, respectively, from ultrafiltered artichoke wastewaters [30]. This work was aimed at evaluating the potential of an integrated system based on the combination of membrane processes and polymeric resins for the selective purification of polyphenols with desirable biofunctional properties from artichoke wastewaters. In particular, artichoke wastewaters were clarified by UF in order to remove macromolecular compounds and suspended solids. The UF permeate was then submitted to a NF process in order to obtain concentrated fractions of phenolic compounds and sugars and a water permeate stream which can be reused in the artichoke processing industry. The NF retentate was submitted to an adsorption/desorption treatment by using three different macroporous resins in order to purify phenolic compounds, such as chlorogenic acid (CA) and apigenin 7-O-glucoside (AOG) from sugars. Fractions coming from the membrane processes (UF and NF) were analyzed for their content in total antioxidant activity (TAA), low molecular weight polyphenols and sugars, while fractions from adsorption/desorption process were
analyzed in terms of low molecular weight polyphenols and sugars in order to evaluate
the selectivity of each step towards compounds of interest. The performance of UF and
NF membranes was also evaluated in terms of productivity (permeate fluxes) in selected
operating conditions.

2. Material and methods

2.1. Artichoke wastewaters

Artichoke wastewaters coming from the blanching step were supplied by Conservas
Manuel Mateo Candel S.L. (Rafal, Alicante, Spain). Before use, they were filtered
through a cotton fabric filter in order to remove most of suspended solids and foreign
materials. The prefiltered solutions were stored at -17°C and defrosted before
membrane processing. The physico-chemical composition of the UF feed solution is
provided in Table 1.

2.2. Ultrafiltration

Artichoke wastewaters were clarified by using a pilot plant consisting of a 100 L
stainless steel feed tank, a pre-filter system equipped with a 10 μm filter cartridge, a
centrifugal pump, a feed flow meter, a thermometer, two manometers for the measure of
the inlet and outlet pressures and a membrane module. The feed flow-rate and the
transmembrane pressure (TMP) values were regulated by a pressure control valve, on
the retentate side, and by regulating the pump velocity. A tube and shell heat exchanger,
placed after the feed pump, was used to maintain the feed temperature constant.
The plant was equipped with a tubular UF membrane module supplied by Tami
Industries (Nyons, France) whose characteristics are reported in Table 2.
Artichoke wastewaters were clarified in selected operating conditions according to a batch concentration configuration (permeate is collected separately and retentate is recycled to the feed tank). In particular, the UF system was operated at a transmembrane pressure (TMP) of 430 kPa, an axial feed flow rate of 4,000 L/h and a temperature of 25°C. Experimental runs were performed in triplicate. Permeate flux data were expressed as mean ± SD. After each experiment the membrane was cleaned by using a 0.2% NaOH solution at 40°C for 1 h. Then the system was rinsed with tap water for 30 min.

2.3. Nanofiltration

The clarified artichoke wastewaters were submitted to a NF process performed by using a laboratory plant supplied by Matrix Desalination Inc. (Florida, USA). The equipment consists of a feed tank with a capacity of 20 liters, a stainless steel housing for 2.4x21 inches spiral wound membrane module, a high pressure pump, two pressure gauges (0-4000 kPa) for the control of the inlet and outlet pressures, a pressure control valve and a coiling cool fed with tap water used to maintain the feed temperature constant. The plant was equipped with a NF spiral wound membrane module (Filmtec NF 270) supplied by Dow Chemicals (Minneapolis, USA) whose characteristics are reported in Table 2. NF experiments were carried out according to the batch concentration configuration at an operating temperature of 12 °C, an axial feed flow rate of 300 L/h and a TMP of 800 kPa up to reach a weight reduction factor (WRF) of 5. The WRF is defined as the ratio between the initial feed weight and the final retentate weight, according to the following equation:

\[
WRF = \frac{W_f}{W_r} = 1 + \frac{W_p}{W_r}
\]  

(1)
where $W_f$, $W_p$ and $W_r$ are the weight of feed, permeate and retentate, respectively.

Experimental runs were performed in triplicate. Permeate flux data were expressed as mean ± SD.

After the artichoke wastewater treatment, the NF membrane was cleaned with a 0.05 % (w/w) NaOH solution at 40°C for 1h. Then the system was rinsed with tap water for 30 min.

The effect of the UF and NF processes on the recovery of bioactive compounds was measured by the rejection (R) rate according to the following equation:

$$R = (1 - \frac{C_p}{C_f}) \cdot 100$$  \hspace{1cm} (2)

where $C_p$ and $C_f$ are the feed and permeate concentration of compounds of interest.

2.4. Polyphenols purification by resin adsorption

2.4.1. Adsorbents

The final retentate of the NF process was processed by using three different macroporous resins based on polystyrene (Lewatit S 6328 A, Lewatit S 2328 and Lewatit S 7968) from Lanxess (Leverkusen, Germany). The S 6328 A is a strongly basic, macroporous anion exchange resin; the S 2328 is a food grade strongly acidic, crosslinked macroporous cation exchange resin and the S 7968 is a macroporous uncharged adsorbent resin (without functional groups).

The characteristics of the selected resins are reported in Table 3 according to the manufacturer’s information. Before use, adsorbents were activated as follows: 15 g of each resin (wet basis, w.b.) were previously cleaned. For the S 2328 resin, the start-up was performed by a sequence of water cleaning with 6% HCl and back-wash steps. For the start-up of S 6328 A and S 7968 resins, 6% HCl and 4% NaOH solutions were used.
with distilled water washes in between. Finally, for all resins, distilled water was passed through resins as necessary to reach a pH close to the distilled water pH.

2.4.2. Dynamic adsorption and desorption tests

Dynamic adsorption and desorption experiments were conducted in a glass column packed with 15 g (w.b.) of pre-treated resins. The bed volume (BV) of the wet-packed resin was 20 mL. In order to properly compare the performance of the 3 resins, the feed volume of concentrated wastewaters for all of them was fixed at 250 mL. Concentrated artichoke wastewaters were pumped to the column at a flow rate of 2±0.2 mL/min using a peristaltic pump (Peristaltic PR-2003, JP Selecta S.A, Spain). Desorption experiments were carried out with ethanol-water (70:30, v/v) solutions at a flow rate of 1±0.2 mL/min using a fixed volume of ethanol-water of 110 mL. To follow the adsorption and desorption processes, samples were collected at intervals of 30 min approx. during the adsorption tests and 20 min approx. for desorption runs. All samples were later analyzed by HPLC in terms of CA and AOG.

A schematic representation of the processing method investigated is reported in Figure 1.

2.4.3. Quantification of the adsorption and desorption ratios

The adsorption ratio was calculated as adsorbed weight/feed weight (%), while the desorption ratio was calculated as desorbed weight/adsorbed weight (%) of the analyzed polyphenols, CA and AOG.

For the calculation of the feed and adsorbed weight of CA and AOG, the concentration of the feed and the effluent samples during the adsorption process was plotted against the volume passed through the column. The area under the feed line was the weight
passed by the column whereas the area under the effluent samples meant the weight of CA or AOG do not adsorbed. The CA and AOG adsorbed weight was calculated as the difference of these feed and non adsorbed areas. In the desorption process, the CA and AOG desorbed weight was directly calculated as the area of the effluent samples vs. volume of ethanol-water passed through the column.

The total adsorption-desorption yield (TADY) was calculated as the product of both adsorption and desorption ratios. To normalize and compare results obtained for the investigated macroporous resins the total adsorption-desorption capacity for each resin was expressed as TADY/weight of resin (%/g). Moreover, to reduce the effect of the moisture content of resins, total adsorption-desorption capacities were expressed in terms of the dry weight of resins.

2.5. Analytical evaluations

2.5.1 Suspended solids

The suspended solids content was determined by centrifuging at 2000 rpm for 20 min, 10 mL of a pre-weighted sample; the weight of settled solids was determined after removing supernatant.

2.5.2. Total soluble solids (TSS)

Total soluble solids (TSS) were measured by using a hand refractometer (Atago Co., Tokyo, Japan) with scale range of 0-32 °Brix.

2.5.3. Identification and quantification of polyphenols compounds by HPLC

The content chlorogenic acid and apigenin-7-O-glucoside was determined by a Waters Alliance 2695 (Milford, MA, USA) HPLC system, equipped with a vacuum degasser, a
binary pump, an autosampler, a thermostated column compartment, a model 2996 diode array detector (DAD) and a Empower software (Waters Corporation, Milford, Ireland) for data collection.

Chromatographic separation was performed by using a Luna C 18(2) column (250×4.6mm, 5µm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of 0.1% of HCOOH in water (eluent A) and 0.1% of HCOOH in acetonitrile (eluent B). The following gradient system was used: 0 min, 90% A and 10% B; 30 min, 50% A and 50% B; 35 min, 0% A and 100% B. Analyses were stopped after 50 min. The system was equilibrated between runs for 10 min using the start mobile phase composition. The flow was maintained at 1 mL/min and the injection volume was 10 µL. Diode array detection was between 200 and 600 nm.

Prior to HPLC analysis, all samples were filtered by using 0.45 µm nylon filters. All polyphenols were identified by matching the retention time and their spectral characteristics against those of standards. Quantification was made according to the linear calibration curves of standard compounds.

2.5.4. Identification and quantification of glucose, fructose and sucrose

Analyses of sugars were performed by a high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). The separation was performed by using a Metrosep Car B (250×4.6mm, from Metrohn) column. The following conditions were used: flux, 1mL/min; temperature of detector, 32°C; pressure: 9-10MPa; mobile phase, NaOH 0.1 M (isocratic elution). Prior to HPAEC analysis, all samples were filtered by using 0.45 µm nylon filters and diluted 1:25 with bidistilled water.
2.5.5. Antioxidant activity in “vitro”

The total antioxidant activity in samples coming from the UF and NF processes was measured by the DPPH method [31]. An aliquot of 0.2 mL of diluted sample was added to 3.8 mL of DPPH solution (60 μM in Methanol). The absorbance was measured at $t=0$ and $t=30$ min at 515 nm. The antioxidant capacity was expressed as a percentage of inhibition of DPPH radical according to the following equation:

\[
\% \text{ inhibition of DPPH radical} = \left( \frac{A_{c(0)} - A_{a(30)}}{A_{c(0)}} \right) \times 100
\]  

(3)

where $A_{c(0)}$ is the absorbance of the control at $t=0$ min and $A_{a(30)}$ the absorbance of the antioxidant at $t=30$ min. Results were expressed as mM Trolox equivalent.

2.5.6. Determination of the moisture content of resins

For the determination of the moisture content of resins, 15 g (w.b.) of each resin were spread in a petri dish and left stand at room temperature during 24 h. Afterwards, petri dishes were introduced in a vacuum oven at 60°C and weighted regularly until reaching a constant weight.

3. Results and discussion

3.1. Ultrafiltration of artichoke wastewaters

Figure 2 shows the time evolution of the permeate flux referred to the clarification of artichoke wastewaters by UF in the selected operating conditions. The results showed that the permeate flux declined immediately after starting the process, due to the accumulation of artichoke wastewaters components in the pores (membrane fouling) and on the membrane surface (concentration polarization and gel formation). The $J_p$ vs time curve could be divided in three periods: a first step characterised by a rapid decrease of permeate flux from the initial value of 82 kg/m²h; a
second step corresponding to a smaller decrease of permeate flux; a third period
characterised by a small decrease of permeate flux up to a steady-state value of about 20
kg/m²h. A similar behavior was observed by Wallberg and Jonsson [32] in the treatment
of kraft black liquors by using a 15 kDa ceramic UF membrane at lower TMP values
(100 kPa).

The alkaline cleaning of the UF membrane according to the selected protocol (0.2%
NaOH, 40 °C, 1h) produced a water flux recovery of 77% due to an irreversible fouling
component.

The physico-chemical composition of artichoke wastewaters before and after the UF
process is reported in Table 1. The UF membrane retained all suspended solids
producing a clear artichoke wastewaters; the content of TSS and pH remained
unchanged in the UF permeate.

A little decrease of polyphenols was observed in the clarified fraction. Particularly, the
content of apigenin 7-0-glucoside in the UF permeate was 6% lower than the feed
solution while the content of chlorogenic acid remained unchanged. The TAA of the
initial solution was very well preserved after the clarification process: the UF membrane
showed a low rejection towards this parameter (1%). Similar results were obtained by
Galanakis et al. [33,34] in the clarification of high-added value products from olive mill
wastewaters by using polysulphone UF membranes with a MWCO of 25 kDa.

These membranes were able to partially remove the heavier fragments of
hydroxycinnamic acid derivatives and flavonols, and simultaneously to sustain the
antioxidant properties of the phenol containing beverage in the permeate stream.

By referring to the analyses of sugar compounds, a low rejection towards glucose and
fructose was measured (1.5-2.5%, respectively), while the observed rejection towards
sucrose was of 8.57%. Adversely, Gullón et al. [35] measured a higher rejection
towards glucose (about 35%) using a 15 kDa ceramic membrane in the treatment of liquors from Eucalyptus globulus autohydrolysis.

3.2. Nanofiltration of clarified artichoke wastewaters

In order to concentrate the phenolic compounds and sugars in artichoke wastewaters, the UF permeate was processed by NF. The behavior of the permeate flux as function of the operating time and WRF during the NF process, in selected operating conditions, is showed in Figure 3. A decrease in permeate flux (from an initial value of 10.3 kg/m$^2$h up to a steady-state value of about 4 kg/m$^2$h) was observed throughout the time, due to different factors:

1) the osmotic pressure increases due to the increment of the concentration of small molecules, mainly sugars and low molecular weights polyphenols, in the retentate, and consequently on the membrane surface;

2) increasing of the viscosity of the concentrated fraction;

3) fouling phenomena due to the reversible or irreversible adhesion of the molecules on the membrane surface or inside the pores which reduces their diameter.

This behavior was similar to that observed by Xu and Wang [36] in the concentration of flavonoids from aqueous *Ginko biloba* extract by NF. These authors obtained permeate fluxes between 5.9 and 9.5 L/m$^2$h operating at pressures of 1200 kPa and temperatures of 35-40 °C. Similarly, Warczok et al. [37] obtained permeate flux values between 1.8 and 5.9 L/m$^2$h in the concentration of apple juice operating at 1200 kPa and 30 °C. Operating at lower pressures and temperatures (300 kPa and 24 °C, respectively) Negrão Murakami et al. [18] obtained average permeate fluxes of about 4.53 L/m$^2$h in
the concentration of phenolic compounds from an aqueous mate extract by using a spiral-wound NF membrane with a MWCO of 150-300 Da.

A complete recovery (about 99%) of the initial water permeability was obtained after a cleaning of the NF membrane with a 0.05% (w/w) NaOH solution.

In Table 4 the content of low molecular weight polyphenols (chlorogenic acid and apigenin 7-O-glucoside), sugars (glucose, fructose and sucrose) and TAA in the different fractions of the NF process is reported.

The NF membrane presented a very high retention towards phenolic compounds: no phenolics compounds were detected in the NF permeate allowing to verify the efficiency of the membrane concentration process. The obtained results were also confirmed by the HPLC profile (Figure 4): a remarkable increase of the peaks (1-2) corresponding to the chlorogenic acid and apigenin 7-O-glucoside, respectively, was observed. The concentrated fraction showed a high value of the antioxidant activity in “vitro” (43 mM of Trolox in comparison with 13 mM Trolox of the NF feed) as a consequence of the concentration of phenolic compounds. Accordingly, a low antioxidant capacity was detected in the NF permeate due to the absence of phenolic compounds. This is in agreement with results obtained by Gouveia and Castilho [38] which reported a decrease in the antioxidant potential of artichoke dietary supplements due to the absence of phenolic compounds. A correlation between the caffeoylquinic and chlorogenic acids content and the radical scavenging activities of artichoke has been also reported in literature [6,39].

The NF membrane showed also a high retention towards glucose, fructose and sucrose. Consequently, the NF permeate is completely depleted of sugar compounds as showed in Figure 5. This is in agreement with the estimated molecular weight cut-off (250 Da) of the NF membrane and the molecular weight cut-off of the analyzed compounds (in
Therefore, the size exclusion can be considered as the dominant phenomenon during the separation process, while the concepts of polarity resistance and induced concentration polarization affected it to a lesser extent [40]. Cissè et al. [41] reported similar results in terms of high rejection towards TSS and anthocyanins during the treatment of clarified roselle extract by using the same NF membrane. Similarly, Giacobbo et al. [42] suggested a concentration of sugars and polyphenols during the treatment of winery effluents with the NF 270 membrane. A rejection of 90% towards glucose model solutions was also measured with this membrane by Mänttäri et al. [43].

3.3. Selection of macroporous resins for the purification of phenolics compounds

The moisture content for S 2328, S 6328 A and S 7968 adsorption resins was of 67.3±1.6%, 48.6±1.7% and 61.3±1.6%, respectively. The different tested resins showed different results in terms of adsorption ratio and desorption ratio of the analyzed polyphenols (chlorogenic acid and apigenin 7-O-glucoside).

According to data reported in Table 5, the macroporous S 7968 resin presented the highest adsorption ratio for both CA and AOG (81.35% and 100%, respectively). Adversely, the S 2328 resin showed the lowest adsorption ratios (26.65% for CA and 85.70% for AOG), while the S 6328 A resin showed a low adsorption ratio (38.38%) for CA and a high adsorption ratio (99.88%) for AOG. The low adsorption ratio of the S 2328 resin may be explained assuming that the low affinity of the analyzed compounds with cation exchangers. Kammerer et al. [44] showed a low binding rate of phenolic compounds, particularly chlorogenic and caffeic acids, with a cationic Lewatit S 2328 resin as compared to the anion exchange and adsorbent resins.
Results related to the desorption process of CA and AOG by elution with 70% ethanol/water are also reported in Table 5. For CA, good desorption ratios, between 72.74 and 77.92% were detected for all the investigated resins. For AOG the range of desorption ratios was wider (20.78-91.54%) than that observed for CA, with the S 2328 resin showing the highest desorption ratio.

Regarding the total adsorption-desorption yield, for the CA it was found that the S 7968 resin showed the highest performance (63.39%), while the worst TADY was given by the S 2328 resin (19.40%). For the AOG, the S 6328 A was by far the resin with lowest TADY (20.76%), while S 2328 and S 7968 resins, showed not very different performances (78.45% and 68.31%, respectively). In Table 5 are also listed the TADY results in wet and dry basis. According to these data, the S 6328 A resin presented the lowest TADY (d.b.) for both CA and AOG. The S 2328 resin gave the best performance for AOG but it cannot be selected for CA. On the other hand, the S 7968 resin could be considered suitable for the recovery of both CA and AOG.

Table 6 shows the content of sugar compounds in the NF retentate and in the desorbed fractions of each investigated resins. As it can be seen sugar compounds were quite totally recovered in the desorbed fraction independently on the type of resin.

According to these results and considering a NF retentate volume to be treated of 1 L, two options based on the sequential use of both S 2328 and S 7968 resins were proposed for the recovery of CA and AOG. In both cases, some amount of polyphenols get retained by resin matrix (less than 25%). For the first option (Figure 6a), expected values for the recovery of CA and AOG in each stream involved are also reported. The first option consists in a previous use of S 2328 resin in which CA and AOG are fractioned so that in the product of the desorption step, most AOG of the feed is obtained, representing the 43.6% of the total polyphenol content in this desorbed
stream. On the other hand, most CA from the feed is obtained in the not adsorbed stream, representing the 96.4% of the total polyphenols. In case pure CA would be desirable, the use of S 7968 resin would be recommended.

The second option (Figure 6b) is based on the use of the S 7968 resin first and then AOG is completely adsorbed so that a stream of pure CA can be obtained. When desorption of the resin is performed AOG represents only the 17.1% of the total polyphenols. If a major fractionation of CA and AOG is required, the desorbed stream from the S 7968 resin can be used as feed for the S 2328 resin, obtaining a stream of not adsorbed polyphenols with a 96.2% of CA and a desorbed stream containing close to 50% of AOG.

4. Conclusions

An integrated process based on the use of membrane operations such as UF and NF and adsorbents resins, in a sequential form, was proposed for the concentration and purification of phenolic compounds from artichoke wastewaters. Suspended solids and macromolecular compounds were completely removed from the artichoke wastewaters by UF producing a permeate stream enriched in phenolic compounds and sugars.

Phenolic compounds were concentrated by NF with a production of a retentate stream containing about 1.6 g/L of CA and 0.3 g/L of AOG.

Among the three different tested resins, the S 7968 offered the best performance in terms of adsorption/desorption ratio for CA, with a TADY of 63.39%; while for the AOG the S 7968 and the S 2328 resins showed a TADY in the range 68.31-78.45%.

The global results indicate that the integration of membrane operations with adsorbents resins can be an interesting approach for the purification of phenolic compounds from...
artichoke wastewaters. In particular, the combination of an adsorption/desorption system with a membrane filtration system produces a more purified fraction of phenolic compounds if compared to an integrated system fully based on the use of membranes. Although the set-up of the process has been structured on lab scale experiments future developments could lead to its implementation on pilot or industrial scale.

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References


Table I - General composition of artichoke wastewaters before and after the UF process

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Feed</th>
<th>Permeate</th>
<th>Retentate</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.18 ±0.03</td>
<td>4.12 ±0.12</td>
<td>4.16 ±0.60</td>
</tr>
<tr>
<td>TSS (°Brix)</td>
<td>2.3 ±0.1</td>
<td>2.3 ±0.1</td>
<td>2.6 ±0.1</td>
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<tr>
<td>Suspended solids (%)</td>
<td>3.08 ±0.08</td>
<td>n.d.</td>
<td>3.17 ±0.07</td>
</tr>
<tr>
<td>TAA (mM Trolox)</td>
<td>13.2 ±0.2</td>
<td>13.0 ±0.2</td>
<td>13.1 ±0.1</td>
</tr>
<tr>
<td>Chlorogenic acid (ppm)</td>
<td>560.1 ±1.3</td>
<td>555.4 ±1.2</td>
<td>556.20 ±3.0</td>
</tr>
<tr>
<td>Apigenin-7-O-glucoside (ppm)</td>
<td>80.0 ±1.3</td>
<td>75.0 ±0.2</td>
<td>81.0 ±2.1</td>
</tr>
<tr>
<td>Glucose (ppm)</td>
<td>1422.0 ±2.5</td>
<td>1400.0 ±3.2</td>
<td>1450.0 ±1.7</td>
</tr>
<tr>
<td>Fructose (ppm)</td>
<td>614.0 ±2.1</td>
<td>600.0 ±2.3</td>
<td>627.0 ±2.7</td>
</tr>
<tr>
<td>Sucrose (ppm)</td>
<td>350.0 ±2.4</td>
<td>320.0 ±3.1</td>
<td>365.0 ±3.7</td>
</tr>
</tbody>
</table>
**Table 2 - Characteristics of UF and NF membranes**

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Inside Ceram</th>
<th>NF 270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Tami industries</td>
<td>Dow-Filmtec</td>
</tr>
<tr>
<td>Material</td>
<td>TiO$_2$</td>
<td>Polyamide</td>
</tr>
<tr>
<td>Configuration</td>
<td>tubular</td>
<td>spiral-wound</td>
</tr>
<tr>
<td>MWCO (Da)</td>
<td>15,000</td>
<td>200-300</td>
</tr>
<tr>
<td>Membrane surface area (m$^2$)</td>
<td>0.1</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Maximum operating pressure (kPa)</strong></td>
<td><strong>1,000</strong></td>
<td><strong>4,100</strong></td>
</tr>
<tr>
<td>Maximum operating temperature (°C)</td>
<td>350</td>
<td>45</td>
</tr>
<tr>
<td>MgSO$_4$ retention (%)</td>
<td>-</td>
<td>&gt;97</td>
</tr>
<tr>
<td>pH range</td>
<td>0-14</td>
<td>3-10</td>
</tr>
<tr>
<td>Resin type</td>
<td>S 6328 A</td>
<td>S 2328</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Lewatit</td>
<td>Lewatit</td>
</tr>
<tr>
<td>Matrix</td>
<td>Crosslinked polystyrene</td>
<td>Crosslinked polystyrene</td>
</tr>
<tr>
<td>Structure</td>
<td>Macroporous</td>
<td>Macroporous</td>
</tr>
<tr>
<td>Ionic form as shipped</td>
<td>Cl⁻</td>
<td>H⁺</td>
</tr>
<tr>
<td>Functional group</td>
<td>Quaternary ammine</td>
<td>Sulphonic acid</td>
</tr>
<tr>
<td>Stability pH range</td>
<td>0-14</td>
<td>0-14</td>
</tr>
</tbody>
</table>
Table 4 - General composition of artichoke wastewaters before and after the NF process

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Feed</th>
<th>Permeate</th>
<th>Retentate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (°Brix)</td>
<td>2.4±0.2</td>
<td>0.6±0.1</td>
<td>6.8±0.2</td>
</tr>
<tr>
<td>TAA (mM Trolox)</td>
<td>13.5±1.5</td>
<td>1.0±0.1</td>
<td>43.0±1.6</td>
</tr>
<tr>
<td>Chlorogenic acid (ppm)</td>
<td>550.0±5.0</td>
<td>n.d.</td>
<td>1620.0±7.1</td>
</tr>
<tr>
<td>Apigenin-7-O-glucoside (ppm)</td>
<td>70.0±4.5</td>
<td>n.d.</td>
<td>310.0±5.6</td>
</tr>
<tr>
<td>Glucose (ppm)</td>
<td>1381.0±2.4</td>
<td>n.d.</td>
<td>5771.0±2.2</td>
</tr>
<tr>
<td>Fructose (ppm)</td>
<td>530.0±5.1</td>
<td>n.d.</td>
<td>2704.0±4.3</td>
</tr>
<tr>
<td>Sucrose (ppm)</td>
<td>274.0±2.7</td>
<td>n.d.</td>
<td>1360.0±3.4</td>
</tr>
</tbody>
</table>
Table 5 - Adsorption and desorption ratios of Chlorogenic acid and Apigenin-7-O-glucoside for all tested resins. Total adsorption-desorption (TYAD) yields

<table>
<thead>
<tr>
<th>Resin</th>
<th>Adsorption (%)</th>
<th>Desorption (%)</th>
<th>TADY (%)</th>
<th>TADY (%)/g resin (w.b.)</th>
<th>TADY (%)/g resin (d.b.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorogenic acid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewatit S 6328A</td>
<td>38.38±1.47</td>
<td>75.74±3.29</td>
<td>29.07±2.38</td>
<td>1.94±0.04</td>
<td>3.78±0.12</td>
</tr>
<tr>
<td>Lewatit S 2328</td>
<td>26.65±1.54</td>
<td>72.81±8.10</td>
<td>19.40±3.28</td>
<td>1.29±0.05</td>
<td>3.96±1.14</td>
</tr>
<tr>
<td>Lewatit S 7968</td>
<td>81.35±1.91</td>
<td>77.92±1.26</td>
<td>63.39±2.51</td>
<td>4.23±0.06</td>
<td>10.93±0.55</td>
</tr>
<tr>
<td><strong>Apigenin 7-O-glucoside</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewatit S 6328A</td>
<td>99.88±6.79</td>
<td>20.78±0.91</td>
<td>20.76±2.32</td>
<td>1.38±0.07</td>
<td>2.70±0.18</td>
</tr>
<tr>
<td>Lewatit S 2328</td>
<td>85.70±6.42</td>
<td>91.54±4.38</td>
<td>78.45±9.63</td>
<td>5.23±0.21</td>
<td>16.01±1.14</td>
</tr>
<tr>
<td>Lewatit S 7968</td>
<td>100±6.23</td>
<td>68.31±1.55</td>
<td>68.31±5.81</td>
<td>4.55±0.12</td>
<td>11.78±0.55</td>
</tr>
</tbody>
</table>
Table 6 - Concentration of glucose, fructose and sucrose in samples before and after the treatment with the tested resins

<table>
<thead>
<tr>
<th>Resin</th>
<th>NF retentate (ppm)</th>
<th>After treatment with resin (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewatit S 6328A</td>
<td>5341.0±10.4</td>
<td>5311.0±15.6</td>
</tr>
<tr>
<td>Lewatit S 2328</td>
<td>5523.0±6.3</td>
<td>5496.0±11.6</td>
</tr>
<tr>
<td>Lewatit S 7968</td>
<td>5638.0±8.4</td>
<td>5622.0±9.2</td>
</tr>
<tr>
<td><strong>Fructose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewatit S 6328A</td>
<td>2596.0±6.2</td>
<td>2596.0±5.5</td>
</tr>
<tr>
<td>Lewatit S 2328</td>
<td>2662.0±14.6</td>
<td>2625.0±8.8</td>
</tr>
<tr>
<td>Lewatit S 7968</td>
<td>2685.0±10.4</td>
<td>2634.0±9.4</td>
</tr>
<tr>
<td><strong>Sucrose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewatit S 6328A</td>
<td>1346.0±5.7</td>
<td>1326.0±8.4</td>
</tr>
<tr>
<td>Lewatit S 2328</td>
<td>1355.0±9.4</td>
<td>1348.0±10.5</td>
</tr>
<tr>
<td>Lewatit S 7968</td>
<td>1388.0±12.5</td>
<td>1375.0±9.7</td>
</tr>
</tbody>
</table>
Figure captions

**Figure 1.** General scheme of the investigated process.

**Figure 2.** UF of artichoke wastewaters with 15 kDa ceramic membrane. Time course of permeate flux. (T= 25 °C; Q_f = 4.0 m³/h; TMP = 430 kPa).

**Figure 3.** NF of clarified artichoke wastewaters with Filmtec NF 270 membrane. Time course of permeate flux. (T= 12 °C; Q_f = 300 L/h; TMP 800 kPa).

**Figure 4.** HPLC chromatograms of phenolics compounds detected in feed, permeate and retentate samples coming from the NF process. Peaks: 1, chlorogenic acid (CA); 2, apigenin 7-O-glucoside (AOG).

**Figure 5.** HPAEC chromatograms of sugars detected in feed, permeate and retentate samples coming from the NF process. Peaks: 1, glucose; 2, fructose; 3, sucrose.

**Figure 6.** Proposal for the fractionation, purification and recovery of chlorogenic acid (CA) and apigenin-7-O-glucoside (AOG) from 1 L of NF retentate.
FIGURE 1

Artichoke wastewaters

UF

Suspended solids

NF

NF retentate

Adsorption

Sugars

Desorption

Polyphenols

Ethanol

Water
FIGURE 2
FIGURE 3
FIGURE 4
FIGURE 5

Feed

Permeate

Retentate