

1 **Fractionation and characterisation of dietary fibre from**
2 **blackcurrant pomace**

3 K. Alba^a, W. MacNaughtan^c, A. P. Laws^b, T. J. Foster^c, G. M. Campbell^b, and V.
4 Kontogiorgos^{a*}

5 ^a*Department of Biological Sciences, University of Huddersfield, HD1 3DH, UK*

6 ^b*Department of Chemical Sciences, University of Huddersfield, HD1 3DH, UK*

7 ^c*Division of Food Sciences, School of Biological Sciences, University of Nottingham,*
8 *Sutton Bonington Campus, Loughborough, LE12 5RD, UK*

9

10

11

12

13

14

15

16

17

18

19

20

21

22 *Corresponding author

23 Tel.: +44 1484 472488

24 e-mail: v.kontogiorgos@hud.ac.uk

25

26

27

28 **Abstract**

29 The potential of blackcurrant pomace as a raw material for the extraction of dietary
30 fibre was evaluated using two pomaces one sourced from the UK and one from Poland.
31 A fractionation protocol was designed to isolate and subsequently quantify the soluble
32 and insoluble dietary fibre fractions. Blackcurrant pomace and isolated pectins,
33 hemicelluloses and celluloses were assessed by means of sugar compositional analysis,
34 spectroscopy, size exclusion chromatography and dilute solution viscometry. The
35 blackcurrant pomaces presented considerable amounts of dietary fibre with soluble
36 fibre ranging from 25-30% w/w and insoluble dietary fibre accounting for about 47%
37 w/w for both pomaces. Blackcurrant pomaces differed in the amount of extracted
38 pectins with an almost two times higher pectin yield obtained from blackcurrant
39 pomace sourced from Poland. The hemicellulosic polysaccharide content was 15% w/w
40 whereas the amount of cellulosic fraction varied from 14-17% w/w. Pectins isolated
41 from both blackcurrant pomaces were LM pectins with a degree of esterification in the
42 range of 11-38%. The work has identified that dietary fibres obtained from blackcurrant
43 pomace had desirable ratio of insoluble to soluble fibre and are a potential new source
44 of dietary fibre.

45

46

47 *Keywords: blackcurrant; dietary fibre; pectin; cellulose; NMR*

48

49

50

51

52

53 *Abbreviations:*

54 POMUK – pomace from UK

55 POMPOL – pomace from Poland

56 pIDFUK – pure insoluble dietary fibre from UK (fraction that was obtained after
57 extraction of acid-soluble and Ca²⁺-bound pectins)

58 pIDFPOL – pure insoluble dietary fibre from Poland

59 APUK – acid-soluble pectin isolated from POMUK

60 APPOL – acid-soluble pectin isolated from POMPOL

61 CBPUK – calcium-bound pectin isolated from POMUK

62 CBPPOL – calcium-bound pectin isolated from POMPOL

63 HEMUK – hemicellulose isolated from POMUK

64 HEMPOL – hemicellulose isolated from POMPOL

65 CELUK – cellulose isolated from POMUK

66 CELPOL – cellulose isolated from POMPOL

67 DF – dietary fibre

68 SDF – soluble dietary fibre

69 IDF – insoluble dietary fibre

70 DE – degree of esterification

71 HBSS – hot buffer soluble solids

72 CHSS – chelator soluble solids

73 HG – homogalacturonan

74 RG-I – rhamnogalacturonan-I

75 GalA – galacturonic acid

76 MWCO – molecular weight cut-off

77 LM pectin – low-methylated pectin

78 **1. Introduction**

79 Processing of fruits and vegetables results in a large amount of agricultural
80 waste with significant potential for the recovery of functional materials. For instance,
81 about 15% of grapes or 20% of soft berries used in winemaking or juice manufacturing
82 are discarded in the form of pomace (Makris, Boskou, & Andrikopoulos, 2007;
83 Minjares-Fuentes, Femenia, Garau, Meza-Velazquez, Simal, & Rossello, 2014a).
84 Commonly, fruit or vegetable pomace is discarded (e.g. into soil or landfills), however,
85 the pomace obtained after juice pressing could also be of great interest to food,
86 pharmaceutical and cosmetic industries due to the high carbohydrate fraction that can
87 be utilised as a source of dietary fibre (DF), structuring components and/or bioactive
88 compounds (Femenia, 2007; Rohm, Brennan, Turner, Günther, Campbell, Hernando,
89 Struck, & Kontogiorgos, 2015; Quiles, Campbell, Struck, Rohm & Hernando, 2016).

90 DFs are often carbohydrate polymers that are resistant to digestion and
91 absorption in the human small intestine and undergo complete or partial fermentation
92 in the human large intestine (DeVries, Camire, Cho, Craig, Gordon, Jones, Li,
93 Lineback, Prosky, & Tunland, 2001). DFs are classified based on their solubility in
94 water into soluble fibre (e.g., pectin and some hemicelluloses) or insoluble fibre (e.g.,
95 cellulose or lignin). Adequate consumption of DF has been linked to diverse nutritional
96 and health benefits. It has been shown that DFs from various sources have the capacity
97 to regulate food intestinal transit, with concomitant benefits to health including reduced
98 risks of diabetes, cardiovascular diseases and obesity (Kendall, Esfahani, & Jenkins,
99 2010; Mann & Cummings, 2009).

100 Generally, DFs derived from fruit and vegetable co-products contain a higher
101 content of soluble DF (SDF) than those obtained from cereals, present insoluble-to-
102 soluble fibre ratios in the range 1 to 3 that are technologically desirable (Larrauri, 1999;

103 Vergara-Valencia, Granados-Pérez, Agama-Acevedo, Tovar, Ruales, & Bello-Pérez,
104 2007) and therefore have superior functional properties (e.g., water holding or swelling
105 capacity, viscosity enhancement or gel formation). The presence of a soluble fibre
106 fraction in DF may provide improved physiological functions in addition to the
107 functionality provided by the insoluble DF (IDF) fraction. Moreover, fruit DF also
108 contains considerable amounts of various bioactive compounds (e.g., polyphenols) that
109 can provide additional health benefits (Struck, Plaza, Turner, & Rohm, 2016). DF
110 content of wheat flour is limited to 2-4% and therefore incorporation of DF from
111 alternative sources such as whole grain cereals and fruits in food formulations could
112 increase the nutritional value of the final products (Jenkins, Kendall, & Ransom, 1998;
113 Pelucchi, Talamini, Galeone, Negri, Franceschi, Dal Maso, Montella, Conti, & La
114 Vecchia, 2004). The effectiveness of DF to deliver or promote health benefits depends
115 not only on intake but also on the source of DF and its structural and chemical
116 composition. In addition, the incorporation of DF into food requires a substantial
117 understanding of its chemical structure due to the interactions between DF and other
118 ingredients (e.g., gluten) that can considerably alter microstructure and acceptability
119 characteristics of the final product.

120 Blackcurrant (*Ribes nigrum*) is primarily used in juice manufacturing,
121 generating several thousand tonnes per annum of pomace with the potential for
122 recovery of novel functional DF. The application of modern extraction methods for
123 fractionation of blackcurrant pomace into its constituent components (pectin,
124 hemicellulose, cellulose and lignin) with specified chemical compositions and physical
125 properties might improve its functionality and promote the development of value-added
126 ingredients for human consumption. The premise of the current work is that
127 blackcurrant pomace, if characterised and fractionated appropriately, could be adopted

128 by the food industry as a source of functional DF. Chemical composition and
129 macromolecular properties may vary depending on the blackcurrant cultivar, growth
130 conditions, climate, and processing steps, thus influencing the techno-functional (e.g.,
131 product stability) and bio-functional properties (e.g., digestion). The aims of this study,
132 therefore, were to fractionate and characterise the carbohydrate polymers present in
133 blackcurrant pomaces from two distinct regions (UK and Poland), with the goal to
134 evaluate in more general terms the potential of blackcurrant pomace as a raw material
135 for the production of functional DF fractions.

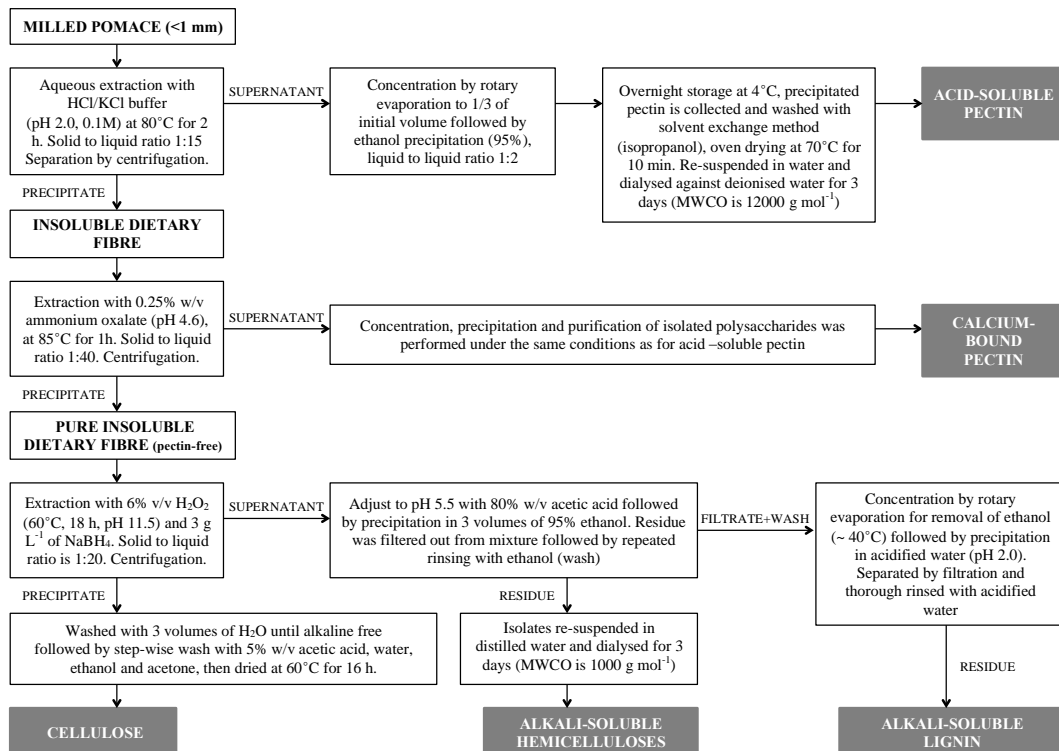
136 **2. Materials and methods**

137 *2.1 Materials*

138 Two dried blackcurrant pomaces (*Ribes nigrum*) consisting of stems, seeds and
139 exocarp were obtained from Lucozade-Ribena-Suntory (LRS, UK) and GreenField
140 Natural Ingredients (Warsaw, Poland). All analytical reagents and standards reported
141 in the experimental sections were purchased from Sigma-Aldrich (Poole, UK). Pectin
142 standards with 10% and 70% degree of esterification (DE) were obtained from CP
143 Kelco UK Ltd.

144 *2.2 Fibre fractionation*

145 Blackcurrant pomaces were milled to an average particle size of < 1 mm prior
146 to fibre extraction. The fractionation protocol of pectins, hemicelluloses, cellulose and
147 lignin is shown in Figure 1. Fractionation of pomace was performed using a batch
148 extraction approach (200 g of milled pomace) on a laboratory scale. Isolated pectins
149 and hemicelluloses were dialysed against distilled water in order to remove impurities
150 such as low molecular weight sugars and oligomers, amino acids, organic acids and
151 inorganic salts. Pectin and hemicellulose fractions were freeze-dried after dialysis for
152 24 h.



154
155
156
157
158

Figure 1. Sequential fractionation protocol of blackcurrant pomace into soluble and insoluble DFs. Five fractions were separated from the raw material including acid-soluble and calcium-bound pectins, celluloses, hemicelluloses and lignin.

159 2.3 Characterisation of blackcurrant pomaces and DFs

160 Proximate analysis (i.e., protein, ash, moisture, fat and carbohydrate) of
161 blackcurrant pomaces was conducted by International Laboratory Services (ILS
162 Limited, Derby, UK). Yields of pectin (acid-soluble and calcium-bound),
163 hemicellulose, cellulose and alkali-soluble lignin were determined gravimetrically
164 following the fractionation protocol. Klason lignin was determined in the following
165 samples: pomaces, pure insoluble DF (pIDF) and isolated celluloses after a two-step
166 Saeman hydrolysis with sulphuric acid (Englyst & Cummings, 1988). Hydrolysis was
167 performed as follows: 50 mg of sample was pre-hydrolysed with 12M H₂SO₄ for 1 h at
168 35 °C followed by dilution to 1M H₂SO₄ by addition of H₂O and boiling at 100 °C for
169 2 h. The insoluble residue was filtered out of the mixture, dried at 102 °C and ashed for
170 4 h at 425 °C. The filtrate was collected and used for spectrophotometric determination

171 of uronic acids by the *m*-hydroxydiphenyl method (Filisetti-Cozzi & Carpita, 1991) in
172 pomaces, pIDF fractions and celluloses. Klason lignin was then calculated using:

$$173 \quad \text{lignin (\% w/w)} = \frac{\text{mass after drying} - \text{mass after ashing}}{\text{mass of sample before hydrolysis}} \cdot 100\% \quad (1)$$

174 The content of uronic acids in soluble DF fractions (i.e., acid-soluble pectin,
175 calcium-bound pectin and hemicelluloses) was determined using the aforementioned
176 *m*-hydroxydiphenyl method. Neutral sugars were analysed as alditol acetates after
177 trifluoroacetic acid (TFA) hydrolysis in soluble and insoluble fractions. Soluble DF
178 fractions (i.e., acid-soluble pectin, calcium-bound pectin and hemicelluloses) were
179 hydrolysed with 4 M TFA at 120 °C for 2 h. Insoluble DFs (i.e., pomace, pIDF fraction
180 and cellulose) were submitted to sequential hydrolysis with varying concentrations of
181 TFA (100, 85, 67, 46 and 30% w/v) (Rowland & Howley, 1989). Hydrolysed
182 polysaccharides were then derivatised to volatile alditol acetates and analysed using an
183 Agilent 7890A GC system (Santa Clara, CA, USA) coupled to an Agilent 5975C
184 quadrupole MS. The samples were eluted from an HP-5 column (30 m × 0.25 mm, 0.25
185 µm film) using helium as a carrier gas at a flow rate of 1 mL min⁻¹ by applying the
186 following temperature settings: start temperature 140 °C, hold time 1 min and final
187 column temperature 220 °C with 25 °C min⁻¹ gradient.

188 *2.4 Spectroscopic analysis*

189 *2.4.1 FT-IR spectroscopy*

190 FT-IR spectra were obtained between 500 and 4000 cm⁻¹ for pomaces, soluble
191 and insoluble fractions at a resolution of 4 cm⁻¹ using 128 scans (Nicolet 380, Thermo
192 Scientific, UK). Spectral smoothing was applied using instrument software (OMNIC
193 3.1). FT-IR spectra were also utilized for determination of degree of esterification of

194 isolated pectins (Manrique & Lajolo, 2002; Monsoor, Kalapathy, & Proctor, 2001). DE
195 of pectins is proportional to the ratio:

$$196 \quad DE = \frac{\text{area of esterified carboxyl groups}}{\text{area of total carboxyl groups}} \cdot 100 \quad (2)$$

197 The calibration curve was constructed using pectin standards with known DE values
198 and by correlating the DE values with the area ratio of esterified carboxylic groups over
199 the total carboxyl groups (esterified bands are centred around 1740 and non-esterified
200 around 1630 cm⁻¹).

201 2.4.2 ¹³C CPMAS NMR spectroscopy

202 ¹³C CPMAS NMR spectra were recorded on a Bruker AVANCE III 600 NMR
203 spectrometer with narrow bore magnet and 4-mm triple resonance probe. The
204 parameters and conditions used in ¹³C CPMAS NMR experiments were: proton 90°
205 pulse length 3 μs, field strength of the proton and spin locking fields during the contact
206 period 83 kHz. The samples were packed into 4-mm rotors and spun at 10 kHz.
207 Chemical shifts (ppm) scales were referenced to the upfield peak of adamantane (29.5
208 ppm) run as an external standard. Proton decoupling was provided by a spinal-64
209 sequence and the proton power levels during the contact time and decoupling stage
210 could be varied independently to provide optimum signal to noise levels. The highest
211 intensity signal for all types of bonded carbons in these materials lies between a contact
212 time of 1 and 2 ms. For all CPMAS experiments a value of 2 ms was used and recycle
213 delay was 2 s. Approximately 5000 data points were normally recorded. On data
214 processing this data set was zero-filled by at least a factor of 2. A Lorentzian line
215 broadening (15 Hz) was then applied. The data were Fourier-transformed and phased
216 with zero and first order corrections. Baseline fitting routines were applied to all
217 spectra.

218 2.4.3 Two-dimensional NMR spectroscopy

219 Two-dimensional (2D) NMR experiments were carried out on a Bruker 800
220 MHz Avance III spectrometer equipped with a QCI cryoprobe. For each sample the
221 90° pulse and transmitter frequency were calibrated. The number of scans collected in
222 each dimension for each experiment was determined by the sample concentration. The
223 2D ¹³C[¹H] HSQC spectra were acquired over a spectral width of 14 ppm in the ¹H
224 dimension and 200 ppm in the ¹³C dimension. The transmitter frequency for carbon was
225 centred at 100 ppm and between 16 and 64 scans were acquired, with 128 complex
226 points in f1. Quadrature detection in the carbon channel was achieved using the States-
227 TPPI method. Data acquisition and processing were carried out using Topspin 3.5
228 software. For 2D datasets a shifted squared sine bell was used with the offset being
229 optimized to achieve the best balance between resolution and signal to noise ratio. All
230 data were zero-filled by at least a factor of 2. For heteronuclear dimensions linear
231 prediction was employed.

232 2.5 Macromolecular characteristics of soluble DFs

233 Weight-average molecular weight (M_w) of pectins and hemicelluloses was
234 determined using size exclusion chromatography coupled to multi-angle laser light
235 scattering (SEC-MALLS) at 25°C. Soluble fractions were stirred overnight in 0.1M
236 NaNO₃ solution (3-5 mg mL⁻¹) at room temperature. Solubilized fractions were injected
237 onto an SEC system (15 µm particle size, 25 cm × 4 mm, Agilent, Oxford, UK) that
238 consisted of a PL Aquagel guard column linked in series with PL Aquagel-OH 60, PL
239 Aquagel-OH 50 and PL Aquagel-OH 40. Fractions were eluted with 0.1M NaNO₃
240 solution at a flow rate of 0.7 mL min⁻¹. The eluent was then detected online firstly by a
241 DAWN EOS light scattering detector (Wyatt Technology, Santa Barbara, U.S.A.) and
242 finally by a rEX differential refractometer (Wyatt Technology, Santa Barbara, U.S.A.).

243 The refractive index increment, dn/dc was taken to be 0.146 mL g^{-1} for pectins and
244 0.060 mL g^{-1} for hemicelluloses (Chapman, Morris, Selvendran, & O'Neill, 1987;
245 Morris, de al Torre, Ortega, Castile, Smith, & Harding, 2008; Morris, Foster, &
246 Harding, 2000; Xu, Leppanen, Eklund, Holmlund, Sjöholm, Sundberg, & Willfor,
247 2010).

248 Intrinsic viscosity measurements were performed on pectins and
249 hemicelluloses. Samples were dispersed at $0.1\text{-}2.0 \text{ g dL}^{-1}$ in 0.1 M NaCl at pH 7.0 in
250 Sorensen's phosphate buffer with $0.02 \text{ g dL}^{-1} \text{ NaN}_3$ and were stirred overnight. An
251 Ubbelohde capillary glass viscometer (PSL Rheotek OB. C 80705) and the Huggins
252 equation was used to estimate the intrinsic viscosities of the isolated fractions at $20 \pm$
253 0.1°C .

254 **3. Results and discussion**

255 *3.1 Fractionation of DFs from blackcurrant pomace*

256 An isolation protocol (Figure 1) was designed to fractionate blackcurrant
257 pomace into its constituent soluble and insoluble fractions. Fractionation commences
258 with the isolation of acid-soluble pectin at 80°C (pH 2.0) followed by isolation of
259 calcium-bound pectin with ammonium oxalate at 85°C (pH 4.6). Hot acid treatment is
260 frequently utilized for pectin extraction from pomaces (Beres, Simas-Tosin, Cabezudo,
261 Freitas, Iacomini, Mellinger-Silva, & Cabral, 2016; Minjares-Fuentes, Femenia, Garau,
262 Meza-Velázquez, Simal, & Rosselló, 2014b) usually resulting in high yields of high
263 molecular weight pectic polysaccharides (Alba & Kontogiorgos, 2017). The calcium-
264 bound pectin fraction is insoluble in hot acid and requires the presence of a chelating
265 agent to remove calcium from intermolecular linkages and allow pectin to solubilise.

266 The insoluble residue that remained after the extraction of pectic
267 polysaccharides represented the lignocellulosic biomass that was further fractionated

268 into three major components, hemicellulose, cellulose and lignin (Figure 1). It has been
269 shown that strong alkali (4M KOH) was more efficient in dissolving hemicelluloses
270 and resulted in higher extraction yields compared to 2M KOH (Minjares-Fuentes,
271 Femenia, Garau, Candelas-Cadillo, Simal, & Rosselló, 2016; Prozil, Costa, Evtuguin,
272 Lopes, & Domingues, 2012). Other authors reported higher yields of hemicelluloses
273 isolated with H₂O₂ rather than with direct alkaline extraction due to high reactivity of
274 hydroxyl radicals that cleave ester links between lignin and hemicelluloses (Rabetafika,
275 Bchir, Blecker, Paquot, & Wathelet, 2014). Therefore, to optimise the yield, isolation
276 of hemicelluloses was performed with 6% v/v H₂O₂ under alkaline conditions. Note
277 that isolated pectins and hemicelluloses represent the fractions that can be used as
278 water-soluble dietary fibres based on their solubility in water after extraction from
279 pomace. Cellulose was separated from lignocellulose after hemicellulose extraction in
280 the form of a solid residue, whereas lignin was recovered by precipitation with acidified
281 water (Figure 1). Generally, yield, purity and macromolecular characteristics of
282 extracted cellulose and lignin vary depending on the isolation method, as described in
283 the following sections.

284 *3.2 Characterisation of blackcurrant pomace*

285 Proximate analysis of blackcurrant pomaces (Table 1) demonstrated
286 comparable amounts of both proteins and carbohydrates in the two pomace samples.
287 Higher protein content (17% w/w) has been previously reported for blackcurrant and
288 bilberry pomaces (Hilz, Bakx, Schols, & Voragen, 2005), however, analysis of grape
289 pomaces revealed a considerably lower amount of protein (2.7-3.8 % w/w) (González-
290 Centeno, Rosselló, Simal, Garau, López, & Femenia, 2010). POMUK had a higher fat
291 content (10.8%) than POMPOL (5.9%), possibly due to higher seed content. These

292 values were much higher than reported fat contents of grape pomaces (0.3-1.0 % w/w)
 293 (Rabetafika, Bchir, Aguedo, Paquot, & Blecker, 2013).

294 Compositional analysis demonstrated the predominance of insoluble DF in both
 295 pomaces (~ 47% w/w) (Table 1). Comparable values of insoluble dietary fibre were
 296 reported for blackcurrant, bilberry, chokeberry and raspberry pomaces or berries (56-
 297 66% w/w) (Jakobsdottir, Nilsson, Blanco, Sterner, & Nyman, 2014; Jaroslawska,
 298 Wroblewska, Juskiewicz, Brzuzan, & Zdunczyk, 2016; Wawer, Wolniak, &
 299 Paradowska, 2006).

300 **Table 1.** Proximate analysis, fibre composition of blackcurrant pomaces and the yield
 301 of constituent DF fractions.

	POMPOL	POMUK
Protein (N × 6.25) ^a	11.1	13.3
Ash ^a	3.3	2.8
Moisture	7.5	3.2
Fat ^a	5.9	10.8
Carbohydrate ^a	71.9	69.8
Total soluble DF ^b	30.0±1.5	25.1±1.0
Acid-soluble pectin ^c	5.8±1.6	2.9±1.0
Calcium-bound pectin ^c	9.8 ±0.1	7.7±0.4
Alkali-soluble hemicelluloses ^c	14.4 ±3.0	14.5±1.5
Total insoluble DF ^d	46.9±4.6	47.4±5.4
Cellulose ^c	17.2±1.8	13.6±2.8
Alkali-soluble lignin ^c	0.4±0.2	0.2±0.1
Ash ^c	3.7±0.6	3.0±0.1
Klason lignin ^c	37.9±9.0	35.7±11.3
Pure insoluble DF ^c	61.3±4.6	61.9±5.4

302 ^a Values are expressed as % wet basis.

303 ^b Values were calculated by adding acid-soluble pectin, calcium-bound pectin and
 304 alkali-soluble hemicellulose. Values are expressed as % w/w (g/100 g of dry pomace).

305 ^c Values are expressed as % w/w (g/100 g of dry pomace).

306 ^d Values were calculated by subtracting the alkali-soluble hemicellulose content
 307 (HEMUK, HEMPOL) from the amount of pure insoluble DF (pIDFUK, pIDFPOL).
 308 Values are expressed as % w/w (g/100 g of dry pomace).

309

310 The amount of cellulose was 13.6 and 17.2% w/w for the two samples higher than a
 311 previously reported value of 12.0% w/w (Nawirska & Kwaśniewska, 2005). Klason
 312 lignin was the main cell wall component and also the major insoluble DF fraction in
 313 both pomaces (Table 1) and was present in lower amounts compared to lignin values

314 previously reported for blackcurrant or grape pomaces that ranged between 41.9 and
315 59.3% w/w (Jakobsdottir, et al., 2014; Nawirska, et al., 2005; Valiente, Arrigoni,
316 Esteban, & Amado, 1995). Generally there is considerable variability in the composition
317 of the insoluble fibres depending on the source of extraction (e.g., berry or fruit co-
318 products) (Aguedo, Kohnen, Rabetafika, Vanden Bossche, Sterckx, Blecker, Beauve,
319 & Paquot, 2012; Rabetafika, et al., 2013; Rabetafika, et al., 2014).

320 Both samples had comparable contents of total soluble DF (25-30% w/w) in
321 contrast to other studies that have reported considerably lower amounts (5.4-7.8% w/w)
322 (Jakobsdottir, et al., 2014). The isolation protocol used in the current work includes not
323 only acid-soluble pectins but also calcium-bound pectin and hemicelluloses, which
324 result in higher contents of total soluble fibre. The total pectin content (acid-soluble and
325 Ca²⁺-bound pectin, Table 1) was greater in POMPOL than in POMUK but substantially
326 higher than that previously reported from blackcurrant (2.7 % w/w) (Nawirska, et al.,
327 2005) or grape (2.0-6.2 % w/w) pomaces (González-Centeno, et al., 2010). The yields
328 of acid-soluble pectins (APUK 2.9% w/w, APPOL 5.8% w/w) in the current work were
329 lower than the yields reported for pectic HBSS fraction of blackcurrants (12.1% w/w)
330 or bilberries (6.0% w/w) (Hilz, et al., 2005). Those results suggest that part of the pectic
331 polysaccharide fraction has been removed during blackcurrant processing (e.g.,
332 enzymic treatment during juice production) resulting in low contents of acid-soluble
333 pectin in both pomaces. Lower yields of pectic polysaccharides have been previously
334 reported from cherry pomace compared with cherries (Kosmala, Milala,
335 Kolodziejczyk, Markowski, Mieszczakowska, Ginies, & Renard, 2009). On the other
336 hand, higher yields (22.0% w/w) of acid-soluble pectins were obtained from grape
337 pomace under comparable isolation conditions (Minjares-Fuentes, et al., 2014a). In
338 addition, the amounts of calcium-bound pectin were 7.7 and 9.8% w/w higher than

339 reported for CHSS fractions isolated from unprocessed blackcurrant (4.1% w/w) and
340 bilberries (4.0% w/w) (Hilz, et al., 2005).

341 With regards to hemicelluloses, the extraction protocol resulted in isolation of
342 comparable amounts of hemicellulosic polysaccharides from both samples (14.5 and
343 14.4% w/w) lower than previously reported for blackcurrant pomace (25.3% w/w)
344 (Nawirska, et al., 2005). The yield of hemicelluloses isolated from grape pomace using
345 alkaline extractions was in the range 5.4-8.0% w/w and was considerably lower than
346 reported yields of those isolated from apple (13.5% w/w) or pear (20.2, 22.1% w/w)
347 (Aguedo, et al., 2012; Minjares-Fuentes, et al., 2016; Rabetafika, et al., 2014).

348 The physiological and technological properties of DF largely depend on the
349 relative amounts of total soluble (SDF) and insoluble (IDF) fibre components, and the
350 IDF/SDF ratio should range from 1.0 to 3.0 in order to yield optimal health benefits
351 and functionality (Gomez, Ronda, Blanco, Caballero, & Apesteguia, 2003). The
352 IDF/SDF ratio was 1.9 for POMUK and 1.6 for POMPOL, indicating the suitability of
353 these blackcurrant pomaces to be utilized as sources of DF.

354 *3.3 Chemical characterisation of pomaces and fractions*

355 Having established the overall composition of pomaces we proceeded with
356 detailed carbohydrate analysis of both samples and their fractions. The two pomaces
357 differed in the amount of total sugars, with higher values obtained for POMPOL (Table
358 2). These values are lower than total sugar contents reported for blackcurrant and
359 bilberry press cakes (34.0 and 36.0 % w/w) (Hilz, et al., 2005) but comparable with
360 those from grape pomaces (15.6-32.5 % w/w) (González-Centeno, et al., 2010).
361 Blackcurrant pomaces were primarily composed of uronic acids and the major neutral
362 sugars were glucose and galactose, with other sugars present in lower proportions
363 (Table 2) in contrast to those previously reported (glucose > mannose > uronic acids)

364 (Hilz, et al., 2005). Additionally, grape pomaces have shown variable sugar
365 composition (glucose > uronic acids > xylose or uronic acids > glucose) exemplifying
366 the influence of botanical variety on sugar profile and molecular structure of pomace
367 polysaccharides (González-Centeno, et al., 2010).

368 APUK and APPOL fractions demonstrated comparable contents of uronic
369 acids, with major neutral sugars being galactose and arabinose while the third major
370 neutral sugar was glucose for APUK and rhamnose for APPOL (Table 2). CBPUK and
371 CBPPOL had similar uronic acid content but differed in neutral sugar composition with
372 galactose being the major neutral sugar in CBPUK (Table 2). Previous reports have
373 shown that isolation of pectic polysaccharides using various extraction solvents from
374 blackcurrant, cherry and cherry pomace yields samples containing mostly arabinose
375 and galactose (Hilz, et al., 2005; Kosmala, et al., 2009). The molecular structure of
376 isolated pectins was modelled using sugar molar ratios and calculated based on the
377 neutral sugar mol% (Table 3). Ratios 1, 2 and HG/RG-I highlight the prevalence of
378 linear segments in the structure of APPOL, whereas other pectins exhibited higher
379 levels of branching (Denman & Morris, 2015). In addition, ratio 3 demonstrates
380 moderate differences in the size of the branching of side chains. Previous studies have
381 also reported the isolation of homogalacturonan-rich pectins from blackcurrants and
382 bilberries (Hilz, et al., 2005). The same group also demonstrated the presence of large
383 amounts of rhamnogalacturonan–II (RG-II) in blackcurrant press cake, based on the
384 presence of several diagnostic sugars such as fucose and xylose (Hilz, Williams, Doco,
385 Schols, & Voragen, 2006). Pectins isolated in the current work did not contain fucose
386 and had negligible amounts of xylose, suggesting that RG-II was not present.

387

388 **Table 2.** Neutral sugar, uronic acid composition and Klason lignin of pomaces (POM)
 389 and its soluble (AP, CBP, HEM) and insoluble fractions (pIDF, CEL) (% w/w, wet
 390 basis). Values in brackets are mol% of each neutral sugar and uronic acids.

	Rha	Ara	Xyl	Man	Gal	Glu	Uronic acids	Klason lignin
POL								
POM	0.05±0.14 (0.3)	0.11±0.01 (0.6)	0.13±0.01 (0.7)	0.11±0.05 (0.5)	0.45±0.02 (2.1)	4.67±0.03 (21.4)	17.68±4.72 (74.5)	37.91± 9.06
pIDF	0.15±0.02 (1.3)	0.02±0.04 (0.2)	0.04±0.02 (0.4)	0.25±0.06 (1.9)	0.37±0.01 (2.8)	7.23±0.03 (54.7)	5.57±1.81 (38.8)	42.23± 2.47
AP	1.22±0.08 (2.4)	4.29±0.25 (9.4)	0.32±0.02 (0.7)	-	2.95±0.29 (5.3)	1.05±0.10 (1.9)	48.60±4.88 (80.2)	-
CBP	2.24±0.11 (3.8)	5.58±0.07 (10.6)	0.88±0.02 (1.7)	-	5.12±0.12 (7.9)	2.78±0.02 (4.3)	50.45±3.03 (71.7)	-
HEM	1.07±0.03 (2.7)	5.34±0.27 (14.8)	9.32±0.19 (25.9)	1.37±0.14 (3.1)	6.68±0.29 (15.1)	6.20±0.06 (14.0)	11.71±0.75 (24.4)	-
CEL	-	0.32±0.47 (0.9)	1.86±0.83 (5.5)	-	0.19±0.28 (0.5)	35.1±1.35 (84.3)	3.98±0.31 (8.8)	14.17± 3.26
UK								
POM	0.29±0.02 (2.4)	0.44±0.05 (4.1)	0.45±0.03 (4.2)	0.39±0.01 (2.9)	1.00±0.01 (7.5)	2.96±0.04 (22.3)	8.13±2.69 (56.5)	35.71± 11.31
pIDF	0.05±0.02 (0.5)	0.06±0.01 (0.6)	0.16±0.04 (1.6)	0.38±0.03 (3.1)	0.47±0.03 (3.8)	6.52±0.06 (53)	5.01±0.33 (37.5)	44.39± 3.83
AP	2.12± 0.05 (3.2)	5.23±0.05 (8.7)	1.10±0.03 (1.8)	-	7.57± 0.18 (10.3)	3.0±0.07 (4.1)	57.28±0.95 (71.8)	-
CBP	1.79±0.43 (3.4)	3.86±0.76 (8.1)	1.45±0.30 (3.0)	-	5.13±0.13 (8.8)	3.81±0.25 (6.5)	44.53±10.45 (70.1)	-
HEM	2.61±0.17 (9.2)	4.36±0.36 (17.1)	5.57±0.17 (21.8)	2.37±0.28 (7.6)	5.17±0.58 (16.5)	2.60±0.26 (8.3)	6.68±0.68 (19.6)	-
CEL	-	0.67±0.25 (1.3)	10.1±0.76 (20.4)	-	7.27±0.85 (11.9)	39.1±2.6 (64)	1.60±0.66 (2.4)	18.13± 4.51

391

392

393

394 **Table 3.** Sugar molar ratios (%) of pectins are shown as R1 = GalA/(Rha + Ara + Gal);
 395 R2 = Rha/GalA; R3 = (Ara + Gal)/Rha; HG (mol %) = GalA (mol %) – Rha (mol %);
 396 RG-I (mol %) = 2 × Rha (mol %) + Ara (mol %) + Gal (mol %). GalA is expressed as
 397 uronic acids shown in Table 2.

398

	APPOL	CBPPOL	APUK	CBPUK
R1 ^a	4.70	3.22	3.23	3.45
R2 ^a	0.03	0.05	0.05	0.05

R3 ^b	6.13	4.87	5.93	4.97
HG	77.80	67.90	68.60	66.70
RG-I	19.50	26.10	25.40	23.70
HG/RG-I	3.99	2.60	2.70	2.81
DE (%) ^c	38.2 ±1.1	11.3 ±2.8	33.2 ±2.1	16.5 ±2.5

399 ^aLinearity

400 ^bDegree of branching of RG-I

401 ^cDegree of esterification

402

403 All pectin fractions isolated in this work were LM pectins and their degrees of

404 esterification varied depending on the isolation conditions (Table 3). Both APUK (33

405 % DE) and APPOL (38 % DE) demonstrated comparable DE values that were higher

406 than those obtained with chelating agent (CBPUK (16 % DE) and CBPPOL (11 %

407 DE)). Chelating agents result in extraction of calcium-bound pectins that typically have

408 a low degree of methylation (Ralet, Crépeau, Buchholt, & Thibault, 2003). The

409 isolation of LM pectins has been previously reported from grape (21-47 %), cherry

410 pomace (10-19 %), blackcurrants (49.2 %), raspberry (45.8 %) and strawberry (46.0 %)

411 (González-Centeno, et al., 2010; Kosmala, et al., 2009; Mierczynska, Cybulska, &

412 Zdunek, 2017; Minjares-Fuentes, et al., 2014a). In contrast, pectic polysaccharides

413 isolated from blackcurrants and bilberries exhibited high degrees of methylation (Hilz,

414 et al., 2005). Lower values of DE in the present work could be attributed to the

415 extraction or processing conditions that result in losses of methyl groups of GalA. For

416 instance, treatment of blackcurrant pomaces with various pectic enzymes (e.g., pectin

417 methylesterases) prior to juice pressing results in lower number of methyl groups in

418 blackcurrant pomaces compared to blackcurrants, leading to reduction of DE of isolated

419 pectins. Losses of methyl groups have been also demonstrated for pectins isolated from

420 sour cherry and sour cherry pomace (Kosmala, et al., 2009) or grapes and grape pomace

421 (González-Centeno, et al., 2010).

422 Hemicelluloses were similar in terms of composition of neutral sugars and were

423 mainly composed of xylose, arabinose and galactose indicating the presence of several

424 hemicellulosic fractions (Table 2). Uronic acids were also identified in both fractions
425 and relatively higher amounts were detected in HEMPOL than in HEMUK. These
426 uronic acids could be a result of either oxidation of neutral sugars leading to the
427 formation of carboxylic acids in the presence of hydrogen peroxide or co-extracted
428 pectic polysaccharides. Hemicelluloses previously isolated from blackcurrant were
429 composed mainly of xylose, glucose and mannose and were identified as xyloglucans,
430 xylans and galactomannans (Hilz, de Jong, Kabel, Schols, & Voragen, 2006) whereas
431 hemicelluloses isolated from grape pomace were mainly composed of xyloglucans,
432 mannans and xylans (Minjares-Fuentes, et al., 2016).

433 Glucose was the major sugar in CELUK and CELPOL, indicating that the
434 extraction protocol resulted in the efficient isolation of cellulosic materials. The
435 presence of galactose and xylose in both fractions suggests the co-extraction of pectic
436 and/or hemicellulosic polysaccharides with considerably lower contents of
437 contaminants in CELPOL fraction than in CELUK (Table 2). Previous work has also
438 demonstrated the presence of galactans in the cellulose fraction isolated from onion cell
439 wall (Foster, Ablett, McCann, & Gidley, 1996). To confirm the structural and
440 compositional identity of isolates, detailed spectroscopic analysis (FTIR and NMR)
441 was carried out as described in the next sections.

442 *3.4 FT-IR spectroscopy of DFs*

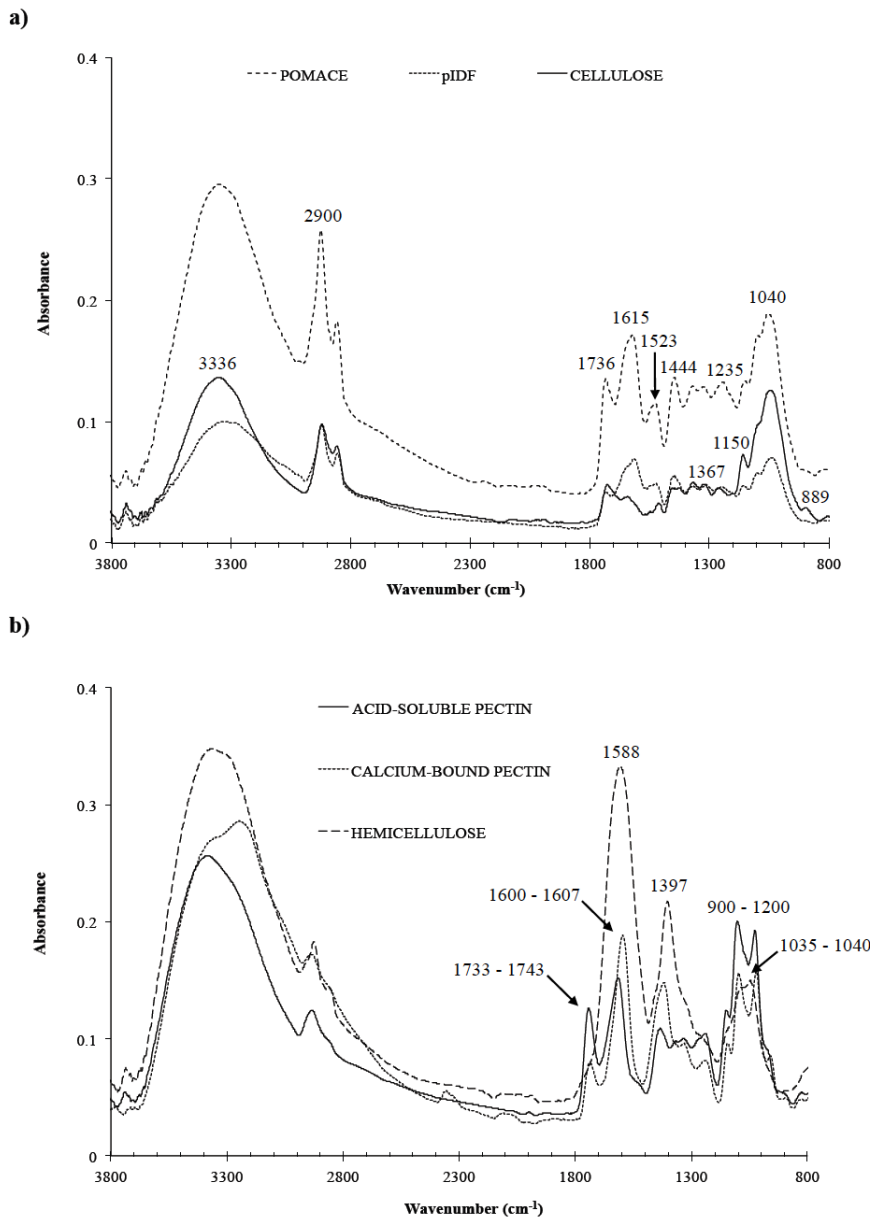
443 Figure 2a illustrates typical FT-IR spectra of pomace, pure insoluble DF and the
444 cellulosic fraction. The prominent peaks at 1736 and 1615 cm^{-1} in the blackcurrant
445 pomaces have been assigned to either acetyl groups or C=O bonds that are characteristic
446 for hemicelluloses, and methyl-esterified or free carboxyl groups that are specific for
447 pectin (Alemdar & Sain, 2008). Those assignments were further corroborated by less
448 pronounced peaks in cellulose spectra due to the removal of the majority of the pectic

449 and hemicellulosic polysaccharides during the extraction stages (Figure 2a). The peak
450 at 1523 cm^{-1} indicated the presence of lignin and was assigned to the aromatic skeletal
451 vibrations of lignin (Peng, Ren, Xu, Bian, Peng, & Sun, 2009). Absorbances at 1367,
452 1150 and 1040 cm^{-1} correspond to vibrations specific for cellulose or hemicellulose
453 (Sena Neto, Araujo, Souza, Mattoso, & Marconcini, 2013).

454 Spectroscopic profiles of most bands of CELUK were comparable to those observed in
455 CELPOL, indicating similarities in the chemical structure of two samples. Absorbances
456 at 3336, 2900, 1641, 1367, 1319, 1257, 1150, 1040 and 889 cm^{-1} are typically
457 associated with native cellulose (Zhang, Dong, Ma, Zhang, Wang, & Hu, 2015).

458

459



460

461 **Figure 2.** FT-IR spectra of POMUK and its fractions: (a) pomace, and insoluble
 462 fractions (i.e., pure insoluble DF (pIDF) and cellulose), and (b) soluble fractions (i.e.,
 463 acid soluble pectin, calcium-bound pectin and hemicellulose). POMPOL and its
 464 fractions showed similar spectra.
 465

466 Absorbance at around 1257 cm⁻¹ is typically associated with O-H in plane bending of
 467 cellulose. A broad peak at 1040 cm⁻¹ originates from stretching of C-O-C bond
 468 indicating the presence of pyranose rings. The minor peak at 889 cm⁻¹ corresponds to
 469 the deformation of glycosidic C₁-H or ring vibrations that are specific for β-glycosidic
 470 linkages between glucose monomers in cellulose (Zhang, et al., 2015). Both peaks are

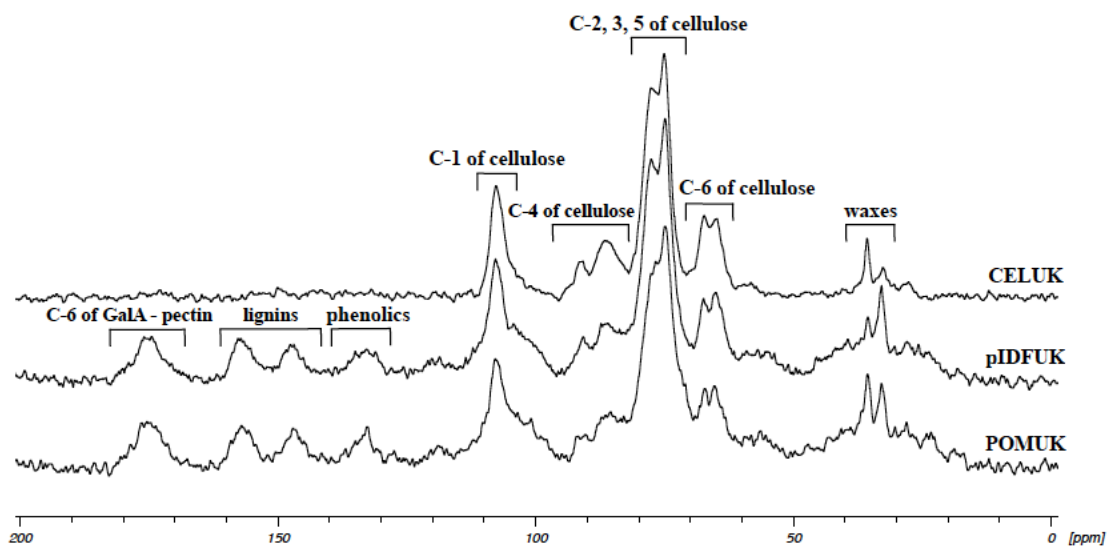
471 indicative of the cellulosic nature of fractions CELUK and CELPOL. A minor peak at
472 1506 and 1423 cm^{-1} corresponded to the C=C stretching and skeletal vibrations of
473 aromatic rings, and indicates the presence of negligible amounts of associated lignin in
474 the cellulosic fraction (Lan, Liu, & Sun, 2011).

475 The spectra of all pectic fractions showed the presence of characteristic peaks
476 for pectins and also indicated moderate structural differences between isolated
477 polysaccharides (Figure 2b). The two major peaks for all four samples at around 3500
478 cm^{-1} and 2500 cm^{-1} correspond to the O-H stretching absorption due to inter- and intra-
479 molecular hydrogen bonding of uronic acids and the C-H absorption of the rings,
480 respectively. Peaks at around 1733-1743 cm^{-1} were attributed to the ester carbonyl
481 (C=O) and those at 1600-1607 cm^{-1} were assigned to stretching of carboxylate anion of
482 GalA (Manrique, et al., 2002). The spectral region in the range of 900-1200 cm^{-1}
483 corresponds to skeletal C-O and C-C vibration bands of glycosidic bonds and pyranose
484 rings and reflects similarities in the neutral sugar composition of the isolated pectic
485 polysaccharides (Grassino, Halambek, Djaković, Rimac Brnčić, Dent, & Grabarić,
486 2016) also supported by sugar composition analysis. The absorption bands at 1413-
487 1427 cm^{-1} suggest the presence of aliphatic or aromatic C-H groups possibly arising
488 from lignin. Minor peaks in the range 1320-1327 and 1232-1237 cm^{-1} were attributed
489 to -OH and C-O bending vibrations (Bian, Peng, Peng, Xu, Sun, & Kennedy, 2012)
490 that represent negligible amounts of co-extracted hemicellulosic compounds that have
491 also been shown in compositional analysis (Table 2). Prominent absorption bands of
492 hemicelluloses at 1035 and 1040 cm^{-1} were assigned to C-O, C-C, and the glycosidic
493 C-O-C stretching that are typically reported for arabinoxylans and xylans (Peng, et al.,
494 2009; Sun, Wen, Ma, & Sun, 2013). In addition, the band centred at $\sim 1397 \text{ cm}^{-1}$ with
495 shoulders at around 1323 and 1249 cm^{-1} represents C-H stretching, O-H or C-O bending

496 vibrations in hemicelluloses (Bian, et al., 2012). The signal at $\sim 1588\text{ cm}^{-1}$ was assigned
497 to carboxylates of uronic acids, the presence of which was also evidenced by
498 compositional analysis of hemicellulosic fractions (Table 2). Other reports assign the
499 band at $\sim 1592\text{ cm}^{-1}$ to symmetric stretching of $-\text{COO}$ salts in 4-*O*-methyl- α -D-
500 glucuronic acid (Sun, et al., 2013). Generally, the spectral profiles of HEMUK and
501 HEMPOL were comparable, indicating structural similarities between fractions
502 obtained from two different blackcurrant pomaces.

503 *3.5 CP/MAS ^{13}C -NMR of insoluble and HSQC of soluble DFs*

504 CP/MAS ^{13}C -NMR spectra were obtained for the blackcurrant pomaces, the
505 pure IDF fractions and the celluloses (Figure 3). In the up-field region of the spectra of
506 cellulose samples, the duplets between 61.4 and 70.6 ppm were attributed to the CH_2
507 group of the C-6 atom. The next cluster of resonances, between 70.6 and 82.0 ppm, is
508 assigned to the ring carbons (C-2, C-3 and C-5) other than those involved in the
509 glycosidic linkage (Atalla & VanderHart, 1999; Kéri, Palcsu, Túri, Heim, Czébély,
510 Novák, & Bányai, 2015; Wawer, et al., 2006). Resonances of carbons involved in the
511 glycosidic linkages appear at around 82.0 to 95.6 ppm (C-4) and the resonance at 107.6
512 ppm is of the anomeric carbon C-1. The appearance of C-6 and C-4 as duplets suggests
513 the presence of crystalline and disordered amorphous regions of cellulose (Wang,
514 Yang, Kubicki, & Hong, 2016). Signals in the up-field region occurring at around 32.6
515 and 35.8 ppm were attributed to the aliphatic groups associated with extracted lipids.
516 Generally, the CP/MAS ^{13}C -NMR spectra of cellulose showed that the isolated
517 fractions were free from hemicellulose and lignin due to the absence of any resonances
518 with typical chemical shifts corresponding to those compounds further confirming the
519 suitability of the fractionation protocol for isolation of pure cellulose.



520

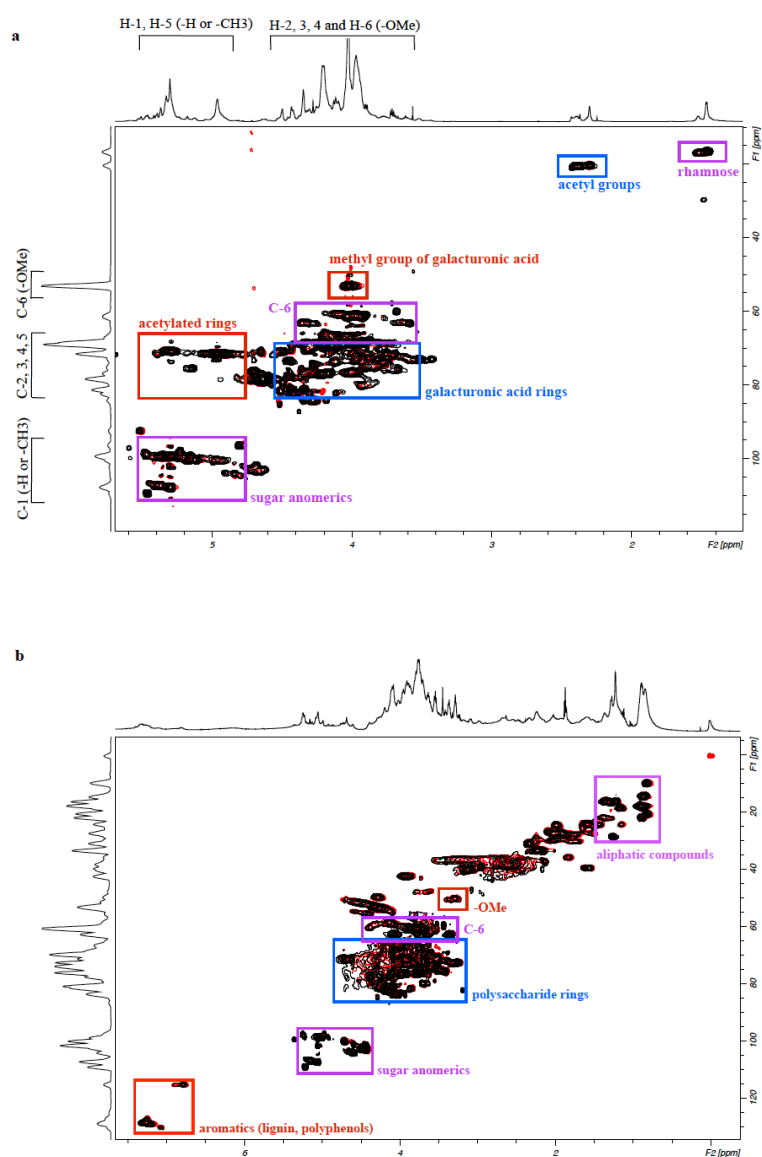
521 **Figure 3.** CP/MAS ^{13}C -NMR spectra of CELUK, pIDFUK and POMUK. Spectra from
 522 pomace obtained from Poland showed similar traces. Chemical shifts were referenced
 523 to the upfield peak of adamantane (29.5 ppm) run as an external standard.
 524

525 Cellulose-like resonances were also identified in the pIDF and pomace spectra
 526 indicating the presence of cellulose in those fractions (Figure 3). A new signal was
 527 observed at 175.3 ppm in the carbonyl region of spectra that corresponded to the C-6
 528 of a methyl-esterified galacturonic acid. This shift is a characteristic signal for pectins
 529 and therefore highlights the presence of pectic compounds in pomace and pIDF. The
 530 presence of lignin in pIDF and pomace is evidenced by the signals occurring at 157.3
 531 ppm and 147.5 ppm that were identified as syringyl and quaiacyl (Wawer, et al., 2006).
 532 Resonances in the aromatic region (centred on 132.9 ppm) and also in the aliphatic
 533 region (31.3-38.0 ppm) of pomace and pIDF spectra (Figure 3) were attributed to the
 534 phenolic compounds and cutin that were previously reported for blackcurrant (Wawer,
 535 et al., 2006). Hemicellulose residues were not identified in the CP/MAS ^{13}C -NMR
 536 spectra of pomace or pIDF due to the possible signal overlaps with intense resonances
 537 from cellulose and pectins.

538 The analysis of HSQC spectra of soluble fractions revealed the presence of
539 signals typical for pectic compounds (Figure 4a). The cluster of $^1\text{H}/^{13}\text{C}$ cross-peaks in
540 the range of 3.6-4.6/69.0-84.1 ppm was assigned to protons (H-2, 3, 4, 5) and carbons
541 (C-2, 3, 4, 5) of galacturonic acid rings. Additional resonances of galacturonic acid
542 rings were identified at around 4.90-5.5/65.0-84.1 ppm due to the signals shift caused
543 by acetylation of the attached -OH. The group of resonances at $\delta_{\text{H}}/\delta_{\text{C}}$ 4.8-5.5/95.9-110.6
544 ppm shows the presence of a number of polysaccharide anomeric protons (H-1) and
545 carbons (C-1). The $-\text{OCH}_3$ of galacturonic acid was identified at $\delta_{\text{H}}/\delta_{\text{C}}$ 3.97/53.1 ppm,
546 whereas non-bonded C-6 of neutral sugars appeared at $\delta_{\text{H}}/\delta_{\text{C}}$ 3.6-4.4/59.8-69.0 ppm. In
547 the up-field region of the HSQC spectra, cross-peaks at $\delta_{\text{H}}/\delta_{\text{C}}$ 1.46, 1.52/16.6 ppm were
548 assigned as belonging to the $-\text{CH}_3$ of rhamnose and signals at $\delta_{\text{H}}/\delta_{\text{C}}$ 2.30, 2.4/20.1 ppm
549 were identified as the COCH_3 of acetyl groups.

550 Signals of the hemicellulosic fractions ($\delta_{\text{H}}/\delta_{\text{C}}$ 6.7-7.4/114.3-131.4) in the
551 aromatic region indicate the presence of moderate amounts of lignin in the isolated
552 samples that can be attributed to guaiacyl, syringyl or *p*-hydroxycinnamic acid units
553 that typically occur at $\delta_{\text{H}}/\delta_{\text{C}}$ 6.7-7.0/103-119, 6.7/103 and 7.3/130 ppm (Figure 4b)
554 (Foston, Samuel, & Ragauskas, 2012). Well-resolved resonances were observed in the
555 anomeric region ($\delta_{\text{H}}/\delta_{\text{C}}$ 4.4-5.4/96.6-110.9 ppm) that can be tentatively assigned to H-
556 1 and C-1 of arabinose and galactose with typical $\delta_{\text{H}}/\delta_{\text{C}}$ in the range of 5.1-5.4/108.5-
557 110.4 and 4.4/102.6 ppm (Das, Mondal, Roy, Maiti, Bhunia, Maiti, & Islam, 2009;
558 Fischer, Yu, Gray, Ralph, Anderson, & Marlett, 2004). In the carbohydrate ring region,
559 complex signals were observed and some of the cross-peaks were attributed to
560 arabinose (3.8/76.2 ppm), xylose (3.6/74.0 ppm), glucose (3.3/72.8 ppm) and mannose
561 (4.6, 4.7/71.3 ppm) units by comparing chemical shifts with those published in

562 literature (Kang, Cui, Phillips, Chen, Guo, & Wang, 2011). Cross-peak centred at δ_H/δ_C
 563 3.3/51.0 ppm originated from $-\text{OCH}_3$, highlights the presence of sugar units



564

565 **Figure 4.** (a) HSQC spectra of CBPUK (black) and CBPPOL (red) recorded in D_2O at
 566 50°C . Similar spectra and correlation of proton/carbon signals were observed for
 567 APUK and APPOL (data not shown), (b) HSQC spectra of HEMUK (black) and
 568 HEMPOL (red) recorded in D_2O at 50°C . In the carbohydrate ring region (δ_H/δ_C 3.1-
 569 4.8/65.0-85.7 ppm), signals originated from arabinose, xylose, glucose and mannose.
 570

571 with $O\text{-Me}$ bonded to carboxyl groups. Three cross-peaks at δ_H/δ_C 3.5-4.3/41.3-52.3
 572 ppm, all originated from $-\text{OCH}_3$, highlight the presence of at least three different sugar
 573 units with $O\text{-Me}$ bonded to carboxyl groups. The cluster of resonances at δ_H/δ_C 0.8-

574 1.5/8.6-30.6 ppm indicates the presence of aliphatic compounds, such as cutin that has
575 been previously reported at 30.0-32.5 ppm (Wawer, et al., 2006). Combining
576 information from three different analytical techniques (sugar analysis, FTIR and NMR)
577 it becomes evident that the present protocol results in a rather heterogeneous
578 hemicellulosic fraction, as it was not possible to identify a particular type of
579 hemicellulose (e.g., xyloglucans, mannans or xylans). As the solution behaviour of the
580 soluble fibres affect their functional and physiological properties, it is imperative to
581 understand their macromolecular characteristics; these are described in the next section.

582 *3.6 Macromolecular characteristics of soluble DFs*

583 The weight-average molecular weights (M_w) of blackcurrant pectins ranged
584 from $29.4 \times 10^3 \text{ g mol}^{-1}$ to $109.6 \times 10^3 \text{ g mol}^{-1}$ with the lowest values recorded for
585 calcium-bound pectins (Table 4). Previous investigations have reported comparable M_w
586 for HBSS and CHSS pectin fractions isolated from blackcurrant and bilberry pomaces
587 (Hilz, et al., 2005). In contrast to the current results, pectins isolated with chelating
588 agents from citrus fruits, mango and banana had considerably higher M_w and intrinsic
589 viscosities than those obtained with acid extractions (Kaya, Sousa, Crépeau, Sorensen,
590 & Ralet, 2014; Koubala, Kansci, Mbome, Crépeau, Thibault, & Ralet, 2008).
591 Variations in M_w between different pectin fractions could be attributed to the isolation
592 of smaller polymers since some of the pectic polysaccharides may have been already
593 removed from pomace during blackcurrant juice manufacturing or due to the partial
594 acid hydrolysis of polysaccharides that occurs at elevated temperatures.

595 The highest M_w was recorded for APUK ($109.6 \times 10^3 \text{ g mol}^{-1}$) and pectins with
596 comparable M_w ($109.6-132.3 \times 10^3 \text{ g mol}^{-1}$) have been previously extracted from grape
597 pomace under similar isolation conditions (Minjares-Fuentes, et al., 2014a). Molecular
598 weight of APUK was higher than the rest of pectin samples, despite the fact that its $[\eta]$

599 values were lower, thus indicating differences in chain flexibility. This is in agreement
600 with the structural analysis (Table 3) that highlights the role of branching particularly
601 for APUK. Therefore higher flexibility, as demonstrated by APUK fractions, is due to
602 the presence of RG-I regions resulting in formation of compact structures with lower
603 hydrodynamic volume. It should be noted that the intrinsic viscosity of isolated pectin
604 fractions was considerably lower than previously reported for citrus (e.g., orange,
605 lemon, lime and grapefruit, ~ 4.0-8.0 dL g⁻¹), sugar beet (2.1-4.1 dL g⁻¹), okra (2.9-5.1
606 dL g⁻¹) or passionfruit pectins (4.7-5.8 dL g⁻¹) (Kaya, et al., 2014; Kpodo, Agbenorhevi,
607 Alba, Bingham, Oduro, Morris, & Kontogiorgos, 2017; Levigne, Ralet, & Thibault,
608 2002; Yapo & Koffi, 2008). The weight-average molecular weight (M_w) of
609 hemicellulosic polysaccharides were 1059 ×10³ g mol⁻¹ and 1167 ×10³ g mol⁻¹ while
610 [η] was 0.52-0.56 dL g⁻¹ for HEMUK and HEMPOL, respectively (Table 4).
611 Hemicelluloses were of particularly high M_w, similar to those extracted from pear
612 pomace also using hydrogen peroxide (Rabetafika, et al., 2014). This has been
613 attributed to the ability of hydrogen peroxide to favour the isolation of high molecular
614 weight polysaccharides or to the strong tendency of hemicelluloses to form aggregates
615 in aqueous solutions that could contribute to overestimation of M_w.

616 **Table 4.** Macromolecular characteristics of soluble DFs.

	APUK	CBPUK	APPOL	CBPPOL	HEMUK	HEMPOL
M _w × 10 ³ , g mol ⁻¹	109.6	31.3	45.5	29.4	1059	1167
M _w /M _n	3.0	3.0	2.9	2.8	10.2	10.4
[η], dL g ⁻¹	0.38	0.58	0.66	0.57	0.52	0.56

617 **4. Conclusions**

618 Molecular characterization and spectroscopy revealed that fractions isolated
619 from blackcurrant pomaces corresponded to pectin, hemicellulose and cellulose.

620 Isolated pectin samples contained 48-57% w/w and 45-50% w/w of galacturonic acid
621 for acid-soluble and calcium-bound pectins, respectively. Chemical analysis of acid-
622 soluble and calcium-bound pectins revealed small amounts of neutral sugars, primarily
623 galactose and arabinose, indicating the predominantly branched nature of the
624 biopolymer backbone, except for acid-soluble pectin isolated from POMPOL that was
625 more linear. Isolated pectins had low degree of esterification with the lowest values
626 obtained for calcium-bound pectin. Weight average molecular weight of pectin ranged
627 between about $30-110 \times 10^3 \text{ g mol}^{-1}$. Hemicelluloses were mainly composed of xylose
628 and galactose with particularly high molecular weight. The IDF/SDF ratio for POMUK
629 was 1.9 and for POMPOL was 1.6, indicating the suitability of blackcurrant pomaces
630 to be utilized as a source of DF in food formulations. Overall, the current work showed
631 that blackcurrant processing waste streams are a potential source of both soluble and
632 insoluble DFs, confirming our initial hypothesis that this raw material could be
633 successfully fractionated into fibre streams with distinct compositions and properties
634 and hence end-uses. Blackcurrant pomace could be used to obtain new functional
635 ingredients or to enhance the fibre content of foods, with minor differences observed
636 between the two places of origin (UK or Poland) and processing conditions.

637 **4. Acknowledgements**

638 The research project was approved during the 2nd SUSFOOD ERA-Net call
639 (www.susfood-era.net). The authors wish to acknowledge the financial support of
640 DEFRA, UK (Grant #FO0319). We thank H.E.L. Williams for help with the processing
641 of NMR spectroscopic data.

642 **5. References**

643 Aguedo, M., Kohnen, S., Rabetafika, N., Vanden Bossche, S., Sterckx, J., Blecker, C.,
644 Beauve, C., & Paquot, M. (2012). Composition of by-products from cooked

- 645 fruit processing and potential use in food products. *Journal of Food*
646 *Composition and Analysis*, 27, 61-69.
- 647 Alba, K., & Kontogiorgos, V. (2017). Pectin at the oil-water interface: Relationship of
648 molecular composition and structure to functionality. *Food Hydrocolloids*, 68,
649 211-218.
- 650 Alemdar, A., & Sain, M. (2008). Isolation and characterization of nanofibers from
651 agricultural residues: wheat straw and soy hulls. *Bioresour Technol*, 99, 1664-
652 1671.
- 653 Atalla, R. H., & VanderHart, D. L. (1999). The role of solid state ¹³C NMR
654 spectroscopy in studies of the nature of native celluloses. *Solid State Nuclear*
655 *Magnetic Resonance*, 15 1–19.
- 656 Beres, C., Simas-Tosin, F. F., Cabezudo, I., Freitas, S. P., Iacomini, M., Mellinger-
657 Silva, C., & Cabral, L. M. C. (2016). Antioxidant dietary fibre recovery from
658 Brazilian Pinot noir grape pomace. *Food Chemistry*, 201, 145-152.
- 659 Bian, J., Peng, F., Peng, X.-P., Xu, F., Sun, R.-C., & Kennedy, J. F. (2012). Isolation
660 of hemicelluloses from sugarcane bagasse at different temperatures: Structure
661 and properties. *Carbohydrate Polymers*, 88, 638-645.
- 662 Chapman, H. D., Morris, V. J., Selvendran, R. R., & O'Neill, M. A. (1987). Static and
663 dynamic light-scattering studies of pectic polysaccharides from the middle
664 lamellae and primary cell walls of cider apples. *Carbohydrate Research*, 165,
665 53-68.
- 666 Das, D., Mondal, S., Roy, S. K., Maiti, D., Bhunia, B., Maiti, T. K., & Islam, S. S.
667 (2009). Isolation and characterization of a heteropolysaccharide from the corm
668 of *Amorphophallus campanulatus*. *Carbohydrate Research*, 344, 2581-2585.
- 669 Denman, L. J., & Morris, G. A. (2015). An experimental design approach to the
670 chemical characterisation of pectin polysaccharides extracted from *Cucumis*
671 *melo Inodorus*. *Carbohydr Polym*, 117, 364-369.
- 672 DeVries, J. W., Camire, M. E., Cho, S., Craig, S., Gordon, D., Jones, J. M., Li, B.,
673 Lineback, D., Prosky, L., & Tunland, B. C. (2001). The definition of dietary
674 fiber. *Cereal Foods World*, 46, 112-129.
- 675 Englyst, H. N., & Cummings, J. H. (1988). Improved method for measurement of
676 dietary fiber as non-starch polysaccharides in plant foods. *Journal of the*
677 *Association of Official Analytical Chemists*, 71, 808-814.
- 678 Femenia, A. (2007). High-value co-products from plant foods: cosmetics and
679 pharmaceuticals. . In K. Waldron (Ed.), *Handbook of waste mangement and*
680 *coproduct recovery in food processing*, (Vol. 1, pp. 470-501). Cambridge:
681 Woodhead Publishing Limited.
- 682 Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids
683 without interference from neutral sugars. *Analytical Biochemistry*, 197, 157-
684 162.

- 685 Fischer, M. H., Yu, N., Gray, G. R., Ralph, J., Anderson, L., & Marlett, J. A. (2004).
686 The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk).
687 *Carbohydrate Research*, 339, 2009-2017.
- 688 Foster, T. J., Ablett, S., McCann, M. C., & Gidley, M. J. (1996). Mobility-resolved
689 ¹³C-NMR spectroscopy of primary plant cell walls. *Biopolymers*, 39, 51-66.
- 690 Foston, M., Samuel, R., & Ragauskas, A. J. (2012). ¹³C cell wall enrichment and ionic
691 liquid NMR analysis: progress towards a high-throughput detailed chemical
692 analysis of the whole plant cell wall. *Analyst*, 137, 3904-3909.
- 693 Gomez, M., Ronda, F., Blanco, C. A., Caballero, P. A., & Apesteguia, A. (2003). Effect
694 of dietary fibre on dough rheology and bread quality. *European Food Research
695 and Technology*, 216, 51-56.
- 696 González-Centeno, M. R., Rosselló, C., Simal, S., Garau, M. C., López, F., & Femenia,
697 A. (2010). Physico-chemical properties of cell wall materials obtained from ten
698 grape varieties and their byproducts: grape pomaces and stems. *LWT - Food
699 Science and Technology*, 43, 1580-1586.
- 700 Grassino, A. N., Halambek, J., Djaković, S., Rimac Brnčić, S., Dent, M., & Grabarić,
701 Z. (2016). Utilization of tomato peel waste from canning factory as a potential
702 source for pectin production and application as tin corrosion inhibitor. *Food
703 Hydrocolloids*, 52, 265-274.
- 704 Hilz, H., Bakx, E. J., Schols, H. A., & Voragen, A. G. J. (2005). Cell wall
705 polysaccharides in black currants and bilberries—characterisation in berries,
706 juice, and press cake. *Carbohydrate Polymers*, 59, 477-488.
- 707 Hilz, H., de Jong, L. E., Kabel, M. A., Schols, H. A., & Voragen, A. G. (2006). A
708 comparison of liquid chromatography, capillary electrophoresis, and mass
709 spectrometry methods to determine xyloglucan structures in black currants. *J
710 Chromatogr A*, 1133, 275-286.
- 711 Hilz, H., Williams, P., Doco, T., Schols, H. A., & Voragen, A. G. J. (2006). The pectic
712 polysaccharide rhamnogalacturonan II is present as a dimer in pectic
713 populations of bilberries and black currants in muro and in juice. *Carbohydrate
714 Polymers*, 65, 521-528.
- 715 Jakobsdottir, G., Nilsson, U., Blanco, N., Sterner, O., & Nyman, M. (2014). Effects of
716 Soluble and Insoluble Fractions from Bilberries, Black Currants, and
717 Raspberries on Short-Chain Fatty Acid Formation, Anthocyanin Excretion, and
718 Cholesterol in Rats. *Journal of Agricultural and Food Chemistry*, 62, 4359-
719 4368.
- 720 Jaroslawska, J., Wroblewska, M., Juskiewicz, J., Brzuzan, L., & Zdunczyk, Z. (2016).
721 Protective effects of polyphenol-rich blackcurrant preparation on biochemical
722 and metabolic biomarkers of rats fed a diet high in fructose. *J Anim Physiol
723 Anim Nutr (Berl)*, 100, 136-145.

- 724 Jenkins, D. J. A., Kendall, C. W. C., & Ransom, T. P. P. (1998). Dietary fiber, the
725 evolution of the human diet and coronary heart disease. *Nutrition Research*, *18*,
726 633-652.
- 727 Kang, J., Cui, S. W., Phillips, G. O., Chen, J., Guo, Q., & Wang, Q. (2011). New studies
728 on gum ghatti (*Anogeissus latifolia*) part II. Structure characterization of an
729 arabinogalactan from the gum by 1D, 2D NMR spectroscopy and methylation
730 analysis. *Food Hydrocolloids*, *25*, 1991-1998.
- 731 Kaya, M., Sousa, A. G., Crepeau, M. J., Sorensen, S. O., & Ralet, M. C. (2014).
732 Characterization of citrus pectin samples extracted under different conditions:
733 influence of acid type and pH of extraction. *Annals of Botany*, *114*, 1319-1326.
- 734 Kendall, C. W. C., Esfahani, A., & Jenkins, D. J. A. (2010). The link between dietary
735 fibre and human health. *Food Hydrocolloids*, *24*, 42-48.
- 736 Kéri, M., Palcsu, L., Túri, M., Heim, E., Czébely, A., Novák, L., & Bányai, I. (2015).
737 ¹³C NMR analysis of cellulose samples from different preparation methods.
738 *Cellulose*, *22*, 2211-2220.
- 739 Kosmala, M., Milala, J., Kolodziejczyk, K., Markowski, J., Mieszczakowska, M.,
740 Ginies, C., & Renard, C. M. (2009). Characterization of cell wall
741 polysaccharides of cherry (*Prunus cerasus* var. Schattenmorelle) fruit and
742 pomace. *Plant Foods Hum Nutr*, *64*, 279-285.
- 743 Koubala, B. B., Kansci, G., Mbome, L. I., Crépeau, M. J., Thibault, J. F., & Ralet, M.
744 C. (2008). Effect of extraction conditions on some physicochemical
745 characteristics of pectins from “Améliorée” and “Mango” mango peels. *Food*
746 *Hydrocolloids*, *22*, 1345-1351.
- 747 Kpodo, F. M., Agbenorhevi, J. K., Alba, K., Bingham, R. J., Oduro, I. N., Morris, G.
748 A., & Kontogiorgos, V. (2017). Pectin isolation and characterization from six
749 okra genotypes. *Food Hydrocolloids*, *72*, 323-330.
- 750 Lan, W., Liu, C.-F., & Sun, R.-C. (2011). Fractionation of Bagasse into Cellulose,
751 Hemicelluloses, and Lignin with Ionic Liquid Treatment Followed by Alkaline
752 Extraction. *Journal of Agricultural and Food Chemistry*, *59*, 8691-8701.
- 753 Larrauri, J. A. (1999). New approaches in the preparation of high dietary fibre powders
754 from fruit by-products. *Trends in Food Science and Technology*, *10*, 3-8.
- 755 Levigne, S., Ralet, M.-C., & Thibault, J.-F. (2002). Characterization of pectins
756 extracted from fresh sugar beet under different conditions using an experimental
757 design. *Carbohydrate Polymers*, *49*, 145-153.
- 758 Makris, D. P., Boskou, G., & Andrikopoulos, N. K. (2007). Polyphenolic content and
759 in vitro antioxidant characteristics of wine industry and other agri-food solid
760 waste extracts. *Journal of Food Composition and Analysis*, *20*, 125-132.
- 761 Mann, J. I., & Cummings, J. H. (2009). Possible implications for health of the different
762 definitions of dietary fibre. *Nutr Metab Cardiovasc Dis*, *19*, 226-229.

- 763 Manrique, G. D., & Lajolo, F. M. (2002). FT-IR spectroscopy as a tool for measuring
764 degree of methyl esterification in pectins isolated from ripening papaya fruit.
765 *Postharvest Biology and Technology*, 25, 99-107.
- 766 Mierczynska, J., Cybulska, J., & Zdunek, A. (2017). Rheological and chemical
767 properties of pectin enriched fractions from different sources extracted with
768 citric acid. *Carbohydr Polym*, 156, 443-451.
- 769 Minjares-Fuentes, R., Femenia, A., Garau, M. C., Candelas-Cadillo, M. G., Simal, S.,
770 & Rosselló, C. (2016). Ultrasound-assisted extraction of hemicelluloses from
771 grape pomace using response surface methodology. *Carbohydrate Polymers*,
772 138, 180-191.
- 773 Minjares-Fuentes, R., Femenia, A., Garau, M. C., Meza-Velazquez, J. A., Simal, S., &
774 Rossello, C. (2014a). Ultrasound-assisted extraction of pectins from grape
775 pomace using citric acid: a response surface methodology approach. *Carbohydr*
776 *Polym*, 106, 179-189.
- 777 Minjares-Fuentes, R., Femenia, A., Garau, M. C., Meza-Velázquez, J. A., Simal, S., &
778 Rosselló, C. (2014b). Ultrasound-assisted extraction of pectins from grape
779 pomace using citric acid: A response surface methodology approach.
780 *Carbohydrate Polymers*, 106, 179-189.
- 781 Monsoor, M. A., Kalapathy, U., & Proctor, A. (2001). Improved method for
782 determination of pectin degree of esterification by diffuse reflectance Fourier
783 transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry*,
784 49, 2756-2760.
- 785 Morris, G. A., de al Torre, J. G., Ortega, A., Castile, J., Smith, A., & Harding, S. E.
786 (2008). Molecular flexibility of citrus pectins by combined sedimentation and
787 viscosity analysis. *Food Hydrocolloids*, 22, 1435-1442.
- 788 Morris, G. A., Foster, T. J., & Harding, S. E. (2000). The effect of the degree of
789 esterification on the hydrodynamic properties of citrus pectin. *Food*
790 *Hydrocolloids*, 14, 227-235.
- 791 Nawirska, A., & Kwaśniewska, M. (2005). Dietary fibre fractions from fruit and
792 vegetable processing waste. *Food Chemistry*, 91, 221-225.
- 793 Pelucchi, C., Talamini, R., Galeone, C., Negri, E., Franceschi, S., Dal Maso, L.,
794 Montella, M., Conti, E., & La Vecchia, C. (2004). Fibre intake and prostate
795 cancer risk. *International Journal of Cancer*, 109, 278-280.
- 796 Peng, F., Ren, J. L., Xu, F., Bian, J., Peng, P., & Sun, R. C. (2009). Comparative study
797 of hemicelluloses obtained by graded ethanol precipitation from sugarcane
798 bagasse. *J Agric Food Chem*, 57, 6305-6317.
- 799 Prozil, S. O., Costa, E. V., Evtuguin, D. V., Lopes, L. P., & Domingues, M. R. (2012).
800 Structural characterization of polysaccharides isolated from grape stalks of *Vitis*
801 *vinifera* L. *Carbohydr Res*, 356, 252-259.

- 802 Rabetafika, H. N., Bchir, B., Aguedo, M., Paquot, M., & Blecker, C. (2013). Effects of
803 Processing on the Compositions and Physicochemical Properties of Fibre
804 Concentrate from Cooked Fruit Pomaces. *Food and Bioprocess Technology*, *7*,
805 749-760.
- 806 Rabetafika, H. N., Bchir, B., Blecker, C., Paquot, M., & Wathelet, B. (2014).
807 Comparative study of alkaline extraction process of hemicelluloses from pear
808 pomace. *Biomass and Bioenergy*, *61*, 254-264.
- 809 Ralet, M.-C., Crépeau, M.-J., Buchholt, H.-C., & Thibault, J.-F. (2003). Polyelectrolyte
810 behaviour and calcium binding properties of sugar beet pectins differing in their
811 degrees of methylation and acetylation. *Biochemical Engineering Journal*, *16*,
812 191-201.
- 813 Rowland, S. P., & Howley, P. S. (1989). Simplified hydrolysis of cellulose and
814 substituted cellulose: Observations on trifluoroacetic acid hydrolyses. *Journal*
815 *of Applied Polymer Science*, *37*, 2371-2382.
- 816 Sena Neto, A. R., Araujo, M. A. M., Souza, F. V. D., Mattoso, L. H. C., & Marconcini,
817 J. M. (2013). Characterization and comparative evaluation of thermal,
818 structural, chemical, mechanical and morphological properties of six pineapple
819 leaf fiber varieties for use in composites. *Industrial crops and products*, *43*, 529-
820 537.
- 821 Struck, S., Plaza, M., Turner, C., & Rohm, H. (2016). Berry pomace - A review of
822 processing and chemical analysis of its polyphenols. *International Journal of*
823 *Food Science and Technology*, *51*, 1305-1318.
- 824 Sun, S. L., Wen, J. L., Ma, M. G., & Sun, R. C. (2013). Successive alkali extraction
825 and structural characterization of hemicelluloses from sweet sorghum stem.
826 *Carbohydr Polym*, *92*, 2224-2231.
- 827 Valiente, C., Arrigoni, E., Esteban, R. M., & Amado, R. (1995). Grape Pomace as a
828 Potential Food Fiber. *Journal of Food Science*, *60*, 818-820.
- 829 Vergara-Valencia, N., Granados-Pérez, E., Agama-Acevedo, E., Tovar, J., Ruales, J.,
830 & Bello-Pérez, L. A. (2007). Fibre concentrate from mango fruit:
831 Characterization, associated antioxidant capacity and application as a bakery
832 product ingredient. *LWT - Food Science and Technology*, *40*, 722-729.
- 833 Wang, T., Yang, H., Kubicki, J. D., & Hong, M. (2016). Cellulose Structural
834 Polymorphism in Plant Primary Cell Walls Investigated by High-Field 2D
835 Solid-State NMR Spectroscopy and Density Functional Theory Calculations.
836 *Biomacromolecules*, *17*, 2210-2222.
- 837 Wawer, I., Wolniak, M., & Paradowska, K. (2006). Solid state NMR study of dietary
838 fiber powders from aronia, bilberry, black currant and apple. *Solid State Nucl*
839 *Magn Reson*, *30*, 106-113.
- 840 Xu, C., Leppanen, A. S., Eklund, P., Holmlund, P., Sjöholm, R., Sundberg, K., &
841 Willfor, S. (2010). Acetylation and characterization of spruce (*Picea abies*)
842 galactoglucomannans. *Carbohydr Res*, *345*, 810-816.

- 843 Yapo, B. M., & Koffi, K. L. (2008). The polysaccharide composition of yellow passion
844 fruit rind cell wall: chemical and macromolecular features of extracted pectins
845 and hemicellulosic polysaccharides. *Journal of the Science of Food and*
846 *Agriculture*, 88, 2125-2133.
- 847 Zhang, P., Dong, S.-J., Ma, H.-H., Zhang, B.-X., Wang, Y.-F., & Hu, X.-M. (2015).
848 Fractionation of corn stover into cellulose, hemicellulose and lignin using a
849 series of ionic liquids. *Industrial crops and products*, 76, 688-696.
850
- 851 Rohm H., Brennan C. S., Turner C., Günther E., Campbell G. M., Hernando I., Struck
852 S., Kontogiorgos V. (2015). “Adding value to fruit processing waste: Innovative
853 ways to incorporate fibers from berry pomace in baked and extruded cereal-
854 based foods – A SUSFOOD project.” *Foods* 4(4), 690-697.
855
- 856 Quiles A., Campbell G. M., Struck S., Rohm H. and Hernando I. (2016). “Fiber from
857 fruit pomace: A review of applications in cereal-based products.” *Food Reviews*
858 *International*, 34 (2), 162-181.
859
- 860
- 861
- 862
- 863