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

Mingrui Chen, Marc Lahaye

▶ To cite this version:

Mingrui Chen, Marc Lahaye. Natural deep eutectic solvents pretreatment as an aid for pectin extraction from apple pomace. Food Hydrocolloids, 2021, 115, pp.106601. 10.1016/j.foodhyd.2021.106601 . hal-03186525

HAL Id: hal-03186525

https://hal.inrae.fr/hal-03186525

Submitted on 13 Feb 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Version of Record: https://www.sciencedirect.com/science/article/pii/S0268005X21000175
Manuscript_a7cf57d210ffaef65ae48de2f18a0786

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

3 Mingrui Chen, Marc Lahaye*
4 UR 1268 Biopolymères Interactions Assemblages, équipe Paroi Végétale et Polysaccharides
5 Pariétaux (PVPP), INRAE, 44300, Nantes, France
6 *Corresponding author. E-mail address: marc.lahaye@inra.fr (M. Lahaye).

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

27
28

© 2021 published by Elsevier. This manuscript is made available under the CC BY NC user license
https://creativecommons.org/licenses/by-nc/4.0/

1. Introduction

Apple is the most cultivated and consumed fleshy fruit in the world and its associated products, such as juice, cider and sauce are very popular among consumers. Apple processing generates side-streams, such as about10 million tons of pomace each year from the apple juice industry (Alongi, Melchior, & Anese, 2018) which can be valorized to extract pectin (May, 1990). Besides for food gelling ingredient, apple pomace may be source of other valuable polysaccharides, such as hemicellulose and cellulose. Hemicellulose can be applicable as film, drug carrier and stabilizing additive in food, pharmacy and other related industries (Ebringerová, 2005), while cellulose play a paramount role in paper making industry (Ververis, Georghiou, Christodoulakis, Santas, & Santas, 2004). To that end, a biorefinery approach was engaged to isolate apple pomace polysaccharides and, as a first step, pectin extraction was reconsidered. Pectin is made of approximately 65% homogalacturonan (HG), 20–35% rhamnogalacturonan I (RGI) and 10% rhamnogalacturonan II (RGII) structural domains (Mohnen, 2008). HG is formed by repeats of $[\alpha\text{-1,4-D-galacturonic acid}]_n$ units that can be partially esterified by methanol on O-6 (Voragen, Beldman, & Schols, 2001) and acetyl at O-2 and/or O-3 (Atmodjo, Hao, & Mohnen, 2013). It can be further modified at O-3 by xylose to form xylogalacturonan domains (Schols, Bakx, Schipper, & Voragen, 1995). According to the degree of methyl esterification, pectin is classified as high methoxyl (HM) pectins (degree of esterification > 50%) and low methoxyl (LM) pectins (degree of esterification < 50%) (Löfgren & Hermansson, 2007). RG-I pectic domain is built on the disaccharide repeat unit $[\alpha\text{-D-GalA-1,2-}\alpha\text{-L-Rha-1,4}]_n$ on which side-chains made of α-L-Araf and β-D-Galp are branched on O-4 of the rhamnosyl residues (Scheller, Jensen, Sørensen, Harholt, & Geshi, 2007). The minor RG-II structural domains consist of at least 8 α-1,4-D-galacturonic acid units with four types of complex side branches made of 12 different types of sugars (O'Neill, Ishii, Albersheim, & Darvill, 2004). Generally, extraction of pectin from agricultural side-streams is achieved through mild acid, mild alkali or enzyme extraction. Pectin industrial production mainly uses aqueous mineral acid which is responsible for corrosion of equipment and environmental pollution (Wikiera, Mika, Starzyńska-Janiszewska, & Stodolak, 2015). Methods using mild alkali decrease the degree of esterification and the molecular weight of pectin (Yuliarti et al., 2015) while enzymatic extraction affects pectin yield, physicochemical and rheological properties (Ptichkina, Markina, & Rumyantseva, 2008). Microwave and ultrasound techniques provide clear benefits in shortening extraction time and reducing energy consumption (Adetunji, Adekunle, Orsat, & Raghavan, 2017) but if 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 (Benvenutti, 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 (Benvenutti, 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 Gerges, 2019; Benvenutti 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, & Spychaj, 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).

129

130 2.2. Methods

131

132 2.2.1. Pretreatment of apple pomace with NADESs

133 Five g of thawed apple pomace (water content = 68%) were mixed with 40 mL of different
134 NADES (1:8, w/v) and the big particles in suspension were dispersed with a Polytron mixer.
135 The mixture was stirred with a magnetic bar and heated in water bath under different
136 conditions as listed in Table 1. The suspension was then centrifuged at 15000 g for 20 min
137 and the supernatant was collected as NADES supernatant fraction. The remaining pomace
138 was washed twice with deionized water at ambient temperature to reach water pH (pH 6.5),
139 then, the pellet was resuspended in 40 mL of deionized water at 80 °C for 10 min under
140 constant agitation and the water extract was recovered after centrifugation as above. This
141 operation was repeated 5 times. The pooled water washes, referred to as the water fraction,
142 was concentrated to 10 mL with a vacuum rotary evaporator. Polymers in the NADES
143 supernatant and water fractions were precipitated by 4 volumes of ethanol. The precipitates
144 were recovered by centrifugation (15000 g, 20 min) and washed with 40 mL of 70% of
145 ethanol for 10 min (3 times), followed by 40 mL of ethanol and acetone for 10 min (2 times).
146 The ethanol and acetone washings were repeated to obtain low color solutions. The pomace
147 residue underwent the same washing and dehydration process as above. Finally, samples were
148 dried at 40 °C in vacuum oven over P_2O_5 powder for 12 h and stored at 4 °C for later analysis.
149 The extraction was conducted four times and water was used as a control pretreatment
150 condition. Fractions yield was calculated as follows:

151 $$\text{Yield (\%)} = \times 100$$

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

154 **Table 1.** Extraction conditions with NADES

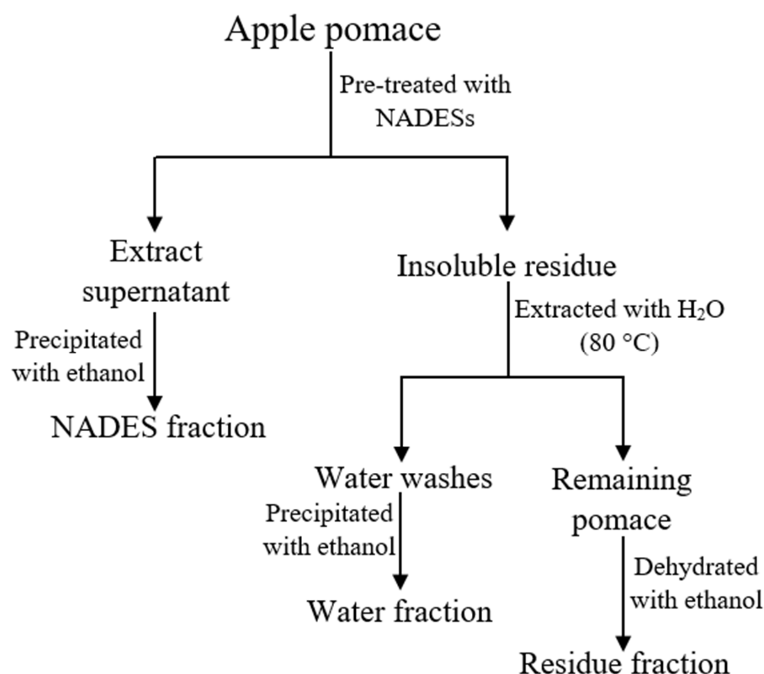
Extraction condition	
Time	Temperature
2 h	80 °C
1 h	80 °C, 60 °C, 40 °C
0.5 h	40 °C

155

156

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 $NaBH_4$ 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, Folsorn, 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. ^{1}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_2O (99.96 atom% D, Sigma-Aldrich) and then lyophilized. This

211 process was repeated twice. Water pre-saturated ^{1}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 $CuSO_4.5H_2O$ 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. H_2SO_4 (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 uL 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 $NaNO_3$ 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}

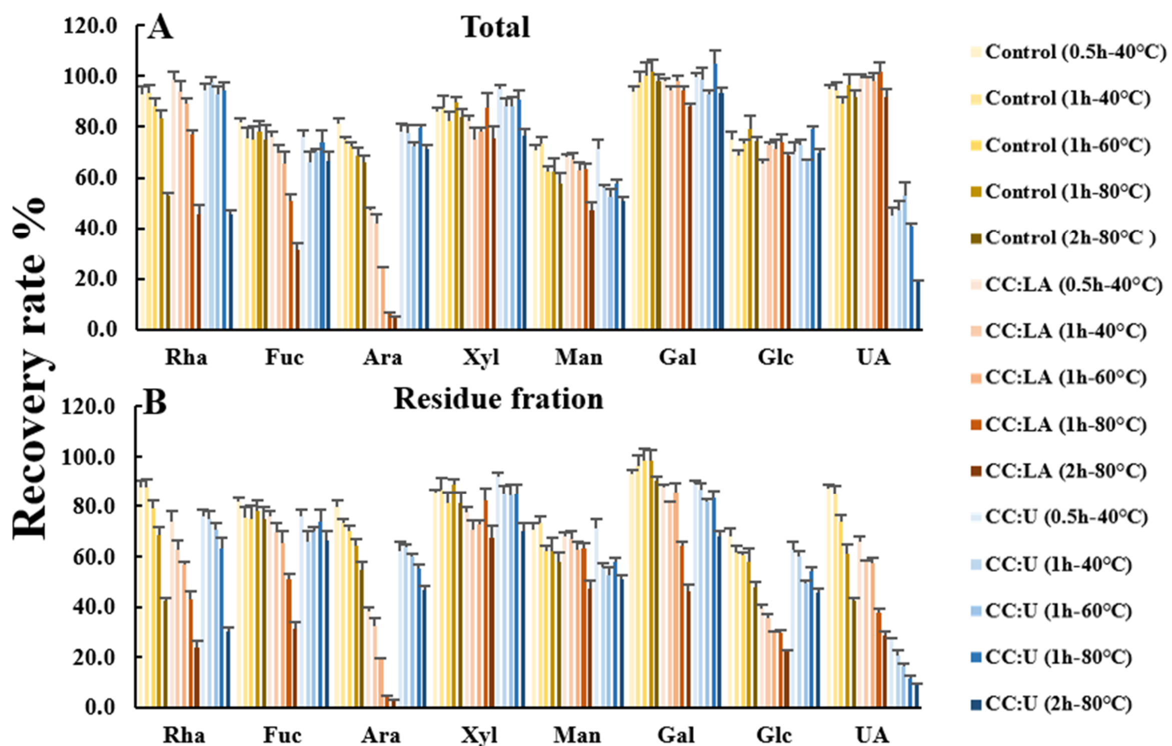
Control: water replacing for NADES; CC:LA, CC:U, CC:OA: choline chloride: lactic acid, urea and oxalic acid, Mean values (± standard deviation, n = 4) with unlike letters were significantly different (P<0.05), significant difference was analyzed in each fraction. a,d,c (row): significantly different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

Extraction yields with the CC:LA, CC:OA and CC:U are depicted in Table 2. The viscosity of the NADES is provided in Table S1 and showed, as reported that it decreased with both temperature and addition of water (Yang et al., 2018; Zdanowicz et al., 2018). Moreover, consistent with other reports (Fisher & Kunz, 2014; Hou et al., 2008)), the three NADES were non-Newtonian with a shear-thinning behavior (data not shown). The initial extraction conditions were set at 80 °C for 2 h to lower viscosity of NADES (viscosity at 10 s^{-1}: from 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 mPas at 80 °C for CC:U; from solid at 40 °C to 37.0 mPas at 80 °C for CC:OA). As mass transfer plays an important role in determining extraction conditions, the lower viscosity at 80 °C with longer duration can facilitate the interaction between NADESs and apple pomace. Morrais et al. (2018) demonstrated that higher hardwood xylan solubilization was achieved with CC:U (1:2) at 80 °C. Moreover, the optimal condition for extracting pectin from *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. Benvenutti 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

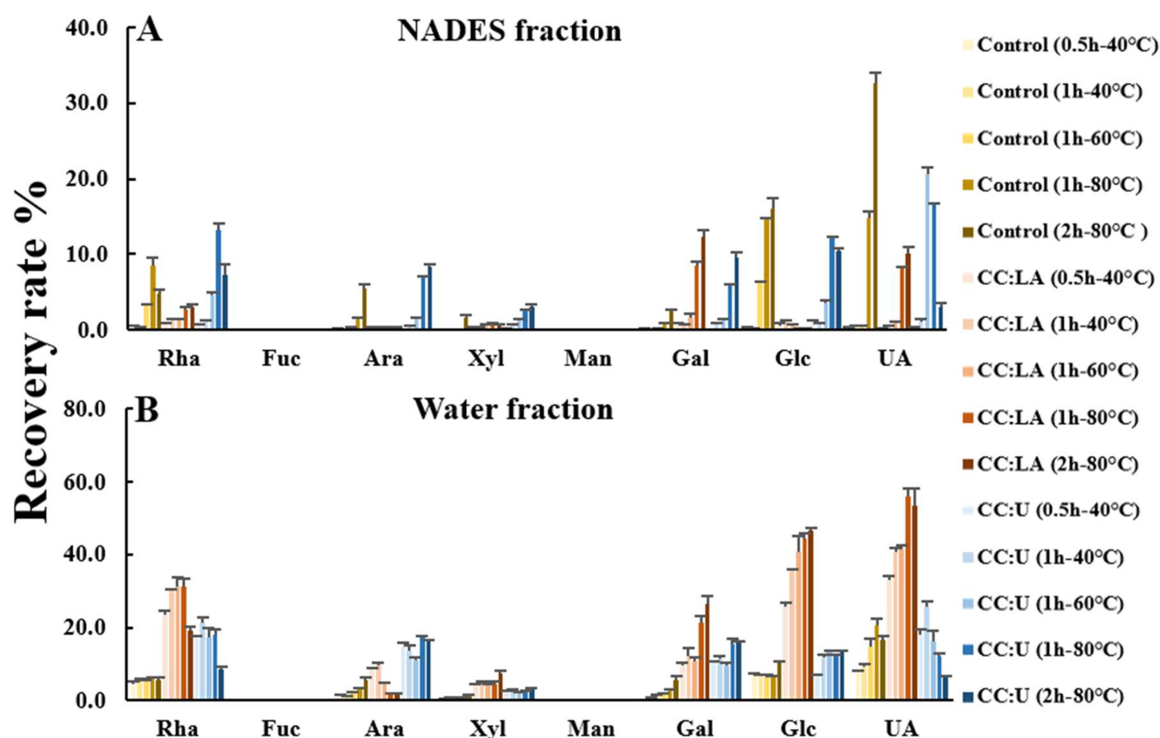
356 cell wall fractions. Arabinose and galactose can also arise from arabinogalactan proteins
357 (AGP) known to be present in apple (Brillouet, Williams, Will, Müller, & Pellerin, 1996;
358 Leszczuk, Szczuka, Wydrych, & Zdunek, 2018). Based on the sugar composition of the total
359 material recovered (Figure 2A-supplementary Table S2), NADES pretreatments had a
360 negative impact on the recovery of rhamnose, arabinose, fucose, galactose, glucose and uronic
361 acids. Compared with water treatment (control), losses of neutral sugars (rhamnose, 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 (rhamnose, 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

409



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.

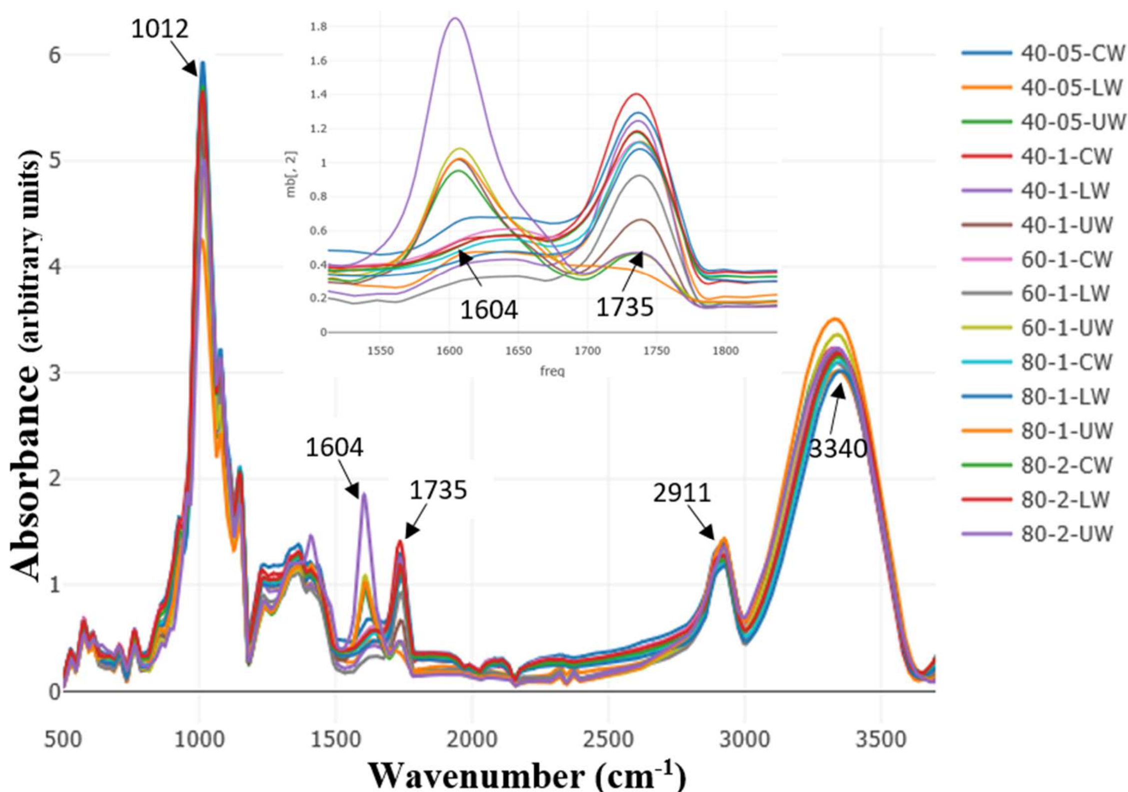
417

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, Prutyanov, Skoblya, & 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, ^{1}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

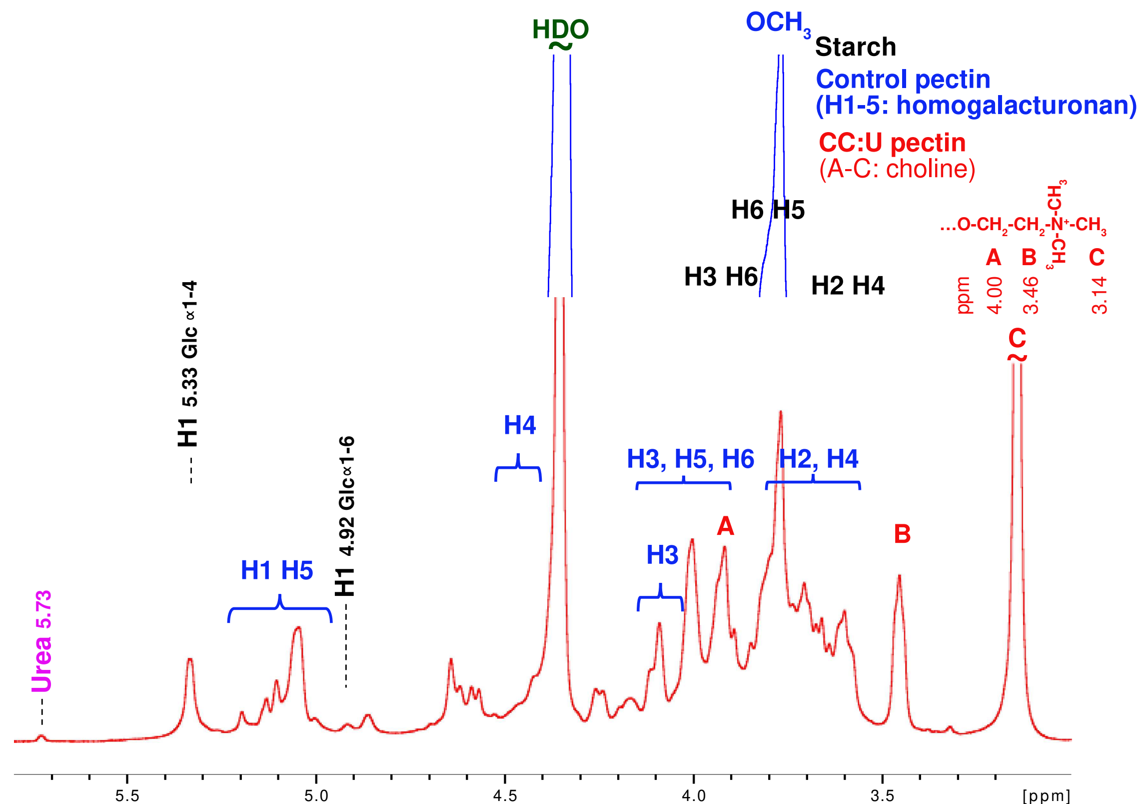
472 **Fig. 4** FTIR spectrum of water soluble polysaccharides from control (CW), CC:LA (LW) and
473 CC:U (UW) treated apple pomace at 80, 60 and 40 °C (80, 60, 40) for 0.5, 1.0 or 2 h (05, 1, 2).
474
475 **Table. 3** Mean nitrogen and carbon contents (% of dry materials, ± standard deviation, n = 4)
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 ± 0.005	40.73 ± 0.89	0.06 ± 0.01	42.29 ± 0.39	3.59 ± 0.44	41.47 ± 0.99
80 °C – 1h	0.01 ± 0.001	38.42 ± 0.13	0.07 ± 0.005	42.18 ± 0.20	0.95 ± 0.13	42.37 ± 0.62
60 °C – 1h	0.01 ± 0.001	39.40 ± 0.25	0.06 ± 0.004	41.52 ± 0.36	0.54 ± 0.11	41.45 ± 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** ^{1}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 & Spychaj, 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 (± 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 ± 2.6			51.6 ± 2.2		
80 °C – 2h		64.8 ± 2.0^{A}	$56.9 \pm 3.6^{*A}$		13.2 ± 2.9^{A}	$27.9 \pm 1.5^{*A}$
80 °C – 1h		73.1 ± 2.1^{B}	$59.1 \pm 3.3^{*A}$		29.9 ± 1.4^{B}	32.0 ± 2.8^{B}
60 °C – 1h		72.9 ± 1.2^{B}	$60.3 \pm 3.3^{*A}$		31.0 ± 3.5^{B}	$48.8 \pm 3.9^{*C}$
40 °C – 1h		75.8 ± 3.5^{B}	77.9 ± 4.3^{B}		45.4 ± 3.7^{C}	46.4 ± 0.8^{C}
40 °C – 0.5h		79.7 ± 0.7^{C}	78.3 ± 1.5^{B}		53.7 ± 3.4^{D}	54.5 ± 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 (raws).
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 (± 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 (× 10^5 Da)			**Viscosity ($cm^3 g^{-1}$)**		
	SP	**Control**	**CC:LA**	**SP**	**Control**	**CC:LA**
	2.8 ± 0.3			8.1 ± 0.7		
80 °C – 2h		1.7 ± 0.2^{A}	2.9 ± 0.4^{*A}		10.0 ± 0.5^{A}	6.7 ± 0.3^{*A}
80 °C – 1h		2.5 ± 0.3^{A}	3.5 ± 0.7^{*AB}		12.1 ± 0.7^{B}	8.3 ± 0.4^{*B}
60 °C – 1h		2.6 ± 0.2^{A}	3.9 ± 0.3^{*B}		12.9 ± 0.1^{C}	12.4 ± 1.3^{C}
40 °C – 1h		4.1 ± 0.2^{B}	7.0 ± 0.7^{*C}		14.2 ± 0.5^{D}	12.6 ± 0.5^{*C}
40 °C – 0.5h		5.3 ± 1.2^{C}	8.3 ± 0.6^{*D}		15.8 ± 0.2^{E}	12.7 ± 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 (raws).
571

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
590 **Acknowledgement**
591 MC is supported in part by a scholarship from the China Scholarship Council. The authors
592 thank J. Vigouroux, S. Durand, L. Saulnier, MJ. Crépeau (INRA-BIA), X. Falourd (INRA-
593 BIBS instrumental platform) for their help in GLC, FTIR, Mw and NMR experiments. R.
594 Bauduin (IFPC, Le Rheu) is thanked for providing apple pomace.
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613

Reference

Achkar, T. El., Fourmentin, S., & Greige-Gerges, H. (2019). Deep eutectic solvents: An overview on their interactions with water and biochemical compounds. *Journal of Molecular Liquids, 288*, 111028. https://doi.org/10.1016/j.molliq.2019.111028

Adetunji, L. R., Adekunle, A., Orsat, V., & Raghavan, V. (2017). Advances in the pectin production process using novel extraction techniques: A review. *Food Hydrocolloids, 62*, 239-250. https://doi.org/10.1016/j.foodhyd.2016.08.015

Alongi, M., Melchior, S., & Anese, M. (2018). Reducing the glycemic index of short dough biscuits by using apple pomace as a functional ingredient. *LWT-Food Science and Technology, 100*, 300-305. https://doi.org/10.1016/j.lwt.2018.10.068

Atmodjo, M. A., Hao, Z., & Mohnen, D. (2013). Evolving views of pectin biosynthesis. *Annual Review of Plant Biology, 64*, 747–779. https://doi.org/10.1146/annurev-arplant-042811-105534

Benvenutti, L., Zielinski, A. A. F., & Ferreira, S. R. S. (2019). Which is the best food emerging solvent: IL, DES or NADES?. *Trends in Food science & Technology, 90*,133-146. https://doi.org/10.1016/j.tifs.2019.06.003

Benvenutti, L., Sanchez-Camargo, A. D. P., Zielinski, A. A. F., & Ferreira, S. R. S. (2020). NADES as potential solvents for anthocyanin and pectin extraction from *Myrciaria cauliflora* fruit by-product: *in silico* and experimental approaches for solvent selection. *Journal of Molecular Liquids*, *315*, 113761. https://doi.org/10.1016/j.molliq.2020.113761

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*(2), 484-489. https://doi.org/10.1016/0003-2697(73)90377-1

Brillouet, J. M., Williams, P., Will, F., Müller, G., & Pellerin, P. (1996). Structural characterization of an apple juice arabinogalactan-protein which aggregates following enzymic dearabinosylation. *Carbohydrate Polymers, 29*(3), 271-275. https://doi.org/10.1016/0144-8617(95)00152-2

Choi, Y. H., Spronsen, J. V., Dai, Y., Verberne, M., Hollmann, F., Arends, I. W. C. E., Witkamp, G. J., & Verpoorte, R. (2011). Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology?. *Plant Physiology, 156*(4), 1701-1705. https://doi.org/10.1104/pp.111.178426

648 Choi, Y. H., & Verpoorte, R. (2019). Green solvents for the extraction of bioactive
649 compounds from natural products using ionic liquids and deep eutectic solvents. *Current*
650 *Opinion in Food Science, 26*, 87-93. https://doi.org/10.1016/j.cofs.2019.04.003
651 Claus-Peter, W. (2011). Urea metabolism in plants. *Plant Science, 180*, 431-438.
652 https://doi.org/10.1016/j.plantsci.2010.11.010
653 Ebringerová, A. (2005). Structural Diversity and Application Potential of Hemicelluloses.
654 *Macromolecular Symposia, 232*(1), 1-12. https://doi.org/10.1002/masy.200551401
655 Dai, Y., Rozema, E., Verpoorte, R., & Choi, Y. H. (2016). Application of natural deep
656 eutectic solvents to the extraction of anthocyanins from catharanthus roseus with high
657 extractability and stability replacing conventional organic solvents. *Journal of*
658 *Chromatography A, 1434*, 50-56. https://doi.org/10.1016/j.chroma.2016.01.037
659 Das, A. K., Sharma, M., Mondal, D., & Prasad, K. (2016). Deep eutectic solvents as efficient
660 solvent system for the extraction of k-carrageenan from *Kappaphycus alvarezii*.
661 *Carbohydrate Polymers, 136*, 930-935. https://doi.org/10.1016/j.carbpol.2015.09.114
662 Elgharbawy, A. A. M., Hayyan, A., Hayyan, M., Mirghani, M. E. S., Salleh, H. M., Rashid, S.
663 N., Ngoh, G. C., Liew, S. Q., Nor, M. R., Zulkifli, M. Z., & Alias, Y. (2019). Natural deep
664 eutectic solvent-assisted pectin extraction from pomelo peel using sonoreactor: Experimental
665 optimization approach. *Processes, 7*(7), 416. https://doi.org/10.3390/pr7070416
666 Finer, E. G., Franks, F., & Tait, M. J. (1972). Nuclear magnetic resonance studies of aqueous
667 urea solutions. *Journal of the American Chemical Society, 94*(13), 4424-4429.
668 https://doi.org/10.1021/ja00768a004
669 Fisher, V., & Kunz, W. (2014). Properties of sugar-based low melting mixtures. *Molecular*
670 *Physics, 112*, 1241-1245. https://doi.org/10.1080/00268976.2014.884249
671 Fry, S. C. (1988). *The growing plant cell wall*. New York, USA: John Wiley & Sons.
672 Garna, H., Mabon, N., Robert, C., Cornet, C., Nott, K., Legros, H., Wathelet, B., & Paquot, M.
673 (2007). Effect of extraction conditions on the yield and purity of apple pomace pectin
674 precipitated but not washed by alcohol. *Journal of Food Science, 72*(1), 1-9.
675 https://doi.org/10.1111/j.1750-3841.2006.00227.x
676 Govindaraju, V., Young, K., & Maudsley, A. A. (2000). Proton NMR chemical shifts and
677 coupling constants for brain metabolites. *NMR in Biomedicine, 13*(3), 129-153.
678 https://doi.org/10.1002/1099-1492(200005)13:3<129::AID-NBM619>3.0.CO;2-V
679 Guillotin, S. E., Bakx, E. J., Boulenguer, P., Schols, H. A., & Voragen, A. G. J. (2007).
680 Determination of the degree of substitution, degree of amidation and degree of blockiness of

681 commercial pectins by using capillary electrophoresis. *Food Hydrocolloids 21*(3), 444-451.
682 https://doi.org/10.1016/j.foodhyd.2006.05.003
683 Guo, N., Kou, P., Jiang, Y. W., Wang, L. T., Niu, L. J., Liu, Z. M., & Fu, Y. J. (2019).
684 Natural deep eutectic solvents couple with integrative extraction technique as an effective
685 approach for mulberry anthocyanin extraction. *Food Chemistry, 296*, 78-85.
686 https://doi.org/10.1016/j.foodchem.2019.05.196
687 Guyot, S., Marnet, N., Laraba, D., Sanoner, P., & Drilleau, J. F. (1998). Reversed-phase
688 HPLC following thiolysis for quantitative estimation and characterization of the four main
689 classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus*
690 *domestica* var. Kermerrien). *Journal of Agricultural & Food Chemistry, 46*(5), 1698-1705.
691 https://doi.org/10.1021/jf970832p
692 Hou, X. D., Li, A. L., Lin, K. P., Wang, Y. Y., Kuang, Z. Y., & Cao, S. L. (2017). Insight into
693 the structure-function relationships of deep eutectic solvents during rice straw pretreatment.
694 *Bioresource Technology, 249*, 261-267. https://doi.org/10.1016/j.biortech.2017.10.019
695 Hou, Y. W., Gu, Y. Y., Zhang, S. M., Yang, F., Ding, H. M., & Shan, Y. K. (2008). Novel
696 binary eutectic mixtures based on imidazole. *Journal of Molecular Liquids, 143*(2-3), 154-
697 159. https://doi.org/10.1016/j.molliq.2008.07.009
698 Huang, Y., Feng, F., Jiang, J., Qiao, Y., Wu, T., Voglmeir, J., & Chen, Z. G. (2017). Green
699 and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic
700 solvents. *Food Chemistry, 221*, 1400-1405. https://doi.org/10.1016/j.foodchem.2016.11.013
701 Jeong, K. M., Lee, M. S., Nam, M. W., Zhao, J., Jin, Y., Lee, D. K., Kwon, S. W., Jeong, J.
702 H., & Lee J. (2015). Tailoring and recycling of deep eutectic solvents as sustainable and
703 efficient extraction media. *Journal of Chromatography A, 1424*, 10-17.
704 https://doi.org/10.1016/j.chroma.2015.10.083
705 Kaya, M., Sousa, A. G., Crepeau, M. J., Sorensen, S. O., & Ralet, M. C. (2014).
706 Characterization of citrus pectin samples extracted under different conditions: influence of
707 acid type and pH of extraction. *Annals of Botany, 114*(6), 1319-1326.
708 https://doi.org/10.1093/aob/mcu150
709 Kim, K. H., Dutta, T., Sun, J., Simmons, B., & Singh, S. (2018). Biomass Pretreatment using
710 Deep Eutectic Solvent from Lignin derived Phenols. *Green Chemistry, 20*(4), 1-7.
711 https://doi.org/10.1039/C7GC03029K
712 Koubala, B. B., Mbome, L. I., Kansci, G., Tchouanguep Mbiapo, F., Crepeau, M. J., Thibault,
713 J. F., & Ralet, M. C. (2008). Physicochemical properties of pectins from ambarella peels

714 (*Spondias cytherea*) obtained using different extraction conditions. *Food Chemistry, 106*(3),
715 1202-1207. https://doi.org/10.1016/j.foodchem.2007.07.065
716 Lachman, J., Martinek, P., Kotíková, Z., Orsák, M., & Šulc, M. (2017). Genetics and
717 chemistry of pigments in wheat grain - A review. *Journal of Cereal Science, 74*, 145-154.
718 https://doi.org/10.1016/j.jcs.2017.02.007
719 Leszczuk, A., Szczuka, E., Wydrych, J., & Zdunek, A. (2018). Changes in arabinogalactan
720 proteins (AGPs) distribution in apple (Malus x domestica) fruit during senescence.
721 *Postharvest Biology and Technology, 138*, 99-106.
722 https://doi.org/10.1016/j.postharvbio.2018.01.004
723 Levigne, S., Thomas, M., Ralet, M. C., Quemener, B., & Thibault, J. F. (2002). Determination
724 of the degrees of methylation and acetylation of pectins using a C18 column and internal
725 standards. *Food Hydrocolloids, 16*(6), 547-550. https://doi.org/10.1016/S0268-
726 005X(02)00015-2
727 Liew, S. Q., Ngoh, G. C., Yusoff, R., & Teoh, W. H. (2018). Acid and Deep Eutectic Solvent
728 (DES) extraction of pectin from pomelo (Citrus grandis (L.) Osbeck) peels. *Biocatalysis and*
729 *Agricultural Biotechnology 13*, 1-11. https://doi.org/10.1016/j.bcab.2017.11.001
730 Löfgren, C., & Hermansson, A. M. (2007). Synergistic rheological behaviour of mixed
731 HM/LM pectin gels. *Food Hydrocolloids, 21*(3), 480-486.
732 https://doi.org/10.1016/j.foodhyd.2006.07.005
733 María, F., Bruinhorst, A. V. D., & Kroon, M. C. (2012). New natural and renewable low
734 transition temperature mixtures (ITTMs): screening as solvents for lignocellulosic biomass
735 processing. *Green Chemistry, 14*, 2153-2157. https://doi.org/10.1039/C2GC35660K
736 May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate*
737 *Polymers, 12*(1), 79–99. https://doi.org/10.1016/0144-8617(90)90105-2
738 McCleary, B. V., Gibson, T. S., & Mugford, D. C. (1997). Measurement of total starch in
739 cereal products by amyloglucosidase–alpha-amylase method: collaborative study. *Journal of*
740 *AOAC International, 80*, 571–579. https://doi.org/10.1093/jaoac/80.3.571
741 Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology,*
742 *11*(3), 266-277. https://doi.org/10.1016/j.pbi.2008.03.006
743 Morais, E. S., Mendonça, P. V., Coelho, J. F. J., Freire, M. G., Freire, C. S. R., Coutinho, J. A.
744 P., & Silvestre, A. J. D. (2018). Deep Eutectic Solvent Aqueous Solutions as Efficient Media
745 for the Solubilization of Hardwood Xylans. *ChemSusChem, 11*(4), 753-762.
746 https://doi.org/10.1002/cssc.201702007

747 Nilsson, G. S., Gorton, L., Bergquist, K. E., & Nilsson, U. (1996). Determination of the
748 Degree of Branching in Normal and Amylopectin Type Potato Starch with ^{1}H-NMR
749 Spectroscopy Improved resolution and two-dimensional spectroscopy. *Starch - Stärke, 48*(10),
750 352-357. https://doi.org/10.1002/star.19960481003
751 O'Neill, M. A., Ishii, T., Albersheim, P., & Darvill, A. G. (2004). Rhamnogalacturonan II:
752 structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annual Review*
753 *of Plant Biology, 55*, 109-139. https://doi.org/10.1146/annurev.arplant.55.031903.141750
754 Passos, H., Tavares, D. J. P., Ferreira, A. M., Freire, M. G., & Coutinho, J. A. P. (2016). Are
755 aqueous biphasic systems composed of deep eutectic solvents ternary or quaternary systems?.
756 *ACS Sustainable Chemistry & Engineering, 4*, 2881-2886.
757 https://doi.org/10.1021/acssuschemeng.6b00485
758 Petersen, B. O., Meier, S., Duus, J. Ø., & Clausen, M. H. (2008). Structural characterization
759 of homogalacturonan by NMR spectroscopy—assignment of reference compounds.
760 *Carbohydrate Research, 343*(16), 2830-2833. https://doi.org/10.1016/j.carres.2008.08.016
761 Ptichkina, N. M., Markina, O. A., & Rumyantseva, G. N. (2008). Pectin extraction from
762 pumpkin with the aid of microbial enzymes. *Food Hydrocolloids, 22*(1), 192-195.
763 https://doi.org/10.1016/j.foodhyd.2007.04.002
764 Ray, S., Vigouroux, J., Quemener, B., Bonnin, E., & Lahaye, M. (2014). Novel and diverse
765 fine structures in LiCl-DMSO extracted apple hemicelluloses. *Carbohydrate Polymers 108*,
766 46-57. https://doi.org/10.1016/j.carbpol.2014.03.017
767 Reitsma, J. C. E., & Pilnik, W. (1989). Analysis of mixtures of pectins and amidated pectins.
768 *Carbohydrate Polymers, 10*(4), 315-319. https://doi.org/10.1016/0144-8617(89)90070-2
769 Rha, H. J., Bae, I. Y., Lee, S., Yoo, S. H., Chang, P. S., & Lee, H. G. (2011). Enhancement of
770 anti-radical activity of pectin from apple pomace by hydroxamation. *Food Hydrocolloids,*
771 *25*(3), 545-548. https://doi.org/10.1016/j.foodhyd.2010.08.010
772 Ruesgas-Ramón, M., Figueroa-Espinoza, M. C., & Durand, E. (2017). Application of Deep
773 Eutectic Solvents (DES) for Phenolic Compounds Extraction: Overview, Challenges, and
774 Opportunities. *Journal of Agricultural and Food Chemistry, 65*(18), 3591-3601.
775 https://doi.org/10.1021/acs.jafc.7b01054
776 Scheller, H. V., Jensen, J. K., Sørensen, S. O., Harholt, J., & Geshi, N. (2007). Biosynthesis
777 of pectin. *Physiologia Plantarum, 129*, 283-295. https://doi.org/10.1111/j.1399-
778 3054.2006.00834.x
779 Scheller, H. V., & Ulvskov, P. (2010). Hemicelluloses. *Annual Review of Plant Biology, 61*,
780 263-289. https://doi.org/10.1146/annurev-arplant-042809-112315

781 Schols, H. A., Bakx, E. J., Schipper, D., & Voragen, A. G. J. (1995). A xylogalacturonan
782 subunit present in the modified hairy regions of apple pectin. *Carbohydrate Research, 279*,
783 265-279. https://doi.org/10.1016/0008-6215(95)00287-1
784 Shafie, M. H., Yusof, R., & Gan, C. Y. (2019). Deep eutectic solvents (DES) mediated
785 extraction of pectin from Averrhoa bilimbi: Optimization and characterization studies.
786 *Carbohydrate Polymers, 216*, 303-311. https://doi.org/10.1016/j.carbpol.2019.04.007
787 Shafie, M. H., & Gan, C. Y. (2020). A comparison of properties between the citric acid
788 monohydrate and deep eutectic solvent extracted Averrhoa bilimbi pectins. *Journal of Food*
789 *Measurement and Characterization*. https://doi.org/10.1007/s11694-020-00533-x
790 Shamsuri, A. A., & Abdullah, D. K. (2010). Protonation and complexation approaches for
791 production of protic eutectic ionic liquids. *Journal of Physical Science, 21*(1), 15-28.
792 Sinitsya, A., Čopíková, J., Prutyanov, V., Skoblya, S., & Machovič, V. (2000). Amidation of
793 highly methoxylated citrus pectin with primary amines. *Carbohydrate Polymers 42*(4), 359-
794 368. https://doi.org/10.1016/S0144-8617(99)00184-8
795 Sirviö, J. A., Ukkola, J., & Liimatainen, H. (2019). Direct sulfation of cellulose fibers using a
796 reactive deep eutectic solvent to produce highly charged cellulose nanofibers. *Cellulose, 26*(4),
797 2303-2316. https://doi.org/10.1007/s10570-019-02257-8
798 Soares, B., Tavares, D. J. P., Amaral, J. L., Silvestre, A. J. D., Freire, C. S. R., & Coutinho, J.
799 A. P. (2017). Enhanced Solubility of Lignin Monomeric Model Compounds and Technical
800 Lignins in Aqueous Solutions of Deep Eutectic Solvents. *ACS Sustainable Chemistry, 5*(5),
801 4056-4065. https://doi.org/10.1021/acssuschemeng.7b00053
802 Thibault, J. F., Guillon, F., & Rombouts, F. M. (1991). Gelation of sugar beet pectin by
803 oxidative coupling. In R. H. Walter (Ed.), *The chemistry and technology of pectin*. San Diego:
804 Academic Press.
805 Van Buren, J. (1970). Fruit phenolics. In A. C. Hulme (Eds.), *The Biochemistry of fruits and*
806 *their products*. (pp. 269-304). London: Academic press.
807 Vanda, H., Dai, Y., Wilson, E. G., Verpoorte, R., & Choi, Y. H. (2018). Green solvents from
808 ionic liquids and deep eutectic solvents to natural deep eutectic solvents. *Comptes Rendus*
809 *Chimie 21*, 628-638. https://doi.org/10.1016/j.crci.2018.04.002
810 Ververis, C., Georghiou, K., Christodoulakis, N., Santas, P., & Santas, R. (2004). Fiber
811 dimensions, lignin and cellulose content of various plant materials and their suitability for
812 paper production. *Industrial Crops and Products, 19*(3), 245-254.
813 https://doi.org/10.1016/j.indcrop.2003.10.006

814 Voragen, F., Beldman, G., & Schols, H. (2001). Chemistry and enzymology of pectins. In B.
815 V. McCleary, & L. Prosky (Eds.), *Advanced dietary fibre technology*. (pp. 379-398). Oxford:
816 Blackwell Science Ltd.
817 Wang, X., Chen, Q., & Lü, X. (2014). Pectin extracted from apple pomace and citrus peel by
818 subcritical water. *Food Hydrocolloids, 38*, 129-137.
819 https://doi.org/10.1016/j.foodhyd.2013.12.003
820 Wikiera, A., Mika, M., Starzyńska-Janiszewska, A., & Stodolak, B. (2015). Development of
821 complete hydrolysis of pectins from apple pomace. *Food Chemistry, 172*, 675-680.
822 https://doi.org/10.1016/j.foodchem.2014.09.132
823 Willberg-Keyriläinen, P., Hiltunen, J., & Ropponen, J. (2017). Production of cellulose
824 carbamate using urea-based deep eutectic solvents. *Cellulose, 25* (1), 195-204.
825 https://doi.org/10.1007/s10570-017-1465-9
826 Yang, L., Brent Friesen, J., McAlpine, J. B., Lankin, D. C., Chen, S. N., & Pauli, G. F. (2018).
827 Natural Deep Eutectic Solvents: Properties, Applications, and Perspectives. *Journal of*
828 *Natural Products, 81*(3), 679-690. https://doi.org/10.1021/acs.jnatprod.7b00945
829 Yiin, C. L., Yusup, S., Quitain, A. T., & Uemura, Y. (2015). Physicochemical Properties of
830 Low Transition Temperature Mixtures in Water. *Chemical Engineering Transactions, 45*,
831 1525-1530. https://doi.org/10.3303/CET1545255
832 Yuliarti, O., Matia-Merino, L., Goh, K. K. T., Mawson, J., Williams, M. A. K., & Brennan, C.
833 (2015). Characterization of gold kiwifruit pectin from fruit of different maturities and
834 extraction methods. *Food Chemistry, 166*, 479-485.
835 https://doi.org/10.1016/j.foodchem.2014.06.055
836 Yu, W., Wang, C., Yi, Y., Zhou, W., Wang, H., Yang, Y., & Tan, Z. (2019). Choline
837 chloride-based deep eutectic solvent systems as a pretreatment for nanofibrillation of ramie
838 fibers. *Cellulose, 26*(5), 3069-3082. https://doi.org/10.1007/s10570-019-02290-7
839 Zdanowicz, M., & Spychaj, T. (2011). Ionic liquids as starch plasticizers or solvents.
840 *Polimery (Warsaw), 56*, 861-864. https://doi.org/10.14314/polimery.2011.861
841 Zdanowicz, M., Wilpiszewska, K., & Spychaj, T. (2018). Deep eutectic solvents for
842 polysaccharides processing. A review. *Carbohydrate Polymers, 200*, 361-380.
843 https://doi.org/10.1016/j.carbpol.2018.07.078
844

Apple pomace

Water extraction

Pectin

Cell wall polysaccharides