1 **Selective extraction of high–value phenolic compounds from distillation**

2 **wastewater of basil (*Ocimum basilicum* L.) by pressurized liquid extraction.**

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22   **Abstract**

23   During the essential oil steam distillation from aromatic herbs, huge amounts of

24   distillation wastewaters (DWWs) are generated. These by-products represent an

25   exceptionally rich source of phenolic compounds such as rosmarinic acid (RA) and

26   caffeic acid (CA). Herein, the alternative use of dried basil DWWs (dDWWs) to

27   perform a selective extraction of RA and CA by pressurized liquid extraction (PLE)

28   employing bio-based solvent was studied. To select the most suitable solvent for PLE,

29   the theoretical modelling of Hansen solubility parameters (HSP) was carried out. This

30   approach allows reducing the list of candidate to two solvents: ethanol and ethyl lactate.

31   Due to the composition of the sample, mixtures of water with those solvents were also

32   tested. An enriched PLE extract in RA (23.90 ±2.06 mg g^{-1} extract) with an extraction

33   efficiency of 75.89 ±16.03 % employing a water-ethanol mixture 25:75 (% v/v) at 50ºC

34   was obtained. In the case of CA, a PLE extract with 2.42 ±0.04 mg g^{-1} extract, having

35   an extraction efficiency of 13.86 ±4.96 % using ethanol absolute at 50ºC was achieved.

36   DWWs are proposed as new promising sources of natural additives and/or functional

37   ingredients for cosmetic, nutraceutical and food applications.

1.   Introduction

Currently, one of the most important worldwide concerns is related to the disposal of agro-industrial wastes. The economic and environmental costs that those wastes generate have become an issue of vital importance for government institutions, in order to respond to the great challenge of the implementation of sustainable environments. The concept of sustainability must be understood as a rational way of improving processes, minimizing the environmental impact. For example, the essential oils play an important commercial function in food, cosmetic and pharmaceutical industries, because they have been traditionally used in many fields such as flavouring and bioactive products [1]. Usually, the essential oils are obtained from raw parts of aromatic plants (leaves, seeds, fruits, bark, roots) by steam distillation [2]. However, the average yields of essential oil distilled is usually below 5 % (w/w) and consequently a huge amount of liquid and solid residues are produced [3] that may cause environmental problems if they are not correctly disposed [4,5]. During the extraction/separation processes, two undesirable products are generated: a) residual plant material and b) distillation wastewaters (DWWs). In particular, this latter one is produced from relapse of condensation water on the vegetable matrix and it is usually discarded, although it is known that is rich in phenolic compounds and represents an unexplored by-product [6]. Zheljazkov and Astatkie have recently proposed the use of DWWs obtained from several aromatic plants as a foliar spray, in order to evaluate their bioactive effect on growth, productivity, essential oil content and composition of peppermint (*Mentha piperita* L.). The results showed that the concentration of menthyl acetate and L-limonene in the essential oil composition of peppermint increased when the DWWs obtained from distillation of *Salvia officinalis* L. and *Rosmarinus officinalis* L. were employed [7]. In addition, Wollinger *et al*., studied the DWWs of rosemary obtained by

steam distillation and hydrodistillation, in terms of rosmarinic acid, carnosic acid and antioxidant activity [8]. Their results showed that during distillation processes a significant amount of rosmarinic acid is unavoidably transferred to DWWs, which can result promising in order to obtain a high added value from this waste. Also, Celano *et al*., characterized the DWWs produced by the distillation of packaged fresh basil, rosemary and sage wastes. HPLC-DAD-HRMS profiling revealed that DWWs contain many water-soluble phenolic compounds, mainly caffeic acid derivatives and flavonoid glycosides, with rosmarinic acid (RA) as predominant component (29–135 mg 100 mL^{-1}). A strong antioxidant activity of the extracts was demonstrated by three *in vitro* AOC methods and it was correlated to the high content of RA and other phenolic compounds [6].

On the other hand, basil is an important *Lamiaceae* aromatic plant, which is cultivated worldwide, especially in Mediterranean area. The basil leaves were used like a spice to give flavour to food and in traditional medicine [9] as carminative, stomachic and antispasmodic [10] and its extracts show antibacterial, antifungal and antioxidant activities [11]. Moreover, basil is characterized by high levels of phenolic compounds [12]. The most important phenolic acids are rosmarinic acid and caffeic acid (CA). RA is the ester that is formed between caffeic acid and 3,4-dihydroxyphenyllattic acid; its biological and functional activities are well known, such as antibacterial, anti-inflammatory, antiviral and antioxidant activities [13]. Likewise, CA is a hydroxycinnamic acid widely distributed in the plant kingdom with antioxidant and free radical scavenging properties [14]. Taking into consideration the need for sustainable processes, the use of green processes have increased for obtaining bioactive compounds, especially natural antioxidants. Pressurized liquid extraction (PLE) is one of them, which is based on use of solvents at elevated temperature (above their atmospheric

88   boiling point) and pressure. At those conditions, the properties of the solvents are

89   modified, causing an enhanced mass-transfer rate [15]. PLE has already been shown as

90   a powerful extraction tool to extract a variety of bioactive compounds from medicinal

91   herbs and food by-products [16, 17]. The solvents normally used to extract polar

92   compounds are water, ethanol or mixture of both. Ethanol has been recognized as safe

93   (GRAS) but also ethyl lactate, a renewable solvent, has been considered as food grade

94   solvent [18]. In the literature, there are several papers regarding PLE using ethanol and

95   water but just few researchers have addressed this issue employing ethyl lactate to

96   extract phenolic compounds [19, 20, 21]. With this perspective, this work focuses on the

97   recovery of bioactive compounds from dried DWWs (dDWWs) of basil (*Ocimum*

98   *basilicum* L.) by PLE using green solvents. In order to improve the selectivity of PLE

99   process, the estimation of the Hansen solubility parameters (HSP) had been successfully

100   employed as a very useful tool to select a suitable solvent for the extraction of target

101   compounds [22, 23, 24, 25, 26]. Thus, this paper presents a new approach to reuse of

102   basil dDWWs seeking to a selective extraction of rosmarinic acid and caffeic acid by

103   PLE using different type of bio-solvents, according to the theoretical modelling of

104   Hansen solubility parameters.

105   **2.   Materials and methods**

106   **2.1   Samples and reagents**

107   Dried basil leaves (*Ocimum basilicum* L., type "Genovese") were obtained from local

108   farms in a south region of Italy (Capaccio, Salerno, Italy) in November 2015. Ultrapure

109   water was obtained using a Millipore system (Billerica, MA, USA), absolute ethanol

110   and ethyl lactate provided from VWR chemicals (Fontenay-sous-Bois, France) were

111   utilized for PLE. Rosmarinic acid (RA ≥98 %), caffeic acid (CA ≥98 %) and sea sand

112   were purchased from Sigma Aldrich (Madrid, Spain). Water ultrapure grade, methanol

113 and formic acid (≥95 %) for HPLC analysis were provided from VWR chemicals

114 (Fontenay-sous-Bois, France).

115

116 **2.2   Steam distillation for essential oil extraction**

117 Steam distillation was performed using a semi-industrial apparatus (Tred Technology,

118 Campobasso, Italy). Five kilograms of dried basil leaves were loaded on a perforated

119 grid in the steam distillation equipment and the essential oil distillation was carried out

120 employing 6 L of water at 75 °C, for an extraction time of 60 min. The DWWs obtained

121 after extraction were filtered with glass fibber filter (Millipore® glass-fibber filters, type

122 2, pore size 1 µm) and stored at -20 °C until freeze-drying. For this last process, a freeze

123 dryer (Alpha 1-2 LD, Christ, Germany) was employed and the yield after lyophilisation

124 was 2.26 g / 100 g of raw DWW.

125

126 **2.3   Solubility parameter estimation: modeling**

127 The Hansen solubility parameters provide a numerical estimate of the degree of

128 interaction between materials, and can be a good indication of their solubility [27].

129 Those parameters are based on the contribution of three cohesion energies: E_D,

130 dispersion energy (related to the Van der Waals forces), E_P, polarity energy (related to

131 dipole moment), and E_H, hydrogen bonding energy. Dividing each energy by the molar

132 volume (V_m) gives the square of the total solubility parameter (δ_T^2) as defined by

133 equation (1):

134

135 $$\delta_T \left(MPa^{\frac{1}{2}} \right) = \sqrt{\left(\frac{E_D}{V_m}\right)^2 + \left(\frac{E_P}{V_m}\right)^2 + \left(\frac{E_H}{V_m}\right)^2} = \sqrt{\delta_D^2 + \delta_P^2 + \delta_H^2} \qquad \text{Eq. (1)}$$

136

137 Based on this approach, the theoretical modelling of solubility parameters was

138 calculated as described by Sánchez-Camargo *et al*., [23]. HSPiP® software (Version

139 5.0, UK) was used for the theoretical estimation of HSP of rosmarinic and caffeic acid

140 in different bio-solvents employing the Yamamoto–Molecular Break (Y-MB) method.

141 To carry out the estimation, the canonical SMILES notation (Simplified Molecular

142 Input Line Syntax) for RA and CA were obtained by the PubMed website database.

143 From the solvent optimizer option in the software, two bio-based solvents (ethanol and

144 ethyl lactate) were selected between 101 solvents, according to the *Ra* term as criteria,

145 which refers to the relative energy distance between solute and solvent as given by

146 Equation (2). The smaller *Ra* corresponds to the greater affinity between solute and

147 solvent. In the equation 2, subscript *i* refers to the solute and *j* refers to the solvent.

148 $$R_a = \sqrt{4\left(\delta_{Di-}\delta_{Dj}\right)^2 + \left(\delta_{Pi-}\delta_{Pj}\right)^2 + \left(\delta_{Hi-}\delta_{Hj}\right)^2} \quad \text{Eq. (2)}$$

149 As described below, bio-based solvents were selected to perform the PLE at different

150 subcritical conditions. In addition, mixtures of water with these bio-solvents were

151 proposed in order to test the possibility of employing crude DWW, without a previous

152 freeze-drying step. Thus, for the HSP estimation at pressurized conditions, Jayasri and

153 Yaseen method [28] was used to evaluate the temperature dependence of the solubility

154 parameter for the phenolic compounds. Since this method employs critical

155 temperatures (Tc), Marrero & Gani [29] group contribution method was used for the

156 estimation of critical data. On the other hand, the physical properties of the subcritical

157 bio-solvents were taken from Pereira *et al*. [18] and were calculated following the

158 Gunn-Yamada method. The effect of pressure and temperature at subcritical conditions

159 was assessed employing the integrated form proposed by Williams *et al*. [30]. For

160 mixtures consisting of water and ethanol or ethyl lactate, the solubility parameters of the

161   mixed fluid were determined by the equation (3), where Φ is the volume fraction of

162   water or ethanol or ethyl lactate.

163      $\delta_{Mix-D,P,H} = \Phi_{H_2O} \times \delta_{H_2O} + \Phi_{Ethanol/Ethyl\ lactate} \times \delta_{Ethanol/Ethyl\ lactate-D,P,H}$ Eq. (3)

164   **2.4   Pressurized liquid extraction**

165   All the extractions were performed using an Accelerated Solvent Extraction system

166   ASE 200 (Dionex, Sunnyvale, CA, USA), equipped with a solvent controller unit. Each

167   extraction was carried out at 10 MPa in 11-mL stainless steel extraction cell containing

168   250 mg of dDDWs and 4 g of sea sand, as dispersive agent. The extraction time (static

169   mode) was set at 20 min after preliminary experiments. PLE was performed using five

170   different percentage of water in ethanol and ethyl lactate (100:0, 75:25, 50:50, 25:75,

171   0:100, % v/v) at three different temperatures, 50, 100, 150 °C. All the experiments were

172   carried out in duplicate. Extracts obtained employing water, ethanol or their mixture

173   were freeze dried after removing organic solvent with vacuum rotary evaporator before

174   chromatographic analysis. Extracts obtained using water and ethyl lactate or mixture of

175   both were reconstituted up to a final volume of 30 mL with water and then submitted to

176   the chromatographic analysis. The extraction yield was determined for each extract

177   gravimetrically using the following equation:

178      $Extraction\ yield\ \% = \frac{g\ PLE\ extract}{g\ dDWW} * 100$ Eq. (4)

179   **2.5   Quantitative analysis by HPLC-UV**

180   Chromatographic analysis was performed using an Accela UHPLC system (Thermo

181   Scientific, San Jose, CA, USA). The chromatographic separation was performed in

182   gradient elution mode at flow rate of 400 μL min^{-1} using a Hipersil Gold C18 column

183   1.9 μm, 2.1x 50 mm (Thermo Scientific, San Jose, CA, USA). A gradient of water (A)

184   and methanol (B), both with 0.1 % formic acid, was applied starting at 95 % A (0-

185   1min), from minute 1 to 5 linearly increase to 80 %A and held to 1.5 min, then increase

186   to 75 % A at min 8, 65 % A at min 10 held for 5 min and finally change to 40 % A at

187   min 20. The column temperature was maintained constant at 30 °C during the entire

188   chromatographic run and the injection volume was 10 μL. The detection was performed

189   at the selective wavelength of 324 nm for CA and 330 nm for RA, but also spectra were

190   recorded in the range 200-600 nm. Peaks were identified by comparing the UV spectra

191   and retention time with standards available. Stock solutions of CA and RA were

192   prepared in methanol at 5 mg mL^{-1} and stored in dark at 4 °C. Calibration curves were

193   carried out in triplicate and they were prepared with appropriate dilutions of the stock

194   solutions in water (CA range 0.5-30 μg mL^{-1}, 11 levels; RA range 1-50 μg mL^{-1}, 11

195   levels). Calibration curves were constructed using external calibration method and

196   linearity was evaluated by ANOVA test (CA y=141467x+12810 R^2=0.9993; RA

197   y=68232x-22892 R^2=0.9998). All the PLE extracts and dDWW were analysed at 1 mg

198   mL^{-1} (filtered before injection). For each experiment, CA and RA content was

199   calculated expressed as mg g^{-1} extract. Also, the percentage of extraction efficiency,

200   was calculated using equation 5:

201   $Extraction\ efficiency\ \% = \frac{mg\ CA\ or\ RA\ extracted\ by\ PLE}{mg\ CA\ o\ RA\ original\ dDWW} * 100$ Eq. (5)

202   where, the numerator was inferred by means of the extraction yield % and mg RA or

203   CA g^{-1} extract in each extract; and the denominator refers the initial content CA or RA

204   per gram of dDWW, as percentage.

205   **3.   Results and discussion**

206   **3.1   HSP approach: A Theoretical selection of the bio-solvents**

207   In the present work, RA and CA were selected as target solutes to estimate their

208   solubility parameters in different green solvents. The bio-based solvents have the

209   advantage of being obtained from renewable resources, thus providing an alternative

210 replace of the petroleum-based products [31]. In this sense, the Hansen approach can be

211 employed in the development of greener selective processes for the recovery of valuable

212 compounds, helping to reduce the number experiments with different solvents [24, 25,

213 27, 32]. As explained in section 2.3, by entering the SMILES values of the target

214 molecules in HSPiP software, the Yamamoto molecular break method can provide a

215 preliminary screening of the more suitable solvents to obtain extracts enriched in RA

216 and CA. Through the software, it was possible to test up to 101 organic solvents,

217 however, according to *Ra* value (Eq. 2) just ethanol and ethyl lactate were selected as

218 promising solvents for the selective extraction of RA and CA. It is worth mentioning

219 that other organic solvents such as benzyl alcohol, tetrahydrofurfuryl alcohol and

220 cyclohexyl alcohol can dissolve RA and CA more effectively than the selected solvents

221 (lower *Ra* value), but due to their toxicity and hazardous conditions, they have not been

222 considered suitable for this study. Table SI-1 (Supporting information) summarizes the

223 HSP estimation of Hansen solubility parameters of RA, CA and bio-based solvents

224 selected at subcritical conditions. According to the molecular structure (Figure SI-1a),

225 the total solubility parameter of RA is highly influenced by the dispersion force

226 (δ_D=20.8), mainly due to the presence of a backbone chain (with a carboxyl group) and

227 two dihydroxyphenyl groups in each end on the molecule. On the other hand, the

228 similar high effect showed by the dispersion (δ_D=20.7) and hydrogen forces (δ_H=19.0)

229 on its total solubility parameter is in agreement with the CA structure (Figure SI-1b). In

230 addition, as can be seen in Table SI-1, the HSP of both phenolic compounds varies only

231 slightly with the temperature, keeping the individual values of each parameter almost

232 invariant. In contrast, for the bio-solvents under subcritical conditions, an increase in the

233 temperature causes a decrease in the partial solubility parameters, and hence, in the total

234 solubility parameter. Regarding the effect of pressure, several authors have point out

235   that it does not cause a significant influence on the individual solubility parameters

236   below its critical point [30, 32], for this reason only values at 10.0 MPa are given. As

237   discussed above, the Ra value gives a good idea about the optimal solvents for a

238   selective enrichment of the target solutes. Due to the polarity of CA and RA, the

239   influence of the addition of a percentage of water has been studied, since in addition, the

240   raw residue (DWW) is in aqueous phase. Ethanol and ethyl lactate are both miscible in

241   water, so it is possible to confirm predictive data with experimental results. The results

242   of Ra for different solvent mixtures are presented in Table 1(a-b). The lighter shades

243   correspond to the lower values of Ra, while the darker shades correspond to the higher

244   values of Ra or less appropriate solvent/solvent mixtures' conditions. From the

245   estimations, pure ethyl lactate at low temperatures (50 ℃) appears to be the best solvent

246   for RA (Table 1a, Ra=11.3), however, ethanol absolute or water: ethyl lactate (25:75 %

247   v/v) mixture can offer similar low values of Ra too (Ra=13.4 and Ra=13.1,

248   respectively). On the other hand, ethanol absolute seems to be the most suitable to

249   solubilize CA (Table 1b). Nevertheless, interestingly, a mixture of ethyl lactate

250   employing 25 % v/v of water (at 50 ℃) shows a lower value of Ra (Ra=10.9),

251   indicating that could be more appropriate as selective solvent. In addition, it can be

252   observed that up to 50 % v/v of water in the mixture, an increment in the temperature

253   raises the Ra value, suggesting a worst affinity of solute-solvent under these conditions.

254   In contrast, employing 75 and 100 % v/v of water, an increase of temperature decreases

255   the Ra value, which could make possible to use water in the solvent mixture.

256

257   **3.2**   **Characterization of dry basil distillation wastewater**

258   In our previous work, a separation methodology was developed for a complete

259   characterization of the chemical profile of this kind of by-product and it was reported

260   the identification of 36 secondary metabolites, mainly CA derivatives and flavone

261   glycosides [6]. Before the PLE experiments, the basil DWW was lyophilized and

262   submitted to chemical characterization, in order to know the composition of the starting

263   material using the chromatographic conditions reported above. The chemical profile

264   showed that RA was the most abundant compound (Figure SI-2, peak at 13.13 min).

265   Moreover, CA is another key compound (Figure SI-2, peak at 4.94 min) and some other

266   peaks correspond to caffeic acid derivatives (Figure SI-2, caftaric acid - 3.08 min,

267   fertaric acid - 4.77 min, chicoric acid - 7.17 min). The main compounds were

268   characterized evaluating retention time and UV spectra. The starting concentration of

269   RA and CA in dDWWs was 13.40 ±0.27 and 0.87 ±0.04 mg g^{-1} extract, respectively.

270   **3.3   Selective extraction of RA and CA acid by PLE**

271   As mentioned previously, based on Hansen solubility parameters, the bio-based green

272   solvents selected for the selective recovery of RA and CA by PLE were ethanol, ethyl

273   lactate and water. Among the variables involved in PLE, the type of solvent and

274   temperature of extraction are the most determinant factors [15]. For these reason three

275   temperatures were tested: 50, 100 and 150 ºC for a 20 min single-cycle at 10.0 MPa.

276   The results on PLE extraction yield (%) are presented in Table 2, for water-ethanol and

277   water-ethyl lactate mixtures. As expected, an increase in the percentage of water in the

278   solvent mixture, increases the extraction yield (%) due the nature of the waste.

279   Maximum extraction yields, close to 100 %, were obtained employing pure water. On

280   the other hand, when raising the temperature, extraction yield also increases, mainly due

281   to an improvement on mass transfer phenomena. In general, at the conditions proposed,

282   both ethanol and ethyl lactate in the aqueous mixtures, exhibited similar values in terms

283   of extraction yield (%).

284 Results regarding the content of target analytes, RA and CA, to be extracted (expressed

285 as mg of compound per g of extract) are reported in Figure 1 (a-b) and Figure 2 (a-b),

286 respectively. For RA, the highest extraction yield was achieved using 75:25 (% v/v)

287 ethanol – water mixture at 50 ℃ (23.90 ±2.06 mg RA g^{-1} extract, Figure 1a). It is worth

288 to note that RA concentration increased in this extract 1.8-fold compared to the

289 corresponding concentration in the original raw material dDWW. In addition, it was

290 observed that an increment in temperature cause an important loss of RA in the final

291 extract, except when pure water is employed, as predicted by HSP approach.

292 Concerning the mixtures employing ethyl lactate, the maximum amount of RA

293 extracted (17.78 ±0.29 mg RA g^{-1} extract) was obtained using 25 % of water in the

294 mixture at 150℃, even though very similar results were evidenced at 50 and 100 ℃

295 (Figure 1b). Surprisingly, this concentration was 25.6 % lower compared to the results

296 obtained employing hydro-alcoholic mixtures. Nevertheless, in order to assess the

297 solvent selectivity, the RA/CA ratio was calculated. Between all the solvent mixtures

298 tested, the highest RA/CA ratio (24.17) was obtained employing pure ethyl lactate at

299 low temperature (50 ℃), according to the HSPs estimations. The effect of temperature

300 did not show a big influence on the RA extraction and similar values were obtained at

301 50, 100 and 150 ℃, except when pure ethyl lactate was employed. This fact may be due

302 to the change in viscosity of ethyl lactate with temperature. The viscosity of ethyl

303 lactate is high (2.4 η/mPa*s at 25 ℃, 1.5 η/mPa*s at 45 ℃ according to literature data

304 [33] and a temperature increase may enhance mass transfer coefficients. It is interesting

305 to note that in both cases, the addition of 25 % of water in the solvent mixture increases

306 the extraction of RA in a greater extent. Nevertheless, higher water contents do not

307 show a significant effect on the extraction of this compound.

308   About the selective extraction of CA (Figure 2, a-b), results showed that pure ethanol at

309   low temperature (50 °C) permits to achieve its maximum concentration in the extracts

310   (2.42 ±0.04 mg g^{-1} extract, 2.8-fold compared to the original concentration in the raw

311   material dDWW). Under these conditions, the highest CA/RA (0.232) ratio was also

312   achieved, being these results in agreement with the above discussed issue. In addition,

313   this value was 1.65-fold higher than the one obtained using water-ethanol mixture 25:75

314   (% v/v) (1.46 ±0.08 mg g^{-1} extract). No significant differences were observed among

315   the other mixtures. For water-ethyl lactate mixtures (Figure 2b), the condition 25:75 (%

316   v/v) at 150 ºC provided the highest CA concentration in the extracts, as estimated by

317   HPS approach (Table 1b). As explained before, an increment in the temperature could

318   cause a decrease of ethyl lactate's viscosity, enhancing the extraction process. The other

319   conditions explored showed similar values of CA concentration and the temperature had

320   no influence, except when pure ethyl lactate was used. It is worthwhile mentioning that

321   some differences observed between experimental and theoretical approach can be

322   explained since HSP are based on thermodynamic data. Kinetics phenomena, which are

323   influenced by diffusion and mass transfer coefficients, whose in turn, are highly

324   dependent to the temperature, are not taken into account in HSP approach. This can be

325   considered the most important limitation of the HPS in the extraction processes. Despite

326   of this, HSP can be employed as a very useful tool to assess the possibility to dissolve a

327   solute in the most suitable solvent for a given application. Taken together, these results

328   suggest two ways of analysis: the enrichment of RA or CA in the extracts or the

329   selective extraction of each of them.

330   **3.4   Extraction efficiency of RA and CA**

331   Once the experimental results in terms of extraction yield (%) and mg RA or CA g^{-1}

332   extract were considered, another important variable that must be taken into account is

333 the extraction efficiency % as defined by the Eq. 5. Table 3 reports the extraction

334 efficiency % of RA and CA employing water-ethanol and water-ethyl lactate mixtures.

335 As can be observed, the addition of 25 % of water in the solvent mixtures improves the

336 extraction recovery from low values up to high recoveries of RA of an average value of

337 80 %. Total recoveries (100 %) were obtained in the other conditions (50, 75 and 100 %

338 v/v) and no significant differences were observed. In any case, pure ethanol achieved

339 higher RA recoveries than pure ethyl lactate. On the other hand, comparable results

340 were observed for CA, that was more efficiently recovered using pure ethanol.

341 Definitely only 25 % of water is enough to confer the correct polarity to the mixture

342 with organic solvent allowing extraction recoveries between 70–95 %. On the other

343 hand, if total recoveries are required, low concentration of RA and CA of the extracts

344 are obtained.

345 **4.   Concluding remarks**

346 Pressurized liquid extraction has proved to be an interesting way for by-product

347 valorisation. Also, the usefulness of Hansen approach for the selection of a selective

348 solvent for target compounds has been demonstrated. Green solvents, ethanol and ethyl

349 lactate, were both capable of extracting CA and RA from basil DWW. In addition, the

350 present work proposed two different scenarios for the extraction of RA from dDWWs:

351 (1) an enriched PLE extract in terms of mg of RA per g extract with an extraction

352 efficiency of 75.89 ±16.03 % employing a water-ethanol mixture 25:75 (% v/v) at 50

353 ºC, and (2) selective extraction in terms of ratio RA/CA (24.17- fold higher CA content)

354 with a extraction efficiency of 1.36 ±0.12 % employing pure ethyl lactate at 50 ºC. On

355 the other hand, PLE extracts with high content of CA , with a recovery of 13.86 ±4.96

356 % using ethanol absolute at low temperatures, allow achieving the maximum selectivity

357 (0.232-fold RA content). Other alternatives studied can consider better recoveries but

358 the concentration of the target compounds decrease considerably. The choice will

359 depend on the particular interest of the research.

364 **Conflict of interest**

365 The authors declare no conflict of interest.

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432   **Figure 1**. PLE extraction efficiency for RA employing ethanol-water (a) or ethyl lactate-water

433   (b) mixtures as solvent, RA content expressed as mg g^{-1} extract (n=3)

434   **Figure 2**. PLE extraction efficiency for CA employing ethanol-water (a) or ethyl lactate-water

435   (b) mixtures as solvent, RA content expressed as mg g^{-1} extract (n=3)

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438   **Table 1.** Ra values for rosmarinic acid (a) and caffeic acid (b) and different subcritical mixtures

439   solvents.

440   a)

T (ºC)	Rosmarinic acid									
	Ethanol - water mixtures					Ethyl lactate - water mixtures				
	Water (% v/v)					Water (% v/v)				
	0	**25**	**50**	**75**	**100**	**0**	**25**	**50**	**75**	**100**
50	13.4	16.9	21.4	26.4	31.7	11.3	13.1	18.2	24.7	31.7
100	15.6	17.5	20.7	24.6	29.1	14.2	14.6	18.0	23.1	29.1
150	18.1	18.7	20.6	23.4	26.9	16.7	16.3	18.3	22.1	26.9

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442   b)

T (ºC)	Caffeic acid									
	Ethanol - water mixtures					Ethyl lactate - water mixtures				
	Water (% v/v)					Water (% v/v)				
	0	**25**	**50**	**75**	**100**	**0**	**25**	**50**	**75**	**100**
50	11.4	12.6	15.9	20.4	25.4	13.4	10.9	13.3	18.7	25.4
100	14.5	14.3	16.0	19.2	23.2	15.9	13.3	14.0	17.8	23.2
150	17.4	16.3	16.7	18.6	21.5	18.0	15.3	15.4	17.4	21.5

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451 **Table 2**. PLE extraction yield (%) for water-ethanol and water-ethyl lactate mixtures, at

452 different extraction conditions.

Temperature (°C)	Water (% v/v)	Extraction Yield % (± SD)	
		Water-ethanol mixtures	Water- ethyl lactate mixtures
	100	97.1 ± 0.5	97.1 ± 0.5
	75	90.4 ± 1.2	83.2 ± 0.4
50	50	75.8 ± 0.8	84.1 ± 4.7
	25	42.5 ± 4.5	52.6 ± 2.0
	0	5.0 ± 1.5	4.829 ± 0.004
	100	96.1 ± 1.6	96.1 ± 1.6
	75	91.7 ± 0.2	90.9 ± 1.2
100	50	83.8 ± 2.1	82.1 ± 1.3
	25	59.2 ± 3.5	56.8 ± 3.1
	0	5.05 ± 0.53	6.5 ± 1.7
	100	97.4 ± 0.3	97.4 ± 0.3
	75	93.9 ± 0.2	99.2 ± 0.4
150	50	86.0 ± 1.7	87.5 ± 8.4
	25	61.1 ± 0.4	61.8 ± 2.9
	0	18.1 ± 0.3	14.7 ± 2.5

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462 **Table 3.** Extraction efficiency (%) of rosmarinic acid and carnosic acid employing water-ethanol mixtures

463 and water- ethyl lactate mixtures in PLE extractions.

Temperature (ºC)	Water (% v/v)	Extraction efficiency % (± SD)			
		Rosmarinic acid		Caffeic acid	
		Water - ethanol mixtures	Water – ethyl lactate mixtures	Water - ethanol mixtures	Water – ethyl lactate mixtures
50	100	71.0 ± 9.2	91.7 ± 12.1	75.2 ± 16.0	88.4 ± 83
	75	104.0 ± 4.8	104.1 ± 3.9	94.2 ± 9.2	94.2 ± 6.2
	50	83.0 ± 10.1	110.6 ± 10.0	85.2 ± 10.9	104.1 ± 10.7
	25	75.9 ± 16.0	65.9 ± 5.9	71.2 ± 15.0	61.9 ± 4.3
	0	3.9 ± 1.3	1.4 ± 0.1	13.9 ± 5.0	1.1 ± 0.2
100	100	93.6 ± 5.4	96.4 ± 5.8	93.3 ± 13.9	117.7 ± 6.2
	75	100.0 ± 12.3	116.1 ± 7.5	92.9 ± 15.7	104.0 ± 9.9
	50	98.3 ± 4.6	107.4 ± 5.8	89.8 ± 7.0	99.1 ± 9.7
	25	86.9 ± 12.0	73.9 ± 5.7	82.5 ± 11.2	73.8 ± 8.3
	0	2.8 ± 0.5	2.1 ± 0.8	7.9 ± 2.5	2.4 ± 0.8
150	100	98.7 ± 9.5	78.7 ± 11.4	95.5 ± 15.5	107.5 ± 9.0
	75	87.6 ± 7.6	118.4 ± 8.7	99.3 ± 7.1	122.6 ± 5.7
	50	88.0 ± 7.1	105.0 ± 13.1	91.9 ± 7.2	101.9 ± 14.6
	25	76.9 ± 10.5	82.0 ± 6.4	94.3 ± 10.4	94.7 ± 10.0
	0	12.6 ± 0.5	6.8 ± 1.4	30.3 ± 2.3	6.8 ± 1.2

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