Food Chemistry 201 (2016) 145–152

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant dietary fibre recovery from Brazilian *Pinot noir* grape pomace

Carolina Beres [a,b], Fernanda F. Simas-Tosin [c], Ignacio Cabezudo [d], Suely P. Freitas [e], Marcello Iacomini [c], Caroline Mellinger-Silva [b,*], Lourdes M.C. Cabral [b]

[a] Instituto de Química, Centro de Tecnologia, Bloco A, Cidade Universitária, Ilha do Fundão, CEP: 21949-900 Rio de Janeiro, RJ, Brazil
[b] Embrapa Agroindústria de Alimentos, Av. das Américas, 29501, 23.020-470, Rio de Janeiro, RJ, Brazil
[c] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná (UFPR), 81.531-980, Curitiba, PR, Brazil
[d] Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ-CONICET), Suipacha 531, Rosario S2002LRK, Argentina
[e] Escola de Química, Centro de Tecnologia, Bloco E, Sala 211, Cidade Universitária, Ilha do Fundão, CEP: 21949-900 Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form 4 January 2016
Accepted 10 January 2016
Available online 11 January 2016

Keywords:
Grape pomace
Antioxidant dietary fibre
Polysaccharide
Hot water extraction
Phenolic compounds
Antioxidant activity

ABSTRACT

Brazilian grape pomace was extracted in hot water, and a factorial experiment was used to evaluate polysaccharide recovery. The dependent variables were the temperature, particle size and solute:solvent ratio. Polysaccharide yields varied from 3% to 10%, and the highest sugar content was observed when extraction was carried out at 100 °C from finely sized particles (⩽249 μm) in a 1:12 solute:solvent ratio. The monosaccharide composition of extracts obtained from flours were, on average, Rha:Ara:Xyl:Man:Gal:Glc:GalA in a 3:32:2:13:11:20:19 M ratio, with varying Glc:GalA ratios. ^{13}C NMR and HSQC spectra confirmed the presence of pectic- and glucose-based polysaccharides in the extracts. Phenolic compounds were found after pomace extraction, and catechin, gallic acid and epicatechin were the principal compounds identified. The extracts also had ABTS radical scavenging capacity (from 8.00 to 46.60 mMol Trolox/100 g pomace). These findings indicate that these grape pomace flours are rich in antioxidant dietary fibre and have a potential use as food ingredients.

1. Introduction

Grapes are the crop with the second highest productivity worldwide. The most well-known grape species is *Vitis vinifera*, which comprises almost all grape varieties and is used in 70% of the total grape production (Breska et al., 2010). Brazil is the third highest fruit producer in the world, and grapes are the fourth most produced fruit in the country. Wine making generates agro-industrial residues comprising grape peels, seeds, stalks, and residual pulp, referred to as grape pomace (González-Centeno et al., 2014; Jara-Palacios, Hernanz, Escudero-Gilete, & Heredia, 2014). It is estimated that approximately 20% of the fruit will be converted into pomace after processing (Spanghero, Salem, & Robinson, 2009), accounting for approximately 290,000 tons of pomace per wine production season in Brazil (Sousa et al., 2014).

Grape pomace is mainly constituted of polysaccharides from plant cell walls, existing as hemicellulose, cellulose and pectin; lignin, protein, fat, and ash are also present. Cell wall polysaccharides are the main dietary fibre constituents and the fibre content obtained from fruit manufacturing usually has better functional quality than residues from grains because of a higher concentration of active compounds, such as phenolics (González-Centeno et al., 2014; Sant'anna, Christiano, Marczak, Tessaro, & Thys, 2014).

These characteristics indicate that the recovery of grape pomace compounds might be useful to enhance human nutrition and/or health. However, the pomace currently discharged is still used to feed animals or as a soil fertilizer (Ferrer et al., 2001). In addition, environmental issues exist regarding the disposal of grape pomace because it has a high organic load and large volumes are generated by the wine industry every growing season (Fontana, Antoniolli, & Bottini, 2013).

Valuable compounds such as phenolics and fibre obtained from different methods of grape pomace recovery are of interest for the development of food ingredients. Previous studies have shown that grape pomace extracts contain bioactive compounds with health potential (Sant'anna et al., 2014; Tseng & Zhao, 2013; Zhu, Du, Zheng, & Li, 2015), or can delay lipid oxidation during refrigeration of meat, extending its shelf-life (Chamorro et al., 2015), or have been used in studies to develop an antifungal preservative for juices

* Corresponding author.
E-mail address: caroline.mellinger@embrapa.br (C. Mellinger-Silva).

http://dx.doi.org/10.1016/j.foodchem.2016.01.039
0308-8146/

(Sagdic et al., 2011). Grape pomace biomass has also been used to produce biogas (Cáceres, Cáceres, Hein, Molina, & Pia, 2012).

Antioxidant dietary fibre (ADF) is defined as "a dietary fibre concentrate containing significant amounts of natural antioxidants associated with non-digestible compounds" (Bravo & Saura-Calixto, 1998; Quirós-Sauceda et al., 2014; Tseng & Zhao, 2013). This concept indicates that ingredients and products might have health benefits from fibre together with the powerful antioxidant activity from secondary metabolites such as the phenolics from grapes.

Therefore, the aim of this work was to extract antioxidant dietary fibre from a Brazilian red grape pomace from the *Pinot noir* grape variety in order to identify the polysaccharide and phenolic content, and to measure its *in vitro* ABTS radical-scavenging capacity to reflect the antioxidant activity.

2. Material and methods

2.1. Materials

Red grape pomace (5 kg) from a white wine process (2014 campaign) was supplied by the Aurora Winery, located in Bento Gonçalves, Rio Grande do Sul, Brazil. The pomace was autoclaved (15 min/121 °C), and oven-dried for 20 h at 60 °C. The dried sample was processed in a depulper (Bonina 0.25 df, Itametal). The seeds were trapped in the strainer and the pomace residue was ground into a powder (pomace flour, PF). The PF was further sieved to obtain fractions with different particle size distributions: coarse (CF; particle diameter >355 μm), medium (MF; particle diameter between 354–250 μm), and fine (FF; particle diameter <249 μm) powders. All pomace flours were conditioned in vacuum sealed plastic bags, at room temperature and protected from light.

All reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA), or Merck (Darmstadt, Germany).

2.2. Chemical composition

Moisture, ash, protein, fat, and total dietary fibre were analysed in duplicate using AOAC methods (1985, 1995, 2007) for grape pomace materials.

2.3. Hot water extraction – experimental design

Hot water extraction with distilled water was used to obtain soluble fibres. The extraction was conducted for 1 h, in a thermostatic bath with agitation (*Dubnoff* NT232 with a TCM 45 *Comtemp* thermostat, Piracicaba, Brazil). To evaluate the effects of the solute: water ratio, particle size and temperature, the experimental composite design was based on 11 trials, combining the three parameters (Table 1). The central points of the design were carried out in triplicate and the other trials are the average values from *duplicate* measurements. After vacuum filtration with Whatman Filter paper No. 1, the final aqueous extracts were lyophilized for further analyses. The dependent variable was the total sugar content in the extracts. The minimum and maximum values for each parameter were chosen based on previous studies on fibre extracted from fruit skin (Bicu & Mustata, 2013; Zhu et al., 2015). Data from the factorial design experiments were evaluated with *Statistica* software (StatSoft, Tulsa, USA).

Table 1
Experimental design with the observed responses for total sugar yield from *Pinot noir* grape pomace.

Trials	Temperature (°C)	Particle size (μm)	Solute:water ratio	Total sugar (%)
1	(−1) 80	(−1) fine	(−1) 1:8	3.15
2	(−1) 80	(+1) coarse	(−1) 1:8	4.27
3	(+1) 100	(−1) fine	(−1) 1:8	2.26
4	(+1) 100	(+1) coarse	(−1) 1:8	4.41
5	(−1) 80	(−1) fine	(+1) 1:12	5.30
6	(−1) 80	(+1) coarse	(+1) 1:12	4.30
7	(+1) 100	(−1) fine	(+1) 1:12	10.93
8	(+1) 100	(+1) coarse	(+1) 1:12	4.86
9	(0) 90	(0) medium	(0) 1:10	4.43
10	(0) 90	(0) medium	(0) 1:10	5.14
11	(0) 90	(0) medium	(0) 1:10	5.48

Code "0" represents the center point of the parameter range, and "±1" were the factorial points.

2.4. Spectrophotometric methods

2.4.1. Total sugar determination by the phenol–sulphuric acid method

All lyophilized samples obtained from hot water extractions were analysed by phenol–sulphuric acid method, as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). D-Glucose was used as a standard, and the analyses were performed at 490 nm.

2.4.2. Uronic acid quantification

The content of uronic acid in lyophilized samples was determined by using the improved *m*-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991). D-Galacturonic acid was used as a standard. Thin layer chromatography (TLC) was used to determine the identity of the uronic acid from samples (data not shown) (Sassaki, Souza, Cirpiani, & Iacomini, 2008).

2.4.3. Antioxidant activity

In vitro antioxidant activity of pomace powder and lyophilized extract was evaluated by the Trolox equivalent antioxidant capacity assay (TEAC). The extraction of compounds was based on the method described by Rufino et al. (2007) and quantification was described by Re et al. (1999). The results are shown as mMol Trolox/100 g of pomace powder or lyophilized extract.

2.4.4. Total phenolics

The total phenolic content of all pomace flours (coarse, medium and fine) and lyophilized extracts was analysed following the method proposed by Singleton and Rossi (1965) and modified by Georgé, Brat, Alter, and Amiot (2005). The results were presented as mg gallic acid/100 g of pomace powder or lyophilized extract.

2.5. Monosaccharide analysis

Samples from lyophilized extracts (2 mg) were hydrolysed with 1 mL of 2 M trifluoroacetic acid (TFA) at 100 °C for 8 h, reduced with $NaBH_4$ (Wolfrom & Thompson, 1963a), and acetylated with acetic anhydride–pyridine (1:1, v/v) (Wolfrom & Thompson, 1963b). The resulting alditol acetate mixtures were analysed with a GC–MS (Varian Saturn 2000R-3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer, Santa Clara, USA), using a DB-225 column (30 m × 0.25 mm, Agilent, Folsom, USA), programmed from 50 to 220 °C at 40 °C/min, using helium as gas carrier. Components were identified by their typical retention times and electron impact spectra (Jansson, Kenne, Liedgren, & Lönngren, 1976). Uronic acid contents were determined as previously described.

2.6. Spectroscopic analysis

^{13}C NMR and HSQC spectra were obtained on a 400 MHz Bruker model DRX Avance spectrometer incorporating Fourier transform (Bruker, Rheinstetten, Germany). Samples were dissolved in D_2O

or NaOD (0.05 M) and examined at 50 °C. Chemical shifts (δ) are expressed in ppm, standardized on the resonance of acetone at δ 30.2 for ^{13}C and 2.224 for ^{1}H.

2.7. High performance liquid chromatography (HPLC)

HPLC analyses of sieved pomace flour extracts (coarse, medium and fine) were performed in an Agilent 1100 series HPLC system equipped with a diode array detector (Agilent, Santa Clara, USA). Prior to analysis, the samples were solubilized in ultrapure water and filtered through a 0.45 μm pore size nylon membrane, and a volume of 50 μL of sample was injected without further preparation. Separation was performed on a Nova-Pak C18 column (250 × 4.6 mm) (Water, Milford, USA). Mobile phase A consisted of 94.9% water, 5% acetonitrile and 0.1% trifluoroacetic acid, and mobile phase B consisted of 99.9% acetonitrile, 0.1% trifluoroacetic acid. The gradient profile was as follows: 0% B (min 0); 0% B (min 10); 32% B (min 45); 90% B (min 50), 90% B (min 60), and 5% B (min 65), with a post-time of 5 min. The flow rate was 0.8 mL min^{-1}. Samples were run in duplicate, and excellent reproducibility was observed. Diode array detection was performed at 280, 320, 365 and 520 nm. Compounds were identified by comparison to the retention times of pure standards: gallic acid (5.779 min), catechin (21.086 min), caffeic acid (23.377 min), epicatechin (26.242 min) and *p*-coumaric acid (28.515 min).

2.8. Statistical analysis

Data obtained from the central composite design experiments (Table 1), were evaluated by response surface methodology using the Statistica 8.0 software. *Fick's* 2nd law was used to fit kinetic data by a non-linear regression algorithm at a significance level of 90% (StatSoft, Tulsa, USA).

3. Results and discussion

3.1. Chemical composition of Brazilian Pinot noir grape pomace

Red *Pinot noir* grape pomace, obtained from white wine processing, was milled to obtain a flour (PF) and further analysed for total protein (13.80 g/100 g), fat (4.21 g/100 g), carbohydrates (19.68 g/100 g), dietary fibre (DF) (51.38 g/100 g), and ash (5.55 g/100 g).

The protein content was slightly higher than that found for *Merlot* (11.26% dry matter (DM)), *Cabernet Sauvignon* (12.34% DM) and *Pinot noir* (12.13% DM) grape pomaces from the United States (Deng, Penner, & Zhao, 2011). However, a high protein content (18.89 g/100 g pomace) was also reported by Basalan, Gungor, Owens, and Yalcinkaya (2011) in red grape stalks, skin and pulp pomace. Fat content in grape pomace consists mainly of the oil from the seeds. In this study, because the seeds were separated from the pomace using a depulper, a low fat composition was found, in accordance with previously reported data (Basalan et al., 2011; Deng et al., 2011; Zhu et al., 2015). Carbohydrates and dietary fibre content had higher values when compared to other primary compounds, as was expected from a material rich in vegetal cell walls (Bravo & Saura-Calixto, 1998; Valiente, Arrigoni, Esteban, & Amado, 1995).

PF consists of more than 50% fibre, which means it already has a great potential for use as a fibre supplementation ingredient because a 10 g portion would afford more than 5 g of total dietary fibre in a meal. Considering the WHO/FAO report (WHO, 2003), the dietary fibre intake of an adult should be of 25 g per day, meaning that a 10 g portion of PF contributes 20% of the recommended daily intake.

3.2. Hot water extraction from sieved flours: surface response methodology

After sieving the PF, three fractions with different particle size distributions were obtained: 71% coarse flour (CF) (particle diameter >355 μm), 14% medium flour (MF) (particle diameter between 354 and 250 μm) and 15% fine flour (FF) (particle diameter <249 μm) (Fig. S1).

The total sugar content in the extract varied from 2.26% to 10.9% (Table 1). These data, collected from 9 trials that had central composite designs, demonstrated that temperature is the parameter that had the highest effect on the yield of polysaccharide extraction. The negative effect of particle size indicates that the smaller the particle, the higher the contact surface between solute and solvent, which might improve the extraction (Fig. 1A). Modelling and analysing the response surface for polysaccharide recovery (Fig. 1B) allowed us to conclude that the most adequate operational conditions was achieved at 100 °C using the smallest particle size (FF) and a solute:solvent ratio 1:12. These conditions were used in Trial 7, with a yield of 10.9% for total sugar (Table 1).

For the response surface analyses, the solute:solvent ratio was fixed at the highest value (1:12), based on a previous study that indicated the importance of a high solvent ratio for sample solubilisation and yield (Zhu & Liu, 2013).

Yields obtained from these trials are in accordance to those from the literature, where high polysaccharide recovery values from vegetal pomaces are approximately 10% (Zhu et al., 2015).

3.3. Polysaccharides identification from hot water extracts

The three sieved flours (CF, MF, FF) were further investigated to identify the main polysaccharides present. Table 2 shows the monosaccharide composition for the trials, with trial 9 being the central composite (90 °C, medium particle size, solute:solvent ratio of 1:10). Fractions, on average, were composed of Rha:Ara:Xyl:Man:Gal:Glc:GalA in a 3:32:2:13:11:20:19 M ratio, respectively.

The main found differences among the trials concerned the Glc and GalA ratios. Flours with medium and large particle sizes had a higher concentration of GalA; over 20% in trials 2, 4, 6, 8, and 9, which might indicate the presence of pectic polysaccharides in these fractions. In contrast, small size particles had a low GalA ratio and higher Glc ratios, the latter being over 20% in trials 1, 3, 5, and 7. These results might indicate that the smaller particle size trials provided a higher water-cell wall polysaccharides interaction and that other hemicelluloses in addition to pectins were extracted.

Trial 7 had the highest glucose ratio (37 mol%) and the highest yield when considering the total sugar (10.9%). For this reason, trial 7 and the central composite trials (9–11), which had the highest GalA amount (31%), were chosen for further spectroscopic analysis.

NMR data for trials 7 and central points (9–11) are shown in Fig. 2. A ^{13}C NMR in trial 9 (Fig. 2A) showed anomeric signals at δ 107.60, 102.74, and 100.09 referred as α-Ara*f*, β-Gal*p*, and α-Gal*p*A, respectively, which probably indicate pectic polysaccharides. The signal at δ 177.11 was assigned to the carboxyl groups of uronic acid residues and the signal at δ 16.62 was assigned to $-CH_3$ of α-Rha*p* units. The well-defined signal at δ 53.90 might be attributed either to the –OCH3 groups of methyl-esterified Gal*p*A units from pectic polysaccharides (Cantu-Jungles et al., 2014; Nascimento et al., 2015), or to the *O*-methyl ester bounds to phenolic compounds, as previously reported (Lu & Foo, 1999; Quirós-Sauceda et al., 2014). Non-related polysaccharide signals were also observed at δ 143.67, 119.15, 116.12, 114.90; these signals might indicate the presence of phenolic compounds, as do signals in high fields of approximately 24–44 ppm (Lu & Foo, 1999).

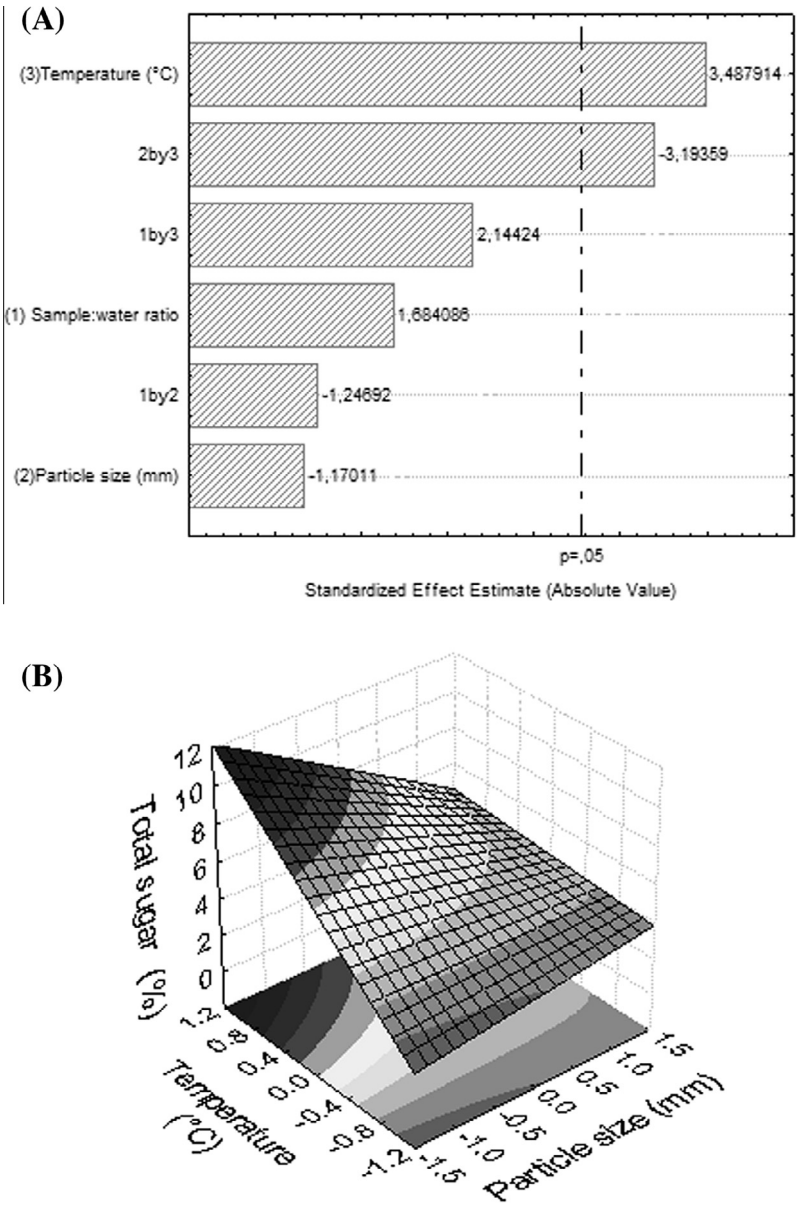

Fig. 1. Statistical analysis of the experimental design applied for the hot water extraction of *Pinot noir* grape pomace. (A) Paretós plot showing the significance of the temperature, particle size and solute–solvent ratio on the total sugar yield. (B) Response surface and contour plots showing the effects of temperature and particle size on the total sugar yield.

HSQC spectra of trials 7 (Fig. 2B) and 9–11 (Fig. 2C) showed very similar profiles, with $^{1}H/^{13}C$ correlations for the anomeric regions at δ 4.96/107.9, 4.98/108.2, 4.95/108.0, 5.04/107.8 attributed to α-Ara*f* units; at δ 5.34/100.5, and 5.21/100.5 for α-L-Rha*p*. Trials 9 to 11 showed two correlations at δ 4.91/99.5 and 4.96/99.5assigned to α-D-Gal*p*A, while trial 7 showed only one at δ 4.80 / 99.5 for α-D-Gal*p*A. The other correlations at δ 4.96/99.5, identified in both spectra, might be from α-Glc*p* (1→4)-linked residues. The main differences from the spectra were the correlations at δ 4.91/100.7 found only in trials 9 to 11, and at δ 4.86/97.5, found only in trial 7 both representing chemical environments of alpha units (Mendes, Prozil, Evtuguin, & Lopes, 2013).

Altogether, these results confirm the presence of pectic polysaccharides in extracts from grape pomace, from either larger or smaller particle sizes, with the latter also being enriched with glucose-based hemicelluloses, which promote a higher yield of total sugar.

3.4. Total phenolic content and structural identification

Total phenolics were investigated in sieved flours (CF, MF, and FF) as in all lyophilized extracts (trials) (Table 3). In pomace flours, phenolic content varied from 21.63 ± 0.14 to 42.38 ± 0.36 mg gallic acid/100 g pomace, while in lyophilized aqueous extracts, the content varied from 9.76 ± 0.25 to 213.08 ± 7.15 mg gallic acid/100 g lyophilized extract.

Phenolic content from pomace flour (FF) of a smaller particle size was approximately twofold higher (42.38 ± 0.36 mg gallic acid/100 g pomace) compared with that from grape flours of a coarse (21.63 ± 0.14 mg gallic acid/100 g pomace) and medium particle size (26.71 ± 0.28 mg gallic acid/100 g pomace). This difference can be related to a higher surface contact between water and the cell wall, which improved the extraction yield.

With the exception of trials 1, 3, and 9, all dried extracts from sieved flours showed an increase in total phenolic content (Table 3).

Table 2
Monosaccharide composition of lyophilized extracts obtained from *Pinot noir* grape pomace using hot water extraction.

Trials	Monosaccharide composition (mol%)[a]						
	Rha[b]	Ara[c]	Xyl[d]	Man[e]	Gal[f]	Glc[g]	GalA[h]
Trial 1	3.0	37.2	2.2	14.9	11.0	21.7	10
Trial 2	3.0	38.4	1.4	14.1	6.7	15.4	21
Trial 3	2.8	35.1	2.1	14.9	10.7	21.4	13
Trial 4	3.0	34.4	1.3	12.9	7.5	13.9	27
Trial 5	4.5	33.0	3.3	13.8	9.7	29.7	6
Trial 6	3.2	23.5	1.7	10.3	28.3	6.0	27
Trial 7	2.0	20.4	3.0	11.8	8.8	37.0	17
Trial 8	3.6	36.4	1.4	14.0	6.6	18.0	20
Trial 9	2.7	30.7	1.2	11.3	7.8	15.3	31

[a] Alditol acetates analysed by gas chromatography, coupled to ESI-MS identification.
[b] Rhamnose.
[c] Arabinose.
[d] Xylose.
[e] Mannose.
[f] Galactose.
[g] Glucose.
[h] Uronic acid determined spectrophotometrically using the *m*-hydroxybiphenyl method, and Galacturonic acid, confirmed by thin layer chromatography (TLC).

Table 3
Total phenolic content and ABTS radical scavenging capacity in coarse, medium and fine flours and hot water extracts from *Pinot noir* grape pomace.

Samples	TP[1]	ABTS[2]
Pomace powder		
Corse	21.63 ± 0.14[a]	23.03 ± 0.93[a]
Medium	26.71 ± 0.28[b]	37.39 ± 2.66[b]
Fine	42.38 ± 0.36[c]	43.80 ± 4.90[c]
Lyophilized aqueous extract		
Trial 1	32.87 ± 0.43[c]	44.02 ± 4.67[d]
Trial 2	29.14 ± 0.08[d]	44.53 ± 5.41[e]
Trial 3	9.76 ± 0.25[b]	46.60 ± 2.03[f]
Trial 4	180.23 ± 16.87[d]	18.67 ± 3.70[g]
Trial 5	213.08 ± 7.15[e]	32.80 ± 2.27[h]
Trial 6	127.98 ± 0.00[f]	22.40 ± 2.00[i]
Trial 7	114.33 ± 1.65[f]	8.00 ± 0.62[j]
Trial 8	177.14 ± 4.36[g]	20.27 ± 1.48[k]
Trial 9	14.06 ± 0.08[g]	28.00 ± 0.96[l]

Different letter in the same column indicates a significant difference using Tukey's test ($\rho = 0.05$).
[1] Total phenolics (mg gallic acid/100 g pomace or lyophilized extract).
[2] ABTS radical scavenging capacity (mMol Trolox/100 g pomace or lyophilized extract).

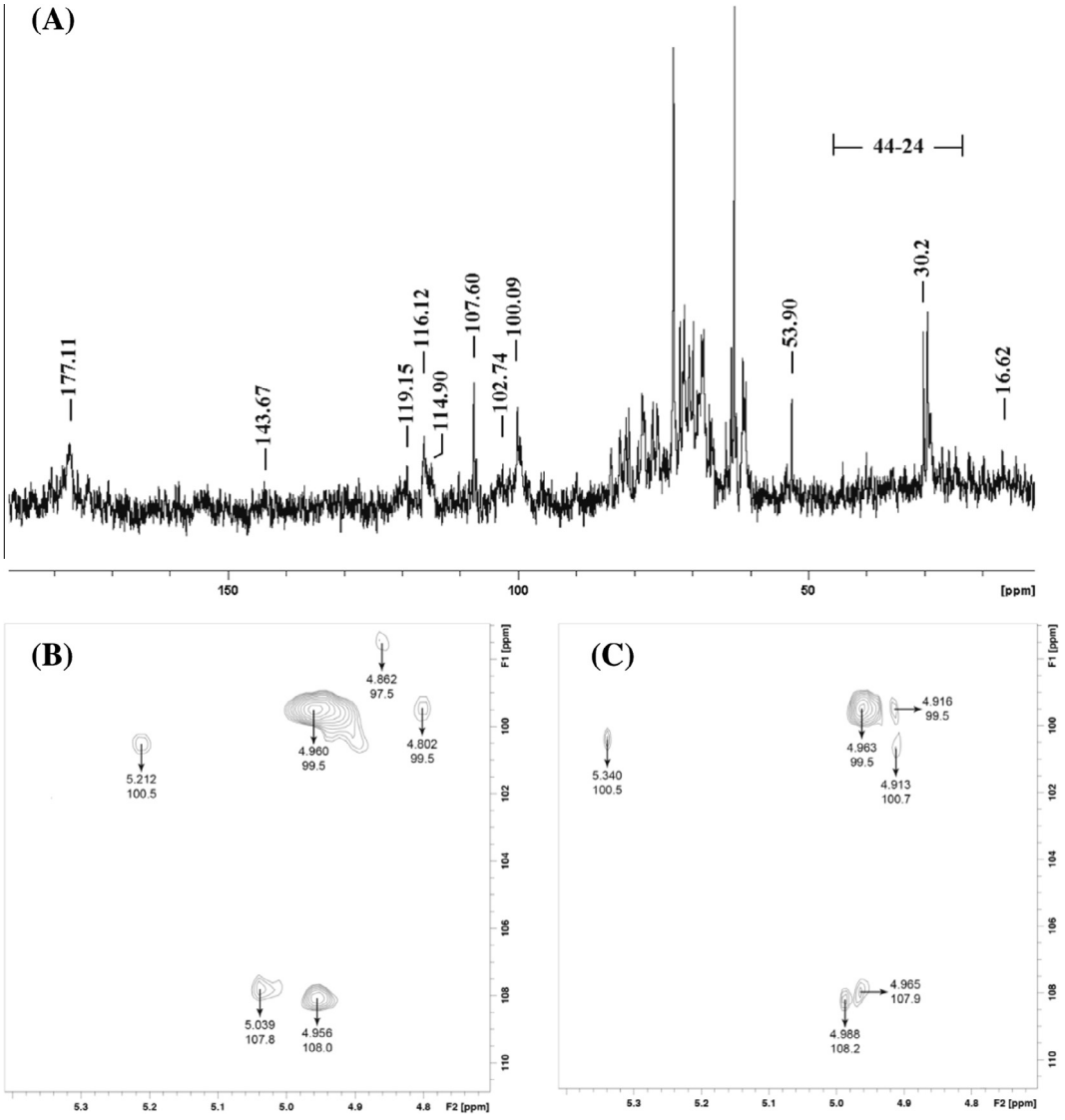

Fig. 2. Spectroscopic data for polysaccharide identification of *Pinot noir* grape pomace extracts. (A) ^{13}C NMR of trial 9. (B) HSQC (anomeric region) of trial 7. (C) HSQC (anomeric region) of trial 9. Chemical shifts are expressed in δ ppm.

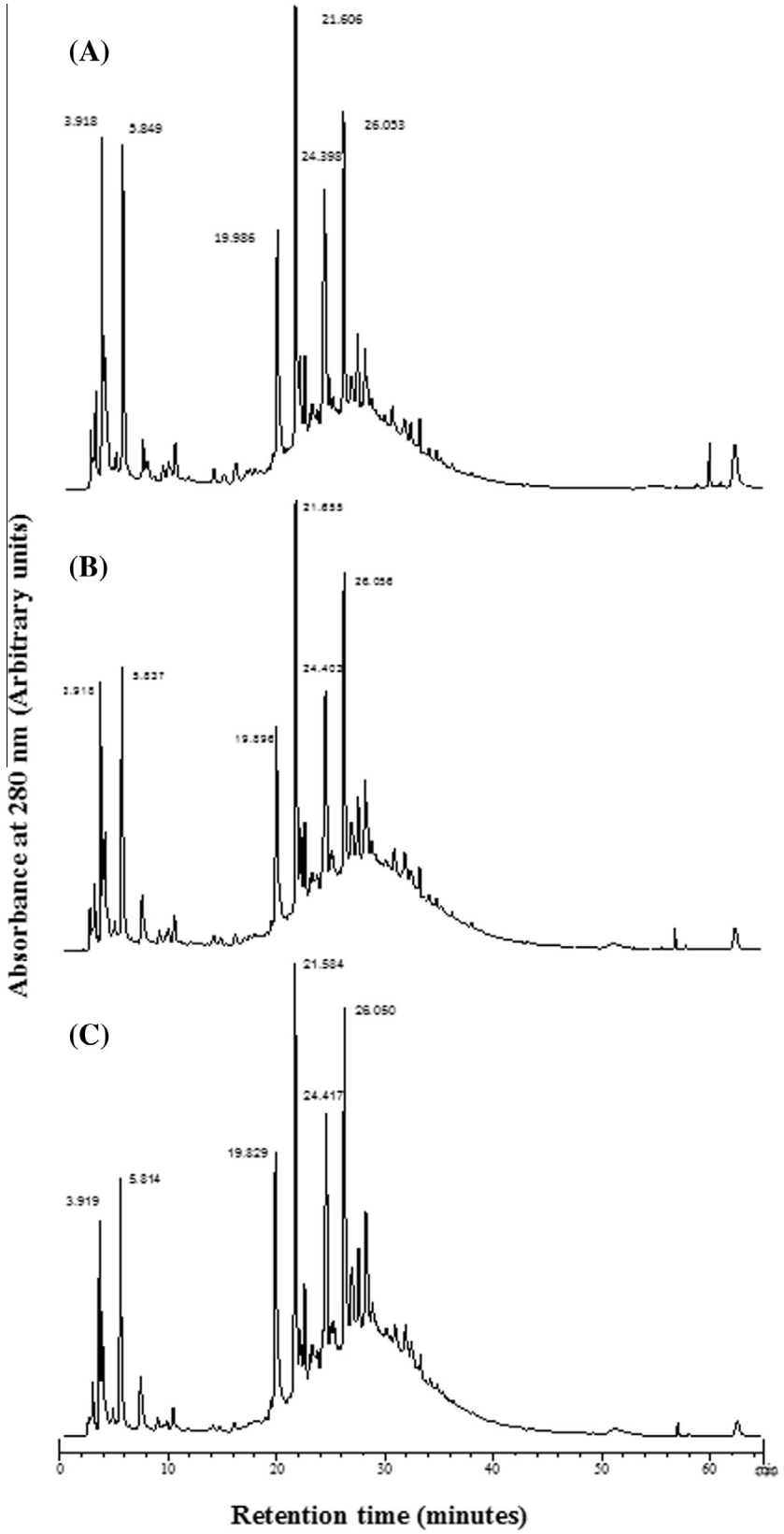

Fig. 3. RP HPLC chromatograms. (A) coarse, (B) medium and (C) fine flours from *Pinot noir* grape pomace.

In trials where the extractions were conducted with coarse particle size flours (2, 4, 6 and 8) an increase in the phenolic content was observed. This was especially observed in trials 4 (180.23 ± 16.87 mg gallic acid/100 g pomace) and 8 (177.14 ± 4.36 mg gallic acid/100 g pomace), both extracted at the highest temperature tested, showing a phenolic content over

eightfold higher. These results indicate that higher particle size flours and higher temperatures might increase the total phenolic content in the extract. This could be due to a higher polysaccharide content, and the phenolic structures could be advantageously protected from temperature degradation because they exist in a fibre-phenolic interaction core formed by hydrogen bonds, hydrophobic interactions or even covalent methyl-esterification (Chamorro et al., 2015).

When the extractions were performed using finely sized particle flours, in trials 5 (80 °C, solute:solvent ratio of 1:12) and 7 (100 °C, solute:solvent ratio of 1:12), a significant increase in total phenolics was observed: approximately five- and threefold, respectively, compared with that using fine pomace flour. These data indicate that a combination of a lower temperature (80 °C), a higher solvent ratio (1:12) and a smaller particle size (<249 μm) results in a higher recovery (213.08 ± 7.15 mg gallic acid/100 g pomace) of total phenolic compounds from lyophilized extracts produced from grape pomace.

Furthermore, coarse, medium, and fine flours were analysed by HPLC to compare and identify the main phenolic structures in sieved flours (Fig. 3). They had very similar phenolic profiles, indicating that phenolic compounds are evenly distributed in pomace flour. Therefore, particle size was not relevant for the selective extraction of specific compounds in this study. Three polyphenol structures were identified in samples as gallic acid (CF:5.849 min; MF: 5.837 min; FF: 5.814 min), (+)-catechin (CF: 21.606; MF: 21.655 min; FF: 21.584 min) and (−)-epicatechin (CF: 26.053 min; MF: 26.056 min; FF: 26.050 min) by comparing the sample retention times to those of pure standards. (+)-Catechin and (−)-epicatechin were the most abundant polyphenols in the pomace flours, as in previous studies (Jara-Palacios, Hernanz, González-Manzano et al., 2014; Sagdic et al., 2011). Significant peaks from 19.829 to 20.098 min and from 24.398 to and 24.417 min indicate compounds not related to the standards used in this work.

3.5. ABTS radical scavenging capacity

In vitro antioxidant activity was measured in coarse, medium and fine sieved pomace flours and in all lyophilized extracts. The results for sieved pomace flours varied from 23.03 ± 0.93 to 43.80 ± 4.90 mMol Trolox/100 g pomace, in accordance with an increase in total phenolic content. We found an approximately 2-fold increase in the antioxidant activity from coarse to medium and fine flours.

Lyophilized extracts did not show a significant increase in *in vitro* antioxidant activity among treatments when compared to crude sieved flours, although all extracts had values in accordance with data reported in the literature (Jara-Palacios, Hernanz, Escudero-Gilete et al., 2014). Varying responses, from 8.00 ± 0.62 to 46.60 ± 2.03 mMol Trolox/100 g lyophilized extract were observed. According to the concept established to consider a material as "antioxidant dietary fibre" (Quirós-Sauceda et al., 2014), these flours and extracts had the characteristics of a product with intrinsic antioxidant activity and could be promising food ingredients.

4. Conclusion

The present study demonstrated a feasible method for the recovery of antioxidant dietary fibre from *Pinot noir* grape pomace. Consequently, these pomace flours could be used to develop a fibre-rich ingredient for foodstuffs that has the benefit of fibre added of antioxidant properties. Further studies are being carried out to ensure the safety and effectiveness of such products for human use.

Acknowledgements

The authors would like to thank the Brazilian agencies *Fundação Carlos Chaga Filho de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ), Coordenação *de* Aperfeiçoamento *de Pessoal de* Nível *Superior* (CAPES), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and *Empresa Brasileira de Pesquisa Agropecuária* (EMBRAPA) for their financial support. The authors also thank Aurora Winery Ltda for supplying the pomace.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.01.039.

References

AOAC (1995). *AOAC official method 960.52. Micro-Kjeldahl method*. Washington DC: Association of Official Analytical Chemists.
AOAC (2007). *AOAC official method 994.13. Total dietary fiber*. Washington DC: Association of Official Analytical Chemists.
AOAC (1985). *Change in methods: Total dietary fiber in foods*. Washington DC: Association of Official Analytical Chemists.
Basalan, M., Gungor, T., Owens, F. N., & Yalcinkaya, I. (2011). Nutrient content and *in vitro* digestibility of Turkish grape pomaces. *Animal Feed Science and Technology, 169*, 194–198.
Bicu, I., & Mustata, F. (2013). Optimization of isolation of cellulose from orange peel using sodium hydroxide and chelating agents. *Carbohydrate Polymers, 98*, 341–348.
Bravo, L., & Saura-Calixto, F. (1998). Characterization of dietary fiber and the *in vitro* indigestible fraction of grape pomace. *American Journal of Enology and Viticulture, 49*, 136–141.
Breska, A. P., III, Takeoka, G. R., Hidalgo, M. B., Vilches, A., Vasse, J., & Ramming, D. W. (2010). Antioxidant activity and phenolic content of 16 raisin grape (*Vitis vinifera* L.) cultivars and selections. *Food Chemistry, 121*, 740–745.
Cáceres, C. X., Cáceres, R. E., Hein, D., Molina, M. G., & Pia, J. M. (2012). Biogas production from grape pomace: Thermodynamic modelo f the process and dynamic model of the power generation system. *International Journal of Hydrogen Energy, 37*(13), 10111–10117.
Chamorro, S., Viveros, A., Rebolé, A., Rica, B. D., Arija, I., & Brenes, A. (2015). Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. *Food Research International, 73*, 197–203.
Cantu-Jungles, T. M., Maria-Ferreira, D., da Silva, L. M., Baggio, C. H., Werner, M. F., Iacomini, M., ... Cordeiro, L. M. (2014). Polysaccharides from prunes: Gastroprotective activity and structural elucidation of bioactive pectins. *Food Chemistry, 146*, 492–499.
Deng, Q., Penner, M. H., & Zhao, Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International, 44*, 2712–2720.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry, 28*, 350–356.
Ferrer, J., Páez, G., Mármol, Z., Ramones, E., Chandler, C., Marín, M., & Ferrer, A. (2001). Agronomic use of biotechnologically processed grape wastes. *Bioresource Technology, 76*, 39–44.
Filisetti-Cozzi, T. M. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugar. *Analytical Biochemistry, 97*, 157–162.
Fontana, A. R., Antoniolli, A., & Bottini, R. (2013). Grape pomace as a sustainable source of bioactive compounds: Extraction, characterization, and biotechnological application of phenolics. *Journal of Agricultural and Food Chemistry, 61*, 8987–9003.
Georgé, S. P., Brat, P., Alter, P., & Amiot, M. J. (2005). Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and Food Chemistry, 29*, 1370–1373.
González-Centeno, M. R., Knoerzer, K., Sabarez, H., Simal, S., Rosselló, C., & Femenia, A. (2014). Effect of acoustic frequency and power density on the aqueous ultrasonic-assisted extraction of grape pomace (*Vitis vinifera* L.) – A response surface approach. *Ultrasonics Sonochemistry, 21*(6), 2176–2184.
Jara-Palacios, M. J., Hernanz, D., Escudero-Gilete, M. L., & Heredia, F. J. (2014). Antioxidant potential of white grape pomace: Phenolic composition and antioxidant capacity measured by spectrophotometric and cyclic voltammetry methods. *Food Research International, 66*, 150–157.
Jara-Palacios, M. J., Hernanz, D., González-Manzano, S., Santos-Buelga, C., Escudero-Gilete, M. L., & Heredia, F. J. (2014). Detailed phenolic composition of white

grape by-products by RRLC/MS and measurement of the antioxidant activity. *Talanta, 125*, 51–57.
Jansson, P. E., Kenne, L., Liedgren, H., & Lönngren, J. (1976). A practical guide to methylation analysis of carbohydrate. *Chemical Communications, 8*, 1–70.
Lu, Y., & Foo, L. Y. (1999). The polyphenol constituents of grape pomace. *Food Chemistry, 65*, 1–8.
Mendes, J. A. S., Prozil, S. O., Evtuguin, D. V., & Lopes, L. P. C. (2013). Towards comprehensive utilization of winemaking residues: Characterization of grape skins from red grape pomace variety *Touriga Nacional*. *Industrial Crops and Products, 43*, 25–32.
Nascimento, G. E., Corso, C. R., Werner, M. F. P., Baggio, C. H., Iacomini, M., & Cordeiro, L. M. C. (2015). Structure of an arabinogalactan from the edible tropical fruit tamarillo (*Solanum betaceum*) and its antinociceptive activity. *Carbohydrate Polymers, 116*, 300–306.
Quirós-Sauceda, A. E., Palafox-Carlos, H., Sáyago-Ayerdi, S. G., Ayala-Zavala, J. F., Bello-Perez, L. A., Álvarez-Parrilla, E., ... González-Aquilar, G. A. (2014). Dietary fiber and phenolic compounds as functional ingredients: INTERACTION and possible effect after ingestion. *Food and Function, 5*, 1063–1072.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.
Rufino, M. S. M., Alves, R. E., Brita, E. S., Morais, S. M., Sampaio, C. G., Pérez-Jiménez, J., & Saura-Calixto, F. D. (2007). *Metodologia Científica: Determinação da atividade antioxidantes total em frutas pela captura do radical livre ABTS*$^{\cdot+}$ Comunicado Técnico 128. Ministério da Agricultura, Pecuária e Abastecimento. ISSN 1679-6535.
Sagdic, O., Ozturk, I., Ozkan, G., Yetim, H., Ekici, L., & Yilmaz, M. T. (2011). RP-HPLC-DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: Evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices. *Food Chemistry, 126*, 1749–1758.
Sant'anna, V., Christiano, F. D. P., Marczak, L. D. F., Tessaro, I. C., & Thys, R. C. S. (2014). The effect of the incorporation of grape marc powder in fettuccini pasta properties. *LWT – Food Science and Technology*, 1–5.
Sassaki, G. L., Souza, L. M., Cirpiani, T. R., & Iacomini, M. (2008). TLC of carbohydrates. In M. Waksmundzka-Hajnos, J. Sherma, & T. Kowalska (Eds.), *Thin layer chromatography in phytochemistry* (pp. 281–304). Boca Raton: CRC Press.
Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic-acid reagents. *American Journal of Enology and Viticulture, 126*, 144–168.
Sousa, E. C., Uchôa-Thomaz, A. M. A., Carioca, J. O. B., Morais, S. M., Lima, A., Martins, C. G., Alexandrino, C. D., Ferreira, P. A. T., Rodrigues, A. L. M., Rodrigues, S. P., Silva, J. N., & Rodrigues, L. L. (2014). Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. *Food Science and Technology, 34*(1), 135–142.
Spanghero, M., Salem, A. Z. M., & Robinson, P. H. (2009). Chemical composition, including secondary metabolites and rumen fermentability of seeds and pulp of California (USA) and Italian grape pomaces. *Animal Feed Science and Technology, 152*, 243–255.
Tseng, A., & Zhao, Y. (2013). Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. *Food Chemistry, 138*, 356–365.
Valiente, C., Arrigoni, E., Esteban, R. M., & Amado, R. (1995). Grape pomace as a potential food fiber. *Journal of Food Science, 60*, 818–820.
WHO (2003). Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO expert consultation. WHO technical report series, 916.
Wolfrom, M. L., & Thompson, A. (1963a). Reduction with sodium borohydride. *Methods Carbohydrates, Chemistry, 2*, 65–67.
Wolfrom, M. L., & Thompson, A. (1963b). Acetylation. *Methods Carbohydrates. Chemistry, 2*, 211–215.
Zhu, C., & Liu, X. (2013). Optimization of extraction process of crude polysaccharides from Pomegranate peel by response surface methodology. *Carbohydrate Polymers, 92*, 1197–1202.
Zhu, F., Du, B., Zheng, L., & Li, J. (2015). Advance on the bioactivity and potential applications of dietary fibre from grape pomace. *Food Chemistry, 186*, 207–212.