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1 **Natural deep eutectic solvents pretreatment as an aid for pectin extraction**
2 **from apple pomace**

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4 UR 1268 Biopolymères Interactions Assemblages, équipe Paroi Végétale et Polysaccharides
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7

8 **Abstract**

9 Three natural deep eutectic solvents (NADES; choline chloride (CC) : lactic acid (LA),
10 CC : oxalic acid (OA), CC : urea (U)) in the ratio 1:2 were tested as a pretreatment of apple
11 pomace prior to hot water extraction of pectin. The pretreatment duration and temperature (for
12 0.5-2h, at 40-80 °C) was adjusted to limit cell wall polysaccharides losses and degradation.
13 Extraction yields and sugar composition were used to evaluate the impact of the pretreatment
14 on pectin extraction and the molecular weight and degree of esterification of pectin were
15 characterized. Results showed that CC:LA and CC:U pretreatments led to close overall
16 recovery yields with the control using water instead of NADES. Instead, CC:OA led to
17 polysaccharides degradation and loss. The cell wall monosaccharides composition was
18 affected after CC:LA and CC:U pretreatments at 60 °C and above, while it was preserved by
19 CC:LA pretreatments at 40 °C for 1h. Moreover, CC:LA pretreatment facilitated hot water
20 extraction of a large amount (33.1%-56.1% of uronic acid recovery) of high Mw HM pectin
21 comparable with pectin obtained through classical method. CC:U pretreatment led to
22 saponification and affected pectin composition by introducing choline. Thus, CC:LA
23 pretreatment of apple pomace followed by water extraction offers a “green” alternative for
24 mineral acid pectin extraction while CC:U allows functionalizing pectin in apple pomace
25 prior to hot water extraction.

26 **Keywords** Natural deep eutectic solvents; Apple pomace; Cell wall polysaccharides; Pectin

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28

29 **1. Introduction**

30 Apple is the most cultivated and consumed fleshy fruit in the world and its associated
31 products, such as juice, cider and sauce are very popular among consumers. Apple processing
32 generates side-streams, such as about 10 million tons of pomace each year from the apple juice
33 industry (Alongi, Melchior, & Anese, 2018) which can be valorized to extract pectin (May,
34 1990). Besides for food gelling ingredient, apple pomace may be source of other valuable
35 polysaccharides, such as hemicellulose and cellulose. Hemicellulose can be applicable as film,
36 drug carrier and stabilizing additive in food, pharmacy and other related industries
37 (Ebringerová, 2005), while cellulose play a paramount role in paper making industry
38 (Ververis, Georghiou, Christodoulakis, Santas, & Santas, 2004). To that end, a biorefinery
39 approach was engaged to isolate apple pomace polysaccharides and, as a first step, pectin
40 extraction was reconsidered. Pectin is made of approximately 65% homogalacturonan (HG),
41 20–35% rhamnogalacturonan I (RGI) and 10% rhamnogalacturonan II (RGII) structural
42 domains (Mohnen, 2008). HG is formed by repeats of [α -1,4-D-galacturonic acid]_n units that
43 can be partially esterified by methanol on O-6 (Voragen, Beldman, & Schols, 2001) and
44 acetyl at O-2 and/or O-3 (Atmodjo, Hao, & Mohnen, 2013). It can be further modified at O-3
45 by xylose to form xylogalacturonan domains (Schols, Bakx, Schipper, & Voragen, 1995).
46 According to the degree of methyl esterification, pectin is classified as high methoxyl (HM)
47 pectins (degree of esterification > 50%) and low methoxyl (LM) pectins (degree of
48 esterification < 50%) (Löfgren & Hermansson, 2007). RG-I pectic domain is built on the
49 disaccharide repeat unit [α -D-GalA-1,2- α -L-Rha-1,4]_n on which side-chains made of α -L-
50 Araf and β -D-Galp are branched on O-4 of the rhamnosyl residues (Scheller, Jensen,
51 Sørensen, Harholt, & Geshi, 2007). The minor RG-II structural domains consist of at least 8
52 α -1,4-D-galacturonic acid units with four types of complex side branches made of 12 different
53 types of sugars (O'Neill, Ishii, Albersheim, & Darvill, 2004). Generally, extraction of pectin
54 from agricultural side-streams is achieved through mild acid, mild alkali or enzyme extraction.
55 Pectin industrial production mainly uses aqueous mineral acid which is responsible for
56 corrosion of equipment and environmental pollution (Wikiera, Mika, Starzyńska-Janiszewska,
57 & Stodolak, 2015). Methods using mild alkali decrease the degree of esterification and the
58 molecular weight of pectin (Yuliarti et al., 2015) while enzymatic extraction affects pectin
59 yield, physicochemical and rheological properties (Ptichkina, Markina, & Rummyantseva,
60 2008). Microwave and ultrasound techniques provide clear benefits in shortening extraction
61 time and reducing energy consumption (Adetunji, Adekunle, Orsat, & Raghavan, 2017) but if
62 these techniques are promising at the laboratory scale, their scaling-up and the cost of

63 investment in new equipment needed for their implementation refrain industry to adopt them.
64 Hence, new solvents that can fit in classical processes of pectin extraction providing less
65 adverse effects on the environment, equipment and polysaccharide physicochemical and
66 rheological properties are being looked for.

67 Solvents, such as ionic liquids and more recently natural deep eutectic solvents (NADES)
68 emerged to extract biopolymers and various plant molecules (Vanda, Dai, Wilson, Verpoorte,
69 & Choi, 2018). NADES combine different cellular hydrogen bonding acceptor (HBA) and
70 hydrogen bonding donor (HBD) metabolites (Choi et al., 2011) to form intermolecular
71 networks at the basis of the physicochemical properties of the solvents (Yang et al., 2018).
72 Distinct from the ionic liquids, which are analogues made of organic salts, the starting
73 ingredients of NADES are cheap, biodegradable and eco-friendly natural non-toxic
74 metabolites, such as, sugar, amino acid, organic acid. As deep eutectics, NADES have a lower
75 melting temperature than that of each starting material and some of them form transparent
76 liquids at ambient temperature, which make these solvents easy to prepare and use. High
77 viscous NADES solutions which can impede extraction rate and time can be mitigated by
78 high temperatures and/or a small proportion of co-solvent, such as water (Vanda et al., 2018).
79 All of these traits, which are consistent with the concept of “green chemistry”, have drawn the
80 focus of recent researches for their extraction potential. To date, NADESs were shown to be
81 excellent solvents for the extraction of phenolic compounds or lignin from different
82 biomasses (Kim, Dutta, Sun, Simmons, & Singh, 2018; Ruesgas-Ramón, Figueroa-Espinoza,
83 & Durand, 2017; Soares et al., 2017) and were tested for the extraction of polysaccharides,
84 among which is fruit cell wall pectin (Benvenuti, Sanchez-Camargo, Zielinski, & Ferreira,
85 2020; Liew, Ngoh, Yusoff, & Teoh, 2018; Shafie, Yusof, & Gan, 2019). However, NADESs
86 are less effective in extracting pectin from pomelo compared with mild organic acids in terms
87 of yield, operational attributes and economical features (Liew et al., 2018). Since NADESs
88 are prepared by combining different compounds in various molar ratio, they offer the
89 possibility of being tailored for specific extraction or for pre-extraction to remove unwanted
90 components and ease extraction or modification of valuable biopolymers (Yu et al., 2019).
91 For various NADES combinations, the majority of NADESs are of choline chloride based
92 (Benvenuti, Zielinski, & Ferreira, 2019; Choi & Verpoorte, 2019), while among studied
93 HBDs, lactic acid, oxalic acid and urea were widely applied in extraction of bioactive
94 compounds or pre-extraction of lignocellulosic matrices (Achkar, Fourmentin, & Greige-
95 Geroges, 2019; Benvenuti et al., 2019). NADESs containing these HBDs may have the
96 potential to extract pectin directly or indirectly. Hence, in the present study, three common

97 NADES: choline chloride : lactic acid (CC:LA), choline chloride : oxalic acid (CC:OA),
98 choline chloride : urea (CC:U) were tested as a mean of pre-treating apple pomace prior to hot
99 water extraction of pectin. The extraction yield and sugar composition analysis were used to
100 evaluate NADES pretreatment efficiency and the molecular weight and degree of
101 esterification of pectin recovered were characterized.

102

103 **2. Materials and Methods**

104 2.1. Materials

105

106 2.1.1. Pomace

107 Wet pomace from *Malus domestica* var *Kermerrien* was provided by IFPC (Le Rheu, France).

108 The pomace was stored at -20 °C prior use.

109

110 2.1.2. Chemicals

111 Choline chloride (CAS: 67-48-1, Sigma-Aldrich, France), urea (CAS: 57-13-6, Sigma-
112 Aldrich, France), oxalic acid dihydrate (CAS: 6153-56-6, Sigma-Aldrich, France), DL-lactic
113 acid (CAS: 50-21-5, Sigma-Aldrich, France), ethanol (CAS: 64-17-5, Carlo Erba reagents,
114 France), acetone (CAS: 67-64-1, Carlo Erba reagents, France), nitric acid (Titripur, Germany),
115 ammonium hydroxide solution (CAS: 1336-21-6, Sigma-Aldrich, France), acetic anhydride
116 (CAS: 108-24-7, Sigma-Aldrich, France), 1-methylimidazole (CAS: 616-47-7, Sigma-Aldrich,
117 France) were used in present research.

118

119 2.1.3. Preparation of NADES

120 Three different NADESs were prepared by separately mixing the choline chloride with lactic
121 acid, urea and oxalic acid in a molar ratio (1:2). The molar ratio was chosen as a widely used
122 ratio (may not be the exact ratio for eutectic point) for polysaccharides processing
123 (Zdanowicz, Wilpiszewska, & Szychaj, 2018). The mixtures were stirred with magnetic bar
124 and heated in the oil bath at 100 °C. The mixture was constantly stirred until the clear liquid
125 formed. The solvents were stored at ambient temperature for later use. Due to the non-volatile
126 property of NADES, prior to application, the water content in NADESs (CC:LA=0.91%
127 (w/w); CC:U=0.32% (w/w); CC:OA=1.66% (w/w)) was determined by freeze drying for 24 h
128 until constant weight was reached (Jeong et al., 2015).

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130 2.2. Methods

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2.2.1. Pretreatment of apple pomace with NADESs

Five g of thawed apple pomace (water content = 68%) were mixed with 40 mL of different NADES (1:8, w/v) and the big particles in suspension were dispersed with a Polytron mixer. The mixture was stirred with a magnetic bar and heated in water bath under different conditions as listed in Table 1. The suspension was then centrifuged at 15000 g for 20 min and the supernatant was collected as NADES supernatant fraction. The remaining pomace was washed twice with deionized water at ambient temperature to reach water pH (pH 6.5), then, the pellet was resuspended in 40 mL of deionized water at 80 °C for 10 min under constant agitation and the water extract was recovered after centrifugation as above. This operation was repeated 5 times. The pooled water washes, referred to as the water fraction, was concentrated to 10 mL with a vacuum rotary evaporator. Polymers in the NADES supernatant and water fractions were precipitated by 4 volumes of ethanol. The precipitates were recovered by centrifugation (15000 g, 20 min) and washed with 40 mL of 70% of ethanol for 10 min (3 times), followed by 40 mL of ethanol and acetone for 10 min (2 times). The ethanol and acetone washings were repeated to obtain low color solutions. The pomace residue underwent the same washing and dehydration process as above. Finally, samples were dried at 40 °C in vacuum oven over P₂O₅ powder for 12 h and stored at 4 °C for later analysis. The extraction was conducted four times and water was used as a control pretreatment condition. Fractions yield was calculated as follows:

$$\text{Yield (\%)} = \frac{\sum W_s}{W} \times 100$$

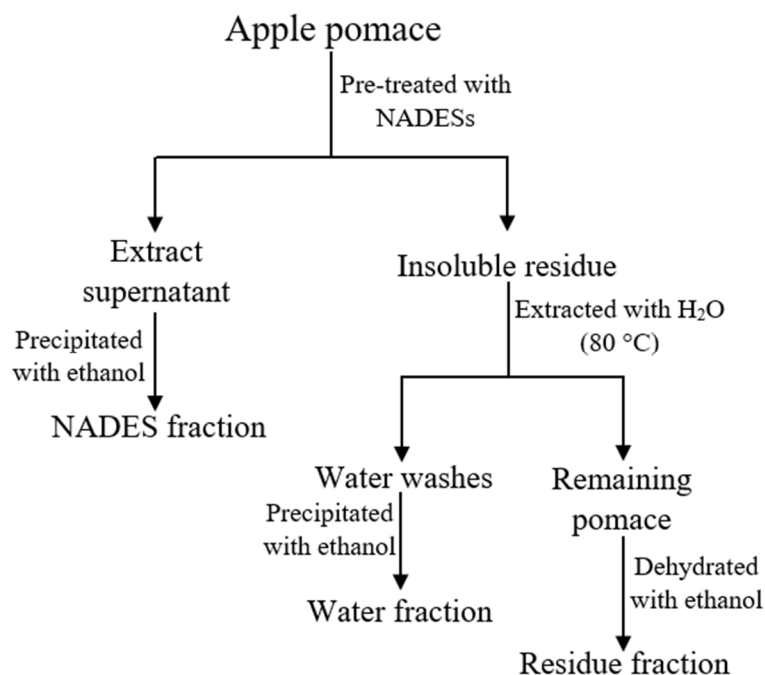
Where the W_s is the sample weight in each fractions and W is the initial wet weight of apple pomace.

Table 1. Extraction conditions with NADES

Extraction condition	
Time	Temperature
2 h	80 °C
1 h	80 °C, 60 °C, 40 °C
0.5 h	40 °C

157 The overall extraction process is summarized in Figure 1.

158



159

160

Fig. 1 Schematic representation of the extraction process with NADES

161

162 2.2.2. Neutral sugars composition and uronic acid analysis

163 To identify and quantify neutral sugars in different fractions, GLC (Gas-liquid chromatograph)

164 analysis was performed according to previously established method (Ray, Vigouroux,

165 Quemener, Bonnin, & Lahaye, 2014). Briefly, sample was dispersed in sulphuric acid (12 M,

166 72%) at 25 °C for 30 min, followed by further hydrolysis with sulphuric acid (2 M) at 100 °C

167 for 2 h. The released sugars were reduced with NaBH₄ solution at 40 °C for 60 min, then

168 acetylated with acetic anhydride and 1-methylimidazole at ambient temperature for 20 min.

169 The obtained alditol acetates were analyzed by GLC (Perkin-Elmer Autosystem) equipped

170 with DB-225 capillary column (J&W Scientific, Folsom, CA, USA) eluted at 205 °C by

171 hydrogen. The split injector and flame ionization detector temperatures were set at 220 °C.

172 Both sugar standard solution and internal standard (inositol) were used for calibration.

173 Uronic acid in the acid hydrolysate was quantified using the m-hydroxydiphenyl colorimetric

174 acid method (Blumenkrantz & Asboe-Hansen, 1973). Galacturonic acid and glucose standard

175 solutions were used for calibration. Sugar composition in each fraction was expressed as

176 recovery rate and was calculated as follows:

177

$$\text{Recovery rate (\%)} = \frac{(P1 \times Y1)}{(P2 \times Y2)} \times 100$$

178 Where P1 is the percentage of each sugar in the extracted sample, Y1 is the extraction yield of
179 the corresponding fraction, P2 is the percentage of each sugar of untreated sample, Y2 is the
180 dry matter percentage of the untreated sample.

181

182 2.2.3. Starch content

183 Starch content in the residue and water fraction was determined by high performance anion
184 exchange chromatograph (HPAEC) according to established method (McCleary, Gibson, &
185 Mugford, 1997). Briefly, 10 mg of sample was swelled in MOPS buffer (200 uL, 50 mM, pH
186 = 7) overnight at ambient temperature. The sample was hydrolyzed by 300 uL of α -amylase
187 (100 U/mL; Megazyme) at 100 °C for 6 min followed by 100 uL of amyloglucosidase (20
188 U/mL; Megazyme) in 400 uL of sodium acetate buffer (200 mM, pH = 4.5) at 50 °C for 30
189 min. Glucose released was quantified by HPAEC equipped with a PA1 column (4 × 250 mm,
190 Dionex) eluted by 500 mM NaOH (eluent A, 20%) and deionized water (eluent B, 80%) at a
191 flow rate of 1 mL min⁻¹. Rhamnose was used as an internal standard in sample and in glucose
192 standard solutions used for calibration. Control was realized with sample without enzyme to
193 correct for eventual free glucose.

194

195 2.2.4. FTIR spectroscopy

196 Infrared spectra of the dried water fractions were collected on a NICOLET IS50
197 spectrophotometer (Thermo scientific). Spectra were collected in the transmission mode on a
198 ATR crystal between 400 and 4000 cm⁻¹ using the Smart iTR ATR sampling accessory. Six
199 spectra were registered for each sample. Data was further processed with R (R Core Team,
200 2014) using the ChemoSpec library
201 (<http://127.0.0.1:10623/library/ChemoSpec/doc/ChemoSpec.pdf>) for normalization,
202 correction and calculation of mean value.

203

204 2.2.5. Nitrogen and carbon content

205 The nitrogen and carbon contents of dry water fractions were analyzed using CNS Vario
206 (Elementar, Germany).

207

208 2.2.6. ¹H NMR spectroscopy

209 Five mg of the dried sample from the water fraction of CC:U treated group and control were
210 dissolved in 0.5 ml of D₂O (99.96 atom% D, Sigma-Aldrich) and then lyophilized. This

211 process was repeated twice. Water pre-saturated ¹H NMR spectra were registered at 60 °C on
212 a Bruker Avance III 400 MHz. Chemical shifts were referred to water assigned to 4.4 ppm.

213

214 2.2.7. Preparation of cholinium polygalacturonate and sodium pectinate

215 Twenty mg of polygalacturonic acid (Sigma-Aldrich) was dissolved in 2 mL of deionized
216 water, neutralized with choline hydroxide solution (45 wt% in methanol, Sigma-Aldrich).

217 Then, the solution was dialyzed against deionized water (MW cutoff: 6000-8000, T2-8030-
218 23, Membrane Filtration Products, Inc.) for 24 h, prior to freeze-drying.

219 Twenty mg of the water fraction from pomace pretreated with CC:U at 80 °C for 2 h was
220 dissolved in 2 mL of deionized water. The pH of solution was adjusted to 2.5-3.0 with
221 hydrochloric acid (0.1M) before dialysis as above. Then, the solution was neutralized with
222 sodium hydroxide (0.1M) and freeze-dried.

223 The freeze-dried cholinium polygalacturonate and Na pectin from CC:U treated pomace were
224 subjected to MHDP colorimetric assay.

225

226 2.2.8. Pectin methylation and acetylation esterification degree

227 Methanol and acetic esters in pectin were measured by HPLC according to previous method
228 (Levigne, Thomas, Ralet, Quemener, & Thibault, 2002). Briefly, samples were saponified for
229 1 h at 4 °C by NaOH (0.5 M) with CuSO₄·5H₂O in isopropanol solution, then centrifuged and
230 the supernatant was filtered through cartridge IC-H (Sstarpure, Maxi-Clean SPE 0.5 ml IC-H
231 50pk) prior to HPLC analysis on C18 (4 mm × 250 mm, Lichrospher 100 RP-18e (5 μm),
232 Interchim, France) at 25 °C. H₂SO₄ (4 mM) was used for isocratic elution at 1.0 mL min⁻¹.

233 Standard solution containing methanol, acetic acid and isopropanol as internal standard was
234 used for calibration. The degree of methyl esterification (DM) and acetyl esterification (DA)
235 were calculated as the number of moles of methanol and acetic acid measured per mole of
236 uronic acid in pectin.

237

238 2.2.9. Molecular weight measurement

239 Molecular weight of pectin was determined by high performance size exclusion
240 chromatography (HPSEC) using Shodex OHpak SB-G 6B pre-column and OHpak SB-805-
241 HQ column (Shodex, Tokyo, Japan) connected to pump (Jasco PU-1580, Tokyo, Japan) and
242 injector (PerkinElmer, series 200 autosampler, Courtaboeuf, France). Prior to injection, starch
243 in water fractions was hydrolyzed by 500 μL of amyloglucosidase (500 U/mL) at 50 °C for 30

244 min. The solution was dialyzed against deionized water (MW cutoff: 6000-8000, T2-8030-23,
 245 Membrane Filtration Products, Inc.) prior to freeze-drying. Glucose content in the sample
 246 was checked by GLC to ensure the removal of starch efficiency. De-starched samples were
 247 dissolved in distilled water overnight at 4 °C, then centrifuged (10 min, 7400 g) and filtered
 248 through 0.45 µm membrane (Millex-HV, PVDF) prior to injection. Elution was performed
 249 with 50 mM NaNO₃ at a flow rate of 0.6 mL min⁻¹ and monitored by i) differential
 250 refractometry (Viscotek VE 3580 RI detector, Malvern Instruments, Orsay, France), ii) light
 251 scattering (LS) detection and iii) differential pressure viscometry (both from Viscotek 270
 252 dual detector, Malvern Instruments, Orsay, France). Molecular weight and viscosity were
 253 obtained using the OmniSEC 4.7.0 software and calibration was done with using pullulan-
 254 P108K (Viscotek, Malvern Instruments, Orsay, France).

255

256 2.2.10. Statistical analysis

257 Data was first verified for homogeneity of variance by Levene's test ($P > 0.05$). Then, they
 258 were subjected to one-way ANOVA and Duncan's multiple range post-hoc test. Besides,
 259 independent-samples T test was carried out to compare the data in esterification degrees and
 260 molecular weight between NADES groups and control group. The SPSS 16.0 statistical
 261 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Differences
 262 were considered significant at $P < 0.05$. Data are presented as mean values with their standard
 263 deviation.

264

265 3. Results and discussion

266 3.1. NADES pretreatment conditions of pomace affect fractions yield

267

268 **Table. 2** Effect of NADES type and NADES treatment temperature and duration on the yield
 269 (% w/w) of total pomace recovery, of extraction residue, of NADES and water fractions.

Extraction Yield	Control	CC:LA	CC:U	CC:OA
Total fraction				
0.5h-40 °C	21.69 ± 0.50 ^{a,A}	21.13 ± 0.52 ^{a,B}	21.26 ± 0.60 ^{a,C}	
1h-40 °C	21.72 ± 0.75 ^{a,A}	21.34 ± 0.30 ^{a,B}	21.80 ± 0.85 ^{a,C}	
1h-60 °C	21.66 ± 0.26 ^{c,A}	20.58 ± 0.34 ^{b,B}	20.03 ± 0.36 ^{a,B}	
1h-80 °C	22.71 ± 0.74 ^{b,B}	18.54 ± 0.62 ^{a,A}	19.39 ± 0.67 ^{a,B}	
2h-80 °C	21.49 ± 0.50 ^{c,A}	17.65 ± 1.23 ^{b,A}	18.10 ± 0.09 ^{b,A}	8.44 ± 0.78 ^a

Residue fraction

0.5h-40 °C	20.23 ± 0.45 ^{c,D}	14.71 ± 0.46 ^{a,E}	18.07 ± 0.47 ^{b,C}	
1h-40 °C	20.10 ± 0.74 ^{c,D}	13.43 ± 0.35 ^{a,D}	17.25 ± 0.46 ^{b,C}	
1h-60 °C	19.21 ± 0.22 ^{c,C}	12.46 ± 0.30 ^{a,C}	15.22 ± 0.24 ^{b,B}	
1h-80 °C	18.18 ± 0.26 ^{c,B}	9.95 ± 0.55 ^{a,B}	14.45 ± 0.83 ^{b,B}	
2h-80 °C	15.51 ± 0.27 ^{c,A}	8.48 ± 0.93 ^{a,A}	13.19 ± 0.86 ^{b,A}	7.70 ± 0.78 ^a

NADES fraction

0.5h-40 °C	0.08 ± 0.02 ^{a,A}	0.26 ± 0.07 ^{b,A}	0.23 ± 0.04 ^{b,A}	
1h-40 °C	0.13 ± 0.02 ^{a,A}	0.27 ± 0.03 ^{b,A}	0.32 ± 0.03 ^{c,A}	
1h-60 °C	0.95 ± 0.05 ^{b,B}	0.29 ± 0.04 ^{a,A}	1.58 ± 0.04 ^{c,B}	
1h-80 °C	2.79 ± 0.34 ^{b,C}	0.57 ± 0.13 ^{a,B}	2.11 ± 0.53 ^{b,B}	
2h-80 °C	3.75 ± 0.15 ^{c,D}	0.69 ± 0.05 ^{a,C}	2.08 ± 0.62 ^{b,B}	0.34 ± 0.08 ^a

Water fraction

0.5h-40 °C	1.37 ± 0.05 ^{a,A}	6.16 ± 0.20 ^{c,A}	2.96 ± 0.11 ^{b,A}	
1h-40 °C	1.49 ± 0.22 ^{a,AB}	7.64 ± 0.12 ^{c,B}	4.23 ± 0.45 ^{b,B}	
1h-60 °C	1.50 ± 0.02 ^{a,AB}	7.83 ± 0.25 ^{c,BC}	3.23 ± 0.18 ^{b,A}	
1h-80 °C	1.75 ± 0.27 ^{a,B}	8.02 ± 0.27 ^{c,C}	2.84 ± 0.17 ^{b,A}	
2h-80 °C	2.23 ± 0.27 ^{b,C}	8.48 ± 0.29 ^{d,D}	2.82 ± 0.33 ^{c,A}	0.49 ± 0.09 ^a

270 Control: water replacing for NADES; CC:LA, CC:U, CC:OA: choline chloride: lactic acid, urea and oxalic acid,
 271 Mean values (± standard deviation, n = 4) with unlike letters were significantly different ($P < 0.05$), significant
 272 difference was analyzed in each fraction. a,d,c (row): significantly different between different groups at same
 273 condition; A,B,C: significantly different at different condition within same group (columns).

274
 275 Extraction yields with the CC:LA, CC:OA and CC:U are depicted in Table 2. The viscosity of
 276 the NADES is provided in Table S1 and showed, as reported that it decreased with both
 277 temperature and addition of water (Yang et al., 2018; Zdanowicz et al., 2018). Moreover,
 278 consistent with other reports (Fisher & Kunz, 2014; Hou et al., 2008)), the three NADES were
 279 non-Newtonian with a shear-thinning behavior (data not shown). The initial extraction
 280 conditions were set at 80 °C for 2 h to lower viscosity of NADES (viscosity at 10 s⁻¹: from
 281 107.6 mPas at 40 °C to 7.9 mPas at 80 °C for CC:LA; from 522.0 mPas at 40 °C to 36.4
 282 mPas at 80 °C for CC:U; from solid at 40 °C to 37.0 mPas at 80 °C for CC:OA). As mass
 283 transfer plays an important role in determining extraction conditions, the lower viscosity at
 284 80 °C with longer duration can facilitate the interaction between NADESs and apple pomace.
 285 Morrais et al. (2018) demonstrated that higher hardwood xylan solubilization was achieved
 286 with CC:U (1:2) at 80 °C. Moreover, the optimal condition for extracting pectin from
 287 *Averrhoa bilimbi* with CC: citric acid was at 80 °C for 2.5 h (Shafie et al., 2019). Extraction

288 with CC:OA for 2h at 80 °C severely affected the total yield of apple pomace with only 8.44 %
289 that did not reach half that of control group pretreated with water. Hence, CC:OA
290 pretreatment was not further studied. Lower losses were observed with CC:LA and CC:U
291 pretreatments and thus, other conditions were tested. Lowering pretreatment temperature from
292 80 °C to 40 °C and duration from 2 h to 0.5 h significantly improved the total recovery yield
293 of pomace after CC:LA and CC:U pretreatments. Moreover, at 40 °C for 0.5 and 1 h, no
294 difference was observed in the total recovery yield of pomace between NADES pretreatments
295 and control ($P>0.05$).

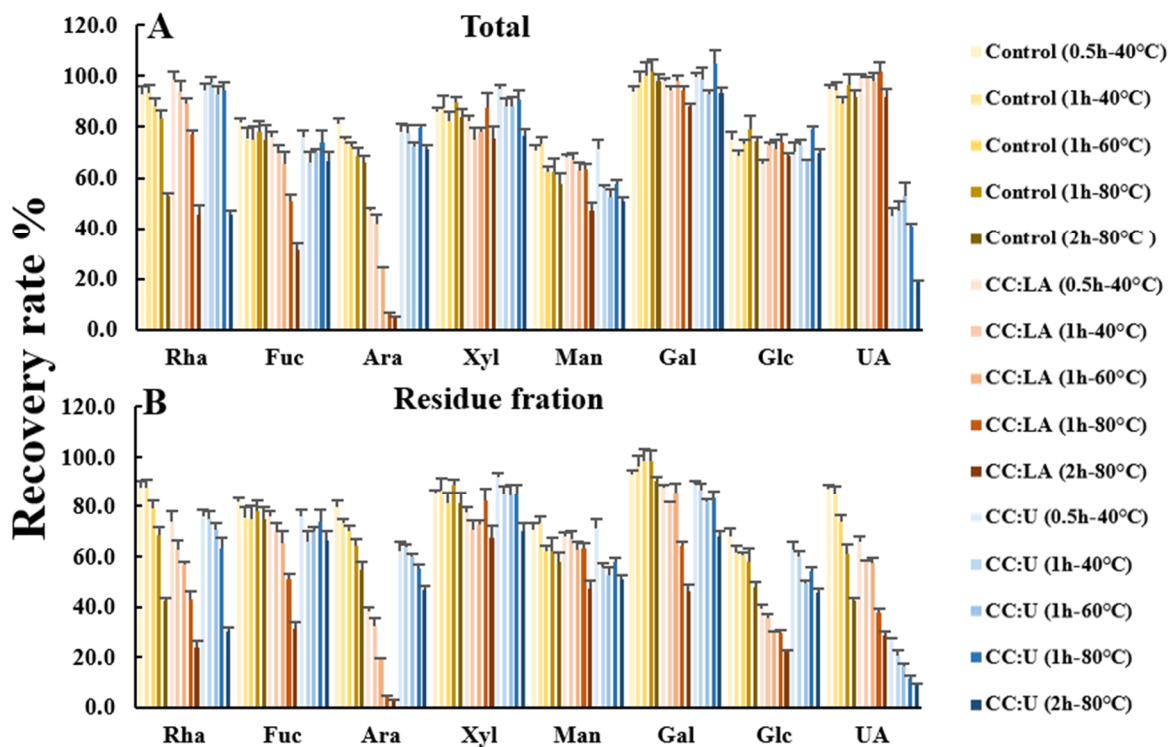
296 Looking at specific fractions, the yield of the NADES fraction increased with higher
297 temperature or extended extraction time. For NADES extraction of pomace, as mentioned
298 above, higher temperature decreased viscosity of NADESs solution. Many studies reported on
299 the inverse relation between extraction yield of bioactive substances and viscosity of DESs
300 (Dai, Rozema, Verpoorte, & Choi, 2016; Guo et al., 2019; Huang et al., 2017). In our study, a
301 similar relation was observed. The significantly high yield of NADES fraction at 80 °C was
302 partly attributed to the low viscosity of solution. In addition, when the extraction temperature
303 was set at 40 °C, the viscosity of NADESs had a more important role in determining
304 extraction yield than duration since the yield of extract was not significantly different at 40 °C
305 for 0.5h or 1h. The relatively higher viscosity (viscosity at 40 °C: 107.6 mPas for CC:LA;
306 522.0 mPas for CC:U) impeded the mass transfer of pectin from cell wall matrix into the
307 solvent even with extended extraction time. Nevertheless, when compared with control, the
308 highest yield (3.75%) was obtained with the control experiment pretreated with water at 80 °C
309 for 2 h. The yield of polymer precipitated by ethanol from CC:LA and CC:U extracts was
310 much lower than that of control at 80 °C. Although their yields were significantly higher at
311 temperature of 40 °C compared with control, the highest yield (0.32%) in CC:U groups still
312 demonstrated that NADESs by themselves were not efficient in extracting polysaccharides
313 from apple pomace. Benvenuti et al. (2020) showed that NADES (citric acid : glucose : water;
314 1:1:3) : water solution (1:9 w/w) offered the highest extraction yield of *Myrciaria cauliflora*
315 pectin. Similarly, the highest pectin yield was attained from pomelo peels with NADES
316 (Choline chloride : glucose : water; 5:2:5) at 60 °C for 2h (Elgharbawy et al., 2019). The
317 disparity from our result may lie in different water content in NADES extraction system
318 (water : NADES=8.5% (v/v), considering 68% of water content in raw pomace), as it affected
319 the extraction ability of DES (Passos, Tavares, Ferreira, Freire, & Coutinho, 2016; Yiin,
320 Yusup, Quitain, & Uemura, 2015). In contrast, the yield of the water fraction after CC:LA
321 pretreatment was at least 6 times higher than that of corresponding NADES fraction.

322 Moreover, the yield of the water fraction following this pretreatment was the highest among
323 all other solvent pretreatments and conditions tested (range from 6.16% to 8.48%, $P < 0.05$).
324 The overall cumulative yield of ethanol precipitated materials did not exceed 23% of the
325 starting weight of raw apple pomace due to its 68% water content. It is also due to partial
326 degradation of apple pomace by NADES as demonstrated by the extract solution colors that
327 ranged from orange to deep red (supplementary Figure S1) from the CC:LA pretreatments. In
328 apple, phenolic compounds are naturally present and their content is more than 10 g kg⁻¹ of
329 flesh weight (Van Buren, 1970). Phenolic compounds of apple mainly consist of
330 hydroxycinnamic acid derivatives, flavan-3-ols, flavonols and dihydrochalcones (Guyot,
331 Marnet, Laraba, Sanoner, & Drilleau, 1998). Condensed tannins are formed by the
332 polymerization of flavan-3-ol compounds, such as catechin or its derivative and are degraded
333 by acid or alkali into water insoluble reddish-colored phlobaphenes (Lachman, Martinek,
334 Kotíková, Orsák, & Šulc, 2017). The color of the CC:LA extract was a strong indication for
335 the degradation of phenolic compounds and their subsequent loss in the ethanol used to
336 precipitate polysaccharides from the extract. On that account, compared with CC:LA
337 pretreatments, the CC:U pretreatments showed lower degradation of phenolic compounds as
338 judged from the solution color (from orange to brown; Figure S1). Such putative phenolic
339 compounds degradation may partly explain the residue yield that was the highest from the
340 control group, followed by the CC:U pretreated pomace and lastly, by the CC:LA pretreated
341 pomace. NADES pH is a known factor influencing extraction efficiency: acidic NADES will
342 break more bonds and may favor the extraction process (Hou et al., 2017). In our result,
343 differences in extraction yield from pomace treated by CC:LA and CC:U may have resulted
344 from acidic pH (pH 1.0) with the former and mild alkaline pH (pH 8.5) with the latter solvent,
345 while hot water in the control replacing for NADES is known to extract cell wall
346 polysaccharides (Fry, 1988).

347

348 3.2. NADES pretreatment affects polysaccharide sugar composition in apple pomace fractions
349 To assess the impact of NADES treatment on apple pomace polysaccharide composition, the
350 recovery rate of cell wall component sugars was determined. Moreover, optimization of
351 extraction condition was conducted not only to improve recovery yields but also to mitigate
352 the negative effects of NADES on cell wall sugar composition. Typical component sugars for
353 HG, RGI pectic domains are uronic acid, rhamnose, arabinose and galactose (Mohnen, 2008)
354 while for hemicellulose they are fucose, xylose, mannose and glucose (Scheller & Ulvskov,
355 2010). Glucose is also typical of cellulose, but can also come from contaminating starch in

356 cell wall fractions. Arabinose and galactose can also arise from arabinogalactan proteins
 357 (AGP) known to be present in apple (Brillouet, Williams, Will, Müller, & Pellerin, 1996;
 358 Leszczuk, Szczuka, Wydrych, & Zdunek, 2018). Based on the sugar composition of the total
 359 material recovered (Figure 2A-supplementary Table S2), NADES pretreatments had a
 360 negative impact on the recovery of rhamnase, arabinose, fucose, galactose, glucose and uronic
 361 acids. Compared with water treatment (control), losses of neutral sugars (rhamnase, arabinose,
 362 fucose, galactose and glucose) in total fraction of CC:LA were particularly observed with
 363 pretreatment conditions of 80 °C-2h ($P<0.05$). Besides, the uronic acids recovery was
 364 significantly lower after CC:U pretreatment. Pretreatment at 40 °C for 1h limited such losses,
 365 except for the arabinose recovery. There was no significant difference in other neutral sugars
 366 (rhamnase, fucose, galactose and glucose) recovery between CC:LA pretreatment and control,
 367 which indicated that most of polysaccharides structure was preserved. However, overall
 368 uronic acids recovery in total fraction after 1h, 40 °C CC:U pretreatment of pomace was less
 369 than 50%. For CC:OA pretreatment, it clearly indicated that CC:OA degraded the pomace
 370 polysaccharides whether as pectin or other type of polysaccharides since both neutral
 371 monosaccharides and uronic acids recoveries in total fraction of CC:OA pretreated pomace
 372 were significantly lower than that of control pomace (supplementary Table S2).
 373



374 **Fig.**

375 **2** Sugar recovery as % weight of individual sugars in dry raw pomace in total fraction (A) and

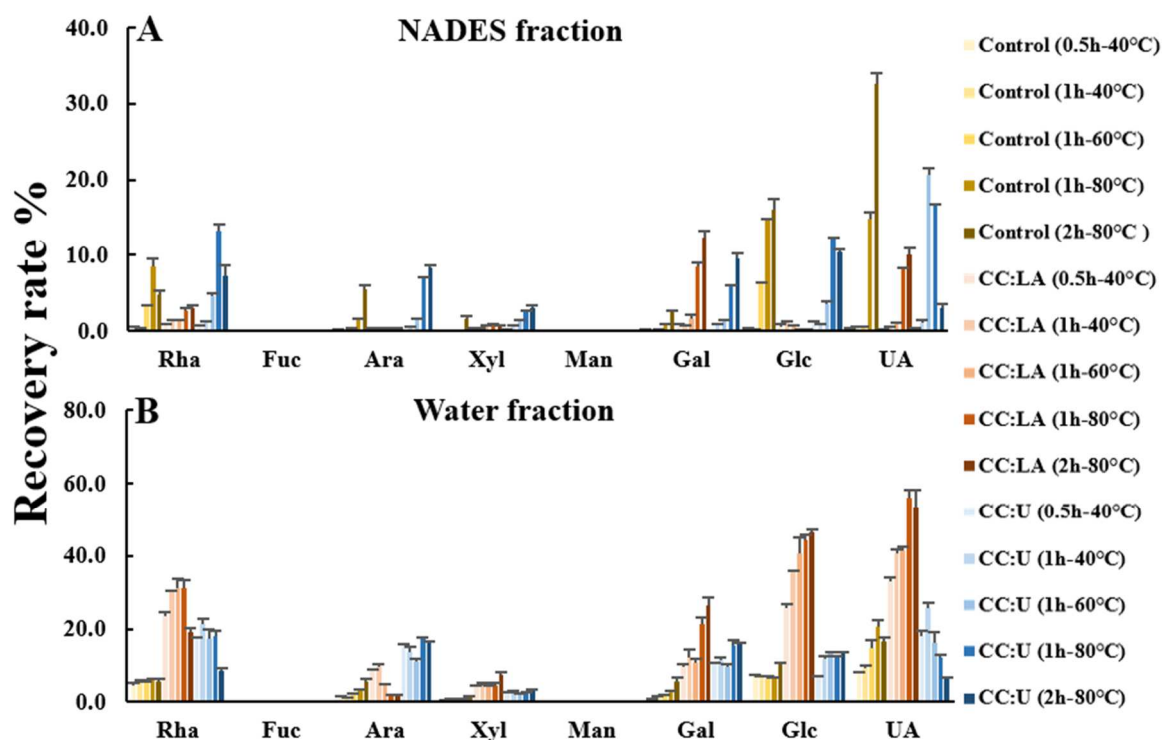
376 residue fraction (B) after CC:LA, CC:U or control pretreatment at different temperatures (40,
377 60, 80 °C) and time (0.5, 1, 2 h) of extraction. Means of four replicates; bar: standard
378 deviation. Rha, Fuc, Ara, Xyl, Man, Gal, Glc, UA: rhamnose, fucose, arabinose, xylose,
379 mannose, galactose, glucose, uronic acids. Detailed data and statistics are provided in
380 supplementary Table S2-3

381

382 Considering pectin-related sugars (arabinose, rhamnose, galactose and uronic acids) in detail,
383 the recovery of uronic acids in the residue fraction of CC:LA treated pomace was much lower
384 than that in the total fraction recovered regardless of pretreatment conditions (Figure 2A,B-
385 supplementary Table S2-3). In fact, most of uronic acids were found in the water fraction with
386 a highest recovery (56.1%) achieved after CC:LA pretreatment at 80 °C for 1h (Figure 3B-
387 supplementary Table S4), which indicated that CC:LA treatment helped in the extraction of
388 pectin. CC:LA likely loosened cell wall structures causing the extraction of pectin by hot
389 water. As mentioned above, CC:LA had negative effects on the total recovery of pectin
390 rhamnose and most notably side chain arabinose which is known to be rapidly cleaved under
391 mild acidic conditions (Thibault, Guillon, & Rombouts, 1991). Similarly, the branch size of
392 pectin obtained with CC: citric acid was found to be less than when pectin was extracted by
393 only a citric acid solution (Shafie & Gan, 2020). The lowest recovery of total rhamnose and
394 arabinose (45.7% for Rha; 4.8% for Ara) was observed at 80 °C-2h. Shortening the CC:LA
395 treatment of pomace to 1 h significantly increased rhamnose recovery in both the water and
396 residue fractions but that of arabinose remained significantly lower compared to control
397 (Figure 2B- supplementary Table S3, Figure 3B- supplementary Table S4). This result
398 indicated that not only pretreatment duration, but also temperature had a significant influence
399 on pectin side-chain structure and/or AGP. The decrease in CC:LA extraction temperature
400 from 80 °C to 60 °C for 1 h or to 40 °C for 1 h or 0.5 h, led to lesser total arabinose losses but
401 still left a majority of the pentose hydrolyzed. Last but not least, despite the fact that uronic
402 acids in the water fraction following CC:LA pretreatment of pomace was significantly higher
403 than that of control regardless of the pretreatment condition, obvious reduction of uronic acids
404 in the water fraction was noticed when the pretreatment temperature was lowered (Figure 3B-
405 supplementary Table S4). This result means lower CC:LA pretreatment temperature may not
406 be sufficient to break bonds in the cell wall to release pectin.

407

408



410
 411 **Fig. 3** Sugar recovery as % weight of individual sugars in dry raw pomace in NADES (A) and
 412 water (B) fractions after CC:LA, CC:U or control pretreatment at different temperatures (40,
 413 60, 80 °C) and time (0.5, 1, 2 h) of extraction. Means of four replicates; bar: standard
 414 deviation. Rha, Fuc, Ara, Xyl, Man, Gal, Glc, UA: rhamnose, fucose, arabinose, xylose,
 415 mannose, galactose, glucose, uronic acids. Detailed data and statistics are provided in
 416 supplementary Table S4-5.

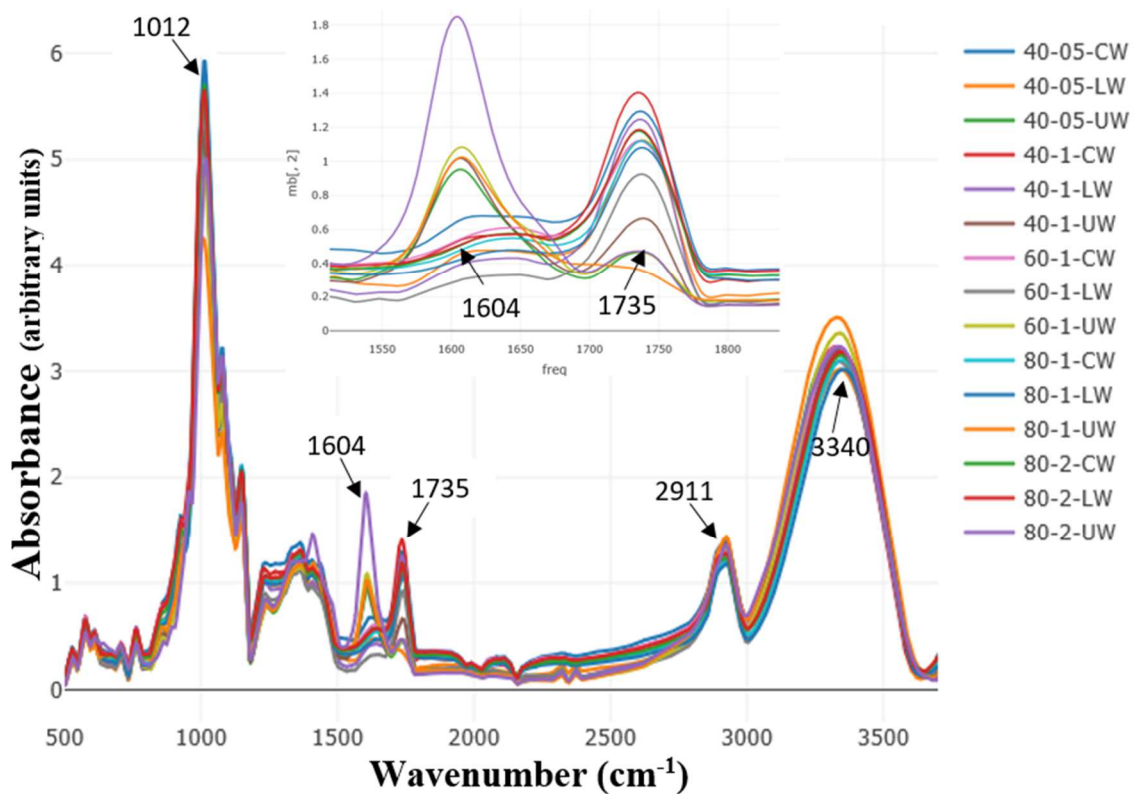
417

418 3.3. Choline chloride: urea treatment functionalizes apple pomace pectin

419 By comparison with the CC:LA, the CC:U pretreatment of pomace had a more profound
 420 impact on uronic acids recovery. It was only 6.5% in the water fraction after 2 h treatment at
 421 80 °C (Figure 3B- supplementary Table S4) and was also low in the NADES (CC:U) and
 422 residue fractions (Figure 3A- supplementary Table S5, Figure 2B- supplementary Table S3).
 423 More importantly, irrespective of pretreatment conditions, the total uronic acids recovery was
 424 not only much lower than the total recovery of other pectin related sugars (rhamnose,
 425 galactose and arabinose), but also significantly lower than that of control. Because the total
 426 recovery yield of fractions following CC:U treatment at 40 °C was not affected on the weight
 427 basis (Table 2), it thus implied that uronic acids quantification by colorimetry using m-
 428 hydroxydiphenyl was affected. Any modification of the uronic acids that change the

429 chemistry of the acid degradation product that complex with the dye may affect color
430 development and thus uronic acids quantitative measurement. For example, amidation of
431 pectin is known to depress the color development (Reitsma & Pilnik, 1989). Carbamate
432 derivatives of cellulose were reported with betain:urea and CC:U solvents (Willberg-
433 Keyriläinen, Hiltunen, & Ropponen, 2017) and similar derivatives were proposed for
434 carrageenan extracted from the red seaweed (Das, Sharma, Mondal, & Prasad, 2016).
435 Furthermore, Sirviö et al. (2019) have shown that sulfate groups were introduced to cellulose
436 with DES rich in urea (sulfamic acid : urea) at 80 °C and above, which indicated that a new
437 functional group can be added to polysaccharides in presence of high concentration of urea.
438 To check for modifications of pectin structure extracted following NADES pretreatment,
439 FTIR spectra of water extracts from control, CC:LA and CC:U treated apple pomace were
440 recorded (Figure 4). The spectra were typical of pectin with -OH and -C-H stretching
441 vibrations at 3340 cm^{-1} and 2911 cm^{-1} , C=O vibration of methyl ester at 1735 cm^{-1} and of the
442 acidic form at 1604 cm^{-1} , -CH-O-CH- stretching at 1012 cm^{-1} (Figure 4) (Guillotin, Bakx,
443 Boulenguer, Schols, & Voragen, 2007; Sinitsya, Čopíková, Prutyánov, Skoblyá, & Machovič,
444 2000). Moreover, by comparing with the characteristic peaks of commercial HM pectin
445 (Sinitsya et al., 2000), both control and CC:LA pectin were classified as HM pectin.
446 The spectra of water soluble pectin from the CC:U treated pomace were distinct from all
447 others with a gradual increase in the absorption band at 1604 cm^{-1} as the extraction condition
448 became harsher, in combination with the decrease in the C=O vibration at 1735 cm^{-1} (Figure
449 4). It thus appears that saponification occurred during the process. This interpretation was
450 supported by the significantly lower degree of methyl-esterification of the pectin from the
451 CC:U treated pomace compared to that of the control and the lowest DM value was measured
452 for pomace treated at 80 °C for 2 h (supplementary Figure S2). In fact, the actual DM is lower
453 than the current value considering the underestimation of uronic acid. This observation
454 provided further clue that the pectin structure was modified after CC:U pretreatment.
455 However, the molecular weight of pectin from CC:U treated pomace was not affected even at
456 80 °C for 2 h (supplementary Table S6), which indicated that CC:U pretreatment did not led
457 to depolymerization by β -elimination. As shown in Table 3, a relatively high nitrogen content
458 was detected in the pectin from the CC:U treated pomace, ranging from 0.54% to 3.59%.
459 Since the typical FTIR amide I and amide II bands at about 1680 and 1595 cm^{-1} were absent
460 from CC:U pectin (Figure 4) (Sinitsya et al., 2000), conversion to amidated pectin by
461 ammonia that could have been produced by hydrothermal degradation of the urea (Claus-
462 Peter, 2011) in the CC:U solvent did not occur. In fact, ^1H NMR spectroscopy (Figure 5)

463 indicated that choline was the likely source of nitrogen. Choline bound to the dissociated
 464 carboxyl group due to the electronic attraction, could have decreased the color development in
 465 the MHDP colorimetric assay of uronic acids. However, cholinium polygalacturonate and
 466 sodium pectinate from the CC:U treated pomace showed no modification in the colorimetric
 467 uronic acids content determination (supplementary Table S7). Hence, ion exchange with
 468 choline was not responsible for interfering with the MHDP colorimetric assay and more
 469 profound changes happened to the galacturonic acid structure.
 470



471
 472 **Fig. 4** FTIR spectrum of water soluble polysaccharides from control (CW), CC:LA (LW) and
 473 CC:U (UW) treated apple pomace at 80, 60 and 40 °C (80, 60, 40) for 0.5, 1.0 or 2 h (05, 1, 2).
 474

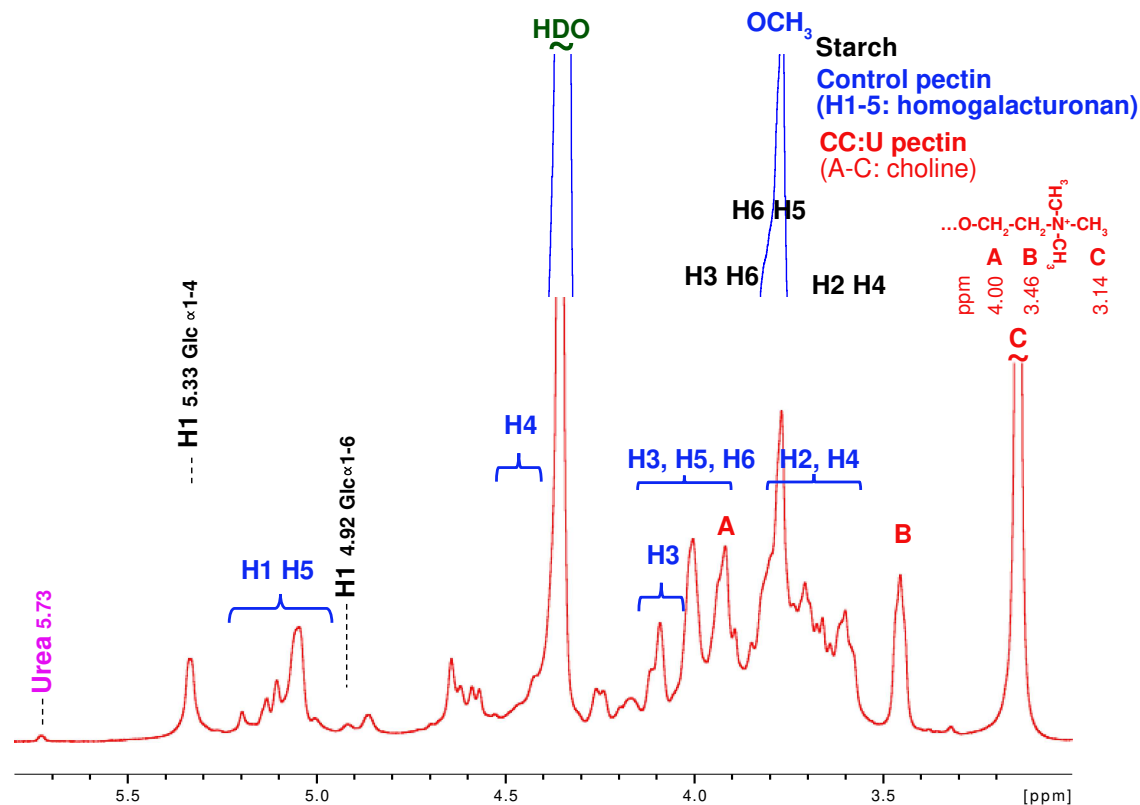
475 **Table. 3** Mean nitrogen and carbon contents (% of dry materials, \pm standard deviation, n = 4)
 476 in dry material recovered in the water fraction after NADES pretreatment

	Control		CC:LA		CC:U	
	N %	C %	N %	C %	N %	C %
80 °C – 2h	0.04 \pm 0.005	40.73 \pm 0.89	0.06 \pm 0.01	42.29 \pm 0.39	3.59 \pm 0.44	41.47 \pm 0.99
80 °C – 1h	0.01 \pm 0.001	38.42 \pm 0.13	0.07 \pm 0.005	42.18 \pm 0.20	0.95 \pm 0.13	42.37 \pm 0.62
60 °C – 1h	0.01 \pm 0.001	39.40 \pm 0.25	0.06 \pm 0.004	41.52 \pm 0.36	0.54 \pm 0.11	41.45 \pm 0.77

40 °C – 1h	0.02 ± 0.003	40.24 ± 0.94	0.07 ± 0.02	42.08 ± 0.47	0.58 ± 0.05	41.64 ± 0.45
40 °C – 0.5h	0.01 ± 0.003	40.52 ± 0.37	0.06 ± 0.001	42.41 ± 0.09	1.47 ± 0.06	41.46 ± 0.77

477

478



479

480 **Fig. 5** ¹H NMR spectroscopy of water fraction from control (blue) and CC:U (red) treated
 481 apple pomace. Starch (black labels), pectin homogalacturonan (blue labels), choline (red
 482 labels) and urea (pink label) signals are within reported region of chemical shifts in the
 483 literature (Finer, Franks, & Tait, 1972; Govindaraju, Young, & Maudsley, 2000; Nilsson,
 484 Gorton, Bergquist, & Nilsson, 1996; Petersen, Meier, Duus, & Clausen, 2008).

485

486 Reducing duration of CC:U treatment of apple pomace to 1 h at 80 °C or down to 40 °C led to
 487 a significantly increased recovery of uronic acids in all fractions (Figure 2, 3-supplementary
 488 Table S2-5), while lower nitrogen content was measured in pectin (Table 3). However, when
 489 pretreatment duration was reduced to 0.5 h, the nitrogen content increased again and the
 490 uronic acids content decreased. There was a negative correlation ($r=-0.75$) between pectin
 491 nitrogen and uronic acids contents after CC:U treatment of apple pomace. The different
 492 impacts of temperature and time on pectin nitrogen content suggest that co-occurring
 493 mechanisms exist in the modification of uronic acids that interfere with their colorimetric
 494 determination. Thus, in the light of the previous report on carbamylation of polysaccharides in

495 urea rich NADES, pretreatment of apple pomace with CC:U functionalizes pectin providing
496 new pectin derivatives. Due to the complexity of the polysaccharides in the water fraction no
497 attempt was made to determine the degree of substitution with choline but based on nitrogen
498 content and degree of methyl-esterification, the different levels of derivation can be achieved
499 by varying conditions.

500

501 3.4. NADES pretreatment facilitates starch extraction

502 Several studies have shown that only 5 wt% to 10 wt% of starch is extracted from different
503 types of agricultural wastes by DES (María, Bruinhorst, & Kroon, 2012; Shamsuri &
504 Abdullah, 2010; Zdanowicz & Szychaj, 2011). In our study, a similar result was found in the
505 CC:U extracts, which contained up to 12.4% of the pomace glucose (Figure 3A-
506 supplementary Table S5) while the CC:LA extracts contained almost no glucose. However, in
507 contrast with previous studies, when the NADES pretreated apple pomace was further
508 extracted by water, high glucose contents were recovered, especially from the CC:LA treated
509 pomace. The comparison between the content in starch and glucose in fractions shows that
510 most if not all glucose in the water fractions of NADES treated groups originated from starch
511 (supplementary Table S8). Moreover, between the two NADES, the CC:LA pomace
512 pretreatment led to a better extraction of starch by hot water and the harsher the pretreatment
513 conditions were, the more starch was extracted. In agreement with Zdanowicz et al. (2011)
514 who reported that DES composed of citric or succinic acid led to the polymer degradation due
515 to the acidic character of the solvents, starch in apple pomace may have been partly degraded
516 by the lactic acid, allowing for its extraction in larger amount by the hot water.

517

518 3.5. Pectin characterization

519 Since a large amount of pectin was extracted with water after CC:LA pretreatment of pomace
520 (33.1%-56.1% of uronic acid recovery), its structural characteristic was further studied. It
521 amounted to 19.3% - 26.6% of the pomace dry weight according to the pretreatment
522 conditions (Table 2 considering 68% water in the wet pomace), which was within the range of
523 values (9.5%-22.0%) (Koubala et al., 2008; Rha et al., 2011; Wang, Chen, & Lü, 2014)
524 reported for apple pomace pectin. Pectin was also extracted from the apple pomace by a
525 conventional method using dilute nitric acid to compare its characteristics with that extracted
526 following NADES pretreatment (SP, extraction method is provided in Supplementary
527 Informations). SP pectin yield (19.1% dry weight basis) and sugar composition were close to
528 that of the water fraction of CC:LA pretreated pomace at 40 °C for 0.5-1 h but contained less

529 uronic acids (supplementary Figure S3). As for pectin from CC:LA pretreated pomace, SP
 530 was contaminated by starch (33.2%). The esterification degree of the different pectin is
 531 shown in Table 4. The DM of pectin from CC:LA pretreated pomace was similar to that of
 532 control when pretreatment temperature was at 40 °C ($P>0.05$). Yet, the DM of pectin from the
 533 CC:LA treated pomace significantly decreased as the pretreatment temperature increased
 534 from 40 °C to 80 °C. The DA is not frequently reported in the study concerning pectic
 535 polysaccharides. The highest DA was obtained when pretreatment was carried out at 40 °C
 536 for 0.5 h in both pectin from CC:LA pretreated pomace and control ($P<0.05$). Close
 537 esterification degrees were also found in SP. The influence of extraction condition on
 538 esterification degree can be mitigated when temperature was decreased to 40 °C. Garna et al.
 539 (2007) studied the influence of different extraction conditions on the yield and chemical
 540 characteristics of apple pomace pectin. They reported that the DM of pectin ranged from 54.5%
 541 to 79.5%, with the highest value recorded for pectin extracted for 1 h at 80 °C and pH 2.0.
 542 Besides, highly acetylated pectin can be obtained from apple pomace extracted with Li Cl-
 543 DMSO, the acetyl ester content reached 3.3% of the weight (Ray et al., 2014), which is in
 544 consistent with our results (acetic acid % in CC:LA 40 °C for 0.5 h = 3.45 %).

545

546 **Table. 4** Mean methyl- and acetyl-esterification degree (\pm standard deviation, n = 4) of
 547 extracted pectin by dilute hot mineral acid (SP), after pomace pretreatment with water
 548 (Control), or choline chloride:lactic acid (CC:LA).

	DM			DA		
	SP	Control	CC:LA	SP	Control	CC:LA
	80.6 \pm 2.6			51.6 \pm 2.2		
80 °C – 2h		64.8 \pm 2.0 ^A	56.9 \pm 3.6 ^{*A}		13.2 \pm 2.9 ^A	27.9 \pm 1.5 ^{*A}
80 °C – 1h		73.1 \pm 2.1 ^B	59.1 \pm 3.3 ^{*A}		29.9 \pm 1.4 ^B	32.0 \pm 2.8 ^B
60 °C – 1h		72.9 \pm 1.2 ^B	60.3 \pm 3.3 ^{*A}		31.0 \pm 3.5 ^B	48.8 \pm 3.9 ^{*C}
40 °C – 1h		75.8 \pm 3.5 ^B	77.9 \pm 4.3 ^B		45.4 \pm 3.7 ^C	46.4 \pm 0.8 ^C
40 °C – 0.5h		79.7 \pm 0.7 ^C	78.3 \pm 1.5 ^B		53.7 \pm 3.4 ^D	54.5 \pm 3.0 ^D

549 Mean values with unlike letters were significantly different ($P<0.05$), A,B,C: significantly different at different
 550 condition within same group (columns), *: significantly different between two groups at same condition (rows).

551

552 Due to the large amount of starch in the water fraction, pectin from different pretreated apple
 553 pomace was treated with amylase before Mw measurement. No glucose content was found in

554 treated pectin, which indicated that starch was totally removed. As is shown in Table 5, the
 555 Mw and intrinsic viscosity of both control and CC:LA pretreated pectin were sensitive to the
 556 pretreatment conditions, they both increased with the decrease in temperature or duration of
 557 pretreatment. Besides, pectin from the CC:LA pretreated pomace had significantly higher Mw
 558 than that of control no matter what the pretreatment conditions were. Although the sugar
 559 composition analysis of extracts and residues indicated that polysaccharide structures were
 560 degraded and lost during the extraction process of apple pomace, the pectin recovered in the
 561 water extract after a pretreatment at 80 °C for 2 h still demonstrated high Mw close to that
 562 obtained with the SP pectin. These results combined with the extraction yield and pectin
 563 chemical composition, clearly showed that hot water extraction after CC:LA pretreatment is a
 564 good candidate to substitute for classic industrial extraction methods of pectin.

565
 566 **Table. 5** Mean molecular weight (\pm standard deviation, n = 4) of pectin extracted by dilute
 567 mineral acid (SP), after pomace pretreatment with water (Control), or choline chloride:Lactic
 568 acid (CC:LA)

	Mw ($\times 10^5$ Da)			Viscosity ($\text{cm}^3 \text{g}^{-1}$)		
	SP	Control	CC:LA	SP	Control	CC:LA
	2.8 \pm 0.3			8.1 \pm 0.7		
80 °C – 2h		1.7 \pm 0.2 ^A	2.9 \pm 0.4 ^{*A}		10.0 \pm 0.5 ^A	6.7 \pm 0.3 ^{*A}
80 °C – 1h		2.5 \pm 0.3 ^A	3.5 \pm 0.7 ^{*AB}		12.1 \pm 0.7 ^B	8.3 \pm 0.4 ^{*B}
60 °C – 1h		2.6 \pm 0.2 ^A	3.9 \pm 0.3 ^{*B}		12.9 \pm 0.1 ^C	12.4 \pm 1.3 ^C
40 °C – 1h		4.1 \pm 0.2 ^B	7.0 \pm 0.7 ^{*C}		14.2 \pm 0.5 ^D	12.6 \pm 0.5 ^{*C}
40 °C – 0.5h		5.3 \pm 1.2 ^C	8.3 \pm 0.6 ^{*D}		15.8 \pm 0.2 ^E	12.7 \pm 0.1 ^{*C}

569 Mean values with unlike letters were significantly different ($P < 0.05$), A,B,C: significantly different at different
 570 condition within same group (columns), *: significantly different between two groups at same condition (rows).

572 4. Conclusion

573 The present study explored the impact of apple pomace pretreatment by three different
 574 NADES on the recovery of water-soluble pectin while allowing for further extractions of
 575 valuable polysaccharides from the residues. Thus, one critical point in the pretreatment
 576 conditions was to keep as much as possible the chemical integrity of hemicellulose and pectin.
 577 Results showed that CC:LA and CC:U treatments led to overall recovery yield comparable
 578 with that of control while CC:OA led to low yield due to polysaccharides degradation. The
 579 polysaccharides structure was well preserved by CC:LA pretreatments when temperature was

580 set at 40 °C but CC:U affected pectin composition by introducing choline and decreased
581 methyl-esterification. CC:LA facilitated extraction of a large amount of hot water-soluble
582 pectin from the pomace and taking extraction yield and pectin structure into consideration,
583 pretreatment at 40 °C for 1 h afforded the recovery of high Mw HM pectin of structural
584 characteristics comparable with classical commercial pectin. Thus, CC:LA pretreatment of
585 apple pomace followed by water extraction offers a “green” alternative to classical pectin acid
586 extraction while CC:U pretreatment open the way to produce low esterified and choline
587 modified pectin that remains to be further characterized either as extracted polyuronan or as
588 semi-refined pectin for technical applications.

589

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Water extraction



Pectin



Cell wall polysaccharides

