

1 **Exploration of steam explosion treatment for the recovery of phenolic compounds**

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26 *Abstract*

27 Steam explosion (SE) is a versatile tool for the pretreatment of lignocellulosic plant  
28 materials and the further separation of their main constitutive components, *i.e.* cellulose,  
29 hemicellulose, lignin, etc. In this study, we propose to evaluate the effects of SE  
30 treatment on the recovery of secondary metabolites. As a case study, the well-known  
31 grape pomace phenolic compounds were considered. Our results demonstrate that the  
32 efficiency of the steam explosion in term of yield (900 mg polyphenols per kg of dry  
33 grape pomace) was relatively similar to conventional maceration methods in alcoholic  
34 media (800 mg/kg). Advantages of SE compared to maceration were highlighted: the  
35 process is organic solvent free, destabilize the biomass structure and release insoluble  
36 bound phenolic compounds. In addition, it offers the possibility to modulate distinct  
37 polyphenols profiles by modifying the process conditions.

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41 *Keywords:* Grape pomace; steam explosion; extraction; polyphenols

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## 44 1 Introduction

45 Steam explosion is a conventional biorefining method usually explored as a  
46 pretreatment procedure for the cracking of lignocellulosic (plant) matrices into their  
47 main constituents, *i.e.* cellulose, hemicellulose and lignin (Jacquet et al., 2015). From a  
48 practical point of view, the raw material is treated in a closed reactor with steam water  
49 at a specified pressure, during a selected retention time. Consequently, the sample  
50 undergoes a modification of both the supramolecular and molecular structures of the  
51 through chemical (mostly auto-hydrolysis of hemicellulose) and physical (phase  
52 change) concomitant phenomena (Li et al., 2007; Han et al., 2010). Auto-hydrolysis is  
53 caused by chemical degradation of acetyl and uronyl groups linked to the  
54 hemicelluloses releasing acetic and uronic acids. These acids catalyze the hydrolysis  
55 hemicelluloses producing the corresponding monosaccharides and oligosaccharides  
56 (Glasser and Wright, 1998). The reactor is then submitted to a sudden depressurization  
57 leading to mechanical modifications of the treated raw material (*i.e.* morphological and  
58 porosity changes). Optimal releasing of phenolic acids is obtained at high temperature  
59 and high pressure through breakdown of the cell wall and degradation of lignin and  
60 hemicelluloses (Tsubaki et al., 2010).

61 Even if steam explosion is envisioned as a suitable cracking methodology, its ability for  
62 the one-step recovery of polyphenols from lignocellulosic matrices remains marginal  
63 (Zitella et al., 2016).

64 Phenolic compounds are found in free, esterified and insoluble-bound forms in the  
65 lignocellulosic biomass (Kurosumi et al.2007; Shahidi and Yeo, 2016). The insoluble-  
66 bound phenolics, localized in cell walls, are linked to structural macromolecules such as  
67 proteins, cellulose, hemicellulose, pectin or lignin (Acosta-Estrada et al., 2014). Lignin  
68 and phenolic acids (hydroxycinnamic and hydroxybenzoic acids) are linked by ether

69 bonds through their hydroxyl groups. Structural carbohydrates and proteins can form  
70 ester linkages through carboxylic groups. Since Adriano Costa de Camargo and co-  
71 workers have highlighted that insoluble-bound phenolics represent the major part of  
72 total phenolics encountered in grape juice and winemaking byproducts. It includes  
73 among others, *p*-coumaric, caffeic and gallic acids (De Camargo et al., 2014). Steam  
74 explosion seems to be a powerful method for the extraction of polyphenols from grape  
75 pomace. Indeed, this technology provides a sufficient breakdown of the lignocellulosic  
76 structure to allow the extraction of bound phenolics and represents then a simple and  
77 eco-friendly alternative to the traditional extraction methods, using highly concentrated  
78 alkaline and acid solvents Liu et al., 2016).

79 Grape pomace was selected as a benchmark for this study due to the marked interest of  
80 this lignocellulosic waste as a valuable source of bioactive compounds (up to 70% of  
81 grape polyphenols could remain in the pomace after wine-making) and the extended  
82 R&D efforts performed in this topic (Beres et al., 2017; Arshadi et al., 2016; Antonioli  
83 et al., 2015).

84

85 2 *Material and methods*

86 2.1 *Raw material*

87 Two varieties of grape (*Vitis vinifera* L. cv Cabernet sauvignon (CS) and *Vitis vinifera*  
88 L. cv Pinot noir (PN)) were grown in Carmel Valley, Monterey county, California  
89 (USA). The corresponding pomaces were sun-dried before being kept at room  
90 temperature in the dark prior to their composition analysis.

91 *Total solids* were determined after the sample was heated to 105°C until a constant  
92 weight was recorded (Sluiter et al., 2008 (1)). *Extractives* were determined after the  
93 samples were successively extracted with water and ethanol in a Soxhlet apparatus  
94 (Sluiter et al., 2005 (1)). *Ash* content was determined after combustion of the samples at  
95 525°C for 4h (Sluiter et al., 2005 (2)). *Protein* content was determined by the Kjeldahl  
96 procedure using a conversion factor of 6.25 (Hames et al., 2008).

97 *Acid insoluble lignin* content was assessed gravimetrically as Klason lignin. Extractible  
98 free samples were hydrolysed with 72% sulphuric acid (30°C for 60 min) followed by  
99 dilution to 4% sulphuric acid with distilled water and hydrolysed in an autoclave (121°C  
100 for 60 min). The mixture was filtered through filtering crucibles, dried at 105°C to a  
101 constant weight and combusted in a muffle furnace at 525°C for 3 hours. Acid insoluble  
102 lignin was measured spectrophotometrically by reading the UV absorbance of the  
103 filtrate at 320 nm. Total lignin content in the sample is assumed to be the sum of the  
104 Klason lignin and the acid soluble lignin (Sluiter et al., 2008 (2)). *Carbohydrate*  
105 composition was determined by gas chromatography (Berchem et al., 2016). Neutral  
106 sugars were determined as alditol acetates. Analyses were carried out with a Hewlett-  
107 Packard (HP 6890) gas chromatograph equipped with a flame ionization detector. The  
108 components were separated using a high performance capillary column, HP1-  
109 methylsiloxane (30 m×320 µm, 0.25 µm, Scientific Glass Engineering, S.G.E. Pty. Ltd.,

110 Melbourne, Australia). Glucose and xylose quantities were converted to the equivalent  
111 amount of polymeric glucan and xylan (anhydro corrections of 0.9 for glucose and 0.88  
112 for xylose are applied).

## 113 2.2 *Polyphenols extraction*

114 The steam explosion assays were carried out on a homemade pilot scale prototype  
115 whose technical configuration has previously been described (Jacquet et al., 2010). This  
116 prototype includes a steam generator (29.4 kW, operating pressure 6.0 MPa), a 50 L  
117 reactor that can operate at a maximum pressure of 5.1 MPa and a cyclone explosion  
118 tank in which the treated product is recovered. A quick-opening ball valve, placed  
119 between the reactor and the explosion cyclone tank, is used to release the steam  
120 accumulated in the reactor, creating a quick decrease in pressure and giving the  
121 explosion effect. Steam explosion experiments were performed on 80 g of grape  
122 pomaces in contact with steam water that was released immediately after the desired  
123 pressure was reached (0.5, 1, 1.5 and 2.5 MPa reached respectively after 0.5, 1, 2 and 3  
124 min.). The phenolic extracts were recovered after filtration on 100µm nylon filter and  
125 freeze-dried prior to further analyses. As a comparison, grape pomaces were also treated  
126 under classical maceration conditions by a direct soaking of the sample in a methanol-  
127 water mixture (80:20 v/v) at 60°C for 60 min with a ratio solid/liquid of 1/10 (w/v)  
128 (Pintac et al., 2018; Benmeziane et al., 2014). The phenolic extracts were recovered  
129 after 10 min. centrifugation at 10,000 g at room temperature.

130 All the experiments were performed in triplicate.

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## 132 2.3 *Polyphenols specific quantification*

133 Polyphenols concentrations were specifically measured by High Performance Liquid  
134 Chromatography, using a HPLC Alliance 2690 (Waters) device coupled with a Waters

135 996 PDA detector. Compounds were separated on a Zorbax 300 sb-C18 (3.5  $\mu\text{m}$ , 4.6  $\times$   
136 150 mm) column at 25°C using a binary mobile phase composed of distilled water with  
137 0,5% acetic acid (A) and acetonitrile with 0,5% acetic acid (B). The total flow rate was  
138 1 mL/min, the injection volume was 15  $\mu\text{L}$  with a specific gradient elution (Istasse et  
139 al., 2016). Briefly, the elution started with 100% A. This proportion was held for 5 min.  
140 then decreased to 85% in 5 min. The proportion of solvent A reached 65% at 30 min,  
141 then 50% at 35 min, and finally cut off to 0% at 36 min. This ratio was held for 4 min.  
142 then the proportion of solvent A was restored to 100% in 1 min then held for 5 min. The  
143 polyphenols absorbances were measured at wavelengths of 280 and 320 nm.  
144

145 3 *Results and discussion*

146 *Cabernet Sauvignon (CS)* and *Pinot Noir (PN)* samples have a similar chemical  
 147 composition (Table 1). The quantities of compounds extracted by water ( $17.49 \pm 0.61$   
 148 and  $17.81 \pm 0.72$ ) and by ethanol ( $11.2 \pm 0.13$  and  $12.04 \pm 0.53$ ) from CS and PN  
 149 respectively are not significantly different.

150

151 Table 1. Compositional analysis of Cabernet Sauvignon (CS) Pinot Noir (PN) pomaces.

	Cabernet sauvignon ( <b>CS</b> )	Pinot noir ( <b>PN</b> )
<b>Lignin</b>	<b>42.62 ± 0.29</b>	<b>41.49 ± 0.38</b>
Klason lignin	38.31 ± 0.11	36.40 ± 0.30
Acid soluble lignin	4.31 ± 0.18	5.09 ± 0.08
<b>Polysaccharides</b>	<b>6.88 ± 0.85</b>	<b>9.74 ± 2.25</b>
Glucan	2.63 ± 0.52	4.5 ± 1.31
Xylan	3.06 ± 0.18	3.3 ± 0.41
Mannan	0.38 ± 0.05	0.66 ± 0.11
Galactan	0.31 ± 0.05	0.45 ± 0.10
Arabinan	0.37 ± 0.02	0.54 ± 0.16
Rhamnan	0.13 ± 0.03	0.29 ± 0.16
<b>Extractives</b>	<b>28.69 ± 0.74</b>	<b>29.85 ± 1.25</b>
Water	17.49 ± 0.61	17.81 ± 0.72
Ethanol	11.2 ± 0.13	12.04 ± 0.53
<b>Proteins</b>	<b>10.23 ± 0.10</b>	<b>10.24 ± 0.05</b>
Extractible proteins	2.20 ± 0.39	1.87 ± 0.44
<b>Ashes</b>	<b>8.51 ± 0.11</b>	<b>9.51 ± 0.54</b>
<b>Total</b>	<b>96.93 ± 2.09</b>	<b>100.83 ± 4.47</b>

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153 A direct maceration of the grape pomaces in a methanol/water mixture at 60°C for 60  
154 min allowed to identify the main presence of gallic acid, catechin, chlorogenic acid, *p*-  
155 coumaric acid, rutin, quercetin and kampferol whose extraction yields varied between  
156 *CS* and *PN* samples mostly for catechin (408 mg/kg of dry grape pomace for *CS*  
157 compared to 592 mg/kg for *PN*) and chlorogenic acid (13 mg/kg for *CS* compared to 23  
158 mg/kg for *PN*) (Fontana et al., 2014).

159 The steam explosion treatment was applied for both *CS* and *PN* samples at different  
160 pressures (from 0.5 to 2.5 MPa).

161 Results are summarized in Table 2 and compared with the aforementioned maceration.

162 The quantity of polyphenols extracted at 0.5 and 1 MPa was quite marginal for both *CS*  
163 and *PN* and did not exceed respectively 4 and 17 mg/kg of dry grape pomace. Catechin  
164 and *p*-coumaric acid were detected in the extracts as the two main recovered phenolic  
165 compounds. At 1.5 MPa, a significant increase in the polyphenols extraction yields was  
166 observed ranging from 56 to 204 mg/kg respectively for *CS* and *PN*. Up to 2.5 MPa, the  
167 concentration of polyphenols extracted using the steam explosion device was noticeable  
168 and culminated up to 900 mg/kg of dry pomace for both samples. This result is superior  
169 to the conventional benchmark maceration where the cumulative yields ranged between  
170 560 mg/kg for *CS* and 820 mg/kg for *PN*. Gallic acid was the major phenolic  
171 compounds detected in the steam-exploded extracts, with yields of about 480 mg/kg for  
172 *CS* and 649 mg/kg for *PN*, while catechin was the main molecule recovered under  
173 maceration conditions representing more than half the total concentration of  
174 polyphenols.

175 Table 2. Main polyphenols recovery after steam explosion processes at 0.5, 1, 1.5 and 2.5 MPa and direct maceration for *CS* (a) and *PN* (b).

176 Results are expressed as mg of polyphenols extracted per kg of dry grape pomace. n.d. stands for “not detected”

	Cabernet sauvignon ( <b>CS</b> )					Pinot noir ( <b>PN</b> )				
	Maceration	SE 5bars	SE 10bars	SE 15bars	SE 25bars	Maceration	SE 5bars	SE 10bars	SE 15bars	SE 25bars
Gallic acid	124.9 ± 5.5	n.d.	n.d.	17.0±7.4	485.1 ± 63.0	140.0 ± 2.13	n.d.	n.d.	67.6 ± 3.0	648.9 ± 42.5
Catechin	408.4 ± 17.4	1.8± 0 .1	3.3 ± 0.0	28.5±5.4	336.06 ± 66.0	592.6 ± 25.0	0.5 ±0.1	14.2 ± 2.1	84.3 ± 2.3	304.8 ± 40.3
Chlorogenic acid	12.7 ± 0.5	n.d.	n.d.	4.0 ± 0.0	15.0 ± 8.3	22.8 ±1.1	n.d.	1.1 ± 0.4	n.d.	21.1 ± 4.7
<i>p</i> -Coumaric acid	n.d.	0.1 ± 0.0	0.1 ± 0.0	6.9 ± 0.5	8.9 ± 0.9	5.7 ± 0.6	n.d.	1.5 ± 0.2	51.7 ± 8.2	14.9 ± 5.5
Rutin	1.6 ± 0.3	n.d.	n.d.	n.d.	n.d.	38.4 ± 5.6	n.d.	n.d.	n.d.	n.d.
Quercetin	11.1 ± 0.5	n.d.	n.d.	n.d.	n.d.	14.4 ±1.1	n.d.	n.d.	n.d.	n.d.
Kaempferol	6.3 ± 0.5	n.d.	n.d.	n.d.	n.d.	8.5 ± 0.2	n.d.	n.d.	n.d.	n.d.

177

178 Regarding the total yield of polyphenols identified in Table 2, it can be highlighted that  
179 steam explosion performed at 2.5 MPa allowed to extract a higher amount of  
180 compounds, especially gallic acid ( $485.1 \pm 63.0$  mg/kg). This is consistent with de  
181 Camargo et al. that found up to 153 and 78 times more gallic acid linked by insoluble  
182 bounds than free and esterified ones respectively in grape juice byproducts (De  
183 Camargo et al., 2014). In regard of treatment time, it is worth noting that the extraction  
184 by steam explosion was performed 10 times faster than the maceration. The exclusive  
185 use of water as extraction solvent set the steam explosion as a competitive technology  
186 from both an economical and an ecological point of view. Moreover, the operating  
187 pressure seemed to enable the selection of the extracted molecules. For instance, the  
188 extraction of gallic acid and chlorogenic acid started from 1.5 MPa whereas catechin  
189 and *p*-coumaric acid were already quantified in 0.5 MPa extracts.

#### 190 4 Conclusion

191 The extraction by steam explosion of secondary metabolites, applied herein on the case  
192 study of grape pomace, appears to be an efficient water-based extraction method. The  
193 process at 2.5 MPa can compete with conventional maceration in term of total  
194 polyphenol yield. Our results highlight as well the potential use of steam explosion as a  
195 tool for selective extraction of secondary metabolites including insoluble bound  
196 phenolic compounds depending on the operating pressure. Further experiments will be  
197 conducted in order to optimize the process according to biomasses composition and  
198 desired profiles. The work can be therefore oriented toward the fate of the main  
199 lignocellulosic compounds and their co-extraction during the process in order to  
200 propose a one-step method for both the biomass fractionation and the recovery of  
201 secondary metabolites.

202 5 *Conflict of interest*

203 The authors declare that they have no conflict of interest

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205 6 *References*

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