

Fractionation by ultrafiltration of an aqueous extract of chestnut obtained by pressurized hot water extraction

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Abstract

Different types, ceramic and polyethersulfone, ultrafiltration membranes were evaluated to purify a wood extract obtained in pressurized hot water conditions. Tests were performed at transmembrane pressure (TMP) from 0.4 to 2.0 bars with membrane molecular weight cut-offs ranging from 5 kDa to 100 kDa. The results showed the permeate fluxes were lower than $15 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$, except for the 100 kDa organic membrane, with no influence of the feed flowrate. The retention of total phenolic compounds highly depends on the membrane cut-off but the retention rates of gallic and ellagic acid were not directly link to the cut-off. The retention rate of gallic acid is lower than 10%, when ellagic acid had retention ranging from 75 to 95 % for the membrane 5 kDa. It is suggested that other phenomena could be involved in solute retention, i.e. Donnan effect or solubility, that could explain so large differences.

Introduction

Phenolic compounds found in wood are valuable products in food and health applications thanks to their antioxidant properties. They were usually recovered by organic solvent extraction like ethanol because of their low solubility in water. Publication recently reported the use of hot water under pressure to decrease the environmental impact of the extraction process. Water is a green solvent whom properties can be modulated with temperature and pressure. It is therefore possible to modify, the solvent-solute interaction and increase the solubility of non-polar molecules (Hartonen et al., 2007). At the temperatures below $150 \text{ }^\circ\text{C}$ and in relatively short extraction times, it is possible to produce an extract with more than 50 % of total phenolic compounds (Beaufils et al., 2021). Nevertheless, the purity is too low for a direct valorisation and therefore, extra-steps of purification are required. In the study of Minhalma and de Pinho (2001), the wastewater from cork manufacturing rich in phenolic acids was purified by 6 kDa and 98 kDa membranes. The retention of phenolic acids was correlated to their hydrophilic/hydrophobic character. The most hydrophobic compound, ellagic acid, was strongly retained independently of the membrane cut-off and the operating conditions. The retention of other phenolic acids shows large variations as a function of transmembrane pressure and feed rate but with a strong membrane clogging (Minhalma and de Pinho, 2001).

In this study, the fractionation of an extract rich in phenolic compounds obtained from a hot water extraction under pressure on chestnut wood has been studied with various ultrafiltration membranes. The work has been managed under different operating conditions in order to characterize the separation mechanisms and its impact on the filtration performances.

Materials and methods

Origin and composition of extract

The extracts were produced from chestnut chips supplied by the company Fibre Excellence Saint Gaudens SAS. The extraction was carried out in a 20L laboratory digester was a heat-insulated batch reactor equipped with a recirculation system allowing the liquid to be circulated and maintained at temperature via a heat exchanger on the recirculation loop. The extraction was carried out at 120°C

for one hour at a pressure of 3.5 bar and a liquid/solid ratio of 5. The compositions of the extract are: total phenolic compounds (10.5 g of gallic acid equivalent/L), gallic acid (352 mg.L⁻¹), and ellagic acid (149 mg.L⁻¹).

Total dissolved solid analysis

The total dissolved solid concentration was determined according to the NREL/TP-510-42621 method. For each sample replicate, a volume between 2 and 5 mL was introduced into a pre-dried crucible. The crucibles were then placed in an oven at 103°C until a constant weight is obtained.

Total phenolics compounds analysis

The total phenolic content of the extracts was determined by the Folin-Ciocalteu method in microplates of 96 wells (Singleton and Rossi, 1965). Each blank, standard and sample were analysed with four replicates. The colour intensity evaluated at 700 nm is proportional to the level of oxidised phenolic compounds. The results are presented in Gallic Acid Equivalent (GAE), gallic acid being the compound used to make the calibration curve. The plates were read in a BMG-Labtech Spectrostar-Nano spectrophotometer.

Phenolics acids analysis

The analysis of phenolic acids was carried out according to the method developed by Canas et al. (2011). The analysis was performed on a Dionex high performance liquid chromatography system, combining a pump (P680), a diode array detector (Dionex (DAD) UVD340U) and an automatic injector (ASI-100). The column used was a Lichrospher RP 18 (5µm) column (40x4mm) equipped with a pre-column. The separation was carried out at 40 °C with a gradient composed of two solutions; solution A: water/formic acid (98:2 vol) and solution B: methanol/water/formic acid (70:28:2 vol). The gradient selected is: 0 %B isocratic for 3min, 0 to 40 %B in 22 min, 40 %B to 60 %B in 18 min, 60 %B isocratic for 12 min, 60 to 80 %B in 5min, 80 %B isocratic for 5 min. Detection is performed using a UV diode array detector at a wavelength of 280 nm. Data acquisition was performed using Chromeleon 6.80 Chromatography Data System software (Dionex).

Membranes apparatus

Filtration has been operated with organic hollow fiber membranes (GE Healthcare) and Kerasep ceramic membrane (Novasep) whom some characteristics are presented in Table 1.

Table 1: Properties of the membranes used

	UFP-5-E-4X2MA	UFP-10-C-4X2MA	UFP-30-C-4X2MA	UFP-100-C-4X2MA	Kerasep
Type of membrane	Hollow Fiber				Tubular
Membrane material	PolyEtherSulfone				Ceramic
Membrane surface (cm ²)	850	1400	1400	1400	1550
Internal diameter of the channels (mm)	1	0.5	0.5	0.5	3.5
Number of fibres/ channels	50	140	140	140	19
MWCO (kDa)	5	10	30	100	5

The extracts were placed in a 5L stainless-steel tank regulate at 25°C. The filtration system was composed of a volumetric diaphragm pump to control the feed flow rate measured by a flowmeter (Danfoss). The pressure was measured, through bourdon-type pressure gauge, at the feed, retentate

and permeate side. Considering that the pressure on the permeate side is equal to atmospheric pressure, the transmembrane pressure (TMP) is calculated as the average pressure at the inlet and outlet of the module. Filtration have been performed in recycling mode, meaning that both permeate and retentate were recycled to the tank in order to reach steady state conditions. Influence of TMP, feed flow-rate and pH has been evaluated at steady state by measuring permeate flowrate and taking samples for off-line analysis.

Results and discussion

A very sharp drop of the flux was observed in the first few minutes for all the organic membranes and then stabilized to values ranging from $1 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$ for the 5kDa membrane to $5 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$ for the 100 kDa membrane. These results show that the filtration of the extract causes a significant clogging of all the membranes, and this limitation is established very quickly, even if the stationary state is only really reached after 30 minutes. The following results were obtained in steady state conditions.

Influence of the hydrodynamic conditions on the filtration performances

The flux values obtained are very low for all the membranes, below $15 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$, except for the 100 kDa membrane, whose flux is between 20 and $50 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$ depending on the pressure. The flux linearly increases with pressure, except for the 100 kDa membrane. In this case, the logarithmic form of the curve indicates the apparition of a polarization layer for a pressure above 0.8 bar. The linear form obtained with the other membranes suggest that the flux is limited by a fouling that reduces the permeability of the membrane.

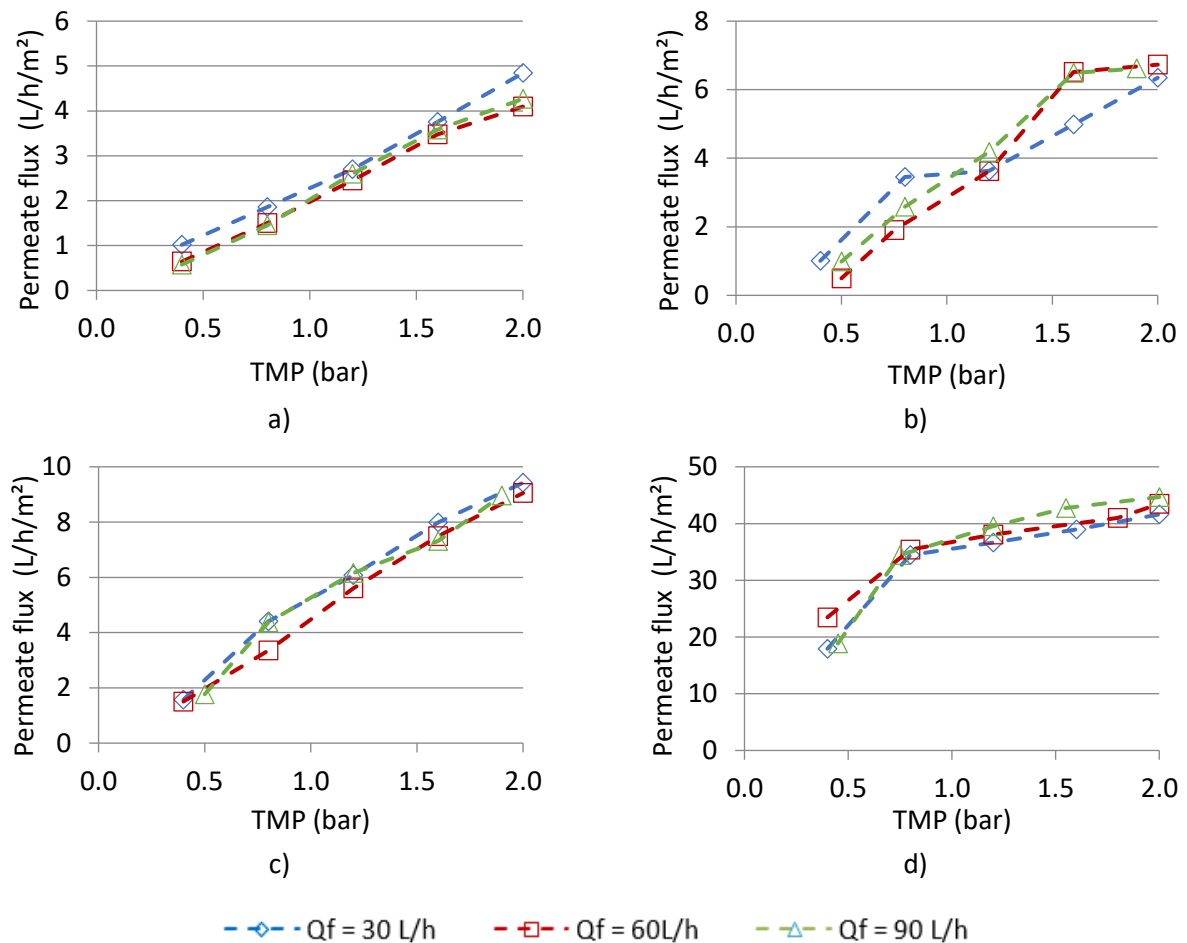


Figure 1: Influence of TMP on permeate flux. a) 5kDa, b) 10kDa, c) 30kDa, d) 100kDa.

The results show that there is no influence of the feed rate on the permeate flow. Particularly, the curves overlap in the majority of the cases and, for the rest, the values are relatively close and the difference can be explained by the experimental error. This result indicates that the shear rate does not impact on the phenomenon that appears. This weak influence of the feed flow rate can be explained by the flow regime. Indeed, the Reynolds number was calculated from the characteristics of the membranes used and for all the flow rates evaluated, the filtration was thus carried out in laminar regime. The permeate flux increases linearly with respect to TMP for membranes of 5-30 kDa, which seems to confirm that a concentration polarisation layer is not formed. The obtained permeabilities are much lower than the water permeabilities measured for each membrane. This means that if the filtration is well performed below the critical flux, the resistance to the passage of the solvent can come from two phenomena, i) pore blockage or ii) adsorption on the surface or in the pores of the membrane. The Reynolds numbers of working conditions ranged from 126.31 to 530.52 meaning that it was laminar conditions that can explain the low influence of the feed flowrate (Shashi Menon, 2015).

Table 2: Reynolds number for each feed rate and for the different organic membranes used. With Qf: feed flowrate, V_{moy} : average speed within a fiber, Re: Reynolds number.

Membrane reference	UFP-5-E-4X2MA			UFP-10-C-4X2MA, UFP-30-C-4X2MA, UFP-100-C-4X2MA		
Qf (L.h ⁻¹)	30	60	90	30	60	90
Qf/fiber (L.h ⁻¹)	0.6	1.2	1.8	0.21	0.43	0.64
V_{moy} (m.s ⁻¹)	0.2	0.4	0.6	0.3	0.6	0.9
Re	176.84	353.68	530.52	126.31	252.63	378.94

Evolution of retention rates of total phenolic compounds

As the extract was rich in polyphenols, the first response monitored is the retention of total phenolic compounds (TPC), for each condition (figure 2). The rejection of total polyphenols increases linearly with the TMP for the 5 kDa, 10 kDa and 30 kDa membranes while it tends towards non-linearity for the 100 kDa membrane. For the 5 kDa membrane, the rate increases from 42 % to 70 % as the TMP increases from 0.4 bar to 2.0 bar, with very little variation as a function of feed rate. For the 10 kDa membrane, the retention of total polyphenols increases from 45 % to 67 %, with retention very close to the 5 kDa membrane. For the 30 kDa membrane, the retention of total polyphenols increased from 34 % to 70 %. The transfer of molecules and solvent would therefore be decoupled, by interactions whose intensity seems to increase with the pressure. This could be, for example, the increase in interactions between solutes or precipitation of the molecules without reducing the water permeability. When the filtration is carried out with the 100 kDa membrane, the retention of total phenolic compounds decreases, since it evolves from 10 % for 0.4 bar to 30 % for 2.0 bar. As TPC are composed of molecules of quite distinct sizes, it is possible that 100 % of a class of molecules is retained while others pass freely.

Evolution of the retention rates of phenolic acids

Two phenolic acids were identified by HPLC in chestnut extract: gallic acid and ellagic acid. These two phenolic acids are the precursors of gallotannins and ellagitannins respectively. Gallic acid is relatively soluble in water, whereas ellagic acid is very slightly soluble in water. Moreover, both molecules are relatively small (170 g.mol⁻¹ for gallic acid and 302 g.mol⁻¹ for ellagic acid).

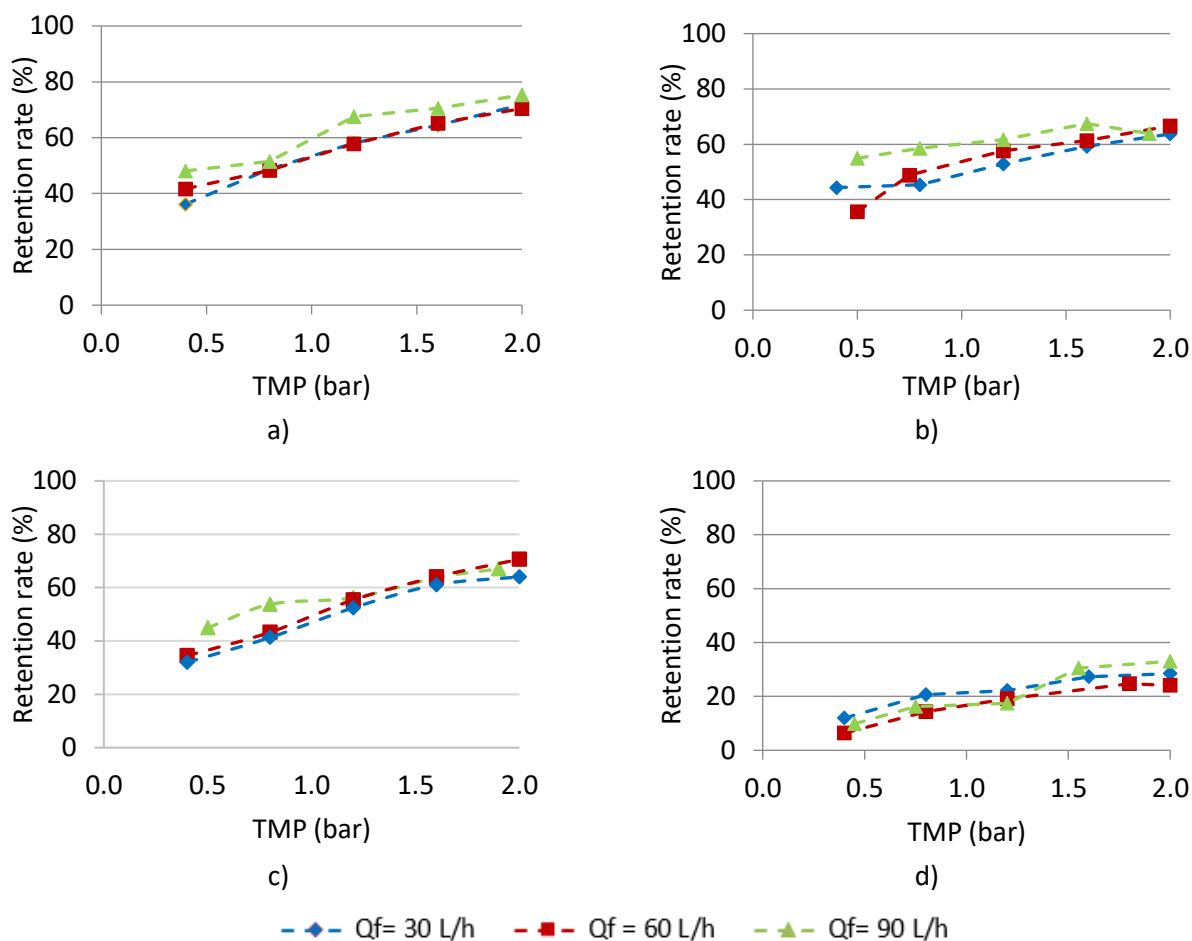


Figure 2: Influence of TMP on the retention rate of total phenolic compounds in chestnut wood extract. a) 5 kDa, b) 10 kDa, c) 30 kDa, d) 100 kDa.

The results show that the rejection rate of gallic acid is low, below 10% for all the membranes, while it is higher for ellagic acid. Furthermore, while it remains constant for all the tested conditions for gallic acid, it increases with pressure for ellagic acid. In some cases, the gallic retention is negative as for the 10 kDa membrane (-3.6 % for a TMP of 0.4 bar and a flow rate of 60 L.h⁻¹) and the 30kDa membrane the rejection rate is -14 % for a TMP of 0.4 bar, a flow rate of 60 L.h⁻¹. Negative retention indicates that an electrical potential appears and accelerates the passage of charged molecules (Donnan effect). Donnan effect arise when two molecules with the same charge present different retention rates. The passage of the molecule with the lowest retention is accelerate to maintain electroneutrality with the counter-ions in the permeate. This result indicates that i) gallic acid is charged in solution and ii) negatively charged molecules are retained by the membrane while positively charged molecules can pass through.

The results also show that the retention depends on the hydrodynamic conditions, which was not visible during the global analysis of phenolic compounds, particularly for ellagic acid. This indicates that the layer formed on the membrane surface is modified in composition. The rejection rate of phenolic compounds could depend on their hydrophobicity/hydrophilicity (Minhalma and de Pinho, 2001). Ellagic acid, which represents the most hydrophobic compound, is strongly retained without influence of TMP, feed rate or membrane cut-off. The reason for this would be the intensity of the hydrophobic interactions between the membrane and the ellagic acid, leading to the formation of an adsorbed layer of ellagic acid on the membrane surface. Another hypothesis could be the solubility of these molecules. Indeed, by increasing the pressure, the concentration of retained molecules increases on the surface

of the membrane. It is then possible that ellagic acid and gallic acid precipitate and modify the structure of the initial layer. A modification of the hydrodynamic conditions can modify the concentration and re-solubilise some molecules. This hypothesis could explain the very high retention of ellagic acid.

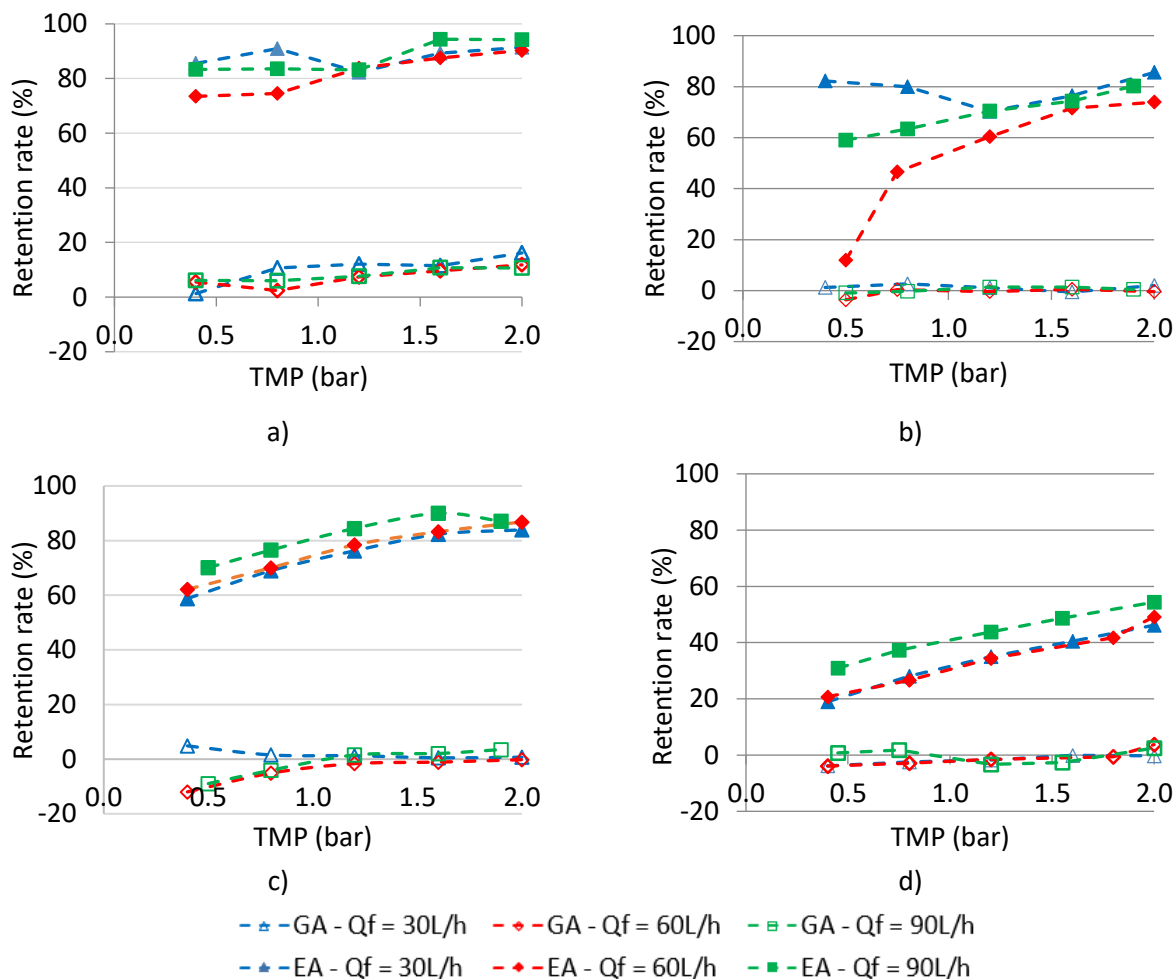


Figure 3: Influence of TMP on the retention rate of gallic acid (GA) and ellagic acid (EA) for different membranes. a) 5k Da, b) 10 kDa, c) 30 kDa, d) 100 kDa. GA: Gallic acid and EA: Ellagic acid

Ceramic membrane

Filtration has been performed with a ceramic membrane to characterize the influence of the solute-membrane interactions on the filtration efficiency. The results show that permeate fluxes were almost three times higher than the fluxes obtained with the PES membranes (figure 4).

Regarding the retention rate, organic and ceramic membranes gave the same rate of total phenolic compounds ranging (data not shown) from around 30 to 70 % and almost no gallic acid is retained. For ellagic acid, ceramic membrane has lower retention capacity than the PES membrane.

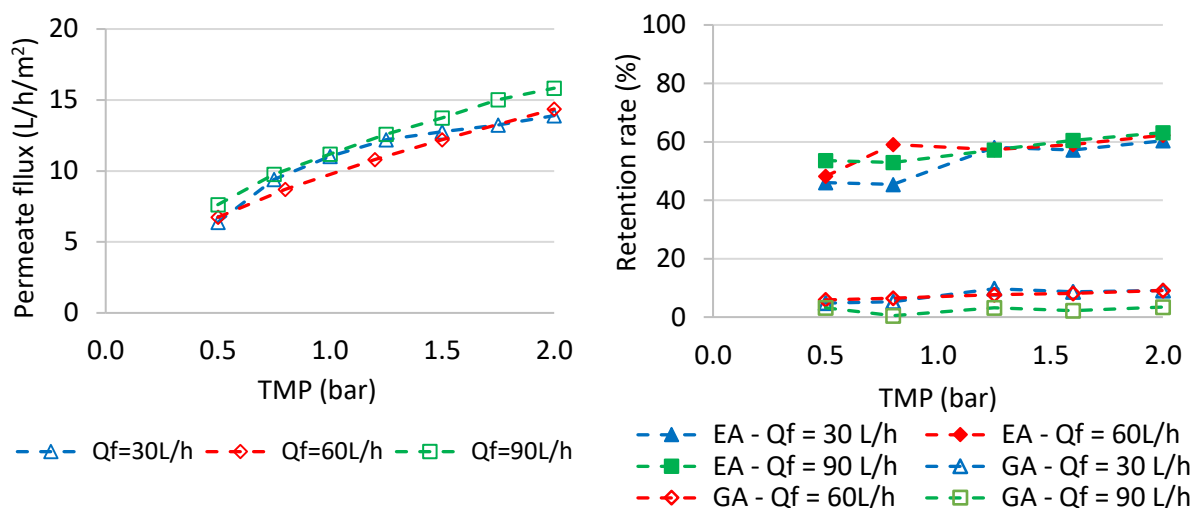


Figure 4: Influence of TMP on permeate flux (A) with different feed flowrates (Qf) and retention rate (B) of ellagic acid (EA) and gallic acid (GA) at pH 3.6 on ceramic membrane 5 kDa as function of TMP with different feed flowrates (Qf)

Effect of pH on retention capacity

The surface charge properties of membrane also impact on the interaction of compounds in the feed and the membrane. The result of Zhang et al., (2009) showed that the isoelectric point of Al₂O₃-TiO₂ was found at around pH 6.1. Hence, different pH values were investigated above and below membrane point of zero charge to assess electrostatic interactions. The pH of the feed solution was adjusted by adding NaOH 1 M to change pH from 3.6 to pH 7.0 and 10.1, respectively.

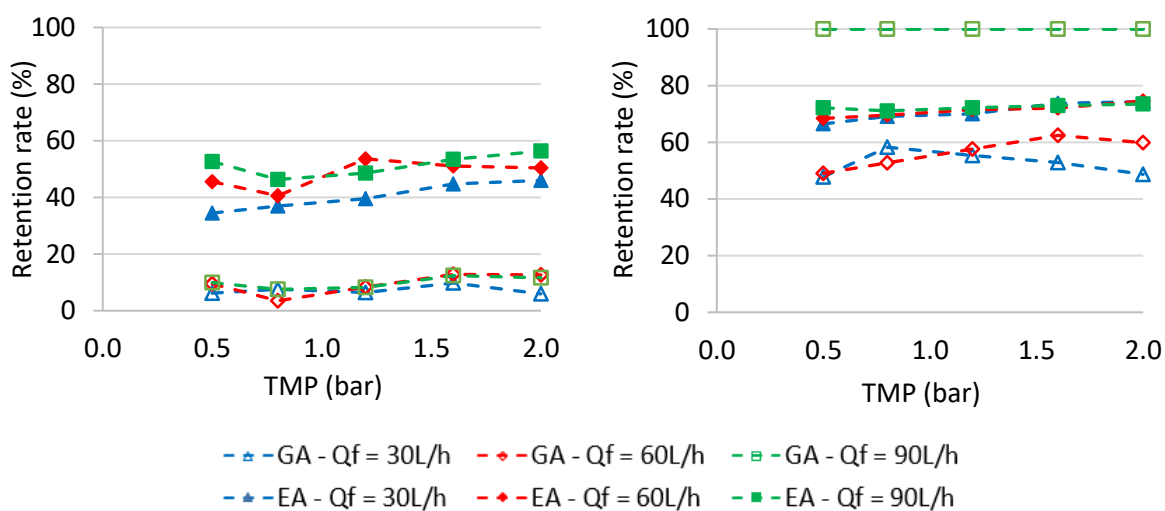


Figure 5: Retention rate of ellagic acid (EA) and gallic acid (GA) at pH 7.0 (left) and 10.1 (right) on ceramic membrane 5 kDa as function of TMP with different feed flowrates (Qf)

The results showed that increasing the pH from 3.6 to 10.1 led to a decrease of the concentration of total phenolic compounds (TPC) in the feed from 16.4 to 12.4 g.L⁻¹ due to chemical transformations. Increasing pH from 3.6 to 7.0 gave a significant decrease of TPC retention rate, followed by a small increase at pH 10.1 (data not shown). The retention rate of ellagic acid and gallic was almost unchanged when increasing the pH from 3.6 to 7.1. But when the pH was 10.1, the rejection rates increased from 50% to 70% for ellagic acid. This increase was sharper for gallic acid with rejection rates passing from 0% to a medium value of 70% that reach almost 100% for the highest flowrate (figure 5).

Conclusion

Ultrafiltration of a chestnut wood extract, obtained under subcritical water conditions, was studied in order to separate different classes of phenolic compounds. During filtration, a flow resistance, related to the compounds in the solution, appeared almost immediately. This resistance is much higher than the hydraulic resistance of the membrane, which strongly reduces the permeate flux values compared to the initial permeability.

The monitoring of free phenolic acids, gallic acid and ellagic acid, showed that, for small compounds, retention is not directly linked to the membrane cut-off. Other phenomena appear involving solute/membrane and solute/solute interactions. In particular, the Donnan effect seems to control the passage of gallic acid, and the layer formed on the surface would have participated in the retention of ellagic acid. But the low solubility of these two molecules could also explain part of the evolution of their retention rate. The comparison of organic and ceramic membranes showed that there was different reaction of ellagic acid with these two membranes. For ceramic membrane, increase the pH led to the transformation of both gallic acid and ellagic acid, especially at pH 10, which contributed to the change of retention rates.

An integrated process could be proposed with two cascaded membranes, a first one to recover and concentrate the phenolic compounds retained by the 100 kDa membrane and a second one to concentrate the intermediate size phenolic compounds in the retentate and produce in the permeate low molecular weight phenolic compounds. This process would lead to the concentration of tannins through the 100 kDa membrane and then the concentration of ellagic acid through the 5 kDa membrane. The permeate containing gallic acid would then need to be concentrated and purified.

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