## 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

1  # Purification of artichoke polyphenols by using

2  # membrane filtration and polymeric resins

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30    **Abstract**

31    The present study aimed at evaluating the potential of an integrated process based on the

32    use of membrane technology and adsorbent resins for the recovery, concentration and

33    purification of phenolic compounds from artichoke wastewaters.

34    In particular, artichoke wastewaters coming from the blanching step were pre-treated by

35    ultrafiltration (UF) in order to remove suspended solids and macromolecular

36    compounds. The UF permeate was submitted to a nanofiltration (NF) process producing

37    a concentrated fraction enriched in phenolic and sugar compounds.

38    Three different macroporous resins were tested through adsorption/desorption methods

39    to produce purified phenolic fractions with high antioxidant activity. Samples produced

40    in UF, NF and adsorption-desorption tests were assayed for phenolic composition

41    (chlorogenic acid and apigenin 7-O-glucoside), sugar composition (fructose, glucose

42    and sucrose) and antioxidant activity.

43    Among the three different tested resins, the S 7968 offered the best performance in

44    terms of adsorption/desorption ratio for chlorogenic acid, with a total

45    adsorption/desorption yield (TADY) of 63.39%; for the apigenin 7-O-glucoside the S

46    7968 and the S 2328 resins showed a TADY in the range 68.31-78.45%.

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48    *Keywords:* Artichoke wastewaters; Ultrafiltration; Nanofiltration; Macroporous resins;

49    Phenolic compounds.

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## 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

76 and water extractions to obtain phenolic-rich extracts [13] and inulin [14] from

77 artichoke agroindustrial wastes have been proposed. Separation methods for the

78 enrichment of phenolic compounds from plant-based materials, including liquid-liquid

79 extraction, ultrasound-assisted extraction, heat treatment, enzyme-assisted extraction,

80 supercritical fluid extraction and chromatography have been recently reviewed by

81 Azmir et al. [15]. Unfortunately, most of these methodologies cause the degradation of

82 the targeted compounds due to high temperature and long extraction times as in solvent

83 extractions, or pose some health-related risks due to the unawareness of safety criteria

84 during irradiation. The requirement of costly and high purity solvents with low selective

85 extractions are additional drawbacks for large scale productions.

86 Membrane processes offer several advantages (low temperature, absence of phase

87 transition and low energy consumption) when compared with conventional technologies

88 for concentrating and/or fractionating bioactive phenolic compounds from different

89 vegetable sources. In particular, pressure-driven membrane technologies, such as

90 ultrafiltration (UF) and nanofiltration (NF), have been widely investigated for the

91 recovery and concentration of bioactive compounds from natural products and by-

92 products of their industrial transformation [16,17]. Successful applications include the

93 concentration by NF of biologically active compounds from mate (*Ilex paraguariensis*)

94 [18], *Sideritis* ssp. L. (an endemic plant of the Balcan Peninsula) [19] and coffee

95 extracts [20], the fractionation of proanthocyanidins from winery extracts [21], the

96 recovery of phenolic compounds from bergamot juice [22] and orange press liquor [23].

97 The integration of UF and NF units have been also proposed for the production of soy-

98 protein hydrolysate with high antioxidant capacity [24], for the concentration of

99 anthocyanin extracts from aronia fruits (black chokeberry) [25] and for the enrichment

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100     of polyphenolic compounds relatively to other compounds such as carbohydrates in

101     ethanolic extracts of *Eucalyptus globulus* bark [26].

102     The combination of membrane operations with other conventional separation

103     technologies (i.e. adsorption, precipitation, crystallization) offers new and interesting

104     perspectives in order to increase the selectivity of the process [27]. For example, the

105     combination of adsorption/desorption with UF and UF-NF coupled processes, have

106     been applied to isolate total polyphenols and caffeic acid from *Green tea leaves* [28]

107     and to purify phenolics compounds in distilled grape pomace press liquors in order to

108     increase the antioxidant capacity of the final products [29].

109     In a previous work the combination of two different NF membranes was proposed in

110     order to obtain two enriched fractions containing phenolic compounds and sugars,

111     respectively, from ultrafiltered artichoke wastewaters [30].

112     This work was aimed at evaluating the potential of an integrated system based on the

113     combination of membrane processes and polymeric resins for the selective purification

114     of polyphenols with desirable biofunctional properties from artichoke wastewaters. In

115     particular, artichoke wastewaters were clarified by UF in order to remove

116     macromolecular compounds and suspended solids. The UF permeate was then

117     submitted to a NF process in order to obtain concentrated fractions of phenolic

118     compounds and sugars and a water permeate stream which can be reused in the

119     artichoke processing industry. The NF retentate was submitted to an

120     adsorption/desorption treatment by using three different macroporous resins in order to

121     purify phenolic compounds, such as chlorogenic acid (CA) and apigenin 7-O-glucoside

122     (AOG) from sugars. Fractions coming from the membrane processes (UF and NF) were

123     analyzed for their content in total antioxidant activity (TAA), low molecular weight

124     polyphenols and sugars, while fractions from adsorption/desorption process were

125 analyzed in terms of low molecular weight polyphenols and sugars in order to evaluate

126 the selectivity of each step towards compounds of interest. The performance of UF and

127 NF membranes was also evaluated in terms of productivity (permeate fluxes) in selected

128 operating conditions.

129

130 **2. Material and methods**

131 *2.1. Artichoke wastewaters*

132 Artichoke wastewaters coming from the blanching step were supplied by Conservas

133 Manuel Mateo Candel S.L. (Rafal, Alicante, Spain). Before use, they were filtered

134 through a cotton fabric filter in order to remove most of suspended solids and foreign

135 materials. The prefiltered solutions were stored at -17°C and defrosted before

136 membrane processing. The physico-chemical composition of the UF feed solution is

137 provided in Table 1.

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139 *2.2. Ultrafiltration*

140 Artichoke wastewaters were clarified by using a pilot plant consisting of a 100 L

141 stainless steel feed tank, a pre-filter system equipped with a 10 μm filter cartridge, a

142 centrifugal pump, a feed flow meter, a thermometer, two manometers for the measure of

143 the inlet and outlet pressures and a membrane module. The feed flow-rate and the

144 transmembrane pressure (TMP) values were regulated by a pressure control valve, on

145 the retentate side, and by regulating the pump velocity. A tube and shell heat exchanger,

146 placed after the feed pump, was used to maintain the feed temperature constant.

147 The plant was equipped with a tubular UF membrane module supplied by Tami

148 Industries (Nyons, France) whose characteristics are reported in Table2.

149    Artichoke wastewaters were clarified in selected operating conditions according to a

150    batch concentration configuration (permeate is collected separately and retentate is

151    recycled to the feed tank). In particular, the UF system was operated at a transmembrane

152    pressure (TMP) of 430 kPa, an axial feed flow rate of 4,000 L/h and a temperature of

153    25°C. Experimental runs were performed in triplicate. Permeate flux data were

154    expressed as mean ± SD.

155    After each experiment the membrane was cleaned by using a 0.2% NaOH solution at

156    40°C for 1 h. Then the system was rinsed with tap water for 30 min.

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158    *2.3. Nanofiltration*

159    The clarified artichoke wastewaters were submitted to a NF process performed by using

160    a laboratory plant supplied by Matrix Desalination Inc. (Florida, Usa). The equipment

161    consists of a feed tank with a capacity of 20 liters, a stainless steel housing for 2.4x21

162    inches spiral wound membrane module, a high pressure pump, two pressure gauges (0-

163    4000 kPa) for the control of the inlet and outlet pressures, a pressure control valve and a

164    coiling cool fed with tap water used to maintain the feed temperature constant.

165    The plant was equipped with a NF spiral wound membrane module (Filmtec NF 270)

166    supplied by Dow Chemicals (Minneapolis, USA) whose characteristics are reported in

167    Table 2.

168    NF experiments were carried out according to the batch concentration configuration at

169    an operating temperature of 12 °C, an axial feed flow rate of 300 L/h and a TMP of 800

170    kPa up to reach a weight reduction factor (WRF) of 5.

171    The WRF is defined as the ratio between the initial feed weight and the final retentate

172    weight, according to the following equation:

173    $WRF = \dfrac{W_f}{W_r} = 1 + \dfrac{W_p}{W_r}$                    (1)

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

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176

177    After the artichoke wastewater treatment, the NF membrane was cleaned with a 0.05 %

178    (w/w) NaOH solution at 40°C for 1h. Then the system was rinsed with tap water for 30

179    min.

180    The effect of the UF and NF processes on the recovery of bioactive compounds was

181    measured by the rejection (R) rate according to the following equation:

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

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

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185    *2.4. Polyphenols purification by resin adsorption*

186    *2.4.1. Adsorbents*

187    The final retentate of the NF process was processed by using three different

188    macroporous resins based on polystyrene (Lewatit S 6328 A, Lewatit S 2328 and

189    Lewatit S 7968) from Lanxess (Leverkusen, Germany). The S 6328 A is a strongly

190    basic, macroporous anion exchange resin; the S 2328 is a food grade strongly acidic,

191    crosslinked macroporous cation exchange resin and the S 7968 is a macroporous

192    uncharged adsorbent resin (without fuctional groups).

193    The characteristics of the selected resins are reported in Table 3 according to the

194    manufacturer's information. Before use, adsorbents were activated as follows: 15 g of

195    each resin (wet basis, w.b.) were previously cleaned. For the S 2328 resin, the start-up

196    was performed by a sequence of water cleaning with 6% HCl and back-wash steps. For

197    the start-up of S 6328 A and S 7968 resins, 6% HCl and 4% NaOH solutions were used

198    with distilled water washes in between. Finally, for all resins, distilled water was passed

199    through resins as necessary to reach a pH close to the distilled water pH.

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201    *2.4.2. Dynamic adsorption and desorption tests*

202    Dynamic adsorption and desorption experiments were conducted in a glass column

203    packed with 15 g (w.b.) of pre-treated resins. The bed volume (BV) of the wet-packed

204    resin was 20 mL. In order to properly compare the performance of the 3 resins, the feed

205    volume of concentrated wastewaters for all of them was fixed at 250 mL. Concentrated

206    artichoke wastewaters were pumped to the column at a flow rate of 2±0.2 mL/min using

207    a peristaltic pump (Peristaltic PR-2003, JP Selecta S.A, Spain). Desorption experiments

208    were carried out with ethanol-water (70:30, v/v) solutions at a flow rate of 1±0.2

209    mL/min using a fixed volume of ethanol-water of 110 mL. To follow the adsorption and

210    desorption processes, samples were collected at intervals of 30 min approx. during the

211    adsorption tests and 20 min approx. for desorption runs. All samples were later analyzed

212    by HPLC in terms of CA and AOG.

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

214    1.

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216    *2.4.3. Quantification of the adsorption and desorption ratios*

217    The adsorption ratio was calculated as adsorbed weight/feed weight (%), while the

218    desorption ratio was calculated as desorbed weight/adsorbed weight (%) of the analyzed

219    polyphenols, CA and AOG.

220    For the calculation of the feed and adsorbed weight of CA and AOG, the concentration

221    of the feed and the effluent samples during the adsorption process was plotted against

222    the volume passed through the column. The area under the feed line was the weight

223 passed by the column whereas the area under the effluent samples meant the weight of

224 CA or AOG do not adsorbed. The CA and AOG adsorbed weight was calculated as the

225 difference of these feed and non adsorbed areas. In the desorption process, the CA and

226 AOG desorbed weight was directly calculated as the area of the effluent samples *vs.*

227 volume of ethanol-water passed through the column.

228 The total adsorption-desorption yield (TADY) was calculated as the product of both

229 adsorption and desorption ratios. To normalize and compare results obtained for the

230 investigated macroporous resins the total adsorption-desorption capacity for each resin

231 was expressed as TADY/weight of resin (%/g). Moreover, to reduce the effect of the

232 moisture content of resins, total adsorption-desorption capacities were expressed in

233 terms of the dry weight of resins.

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235 *2.5. Analytical evaluations*

236 *2.5.1 Suspended solids*

237 The suspended solids content was determined by centrifuging at 2000 rpm for 20 min,

238 10 mL of a pre-weighted sample; the weight of settled solids was determined after

239 removing supernatant.

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241 *2.5.2. Total soluble solids (TSS)*

242 Total soluble solids (TSS) were measured by using a hand refractometer (Atago Co.,

243 Tokyo, Japan) with scale range of 0-32 °Brix.

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245 *2.5.3. Identification and quantification of polyphenols compounds by HPLC*

246 The content chlorogenic acid and apigenin-7-O-glucoside was determined by a Waters

247 Alliance 2695 (Milford, MA, USA) HPLC system, equipped with a vacuum degasser, a

248    binary pump, an autosampler, a thermostated column compartment, a model 2996 diode

249    array detector (DAD) and a Empower software (Waters Corporation, Milford, Ireland)

250    for data collection.

251    Chromatographic separation was performed by using a Luna C 18(2) column

252    (250×4.6mm, 5μm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of

253    0.1% of HCOOH in water (eluent A) and 0.1% of HCOOH in acetonitrile (eluent B).

254    The following gradient system was used: 0 min, 90% A and 10% B; 30 min, 50% A and

255    50% B; 35 min, 0% A and 100% B. Analyses were stopped after 50 min. The system

256    was equilibrated between runs for 10 min using the start mobile phase composition. The

257    flow was maintained at 1 mL/min and the injection volume was 10 μL. Diode array

258    detection was between 200 and 600 nm.

259    Prior to HPLC analysis, all samples were filtered by using 0.45 μm nylon filters. All

260    polyphenols were identified by matching the retention time and their spectral

261    characteristics against those of standards. Quantification was made according to the

262    linear calibration curves of standard compounds.

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264    *2.5.4. Identification and quantification of glucose, fructose and sucrose*

265    Analyses of sugars were performed by a high performance anion exchange

266    chromatography coupled with pulsed amperometric detection (HPAEC-PAD). The

267    separation was performed by using a Metrosep Car B (250×4.6mm, from Metrohn)

268    column. The following conditions were used: flux, 1mL/min; temperature of detector,

269    32°C; pressure: 9-10MPa; mobile phase, NaOH 0.1 M (isocratic elution). Prior to

270    HPAEC analysis, all samples were filtered by using 0.45 μm nylon filters and diluted

271    1:25 with bidistilled water.

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273  *2.5.5. Antioxidant activity in "vitro"*

274  The total antioxidant activity in samples coming from the UF and NF processes was

275  measured by the DPPH method [31]. An aliquot of 0.2 mL of diluted sample was added

276  to 3.8 mL of DPPH solution (60 μM in Methanol). The absorbance was measured at $t$=0

277  and $t$=30 min at 515 nm. The antioxidant capacity was expressed as a percentage of

278  inhibition of DPPH radical according to the following equation:

279  % inhibition of DPPH radical $= \left(\frac{Ac(0)-Aa(30)}{Ac(0)}\right) * 100$          (3)

280  where $A_{c(0)}$ is the absorbance of the control at $t$=0 min and $A_{a(30)}$ the absorbance of the

281  antioxidant at $t$= 30 min. Results were expressed as mM Trolox equivalent.

282

283  *2.5.6. Determination of the moisture content of resins*

284  For the determination of the moisture content of resins, 15 g (w.b.) of each resin were

285  spread in a petri dish and left stand at room temperature during 24 h. Afterwards, petri

286  dishes were introduced in a vacuum oven at 60°C and weighted regularly until reaching

287  a constant weight.

288

289  **3. Results and discussion**

290  *3.1. Ultrafiltration of artichoke wastewaters*

291  Figure 2 shows the time evolution of the permeate flux referred to the clarification of

292  artichoke wastewaters by UF in the selected operating conditions.

293  The results showed that the permeate flux declined immediately after starting the

294  process, due to the accumulation of artichoke wastewaters components in the pores

295  (membrane fouling) and on the membrane surface (concentration polarization and gel

296  formation). The $J_p$ vs time curve could be divided in three periods: a first step

297  characterised by a rapid decrease of permeate flux from the initial value of 82 kg/m$^2$h; a

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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/m$^2$h. 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

323  towards glucose (about 35%) using a 15 kDa ceramic membrane in the treatment of

324  liquors from Eucalyptus globulus autohydrolysis.

325

326  *3.2. Nanofiltration of clarified artichoke wastewaters*

327  In order to concentrate the phenolic compounds and sugars in artichoke wastewaters,

328  the UF permeate was processed by NF. The behavior of the permeate flux as function of

329  the operating time and WRF during the NF process, in selected operating conditions, is

330  showed in Figure 3. A decrease in permeate flux (from an initial value of 10.3 kg/m$^2$h

331  up to a steady-state value of about 4 kg/m$^2$h) was observed throughout the time, due to

332  different factors:

333    1) the osmotic pressure increases due to the increment of the concentration of small

334       molecules, mainly sugars and low molecular weights polyphenols, in the

335       retentate, and consequently on the membrane surface;

336    2) increasing of the viscosity of the concentrated fraction;

337    3) fouling phenomena due to the reversible or irreversible adhesion of the

338       molecules on the membrane surface or inside the pores which reduces their

339       diameter.

340  This behavior was similar to that observed by Xu and Wang [36] in the concentration of

341  flavonoids from aqueous *Ginko biloba* extract by NF. These authors obtained permeate

342  fluxes between 5.9 and 9.5 L/m$^2$h operating at pressures of 1200 kPa and temperatures

343  of 35-40 °C. Similarly, Warczok et al. [37] obtained permeate flux values between 1.8

344  and 5.9 L/m$^2$h in the concentration of apple juice operating at 1200 kPa and 30 °C.

345  Operating at lower pressures and temperatures (300 kPa and 24 °C, respectively)

346  Negrão Murakami et al. [18] obtained average permeate fluxes of about 4.53 L/m$^2$h in

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347  the concentration of phenolic compounds from an aqueous mate extract by using a

348  spiral-wound NF membrane with a MWCO of 150-300 Da.

349  A complete recovery (about 99%) of the initial water permeability was obtained after a

350  cleaning of the NF membrane with a 0.05% (w/w) NaOH solution.

351  In Table 4 the content of low molecular weight polyphenols (chlorogenic acid and

352  apigenin 7-O-glucoside), sugars (glucose, fructose and sucrose) and TAA in the

353  different fractions of the NF process is reported.

354  The NF membrane presented a very high retention towards phenolic compounds: no

355  phenolics compounds were detected in the NF permeate allowing to verify the

356  efficiency of the membrane concentration process. The obtained results were also

357  confirmed by the HPLC profile (Figure 4): a remarkable increase of the peaks (1-2)

358  corresponding to the chlorogenic acid and apigenin 7-O-glucoside, respectively, was

359  observed. The concentrated fraction showed a high value of the antioxidant activity in

360  "vitro" (43 mM of Trolox in comparison with 13 mM Trolox of the NF feed) as a

361  consequence of the concentration of phenolic compounds. Accordingly, a low

362  antioxidant capacity was detected in the NF permeate due to the absence of phenolic

363  compounds. This is in agreement with results obtained by Gouveia and Castilho [38]

364  which reported a decrease in the antioxidant potential of artichoke dietary supplements

365  due to the absence of phenolic compounds. A correlation between the caffeoylquinic

366  and cholorogenic acids content and the radical scavenging activities of artichoke has

367  been also reported in literature [6,39].

368  The NF membrane showed also a high retention towards glucose, fructose and sucrose.

369  Consequently, the NF permeate is completely depleted of sugar compounds as showed

370  in Figure 5. This is in agreement with the estimated molecular weight cut-off (250 Da)

371  of the NF membrane and the molecular weight cut-off of the analyzed compounds (in

the range 180-480 g/mol). 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 cholorogenic and caffeic acids, with a cationic Lewatit S 2328 resin as compared to the anion exchange and adsorbent resins.

396    Results related to the desorption process of CA and AOG by elution with 70%

397    ethanol/water are also reported in Table 5. For CA, good desorption ratios, between

398    72.74 and 77.92% were detected for all the investigated resins. For AOG the range of

399    desorption ratios was wider (20.78-91.54%) than that observed for CA, with the S 2328

400    resin showing the highest desorption ratio.

401    Regarding the total adsorption-desorption yield, for the CA it was found that the S 7968

402    resin showed the highest performance (63.39%), while the worst TADY was given by

403    the S 2328 resin (19.40%). For the AOG, the S 6328 A was by far the resin with lowest

404    TADY (20.76%), while S 2328 and S 7968 resins, showed not very different

405    performances (78.45% and 68.31%, respectively). In Table 5 are also listed the TADY

406    results in wet and dry basis. According to these data, the S 6328 A resin presented the

407    lowest TADY (d.b.) for both CA and AOG. The S 2328 resin gave the best performance

408    for AOG but it cannot be selected for CA. On the other hand, the S 7968 resin could be

409    considered suitable for the recovery of both CA and AOG.

410    Table 6 shows the content of sugar compounds in the NF retentate and in the desorbed

411    fractions of each investigated resins. As it can be seen sugar compounds were quite

412    totally recovered in the desorbed fraction independently on the type of resin.

413    According to these results and considering a NF retentate volume to be treated of 1 L,

414    two options based on the sequential use of both S 2328 and S 7968 resins were

415    proposed for the recovery of CA and AOG. In both cases, some amount of polyphenols

416    get retained by resin matrix (less than 25%). For the first option (Figure 6a), expected

417    values for the recovery of CA and AOG in each stream involved are also reported. The

418    first option consists in a previous use of S 2328 resin in which CA and AOG are

419    fractioned so that in the product of the desorption step, most AOG of the feed is

420    obtained, representing the 43.6% of the total polyphenol content in this desorbed

421      stream. On the other hand, most CA from the feed is obtained in the not adsorbed

422      stream, representing the 96.4% of the total polyphenols. In case pure CA would be

423      desirable, the use of S 7968 resin would be recommended.

424      The second option (Figure 6b) is based on the use of the S 7968 resin first and then

425      AOG is completely adsorbed so that a stream of pure CA can be obtained. When

426      desorption of the resin is performed AOG represents only the 17.1% of the total

427      polyphenols. If a major fractionation of CA and AOG is required, the desorbed stream

428      from the S 7968 resin can be used as feed for the S 2328 resin, obtaining a stream of not

429      adsorbed polyphenols with a 96.2% of CA and a desorbed stream containing close to

430      50% of AOG.

431

432      **4. Conclusions**

433      An integrated process based on the use of membrane operations such as UF and NF and

434      adsorbents resins, in a sequential form, was proposed for the concentration and

435      purification of phenolic compounds from artichoke wastewaters.

436      Suspended solids and macromolecular compounds were completely removed from the

437      artichoke wastewaters by UF producing a permeate stream enriched in phenolic

438      compounds and sugars.

439      Phenolic compounds were concentrated by NF with a production of a retentate stream

440      containing about 1.6 g/L of CA and 0.3 g/L of AOG.

441      Among the three different tested resins, the S 7968 offered the best performance in

442      terms of adsorption/desorption ratio for CA, with a TADY of 63.39%; while for the

443      AOG the S 7968 and the S 2328 resins showed a TADY in the range 68.31-78.45%.

444      The global results indicate that the integration of membrane operations with adsorbents

445      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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**Tables**

| Parameters | Feed | Permeate | Retentate |
|---|---|---|---|
| pH | 4.18 ±0.03 | 4.12±0.12 | 4.16 ±0.60 |
| TSS (°Brix) | 2.3±0.1 | 2.3±0.1 | 2.6±0.1 |
| Suspended solids (%) | 3.08±0.08 | n.d. | 3.17±0.07 |
| TAA (mM Trolox) | 13.2±0.2 | 13.0 ±0.2 | 13.1±0.1 |
| Chlorogenic acid (ppm) | 560.1±1.3 | 555.4±1.2 | 556.20±3.0 |
| Apigenin-7-O-glucoside (ppm) | 80.0±1.3 | 75.0±0.2 | 81.0±2.1 |
| Glucose (ppm) | 1422.0±2.5 | 1400.0±3.2 | 1450.0±1.7 |
| Fructose (ppm) | 614.0±2.1 | 600.0±2.3 | 627.0±2.7 |
| Sucrose (ppm) | 350.0±2.4 | 320.0±3.1 | 365.0±3.7 |

Table 2 - Characteristics of UF and NF membranes

| Membrane type | Inside Ceram | NF 270 |
|---|---|---|
| Manufacturer | Tami industries | Dow-Filmtec |
| Material | $TiO_2$ | Polyamide |
| Configuration | tubular | spiral-wound |
| MWCO (Da) | 15,000 | 200-300 |
| Membrane surface area ($m^2$) | 0.1 | 2.6 |
| Maximum operating pressure (kPa) | 1,000 | 4,100 |
| Maximum operating temperature (°C) | 350 | 45 |
| $MgSO_4$ retention (%) | - | >97 |
| pH range | 0-14 | 3-10 |

Table 3 - Characteristics of the macro-porous resins

| Resin type | S 6328 A | S 2328 | S 7968 |
|---|---|---|---|
| Manufacturer | Lewatit | Lewatit | Lewatit |
| Matrix | Crosslinked polystyrene | Crosslinked polystyrene | Crosslinked polystyrene |
| Structure | Macroporous | Macroporous | Macroporous |
| Ionic form as shipped | $Cl^-$ | $H^+$ | Neutral |
| Functional group | Quaternary ammine | Sulphonic acid | None |
| Stability pH range | 0-14 | 0-14 | 0-14 |

Table 4 - General composition of artichoke wastewaters before and after the NF process

| Parameters | Feed | Permeate | Retentate |
|---|---|---|---|
| TSS (°Brix) | 2.4±0.2 | 0.6±0.1 | 6.8±0.2 |
| TAA (mM Trolox) | 13.5±1.5 | 1.0±0.1 | 43.0±1.6 |
| Chlorogenic acid (ppm) | 550.0±5.0 | n.d | 1620.0±7.1 |
| Apigenin-7-O-glucoside (ppm) | 70.0±4.5 | n.d. | 310.0±5.6 |
| Glucose (ppm) | 1381.0±2.4 | n.d. | 5771.0±2.2 |
| Fructose (ppm) | 530.0±5.1 | n.d | 2704.0±4.3 |
| Sucrose (ppm) | 274.0±2.7 | n.d | 1360.0±3.4 |

| Resin | Adsorption (%) | Desorption (%) | TADY (%) | TADY (%)/g resin (w.b.) | TADY (%)/g resin (d.b.) |
|---|---|---|---|---|---|
| *Chlorogenic acid* | | | | | |
| Lewatit S 6328A | 38.38±1.47 | 75.74±3.29 | 29.07±2.38 | 1.94±0.04 | 3.78±0.12 |
| Lewatit S 2328 | 26.65±1.54 | 72.81±8.10 | 19.40±3.28 | 1.29±0.05 | 3.96±1.14 |
| Lewatit S 7968 | 81.35±1.91 | 77.92±1.26 | 63.39±2.51 | 4.23±0.06 | 10.93±0.55 |
| *Apigenin 7-O-glucoside* | | | | | |
| Lewatit S 6328A | 99.88±6.79 | 20.78±0.91 | 20.76±2.32 | 1.38±0.07 | 2.70±0.18 |
| Lewatit S 2328 | 85.70±6.42 | 91.54±4.38 | 78.45±9.63 | 5.23±0.21 | 16.01±1.14 |
| Lewatit S 7968 | 100±6.23 | 68.31±1.55 | 68.31±5.81 | 4.55±0.12 | 11.78±0.55 |

Table 6 - Concentration of glucose, fructose and sucrose in samples before and after the treatment with the tested resins

|  | Resin | NF retentate (ppm) | After treatment with resin (ppm) |
|---|---|---|---|
| *Glucose* |  |  |  |
|  | Lewatit S 6328A | 5341.0±10.4 | 5311.0±15.6 |
|  | Lewatit S 2328 | 5523.0±6.3 | 5496.0±11.6 |
|  | Lewatit S 7968 | 5638.0±8.4 | 5622.0±9.2 |
| *Fructose* |  |  |  |
|  | Lewatit S 6328A | 2596.0±6.2 | 2596.0±5.5 |
|  | Lewatit S 2328 | 2662.0±14.6 | 2625.0±8.8 |
|  | Lewatit S 7968 | 2685.0±10.4 | 2634.0±9.4 |
| *Sucrose* |  |  |  |
|  | Lewatit S 6328A | 1346.0±5.7 | 1326.0±8.4 |
|  | Lewatit S 2328 | 1355.0±9.4 | 1348.0±10.5 |
|  | Lewatit S 7968 | 1388.0±12.5 | 1375.0±9.7 |

## 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 m$^3$/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.
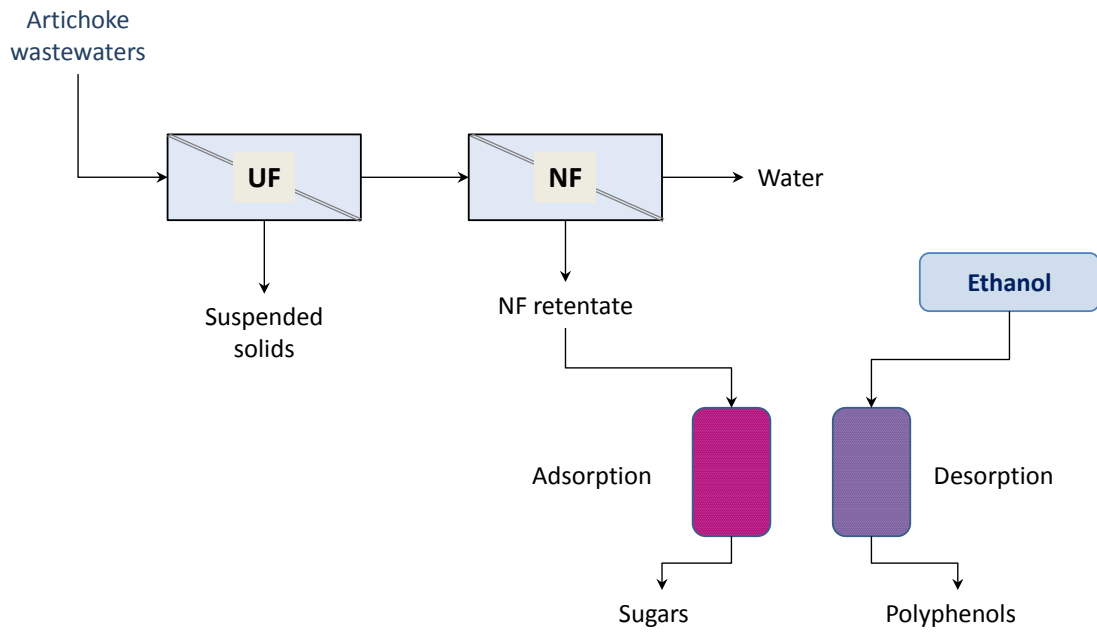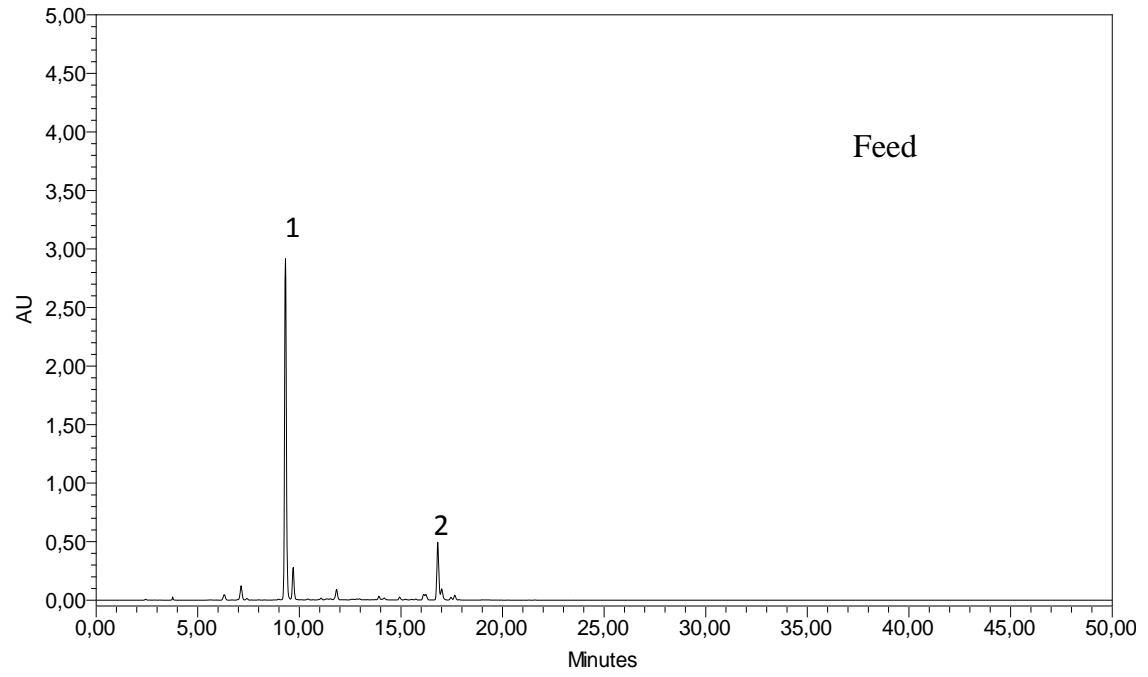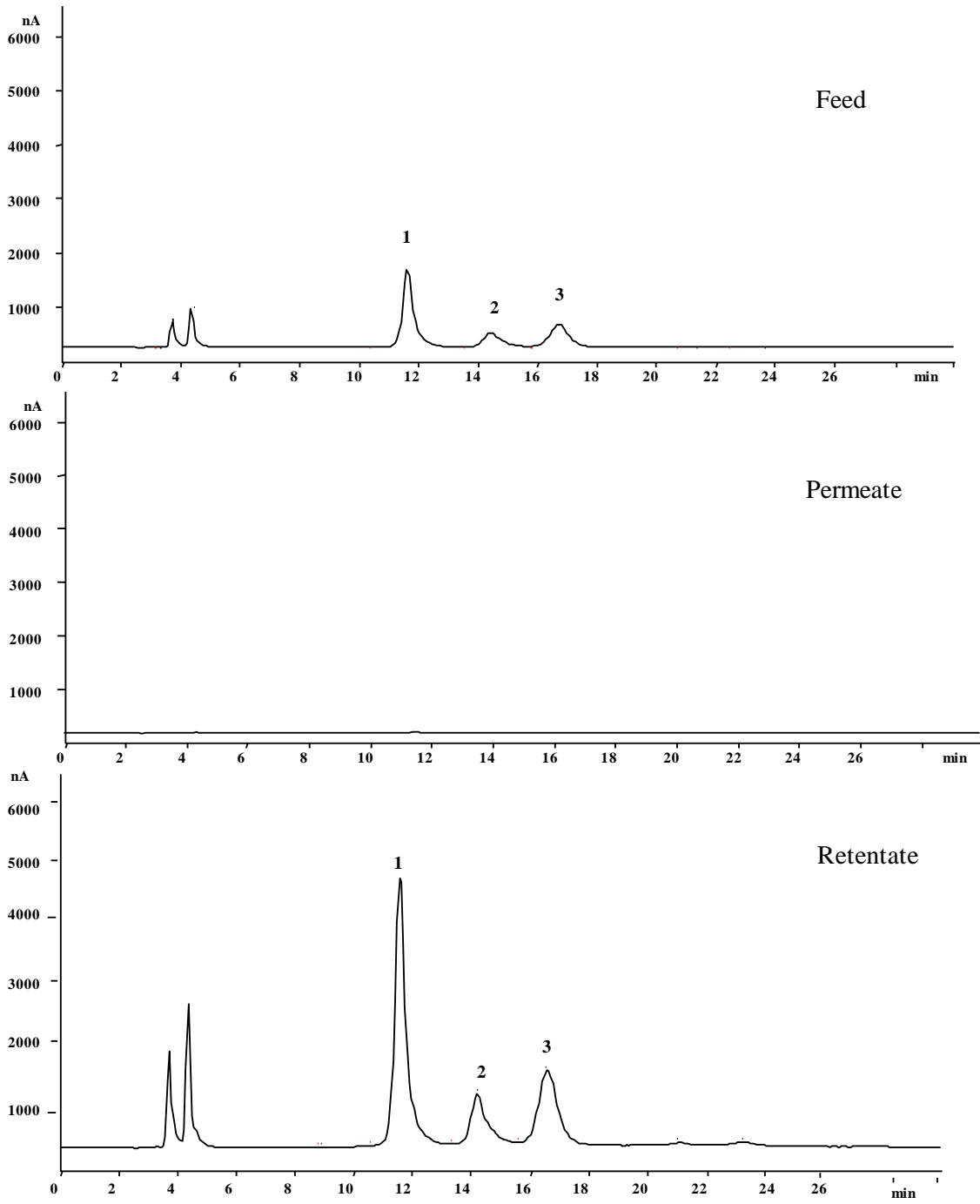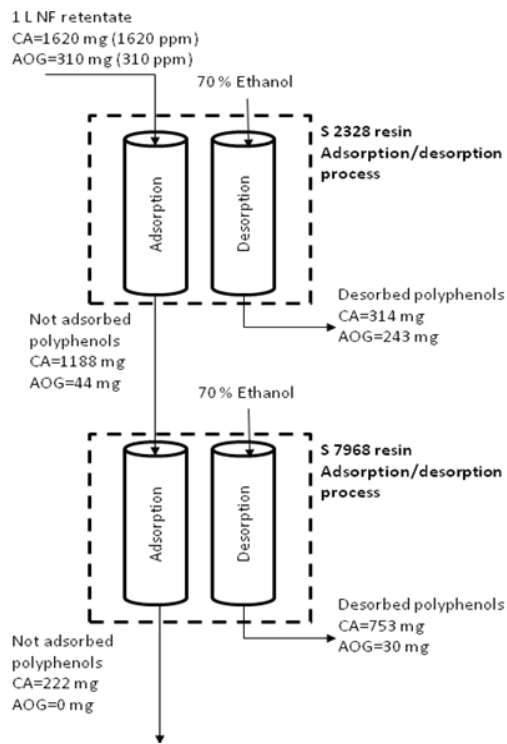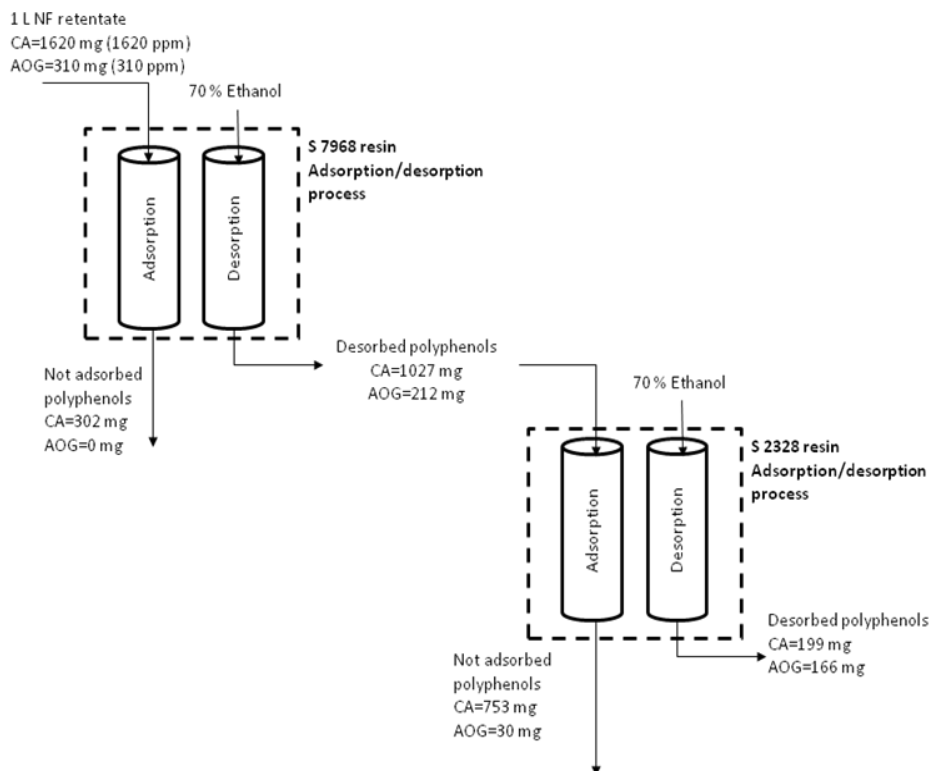
**Figures**
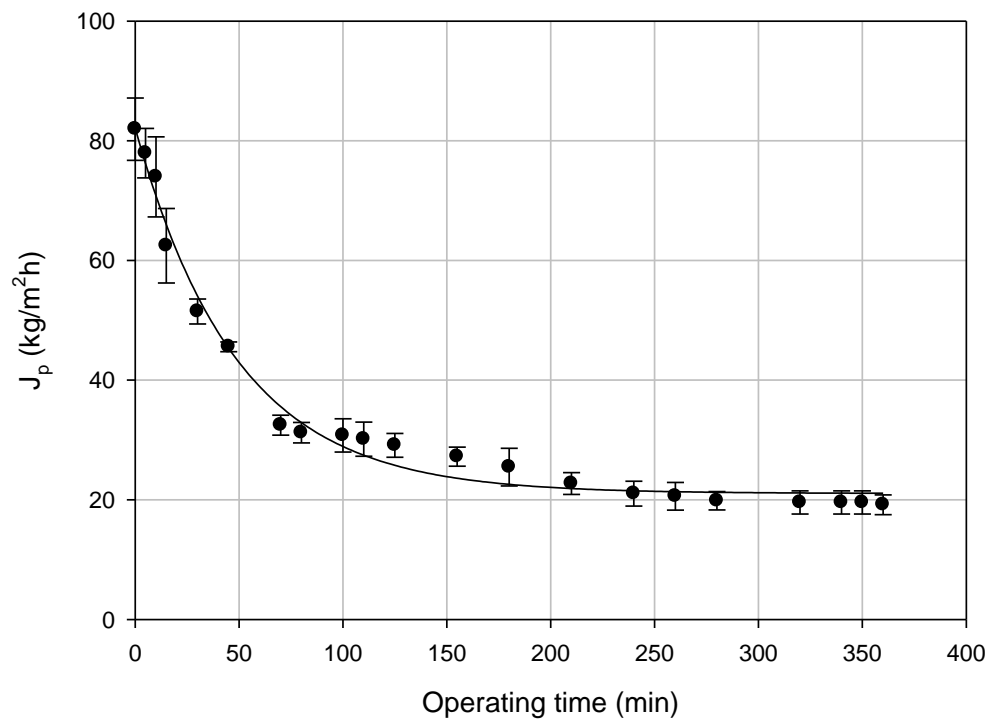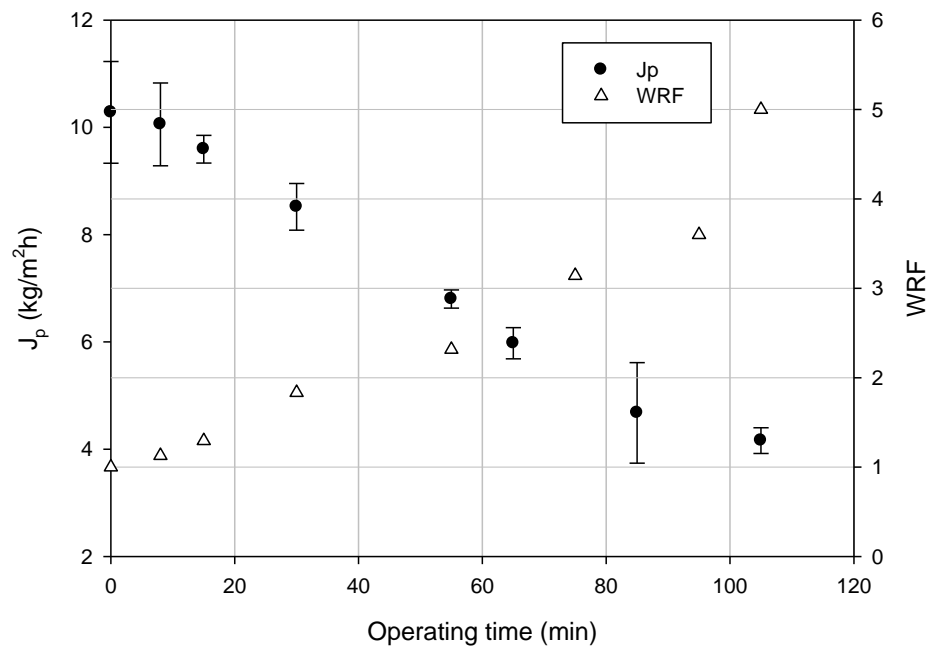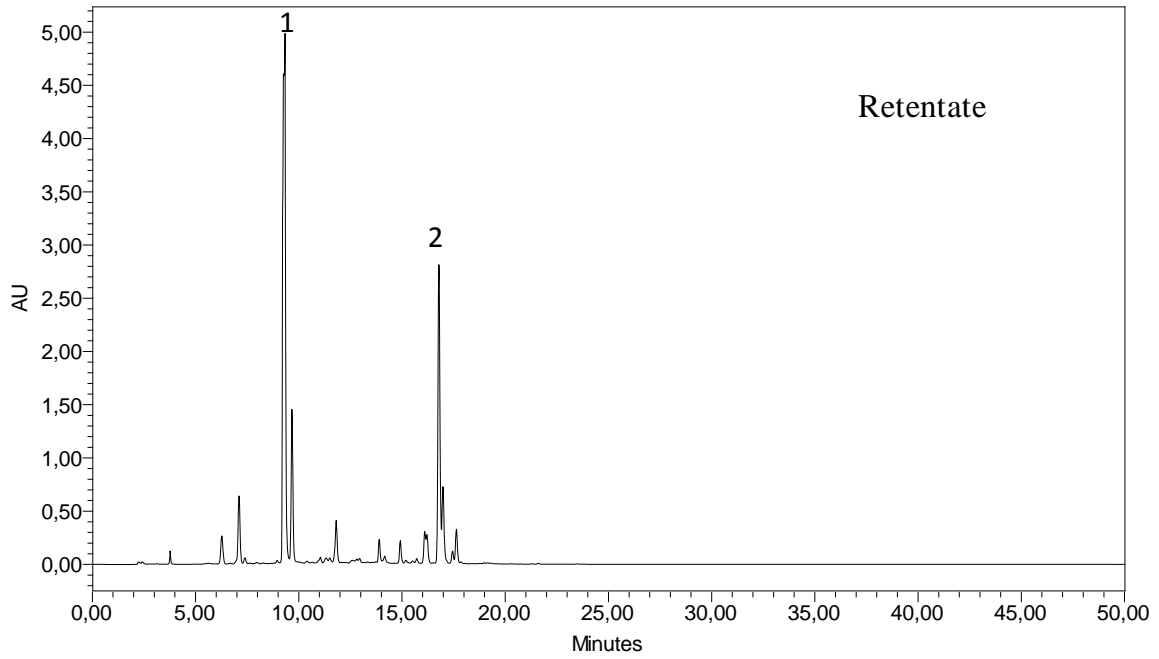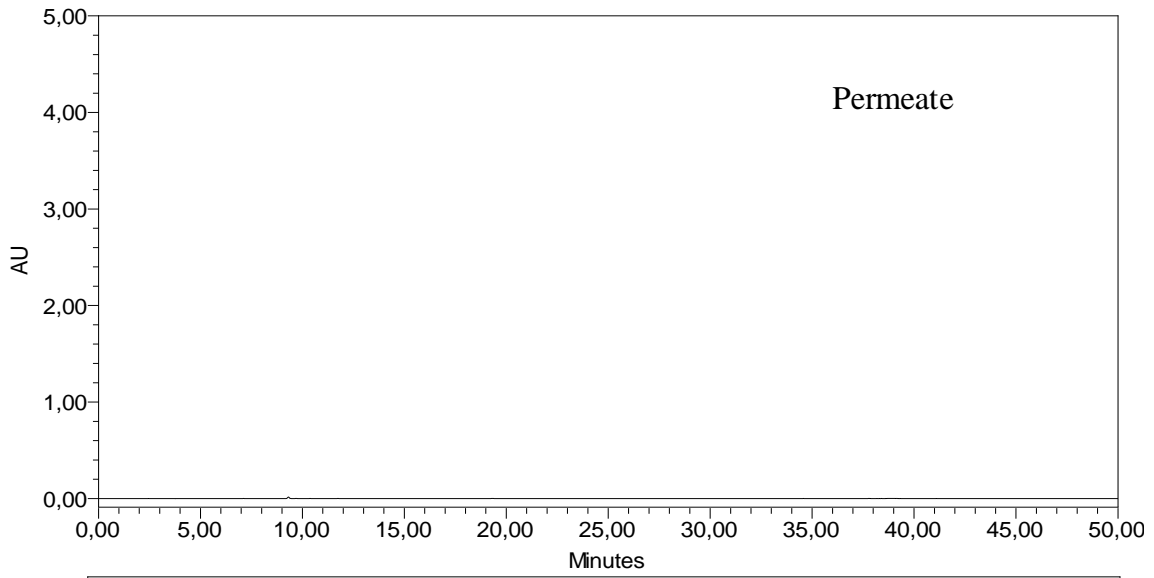


FIGURE 1

<mark>FIGURE 3</mark>

FIGURE 4

FIGURE 5

(a)



(b)

FIGURE 6