Fractionation and characterisation of dietary fibre from blackcurrant pomace

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Abstract

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

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

Abbreviations:

POMUK – pomace from UK

POMPOL – pomace from Poland

pIDFUK – pure insoluble dietary fibre from UK (fraction that was obtained after extraction of acid-soluble and Ca^{2+}-bound pectins)

pIDFPOL – pure insoluble dietary fibre from Poland

APUK – acid-soluble pectin isolated from POMUK

APPOL – acid-soluble pectin isolated from POMPOL

CBPUK – calcium-bound pectin isolated from POMUK

CBPPOL – calcium-bound pectin isolated from POMPOL

HEMUK – hemicellulose isolated from POMUK

HEMPOL – hemicellulose isolated from POMPOL

CELUK – cellulose isolated from POMUK

CELPOL – cellulose isolated from POMPOL

DF – dietary fibre

SDF – soluble dietary fibre

IDF – insoluble dietary fibre

DE – degree of esterification

HBSS – hot buffer soluble solids

CHSS – chelator soluble solids

HG – homogalacturonan

RG-I – rhamnogalacturonan-I

GalA – galacturonic acid

MWCO – molecular weight cut-off

LM pectin – low-methylated pectin

1. Introduction

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

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

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

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

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

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

2. Materials and methods

2.1 Materials

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

2.2 Fibre fractionation

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

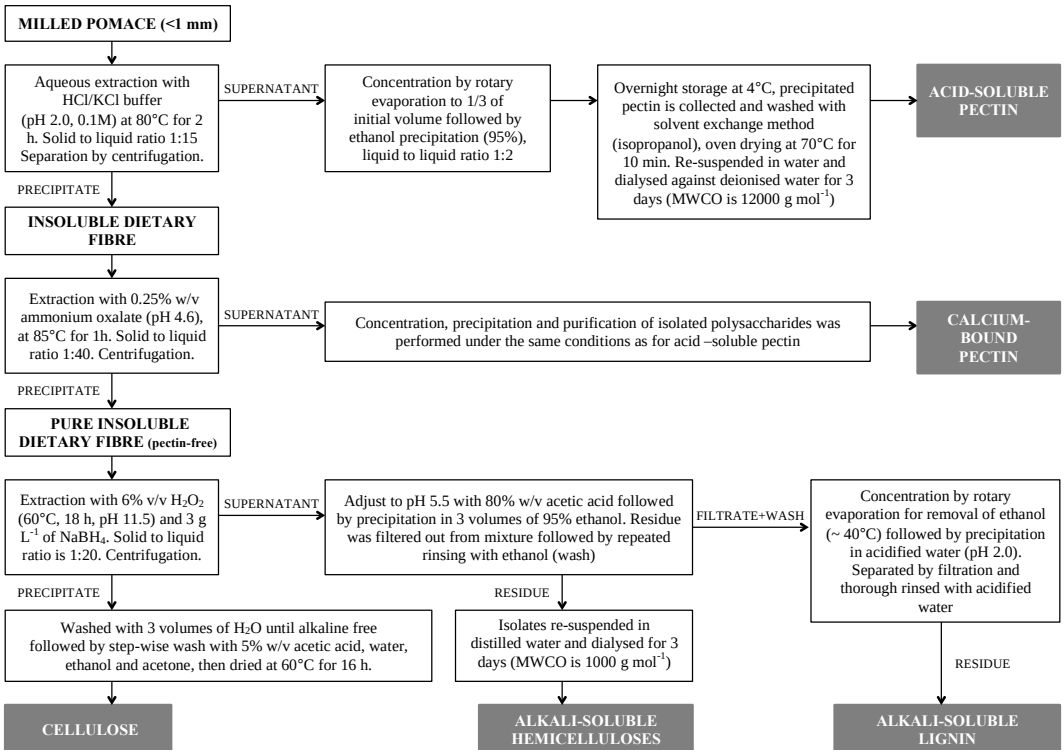


Figure 1. Sequential fractionation protocol of blackcurrant pomace into soluble and insoluble DFs. Five fractions were separated from the raw material including acid-soluble and calcium-bound pectins, celluloses, hemicelluloses and lignin.

2.3 Characterisation of blackcurrant pomaces and DFs

Proximate analysis (i.e., protein, ash, moisture, fat and carbohydrate) of blackcurrant pomaces was conducted by International Laboratory Services (ILS Limited, Derby, UK). Yields of pectin (acid-soluble and calcium-bound), hemicellulose, cellulose and alkali-soluble lignin were determined gravimetrically following the fractionation protocol. Klason lignin was determined in the following samples: pomaces, pure insoluble DF (pIDF) and isolated celluloses after a two-step Saeman hydrolysis with sulphuric acid (Englyst & Cummings, 1988). Hydrolysis was performed as follows: 50 mg of sample was pre-hydrolysed with 12M H_2SO_4 for 1 h at 35 °C followed by dilution to 1M H_2SO_4 by addition of H_2O and boiling at 100 °C for 2 h. The insoluble residue was filtered out of the mixture, dried at 102 °C and ashed for 4 h at 425 °C. The filtrate was collected and used for spectrophotometric determination

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

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

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

2.4 Spectroscopic analysis

2.4.1 FT-IR spectroscopy

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

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

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

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

2.4.2 ^{13}C CPMAS NMR spectroscopy

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

2.4.3 Two-dimensional NMR spectroscopy

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

2.5 Macromolecular characteristics of soluble DFs

Weight-average molecular weight (M_w) of pectins and hemicelluloses was determined using size exclusion chromatography coupled to multi–angle laser light scattering (SEC-MALLS) at 25°C. Soluble fractions were stirred overnight in 0.1M $NaNO_3$ solution (3-5 mg mL^{-1}) at room temperature. Solubilized fractions were injected onto an SEC system (15 µm particle size, 25 cm × 4 mm, Agilent, Oxford, UK) that consisted of a PL Aquagel guard column linked in series with PL Aquagel-OH 60, PL Aquagel-OH 50 and PL Aquagel-OH 40. Fractions were eluted with 0.1M $NaNO_3$ solution at a flow rate of 0.7 mL min^{-1}. The eluent was then detected online firstly by a DAWN EOS light scattering detector (Wyatt Technology, Santa Barbara, U.S.A.) and finally by a rEX differential refractometer (Wyatt Technology, Santa Barbara, U.S.A.).

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

Intrinsic viscosity measurements were performed on pectins and hemicelluloses. Samples were dispersed at 0.1-2.0 g dL^{-1} in 0.1 M NaCl at pH 7.0 in Sorensen's phosphate buffer with 0.02 g dL^{-1} NaN_3 and were stirred overnight. An Ubbelohde capillary glass viscometer (PSL Rheotek OB. C 80705) and the Huggins equation was used to estimate the intrinsic viscosities of the isolated fractions at 20 ± 0.1°C.

3. Results and discussion

3.1 Fractionation of DFs from blackcurrant pomace

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

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

into three major components, hemicellulose, cellulose and lignin (Figure 1). It has been shown that strong alkali (4M KOH) was more efficient in dissolving hemicelluloses and resulted in higher extraction yields compared to 2M KOH (Minjares-Fuentes, Femenia, Garau, Candelas-Cadillo, Simal, & Rosselló, 2016; Prozil, Costa, Evtuguin, Lopes, & Domingues, 2012). Other authors reported higher yields of hemicelluloses isolated with H_2O_2 rather than with direct alkaline extraction due to high reactivity of hydroxyl radicals that cleave ester links between lignin and hemicelluloses (Rabetafika, Bchir, Blecker, Paquot, & Wathelet, 2014). Therefore, to optimise the yield, isolation of hemicelluloses was performed with 6% v/v H_2O_2 under alkaline conditions. Note that isolated pectins and hemicelluloses represent the fractions that can be used as water-soluble dietary fibres based on their solubility in water after extraction from pomace. Cellulose was separated from lignocellulose after hemicellulose extraction in the form of a solid residue, whereas lignin was recovered by precipitation with acidified water (Figure 1). Generally, yield, purity and macromolecular characteristics of extracted cellulose and lignin vary depending on the isolation method, as described in the following sections.

3.2 Characterisation of blackcurrant pomace

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

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

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

Table 1. Proximate analysis, fibre composition of blackcurrant pomaces and the yield 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

[a] Values are expressed as % wet basis.
[b] Values were calculated by adding acid-soluble pectin, calcium-bound pectin and alkali-soluble hemicellulose. Values are expressed as % w/w (g/100 g of dry pomace).
[c] Values are expressed as % w/w (g/100 g of dry pomace).
[d] Values were calculated by subtracting the alkali-soluble hemicellulose content (HEMUK, HEMPOL) from the amount of pure insoluble DF (pIDFUK, pIDFPOL). Values are expressed as % w/w (g/100 g of dry pomace).

The amount of cellulose was 13.6 and 17.2% w/w for the two samples higher than a previously reported value of 12.0% w/w (Nawirska & Kwaśniewska, 2005). Klason lignin was the main cell wall component and also the major insoluble DF fraction in 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). Generaly 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 Ca^{2+}-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

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

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

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

3.3 Chemical characterisation of pomaces and fractions

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

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

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

Table 2. Neutral sugar, uronic acid composition and Klason lignin of pomaces (POM) and its soluble (AP, CBP, HEM) and insoluble fractions (pIDF, CEL) (% w/w, wet 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

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

	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 [a]Linearity
400 [b]Degree of branching of RG-I
401 [c]Degree 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

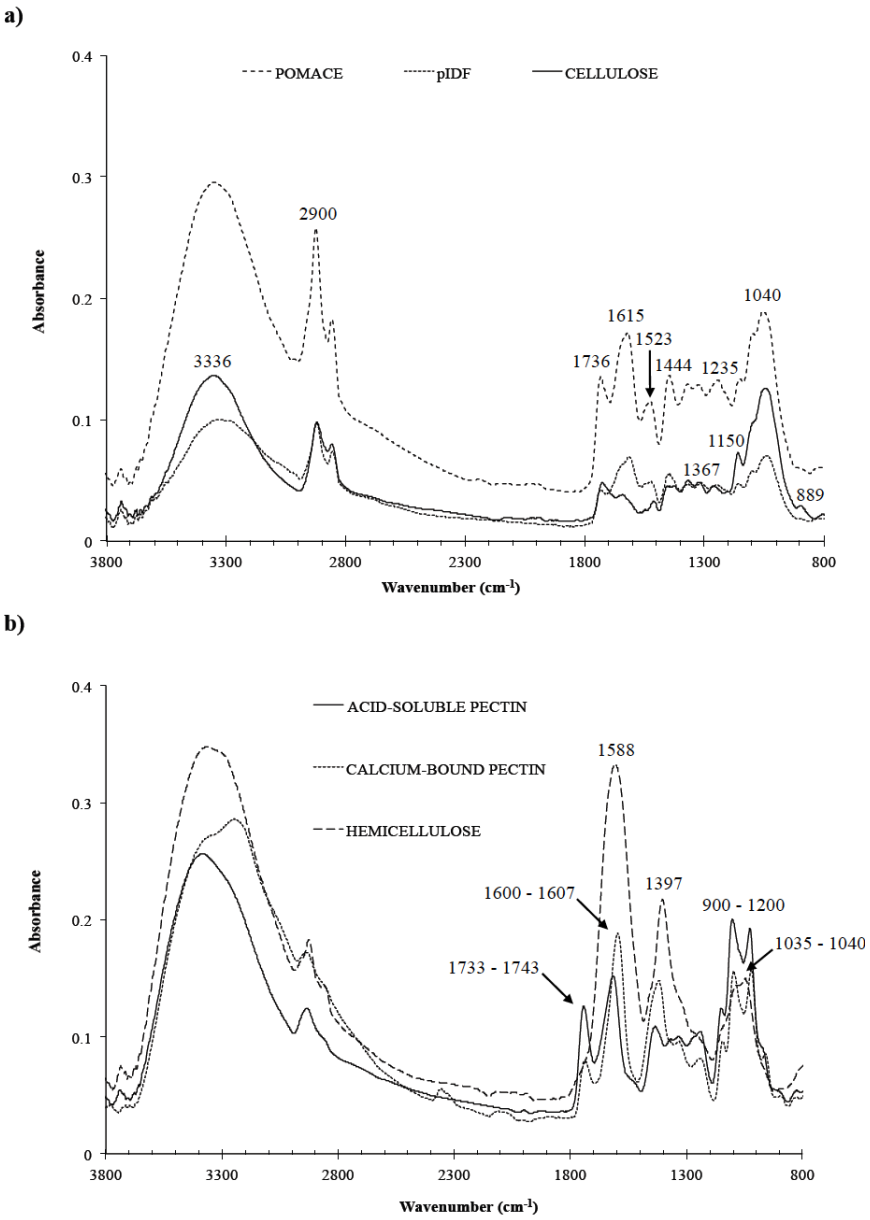
hemicellulosic fractions (Table 2). Uronic acids were also identified in both fractions and relatively higher amounts were detected in HEMPOL than in HEMUK. These uronic acids could be a result of either oxidation of neutral sugars leading to the formation of carboxylic acids in the presence of hydrogen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

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

3.4 FT-IR spectroscopy of DFs

Figure 2a illustrates typical FT-IR spectra of pomace, pure insoluble DF and the cellulosic fraction. The prominent peaks at 1736 and 1615 cm^{-1} in the blackcurrant pomaces have been assigned to either acetyl groups or C=O bonds that are characteristic for hemicelluloses, and methyl-esterified or free carboxyl groups that are specific for pectin (Alemdar & Sain, 2008). Those assignments were further corroborated by less 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 C_1-H or ring vibrations that are specific for β-glycosidic
470 linkages between glucose monomers in cellulose (Zhang, et al., 2015). Both peaks are

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

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

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

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

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

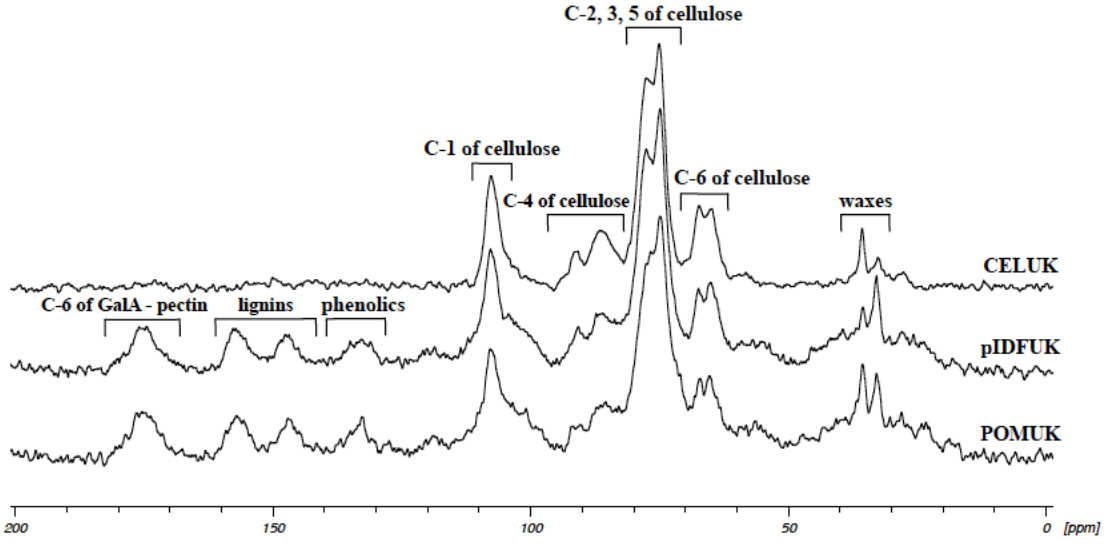


Figure 3. CP/MAS ^{13}C-NMR spectra of CELUK, pIDFUK and POMUK. Spectra from pomace obtained from Poland showed similar traces. Chemical shifts were referenced to the upfield peak of adamantane (29.5 ppm) run as an external standard.

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

The analysis of HSQC spectra of soluble fractions revealed the presence of signals typical for pectic compounds (Figure 4a). The cluster of $^{1}H/^{13}C$ cross-peaks in the range of 3.6-4.6/69.0-84.1 ppm was assigned to protons (H-2, 3, 4, 5) and carbons (C-2, 3, 4, 5) of galacturonic acid rings. Additional resonances of galacturonic acid rings were identified at around 4.90-5.5/65.0-84.1 ppm due to the signals shift caused by acetylation of the attached -OH. The group of resonances at δ_H/δ_C 4.8-5.5/95.9-110.6 ppm shows the presence of a number of polysaccharide anomeric protons (H-1) and carbons (C-1). The $–OCH_3$ of galacturonic acid was identified at δ_H/δ_C 3.97/53.1 ppm, whereas non-bonded C-6 of neutral sugars appeared at δ_H/δ_C 3.6-4.4/59.8-69.0 ppm. In the up-field region of the HSQC spectra, cross-peaks at δ_H/δ_C 1.46, 1.52/16.6 ppm were assigned as belonging to the $–CH_3$ of rhamnose and signals at δ_H/δ_C 2.30, 2.4/20.1 ppm were identified as the $COCH_3$ of acetyl groups.

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

literature (Kang, Cui, Phillips, Chen, Guo, & Wang, 2011). Cross-peak centred at δ_H/δ_C 3.3/51.0 ppm originated from -OCH_3, highlights the presence of sugar units

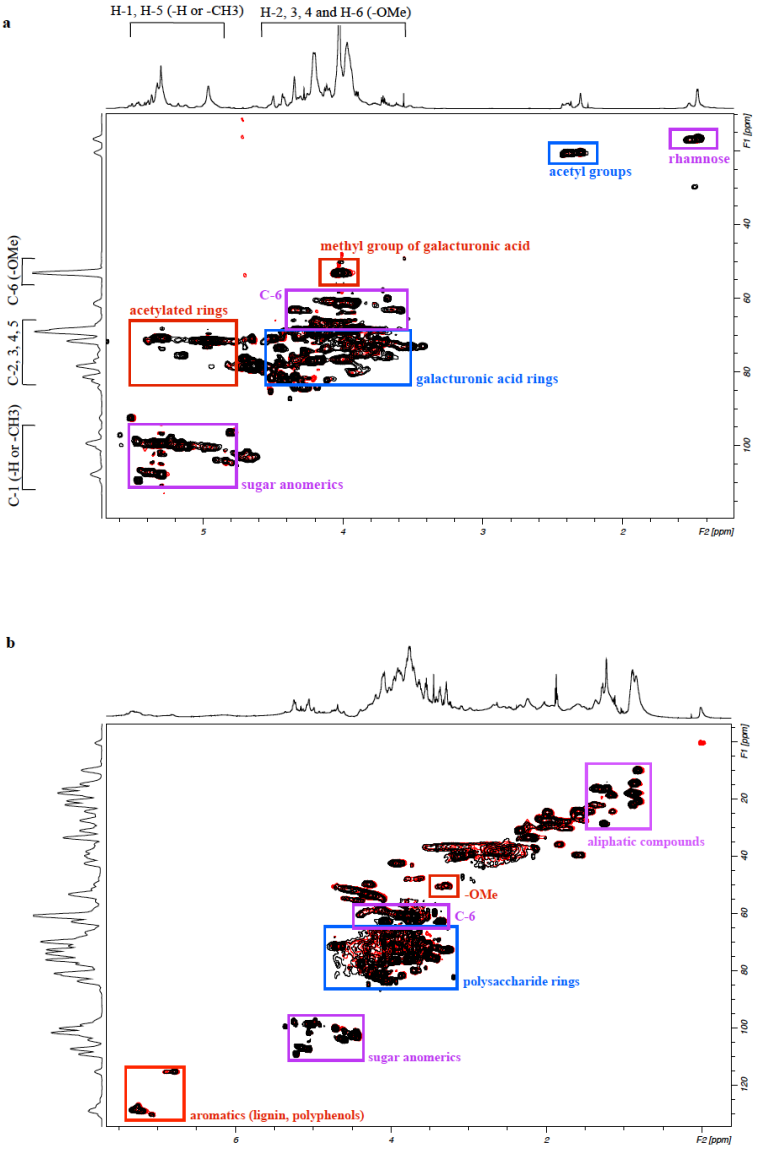


Figure 4. (a) HSQC spectra of CBPUK (black) and CBPPOL (red) recorded in D_2O at 50 °C. Similar spectra and correlation of proton/carbon signals were observed for APUK and APPOL (data not shown), (b) HSQC spectra of HEMUK (black) and HEMPOL (red) recorded in D_2O at 50 °C. In the carbohydrate ring region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

with *O*-Me bonded to carboxyl groups. Three cross-peaks at δ_H/δ_C 3.5-4.3/41.3-52.3 ppm, all originated from -OCH_3, highlight the presence of at least three different sugar units with *O*-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 $\times 10^3$ g mol^{-1} to 109.6 $\times 10^3$ 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, Crepeau, 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 $\times 10^3$ g mol^{-1}) and pectins with
596 comparable M_w (109.6-132.3 $\times 10^3$ 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 [η]

values were lower, thus indicating differences in chain flexibility. This is in agreement with the structural analysis (Table 3) that highlights the role of branching particularly for APUK. Therefore higher flexibility, as demonstrated by APUK fractions, is due to the presence of RG-I regions resulting in formation of compact structures with lower hydrodynamic volume. It should be noted that the intrinsic viscosity of isolated pectin fractions was considerably lower than previously reported for citrus (e.g., orange, 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 dL g^{-1}) or passionfruit pectins (4.7-5.8 dL g^{-1}) (Kaya, et al., 2014; Kpodo, Agbenorhevi, Alba, Bingham, Oduro, Morris, & Kontogiorgos, 2017; Levigne, Ralet, & Thibault, 2002; Yapo & Koffi, 2008). The weight-average molecular weight (M_w) of hemicellulosic polysaccharides were 1059 $\times 10^3$ g mol^{-1} and 1167 $\times 10^3$ g mol^{-1} while [η] was 0.52-0.56 dL g^{-1} for HEMUK and HEMPOL, respectively (Table 4). Hemicelluloses were of particularly high M_w, similar to those extracted from pear pomace also using hydrogen peroxide (Rabetafika, et al., 2014). This has been attributed to the ability of hydrogen peroxide to favour the isolation of high molecular weight polysaccharides or to the strong tendency of hemicelluloses to form aggregates in aqueous solutions that could contribute to overestimation of M_w.

Table 4. Macromolecular characteristics of soluble DFs.

	APUK	CBPUK	APPOL	CBPPOL	HEMUK	HEMPOL
$M_w \times 10^3$, 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

4. Conclusions

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

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

4. Acknowledgements

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

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