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Abstract: Brazilian grape pomace was extracted in hot water using a factorial design to evaluate polysaccharide recovery. Dependent variables were 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 fine particle sizes (≤249 μm), in a 1:12 solute:solvent ratio. Monosaccharide compositions of the flours afforded, in average, Rha,Ara,Xyl,Man,Gal,Glc,GalA in a 3:32:2:13:11:20:19 molar ratio, with varied Glc:GalA ratios. 13C NMR and HSQC spectra confirmed the presence of pectic and glucose-based polysaccharides in the extracts. Phenolic compounds were found after pomace extractions, and cathechin, gallic acid and epicatechin were the main identified compounds. Extracts also presented ABTS radical scavenging capacity (from 8.00 to 46.60 mMol Trolox/100g pomace), which means that these grape pomace flours are rich in antioxidant dietary fiber and have a potential use as food ingredients.

Highlights

- Dietary fibers were recovered from grape pomace through hot water extractions
- Polysaccharides were identified as being pectic and glucose-based polymers
- Phenolics were mainly identified as cathechin, gallic acid and epicatechin
- Extracts presented ABTS radical scavenging capacity, suggesting antioxidant capacity
- Extracts rich in antioxidant dietary fiber have a potential use as food ingredients

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

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Brazilian grape pomace was extracted in hot water using a factorial design to evaluate polysaccharide recovery. Dependent variables were 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 fine particle sizes (≤ 249 μm), in a 1:12 solute:solvent ratio. Monosaccharide compositions of the flours afforded, in average, Rha,Ara,Xyl,Man,Gal,Glc,GalA in a 3:32:2:13:11:20:19 molar ratio, with varied 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 extractions, and cathechin, gallic acid and epicatechin were the main identified compounds. Extracts also presented ABTS radical scavenging capacity (from 8.00 to 46.60 mMol Trolox/100g pomace), which means that these grape pomace flours are rich in antioxidant dietary fiber and have a potential use as food ingredients.

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1. Introduction

Grape culture is the second highest productive crop worldwide. Among the grape species, the most well know is *Vitis vinifera*, which comprises almost all grape varieties, and is used in 70% of the total grape production (Breska III, Takeoka, Hidalgo, Vilches, Vasse & Ramming, 2010). Brazil is the third fruit producer in the

world, being grape the fourth largest fruit culture in the country. Wine making process generates an agro industrial residue consisting of grape peel, seeds, stalks, and residual pulp, referred as grape pomace (González-Centeno, Knoerzer, Sabarez, Simal, Rosselló & Femeria, 2014; Jara-Palacios, Hernanz, Escudero-Gilete & Heredia, 2014a). It is estimated that about 20% of the fruit will be converted into pomace after processing (Spanghero, Salem & Robinson, 2009), which generates about 290,000 tons of pomace per season of wine production in Brazil (Sousa *et al*., 2014).

Grape pomace is mainly constituted by polysaccharides from plant cell walls, distributed as hemicellulose, cellulose and pectin, besides the presence of lignin, protein, fat, and ashes. Cell wall polysaccharides are the main dietary fiber constituents and usually the fiber content obtained from fruit manufacturing has better functional quality when compared to residues from grains, due to a higher concentration of active compounds, such as phenolics (González-Centeno, Knoerzer, Sabarez, Simal, Rosselló & Femeria, 2014; Sant'Anna, Christiano, Marczak, Tessano & Thys, 2014).

In this way, the recovery of grape pomace compounds may enhance human nutrition and/or health matters. However pomace is still currently discharged, used to feed animals or as a soil fertilizer (Ferrer *et al*., 2001). In addition to that, there are environmental issues concerned about the disposal of grape pomace once it has a high organic load and large volumes are generated by wine industries every growing season (Fontana, Antoniolli & Bottini, 2013).

From different alternatives of grape pomace recovery, the obtainment of aggregated-value compounds, such as phenolics and fiber is of interest for the development of food ingredients. Previous studies have already shown grape pomace extracts containing bioactive compounds with health claim (Tseng & Zhao, 2013; Sant'Anna, Christiano, Marczak, Tessano & Thys, 2014; Zhu, Du, Zheng & Li, 2015),

or able to delay lipid oxidation during refrigeration of meat, extending its shelf-life (Chamorro, Viveros, Rebolé, Rica, Arija & Brenes, 2014), or even studies to develop an antifungal preservative for juices (Sagdic, Ozturk, Ozkan, Yetim, Ekici & Yilmaz, 2011) and grape pomace biomass to produce biogas (Cáceres, Cáceres, Hein, Molina & Pia, 2012).

The concept of antioxidant dietary fiber (ADF), is defined as "a dietary fiber concentrate, containing significant amounts of natural antioxidants associated with non-digestible compounds" (Bravo & Saura-Calixto, 1998; Tseng e Zhao, 2013; Quirós-Sauceda *et al*., 2014). From this concept, ingredients and products may carry the health benefits from fiber together with the powerful antioxidant from secondary metabolites, such as the phenolics from grape.

Therefore, the aim of this work was to extract antioxidant dietary fiber from a Brazilian red grape pomace of *Pinot noir* variety; identifying the polysaccharide and phenolic contents with the measure of *in vitro* ABTS radical scavenging capacity, in terms to suggest an antioxidant activity.

2. Material and Methods

2.1. Materials

Red grape pomace (5 kg) from white wine process (2014 campaign) was supplied by Aurora Winery, located in Bento Gonçalves (Rio Grande do Sul, Brazil). The pomace was autoclaved (15 minutes/121 ^{o}C), and oven-dried for 20 hours at 60 ^{o}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 grounded into a powder (pomace flour - PF). Pomace flour was further sieved to obtain fractions with different

127 particle size distribution: coarse (particle diameter > 355 μm), medium (particle
128 diameter beetween 354-250 μm), and fine (particle diameter < 249 μm) powders. All
129 pomace flours were acondicionated in vacuum sealed plastic bags, at room temperature
130 and protected from light.
131
132 *2.2. Chemical composition*
133 Moisture, ash, protein, fat, and total dietary fiber were analyzed in duplicate
134 using AOAC methods (1985, 1995 and 2007) for grape pomace materials.
135
136 *2.3. Hot water extraction – Experimental design*
137 Hot water extraction from distilled water was used to obtain soluble fibers. The
138 extraction was conducted in a thermostatic bath with agitation (*Dubnoff* NT232 with a
139 TCM 45 *Comtemp* thermostat). In order to evaluate the effects of solute: water ratio,
140 particle size and temperature, the experimental composite design was based in 11 trials,
141 combining these three parameters (Table 1). To proceed the analysis, the central points
142 of the design were carried out in triplicate and the other trials are the average values
143 from measurements in duplicate. After vacuum filtration with Whatman Filter paper
144 No.1, the final aqueous extracts were lyophilized for further analyses. The dependent
145 variable was the total sugar content in the extracts. The minimum and maximum values
146 of each parameter were chosen based on previous studies that used fruit skin to extract
147 fiber (Bicu & Mustata, 2013; Zhu, Du, Zheng & Li, 2015). Data from the factorial
148 design experiments were evaluated with the aid of *Statistica* software (Statsoft Inc.,
149 Tulsa, UK).
150

151 *2.4. Spectrophotometric Methods*

152 *Total sugar determination by Phenol-sulfuric acid method*: all lyophilized
153 samples obtained from hot water extractions were analyzed by phenol-sulfuric acid
154 method, described by Dubois, Gilles, Hamilton, Rebers & Smith (1956). D-Glucose was
155 used as standard and the analyses were performed at 490 nm.

156 *Uronic acid quantification:* the content of uronic acid in lyophilized samples
157 was determined by the improved *m*-hydroxybiphenyl method (Filisetti-Cozzi & Carpita,
158 1991). D-Galacturonic acid was used as standard. Thin layer chromatography (TLC)
159 was further used in order to determine the identity of the uronic acid from samples (data
160 not shown) (Sassaki, Sousa, Cirpiani & Iacomini, 2008).

161 *Antioxidant Activity: in vitro* antioxidant activity of pomace powders and
162 lyophilized extracts were evaluated by trolox equivalent antioxidant capacity assay
163 (TEAC). The extraction of compounds was based on the method described by Rufino *et*
164 *al.* (2007) and its quantification was described by Re, Pellegrini, Proteggente, Pannala,
165 Yang & Rice-Evans (1999). Results are shown as mMol Trolox/100g of pomace
166 powder or lyophilized extract.

167 *Total Phenolics:* The pomace flours (coarse, medium and fine) and all
168 lyophilized extracts were analyzed for total phenolic content, following the method
169 proposed by Singleton & Rossi (1965) and modified by Georgé, Brat, Alter & Amiot
170 (2005). Results were presented as g gallic acid/100g of pomace powder or lyophilized
171 extract.

172

173 *2.5. Monosaccharide analysis*

174 Samples from lyophilized extracts (2 mg) were hydrolyzed with 1 mL of 2 M
175 trifluoroacetic acid (TFA) at 100 ^{o}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 examined by GC–MS (Varian Saturn 2000R-3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer), using a DB-225 column (30 m × 0.25mm) programmed from 50 to 220 ^{o}C at 40 ^{o}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 using a 400 MHz Bruker model DRX Avance spectrometer incorporating Fourier transform (Rhein-Stetten). Samples were dissolved in D_2O or NaOD (0.05M) and examined at 50 ^{o}C. Chemical shifts (δ) are expressed in ppm, standardized on the resonance of acetone at δ 30.2.

2.7. High perforce liquid chromatography (HPLC)

HPLC analyses of sieved pomace flour extracts (coarse, medium and fine) were done in an Agilent 1100 series HPLC system equipped with a diode array detector. Prior to analysis, samples were 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 X 4.6 mm). Mobile phase A consisted of 94.9% water, 5% acetonitrile and 0.1% trifluoroacetic acid, and mobile phase B, 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 among them. Diode array

detection proceeded at 280, 320, 365 and 520 nm. Compounds were identified by comparison to pure standards retention time: gallic acid (5.779 min), catechin (21.086 mn), caffeic acid (23.377 min), epicatechin (26.242) 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. The Fick's. 2nd law was considered to fit kinetic data by a non-linear regression algorithm with a significance level of 90% (Statistica 8.0).

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 analyzed for total protein (13.80 g/100g), fat (4.21 g/100 g), carbohydrates (19.68 g/100g), dietary fiber (DF) (51.38 g/100 g), and ash (5.55 g/100 g).

Protein content was slightly higher than the ones 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 grape cultivation (Deng, Penner & Zhao, 2011). However, high protein content (18.89 g/100g pomace) was also reported by Basalan, Gungor, Owens & Yalcinkaya (2011) in red grape stalks, skin and pulp pomace. Fat content in grape pomace consists mainly of oil from the seeds. In this study, the seeds were separated from the pomace using a depulper, so a low fat composition was found, being in accordance to previously reported data (Balasan *et al*., 2011; Deng *et al*., 2011;

Zhu, Du, Zheng & Li, 2015). Carbohydrates and dietary fibers content presented higher values when compared to other primary compounds, as was expected from a vegetal cell wall rich material (Valient, Arrigoni, Esteban & Amado, 1995; Bravo & Saura-Calixto, 1998).

PF consists of more than 50% of fiber, which means it already brings a great potential of use as an ingredient for fiber supplementation, as a 10 g portion would afford more than 5 g of total dietary fiber in a meal. Considering WHO/FAO report (WHO, 2003), the dietary fiber intake of an adult should be of 25 g per day, meaning that the use of a 10 g portion of PF contributes to 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 distribution were obtained: 71% of coarse flour (CF) (particle diameter > 355 µm), 14% of medium flour (MF) (particle diameter beetween 354-250 µm) and 15% of fine flour (FF) (particle diameter < 249 µm) (Fig S1).

Total sugar content in the extract varied from 2.26% to 10.9% (Table 1). These colected data from 9 trials in central composite designs demonstrated that temperature is the parameter that has the higher influence in the yield of polysaccharide extraction. The negative effect of particle size parameter indicates that the smaller the particle is, the higher the contact surface between solute-solvent, what may improve the extraction (Fig. 1A). Modeling and analyzing the response surface for polysaccharide recovery (Fig. 1B) it can be conclude that the most adequate operational conditions was reached at 100 ^{o}C using the smallest particle size (FF) and solute:solvent ratio 1:12. These conditions were carried out in Trial 7, with yield of 10.9 % for total sugar measurement (Table 1).

For the response surface analyses, the solute: solvent ratio was fixed in the highest relation (1:12), decision based on a previous study that showed the importance of high solvent ratio for sample solubilization and yield increase (Zhu & Liu, 2013).

Yields obtained from these trials are in accordance to the ones from the literature, where high results for polysaccharide recovery from vegetal pomaces are around 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 on them. 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 presented, in average, Rha:Ara:Xyl:Man:Gal:Glc:GalA in a 3:32:2:13:11:20:19 molar ratio, respectively.

The main found differences among the trials concerned Glc and GalA ratios. Medium and large particle size flours presented a higher concentration of GalA, over 20% in trials 2, 4, 6, 8, and 9, which may indicate the presence of pectic polysaccharides in these fractions. In opposite, small size particles presented low GalA ratio and higher Glc ratios, the latter being over 20% in trials 1, 3, 5, and 7. These results may indicate that smaller particle size trials provided higher water- cell wall polysaccharides interaction, extracting other hemicelluloses in addition to pectins.

Trial 7, in special, presented the highest glucose ratio (37 mol%) and the highest yield when considering total sugar measure (10.9%) and, besides being formed by small size particles was also extracted in the most rigorous conditions, which means, higher temperature and higher (100 °C) solute:solvent ratio (1:12). For this reason, trial 7 and

central composite trials (9 to 11), which presented the highest GalA amount (31%), were chosen for further spectroscopic analysis.

NMR data for trials 7 and central points (9 to 11) are shown in figure 2. ^{13}C NMR of trial 9 (Fig. 2A) showed anomeric signals at δ 107.6, 102.7, and 100.1 referred as α-Ara*f*, β-Gal*p*, and α-Gal*p*A, respectively, which are probably arranged into pectic polysaccharides. The signal at δ 177.1, referred to the carboxyl groups of uronic acid residues and the signal at δ 16.6 is from –CH_3 of α-Rha*p* units. The well-defined signal at δ 52.9 may be attributed either to the –O*C*H3 groups of methyl-esterified Gal*p*A units from pectic polysaccharides (Cantu-Jungles *et al*., 2014; Nascimento, Corso, Werner, Baggio, Iacomini & Cordeiro, 2015), or to the *O*-methyl ester bounds to phenolic compounds, as previously described (Lu & Foo, 1999; Quirós-Sauceda *et al*., 2014). Non-related polysaccharide signals were also observed at δ 143.67, 119.15, 116.11, 114.90, and may indicate the presence of phenolic compounds, as well as signals in high field, around 24-44 ppm (Lu & Foo, 1999).

HSQC spectra of trials 7 (Fig. 2B) and 9 to 11 (Fig. 2C) showed very similar profiles, with $^{1}H/^{13}C$ correlations for the anomeric regions at δ 4.96/107.8, 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*. Trial 9 to 11 presented two correlations at δ 4.91/99.5 and 4.96/99.5 referred to α-D-Gal*p*A, while trial 7 presented 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, may 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, Evtuguim & Lopes, 2013).

Altogether, these results reinforce the presence of pectic polysaccharides extracted from grape pomace, either from larger or smaller particle sizes, with the

300 addition of the latter being also enriched with glucose-based hemicelluloses, which
301 promoted a higher yield in total sugar evaluation.
302
303 *3.4. Total phenolic content and structural identification*
304 Total phenolics were investigated either in sieved flours (CF, MF, and FF) as in
305 all lyophilized extracts (trials) (Table 3). In pomace flours, phenolic content varied from
306 21.63 ± 0.14 to 42.38 ± 0.36 g gallic acid/100 g pomace, while in lyophilized aqueous
307 extracts the content varied from 9.76 ± 0.25 to 213.08 ± 7.15 g gallic acid/100 g
308 lyophilized extract.
309 Phenolic content from smaller particle size pomace flour (FF) was
310 approximately two fold higher (42.38 ± 0.36 g gallic acid/100g pomace) when
311 compared to coarse (21.63 ± 0.14 g gallic acid/100g pomace) and medium particle size
312 (26.71 ± 0.28 g gallic acid/100g pomace) grape flours. This difference can be related to
313 a higher surface contact between water- cell wall, which improved the extraction yield.
314 With exception of trials 1, 3, and 9, all dried extracts from sieved flours
315 presented an increase in total phenolic content (Table 3). On trials where the extractions
316 were conducted with coarse particle size flours (2, 4, 6 and 8) an increase in the
317 phenolic content was observed. This was specially observed on trials 4 (180.23 ± 16.87
318 g gallic acid/100g pomace) and 8 (177.14 ± 4.36 g gallic acid/100g pomace), both
319 extracted under the highest temperature tested, being phenolic content over eight times
320 higher. Those results mean that, higher particle size flours and higher temperature may
321 increase total phenolic content in the extract. This can be due to higher polysaccharide
322 content, with the advantage that phenolic structures could be protected from temperature
323 degradation as they are into a fiber-phenolic interaction core promoted by hydrogen

bonds, hydrophobic interactions or even covalent methyl-esterification (Chamorro, *et al*. 2012).

When the extractions were performed using fine size particle flours, in trials 5 (80 °C, solute:solvent ratio of 1:12) and 7 (100 °C, solute:solvent ratio of 1:12) a significative increase in total phenolics was observed: about five times and three times, respectively, when compare to fine pomace flour. These data means that the combination of the lower temperature (80^{o}C), the higher solvent ratio (1:12) and the smaller particle size (< 249 µm) results in the higher recovery (213.08 ± 7.15 g Gallic Acid /100g pomace) of total phenolics compounds from the lyophilized extracts, produced from grape pomace.

Further, with the aim to compare and identify the main phenolic structures presented on sieved flours, as coarse, medium, and fine flours were analyzed by HPLC (Fig. 3). They presented very similar phenolic profiles, indicating that phenolic compounds are evenly distributed into pomace flours. So, in this study, particle size was not relevant for the selective extraction of specific compounds. Three polyphenol structures were identified in samples as gallic acid (CF:5.950 min; MF: 5.837 min; FF: 5.814 min), (+)-catechin (MF: 21.655 min; FF: 21.584 min) and (-)-epicatechin (CF: 26.731 min; MF: 26.056 min; FF: 26.050 min) by comparison of samples retention times to pure standards. (+)-Catechin and (-)-epicatechin were the most abundant polyphenols in the pomace flours, in agreement with previous studies (Sagdic *et al*., 2011; Jara-Palacios *et al*., 2014b). The presence of significant peaks at 22.206 and 24.895 minutes indicates 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 generated by the experimental design. Results for sieved pomace flours varied from 23.03 ± 0.93 to 43.80 ± 4.90 mMol Trolox/100g pomace, in accordance to total phenolic content increase, wherein was found an increase in the antioxidant activity from coarse to medium and fine flours of almost 2x.

Lyophilized extracts did not present a significant increase for the *in vitro* antioxidant activity among treatments when compared to crude sieved flours, although all extracts exhibited values that are in accordance to reported data in literature (Jara-Palacios *et al*., 2014a), varying the responses from 8.00 ± 0.62 to 46.60 ± 2.03 mMol Trolox/100g lyophilized extract. According to the concept established to consider a material as "antioxidant dietary fiber" (Quirós-Sauceda *et al*., 2014), these flours and extracts full filled the description of a product with intrinsic antioxidant activity, and could be promising as food ingredients.

4. Conclusion

The present study showed a feasible way to recover antioxidant dietary fiber from *Pinot noir* grape pomace. Consequently, these pomace flours could be used in the development of a fiber-rich ingredient for foodstuffs with the beneficial of fibers added of antioxidant properties. Further studies are being carried out to ensure their safety and effectiveness as products for human use.

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Figure Captions

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

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.

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

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

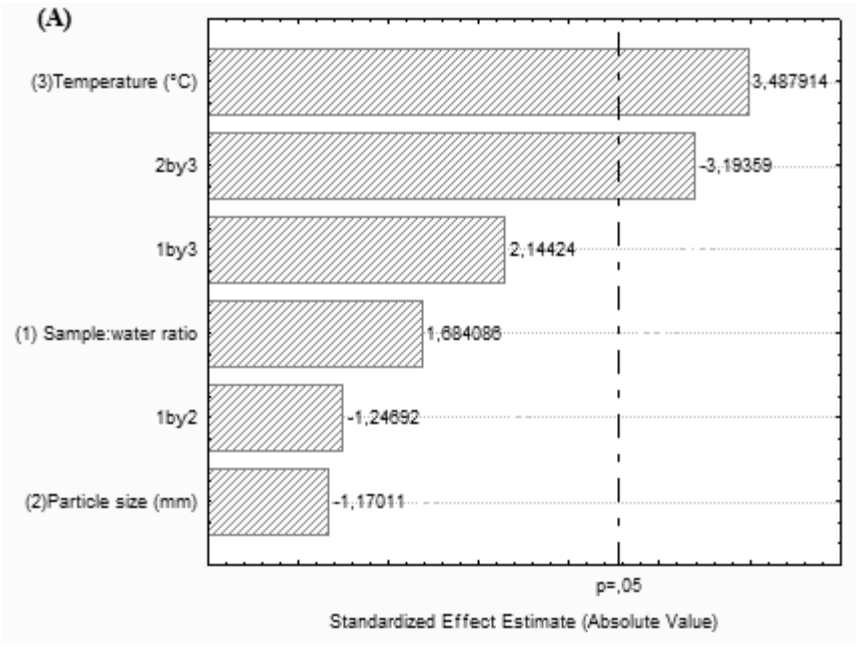
[a]Alditol acetates analyzed by gas chromatography, coupled to ESI-MS identification; [b] Rhamnose; [c] Arabinose;[d] Xylose; [e] Mannose; [f] Galactose; [g] Glucose; [h]uronic acid determined spectrophotometrically using the *m*-hydroxybiphenyl method, and Galacturonic acid, confirmed by thin layer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

[1]Total Phenolics (g gallic acid/100g pomace or lyophilized extract); [2]ABTS radical scavenging capacity (mMol Trolox/100g pomace or lyophilized extract).Different letter in the same column indicates significant difference using Tukey test (ρ=0.05).

Figure 1


(A)
(3)Temperature (°C)
3,487914
2by3
-3,19359
1by3
2,14424
(1) Sample:water ratio
1,684086
1by2
-1,24692
(2)Particle size (mm)
-1,17011
p=,05
Standardized Effect Estimate (Absolute Value)

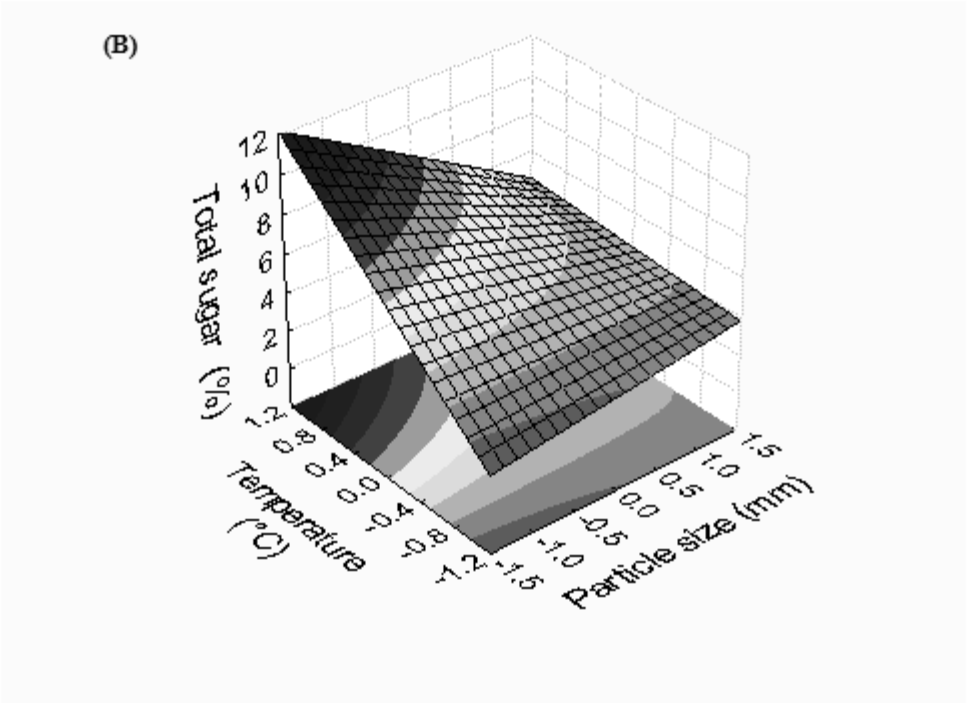
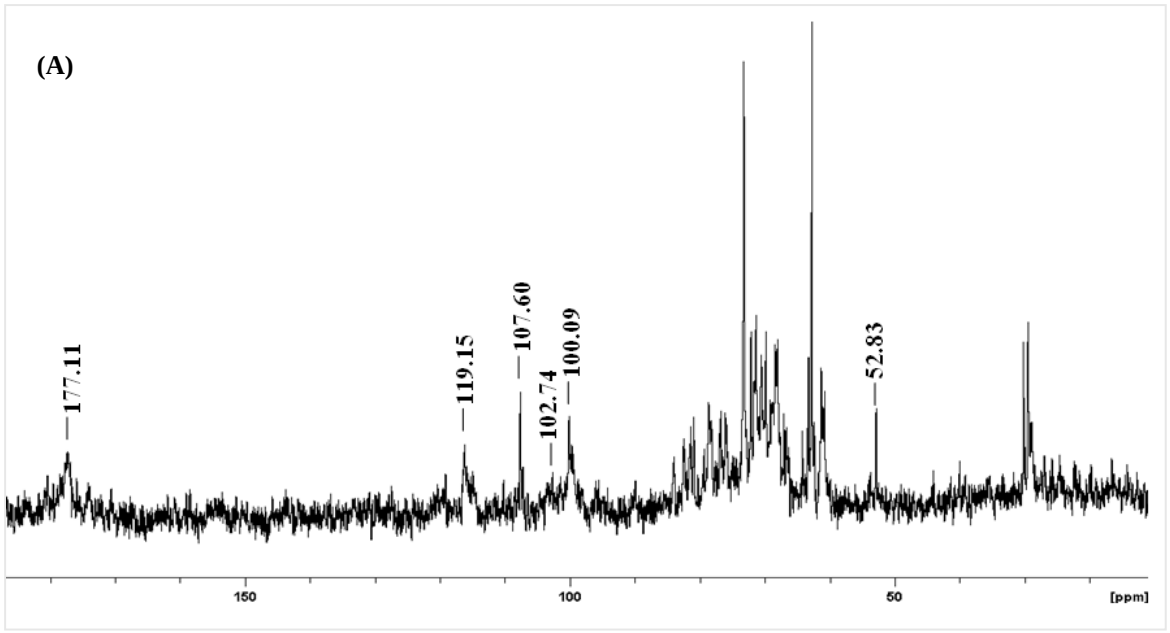
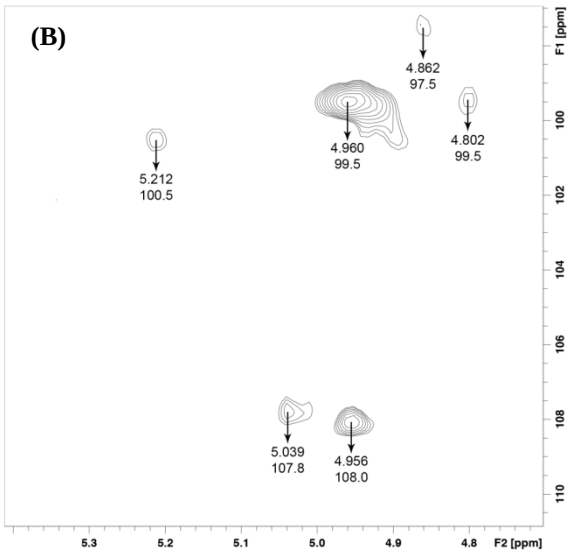

(B)
Total sugar (%)
12
10
8
6
4
2
0
Temperature (°C)
1,2
0,8
0,4
0,0
-0,4
-0,8
-1,2
Particle size (mm)
-1,5
-1,0
-0,5
0,0
0,5
1,0
1,5

Figure 2





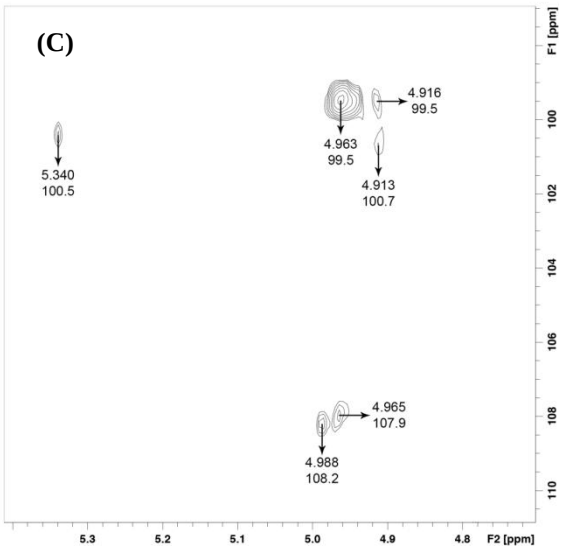


Figure 3

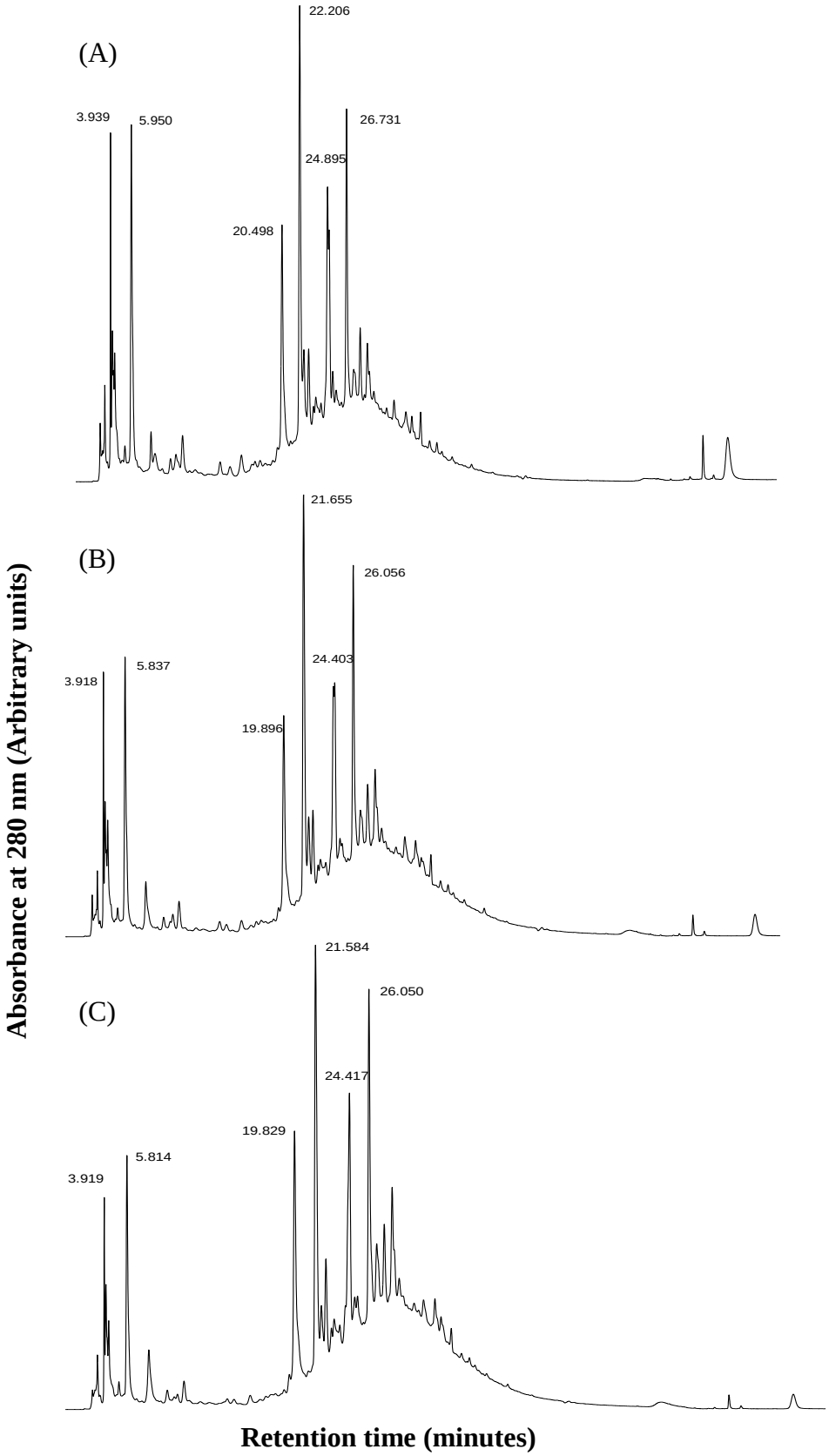

(A)
22.206
3.939
5.950
26.731
24.895
20.498
(B)
21.655
26.056
3.918
5.837
24.403
19.896
(C)
21.584
26.050
24.417
19.829
5.814
3.919
Absorbance at 280 nm (Arbitrary units)
Retention time (minutes)

Figure S1 – Scheme for *Pinot noir* grape pomace processing.