Exploration of steam explosion treatment for the recovery of phenolic compounds

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Abstract

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

Keywords: Grape pomace; steam explosion; extraction; polyphenols

1 Introduction

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

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

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

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

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

2 Material and methods

2.1 Raw material

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

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

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

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

2.2 Polyphenols extraction

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

All the experiments were performed in triplicate.

2.3 Polyphenols specific quantification

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

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

3 Results and discussion

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

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

	Cabernet sauvignon ***(CS)***	Pinot noir (***PN)***
Lignin	**42.62 ± 0.29**	**41.49 ± 0.38**
Klason lignin	38.31 ± 0.11	36.40 ± 0.30
Acid soluble lignin	4.31 ± 0.18	5.09 ± 0.08
Polysaccharides	**6.88 ± 0.85**	**9.74 ± 2.25**
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
Extractives	**28.69 ± 0.74**	**29.85 ± 1.25**
Water	17.49 ± 0.61	17.81 ± 0.72
Ethanol	11.2 ± 0.13	12.04 ± 0.53
Proteins	**10.23 ± 0.10**	**10.24 ± 0.05**
Extractible proteins	2.20 ± 0.39	1.87 ± 0.44
Ashes	**8.51 ± 0.11**	**9.51 ± 0.54**
Total	**96.93 ± 2.09**	**100.83 ± 4.47**

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

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

Results are summarized in Table 2 and compared with the aforementioned maceration. The quantity of polyphenols extracted at 0.5 and 1 MPa was quite marginal for both *CS* and *PN* and did not exceed respectively 4 and 17 mg/kg of dry grape pomace. Catechin and *p*-coumaric acid were detected in the extracts as the two main recovered phenolic compounds. At 1.5 MPa, a significant increase in the polyphenols extraction yields was observed ranging from 56 to 204 mg/kg respectively for *CS* and *PN*. Up to 2.5 MPa, the concentration of polyphenols extracted using the steam explosion device was noticeable and culminated up to 900 mg/kg of dry pomace for both samples. This result is superior to the conventional benchmark maceration where the cumulative yields ranged between 560 mg/kg for *CS* and 820 mg/kg for *PN*. Gallic acid was the major phenolic compounds detected in the steam-exploded extracts, with yields of about 480 mg/kg for *CS* and 649 mg/kg for *PN*, while catechin was the main molecule recovered under maceration conditions representing more than half the total concentration of 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 ***(CS)***					Pinot noir (***PN)***				
	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
p-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.

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

4 Conclusion

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

5 *Conflict of interest*

The authors declare that they have no conflict of interest

6 *References*

Acosta-Estrada B, Gutiérrez-Uribe J, Serna-Saldivar S (2014) Bound phenolics in foods, a review. Food Chem 152:46–55.

Antoniolli A, Fontana AR, Piccoli P, Bottini R (2015) Characterization of polyphenols and evaluation of antioxidant capacity in grape pomace of the cv. Malbec. Food Chem 178:172–178. doi:10.1016/j.foodchem.2015.01.082.

Arshadi M, Attard TM, Lukasik RM, Brncic M, Da Costa Lopes AM, Finell M, Geladi P, Gerschenson LN, Gogus F, Herrero M, Hunt AJ, Ibanez E, Kamm B, Mateos-Aparicio, Matias A, Mavroudis NE, Montoneri E, Morais ARC, Nilsson C, Papaioannoua EH, Richel A, Ruperez P, Skrbic B, Solarov MB, Svarc-Galic J, Waldron K, Yuste F (2016) Pre-treatment and extraction techniques for recovery of added value compounds from wastes throughout the agri-food chain. Green Chem 18:6160–204. doi:10.1039/c6gc01389a.

Benmeziane F, Djamai R, Cadot Y, Seridi R (2014) Optimization of extraction parameters of phenolic compounds from Algerian fresh table grapes, (*Vitis Vinifera*). Int Food Res J 21 (3):1025-1029.

Berchem T, Roiseux O, Vanderghem C, Boisdenghien A, Foucart G, Richel A (2016) Corn stover as feedstock for the production of ethanol: chemical composition of different anatomical fractions and varieties. Biofuels, Bioprod. Biorefin. 6:246–56. doi:10.1002/bbb.

225 Beres C, Costa GNS, Cabezudo I, da Silva-James NK, Teles ASC, Cruz APG,
226 Mellinger-Silva C, Tonon RV, Cabral LMC, Freitas SP (2017) Towards integral
227 utilization of grape pomace from winemaking process: A review. Waste Manag 68:581–
228 94. doi:10.1016/j.wasman.2017.07.017.

229 De Camargo AC, Regitano-D'Arce MAB, Biasoto ACT, Shahidi F (2014) Low
230 molecular weight phenolics of grape juice and winemaking byproducts: Antioxidant
231 activities and inhibition of oxidation of human low-density lipoprotein cholesterol and
232 DNA strand breakage. J Agric Food Chem 62:12159–71. doi:10.1021/jf504185s.

233 Fontana AR, Antoniolli A, Bottini R (2014) Grape pomace as a sustainable source
234 of bioactive compounds : extraction, characterization and biotechnological applications
235 of PubMed Commons. J Agric Food Chem 61:8987–9003. doi:10.1021/jf402586f.

236 Glasser W, Wright R (1998) Steam assisted biomass fractionation. II. Fractionation
237 behavior of various biomass resources. Biomass Bioenerg 14:219–235.

238 Hames B, Scarlata C, Sluiter A (2008) Determination of Protein Content in
239 Biomass. National Renewable Energy Laboratory (NREL)

240 Han G, Deng J, Zhang S, Bicho P, Wu Q (2010) Effect of steam explosion
241 treatment on characteristics of wheat straw. Ind Crops Prod 31:28–33.
242 doi:10.1016/j.indcrop.2009.08.003.

243 Istasse T, Jacquet N, Berchem T, Haubruge E, Nguyen BK, Richel A (2016)
244 Extraction of honey polyphenols: Method development and evidence of cis
245 isomerization. Anal Chem Insights 2016:49–57. doi:10.4137/Aci.s39739.

246 Jacquet N, Maniet G, Vanderghem C, Delvigne F, Richel A (2015) Application of
247 Steam Explosion as Pretreatment on Lignocellulosic Material: A Review. Ind Eng
248 Chem Res 54:2593–8. doi:10.1021/ie503151g.

249 Jacquet N, Vanderghem C, Blecker C, Paquot M (2010) La steam explosion :
250 Application en tant que prétraitement de la matière lignocellulosique. Biotechnol Agron
251 Soc Environ 14:561–6.

252 Kurosumi A, Sasaki C, Kumada K, Kobayashi F, Mtui G, Nakamura Y (2007)
253 Novel extraction method of antioxidant compounds from Sasa palmata (Bean) Nakai
254 using steam explosion. Process Biochem 42:1449–53.
255 doi:10.1016/j.procbio.2007.06.007.

256 Li J, Henriksson G, Gellerstedt G (2007) Lignin depolymerization/repolymerization
257 and its critical role for delignification of aspen wood by steam explosion. Bioresour
258 Technol 98:3061–8. doi:10.1016/j.biortech.2006.10.018.

259 Liu L, Zhao M, Liu X, Zhong K, Tong L, Zhou X, Zhou S (2016) Effect of steam
260 explosion-assisted extraction on phenolic acid profiles and antioxidant properties of
261 wheat bran. J Sci Food Agric 96:3484–91. doi:10.1002/jsfa.7532.

262 Pintac D, Majkic T, Torovic L, Orcic D, Beara I, Simin N, Mimica-Dukic N,
263 Lesjak M (2018) Solvent selection for efficient extraction of bioactive compounds from
264 grape pomace. Ind Crops Prod 111:379-390

265 Shahidi F, Yeo JD (2016) Insoluble-bound phenolics in food. Molecules 21.
266 doi:10.3390/molecules21091216.

Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D (2005) (1) Determination of Extractives in Biomass: Laboratory Analytical Procedure. National Renewable Energy Laboratory (NREL)

Sluiter A, Hames B, Ruiz RO, Scarlata C, Sluiter J, Templeton D (2005) (2) Determination of ash in biomass, National Renewable Energy Laboratory (NREL)

Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J (2008) (1) Templeton D Wolfe J, Determination of total solids in biomass and total dissolved solids in liquid process samples. National Renewable Energy Laboratory (NREL)

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) (2) Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory (NREL)

Tsubaki S, Ozaki Y, Azuma J (2010) Microwave-assisted autohydrolysis of Prunus mume stone for extraction of polysaccharides and phenolic compounds. J Food Sci 75(2):152–159

Zitella P, La Licata B, Boulanger A, Cuzzola V, Morra S, Valetti F, Gilardi G (2016) Extraction of biochemical from the wine industry by-products and their valorization. Environ Eng Manag J 15(9):2049-2056