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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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## 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 [ $\alpha$ -1,4-D-galacturonic acid]<sub>n</sub> 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 [ $\alpha$ -D-GalA-1,2- $\alpha$ -L-Rha-1,4]<sub>n</sub> on which side-chains made of  $\alpha$ -L-  
50 Araf and  $\beta$ -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  $\alpha$ -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, & Romyantseva,  
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 P<sub>2</sub>O<sub>5</sub> 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:

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

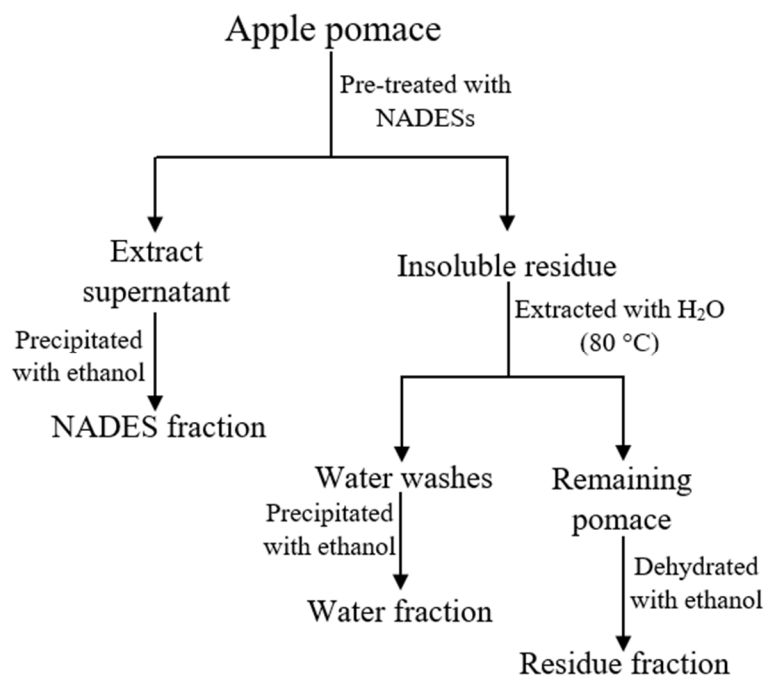
Where the W<sub>s</sub> is the sample weight in each fractions and W is the initial wet weight of apple pomace.

**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 NaBH<sub>4</sub> 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  $\alpha$ -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<sup>-1</sup>. 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<sup>-1</sup> 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. <sup>1</sup>H 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 D<sub>2</sub>O (99.96 atom% D, Sigma-Aldrich) and then lyophilized. This



211 process was repeated twice. Water pre-saturated  $^1\text{H}$  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  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  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  $\times$  250 mm, Lichrospher 100 RP-18e (5  $\mu\text{m}$ ),  
232 Interchim, France) at 25 °C.  $\text{H}_2\text{SO}_4$  (4 mM) was used for isocratic elution at 1.0 mL  $\text{min}^{-1}$ .

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  $\mu\text{L}$  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 NaNO<sub>3</sub> at a flow rate of 0.6 mL min<sup>-1</sup> 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
<b>Total fraction</b>				
0.5h-40 °C	21.69 ± 0.50 <sup>a,A</sup>	21.13 ± 0.52 <sup>a,B</sup>	21.26 ± 0.60 <sup>a,C</sup>	
1h-40 °C	21.72 ± 0.75 <sup>a,A</sup>	21.34 ± 0.30 <sup>a,B</sup>	21.80 ± 0.85 <sup>a,C</sup>	
1h-60 °C	21.66 ± 0.26 <sup>c,A</sup>	20.58 ± 0.34 <sup>b,B</sup>	20.03 ± 0.36 <sup>a,B</sup>	
1h-80 °C	22.71 ± 0.74 <sup>b,B</sup>	18.54 ± 0.62 <sup>a,A</sup>	19.39 ± 0.67 <sup>a,B</sup>	
2h-80 °C	21.49 ± 0.50 <sup>c,A</sup>	17.65 ± 1.23 <sup>b,A</sup>	18.10 ± 0.09 <sup>b,A</sup>	8.44 ± 0.78 <sup>a</sup>

**Residue fraction**

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

**NADES fraction**

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

**Water fraction**

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

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<sup>-1</sup>: 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 ( $P>0.05$ ).

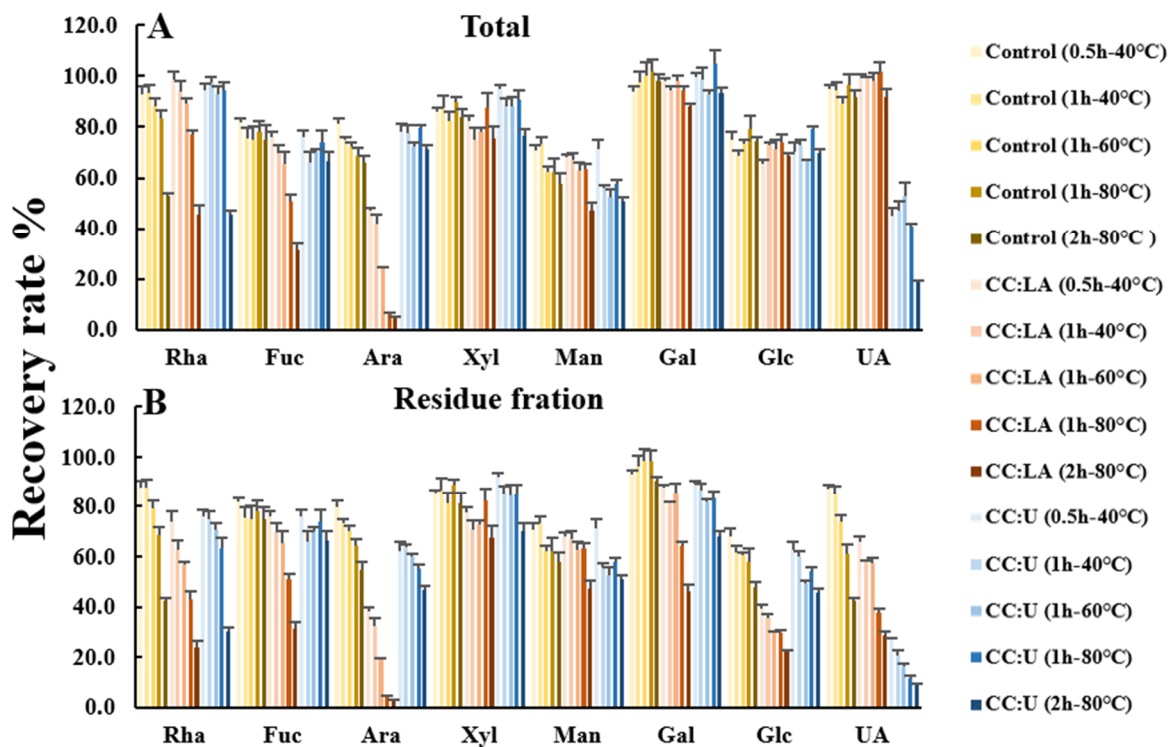
296 Looking at specific fractions, the yield of the NADES fraction increased with higher  
297 temperature or extended extraction time. For NADES extraction of pomace, as mentioned  
298 above, higher temperature decreased viscosity of NADESs solution. Many studies reported on  
299 the inverse relation 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<sup>-1</sup> 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

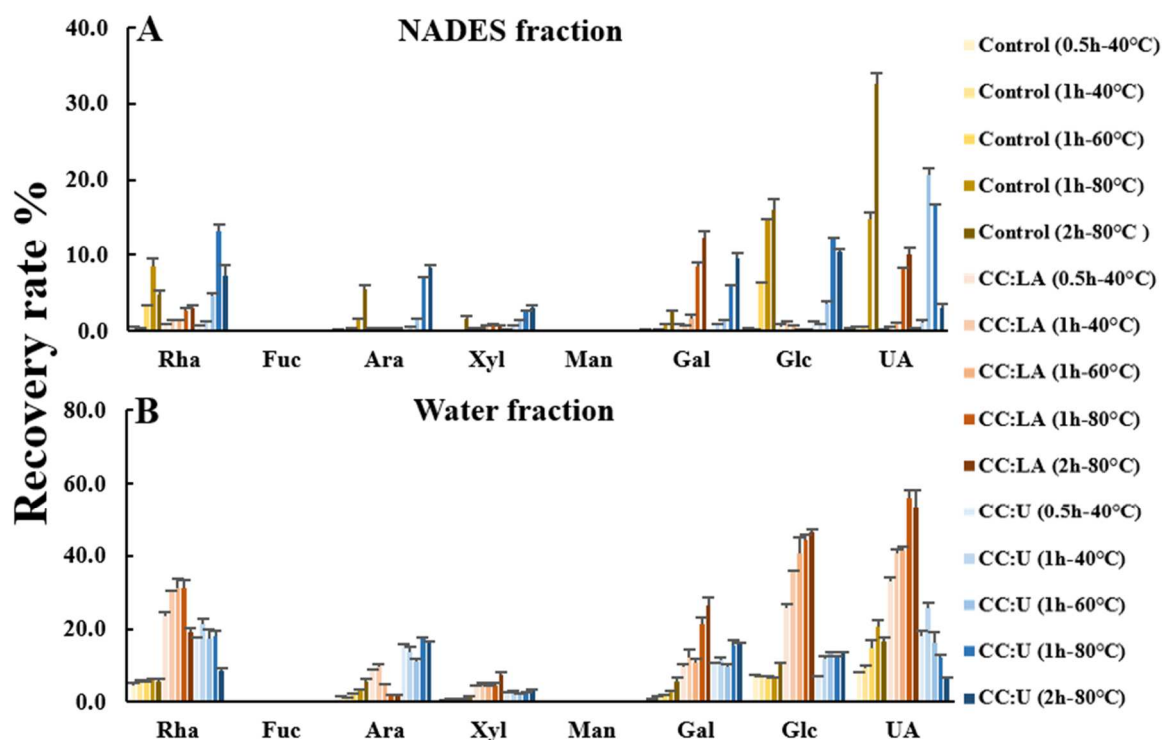
376 residue fraction (B) after CC:LA, CC:U or control pretreatment at different temperatures (40,  
377 60, 80 °C) and time (0.5, 1, 2 h) of extraction. Means of four replicates; bar: standard  
378 deviation. Rha, Fuc, Ara, Xyl, Man, Gal, Glc, UA: rhamnose, fucose, arabinose, xylose,  
379 mannose, 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

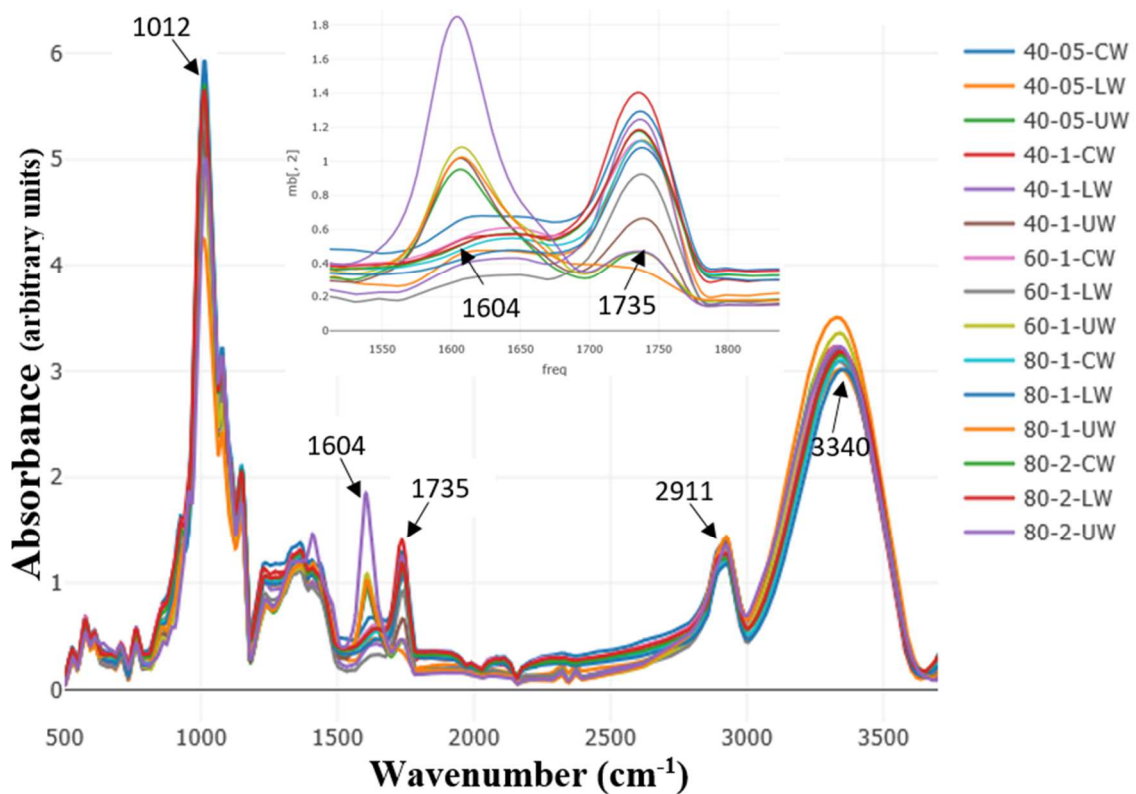
### 418 3.3. Choline chloride: urea treatment functionalizes apple pomace pectin

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<sup>-1</sup> and 2911 cm<sup>-1</sup>, C=O vibration of methyl ester at 1735 cm<sup>-1</sup> and of the  
442 acidic form at 1604 cm<sup>-1</sup>, -CH-O-CH- stretching at 1012 cm<sup>-1</sup> (Figure 4) (Guillotin, Bakx,  
443 Boulenguer, Schols, & Voragen, 2007; Sinitsya, Čopíková, Prutyánov, Skoblyya, & 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<sup>-1</sup> as the extraction condition  
448 became harsher, in combination with the decrease in the C=O vibration at 1735 cm<sup>-1</sup> (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<sup>-1</sup> 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, <sup>1</sup>H 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

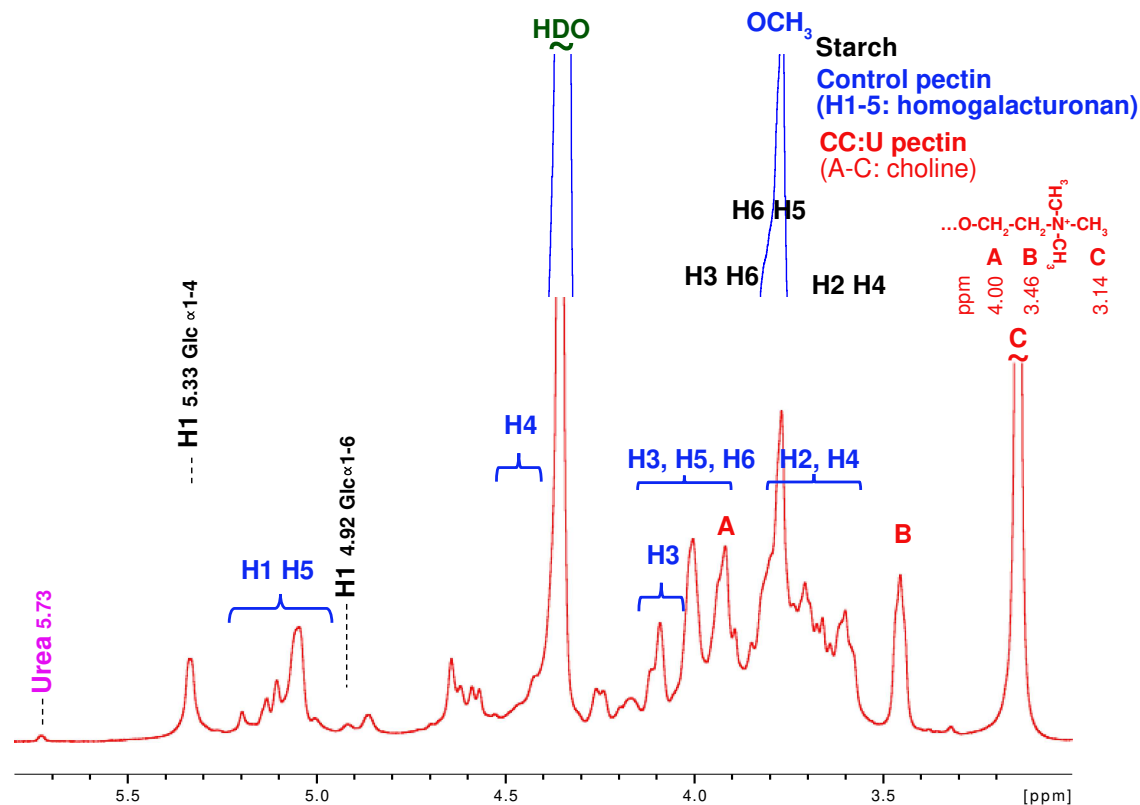
475 **Table. 3** Mean nitrogen and carbon contents (% of dry materials,  $\pm$  standard deviation, n = 4)  
 476 in dry material recovered in the water fraction after NADES pretreatment

	Control		CC:LA		CC:U	
	N %	C %	N %	C %	N %	C %
<b>80 °C – 2h</b>	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
<b>80 °C – 1h</b>	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
<b>60 °C – 1h</b>	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

<b>40 °C – 1h</b>	0.02 ± 0.003	40.24 ± 0.94	0.07 ± 0.02	42.08 ± 0.47	0.58 ± 0.05	41.64 ± 0.45
<b>40 °C – 0.5h</b>	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** <sup>1</sup>H 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 <sup>A</sup>	56.9 $\pm$ 3.6 <sup>*A</sup>		13.2 $\pm$ 2.9 <sup>A</sup>	27.9 $\pm$ 1.5 <sup>*A</sup>
80 °C – 1h		73.1 $\pm$ 2.1 <sup>B</sup>	59.1 $\pm$ 3.3 <sup>*A</sup>		29.9 $\pm$ 1.4 <sup>B</sup>	32.0 $\pm$ 2.8 <sup>B</sup>
60 °C – 1h		72.9 $\pm$ 1.2 <sup>B</sup>	60.3 $\pm$ 3.3 <sup>*A</sup>		31.0 $\pm$ 3.5 <sup>B</sup>	48.8 $\pm$ 3.9 <sup>*C</sup>
40 °C – 1h		75.8 $\pm$ 3.5 <sup>B</sup>	77.9 $\pm$ 4.3 <sup>B</sup>		45.4 $\pm$ 3.7 <sup>C</sup>	46.4 $\pm$ 0.8 <sup>C</sup>
40 °C – 0.5h		79.7 $\pm$ 0.7 <sup>C</sup>	78.3 $\pm$ 1.5 <sup>B</sup>		53.7 $\pm$ 3.4 <sup>D</sup>	54.5 $\pm$ 3.0 <sup>D</sup>

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 <sup>A</sup>	2.9 $\pm$ 0.4 <sup>*A</sup>		10.0 $\pm$ 0.5 <sup>A</sup>	6.7 $\pm$ 0.3 <sup>*A</sup>
80 °C – 1h		2.5 $\pm$ 0.3 <sup>A</sup>	3.5 $\pm$ 0.7 <sup>*AB</sup>		12.1 $\pm$ 0.7 <sup>B</sup>	8.3 $\pm$ 0.4 <sup>*B</sup>
60 °C – 1h		2.6 $\pm$ 0.2 <sup>A</sup>	3.9 $\pm$ 0.3 <sup>*B</sup>		12.9 $\pm$ 0.1 <sup>C</sup>	12.4 $\pm$ 1.3 <sup>C</sup>
40 °C – 1h		4.1 $\pm$ 0.2 <sup>B</sup>	7.0 $\pm$ 0.7 <sup>*C</sup>		14.2 $\pm$ 0.5 <sup>D</sup>	12.6 $\pm$ 0.5 <sup>*C</sup>
40 °C – 0.5h		5.3 $\pm$ 1.2 <sup>C</sup>	8.3 $\pm$ 0.6 <sup>*D</sup>		15.8 $\pm$ 0.2 <sup>E</sup>	12.7 $\pm$ 0.1 <sup>*C</sup>

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

