GRAPE POMACE EXTRACTS FROM WINEMAKING ARTISANAL AND INDUSTRIAL PROCESSES FOR GREEN-BASED PRODUCTS

Catia Santos Branco[1*], Juliane Mascarello[1], Aline Fagundes Cerbaro[1], Alina Concepción-Alvarez[2], Adriano Costa De Camargo[2] & Raquel Bridi[3]

[1] *Biotechnology Institute, Universidade de Caxias do Sul, Caxias do Sul, RS, Brazil.*
[2] *Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, Chile.*
[3] *Facultad de Ciencias Químicas y Farmaceuticas, Universidad de Chile, Santiago, Chile.*
** Corresponding author's email address: csbranc1@ucs.br*

ABSTRACT

Reusing grape pomace, a by-product of the winery sector, has been highlighted as an important strategy in the circular economy. This study aimed to evaluate the physical-chemical composition, antioxidant activity, and phenolic content in the extracts obtained by green extractions without using organic solvents from the "Bordô" grape of artisanal and industrial winemaking. Artisanal pomace showed higher moisture and mineral content ($p<0.05$). Industrial pomace was rich in skins (83%; $p=0.037$) and poor in stalks (below 2%; $p=0.004$) compared to artisanal. Both extracts showed free radical scavenging capacity ($DPPH^{\bullet}$) above 75%. Regarding total phenolic content, 25.58 ± 0.59 mg/100mL was found for artisanal pomace and 35.79 ± 1.35 mg/100mL for industrial ($p<0.05$). Anthocyanin content was twice as high in the artisanal sample ($p<0.05$), so it was chosen to be freeze-dried. By Ultra Efficiency Liquid Chromatography System coupled to Mass Spectrometry (UPLC-MS/MS), seventeen compounds were detected, including chlorogenic acid, sinapic acid, epicatechin, *p*-coumaric acid, and catechin, the five major compounds in the artisanal freeze-dried extract. These results demonstrate that aqueous extraction in the tested conditions is a valuable alternative for extracting phenolic acids, flavonoids, and proanthocyanidins. It can be used to develop high-value-added products.

Keywords: *Vitis labrusca*. Wine. Green extraction. Polyphenols. Bioeconomy.

1 INTRODUCTION

During wine production, residues are generated at various stages, and approximately 20 million tons of winery by-products are discarded each year around the world[1]. In the artisanal process, wine is produced by the farmer or rural family entrepreneur, made following the cultural, historical, and social characteristics, in a maximum quantity of twenty thousand liters annually[2]. Industrial wine is produced on a large scale, depending on more efficient technology. Grape pomace is the main by-product generated during wine production. This is made up of residual pulp, skins, and seeds. The proportion of each component and its chemical composition depends on climate, grape variety, and rootstock [3], among others. Winemaking residues are important sources of phenolic compounds, mainly flavonoids, which can scavenge reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\bullet-}$), due to its antioxidant action[1]. Anthocyanins are a type of flavonol and constitute the largest group of water-soluble pigments that can exhibit significant biological properties, such as preventing neurodegenerative and cardiovascular diseases, some types of cancer, diabetes, and other aging-associated diseases[4]. Until now, there have been no comparative studies on the physical-chemical composition, antioxidant activity, and phenolic content of "Bordô" grape pomace from *Vitis labrusca* made in artisanal or industrial processes extracted under green-based conditions. Therefore, this study aimed to obtain extracts from two different types of by-products in an extraction free of organic solvents, as well as to characterize them and to prospect potential uses.

2 MATERIAL & METHODS

Winery by-products: obtaining and classification

Pomace was obtained from artisanal and industrial wineries (São Marcos, RS, Brazil) during the 2023 harvest. The composition was evaluated through manual separation of each component of the pomace. Project registration (SisGen AD30AE5).

Chemical composition, phenolic profile, and antioxidant activity

Samples were kept in a drying oven (105 ºC) for three hours to obtain moisture content. They were incinerated (500 ºC) for 4 hours to quantify minerals (ash). Protein (Kjeldahl method) and dietary fiber were also quantified. Carbohydrates were obtained by difference and energy value was calculated using conversion (Carbohydrates – 4 cal/g; Proteins – 4 cal/g; Lipids – 9 cal/g)[5]. Fats were estimated by Bligh & Dier, 1959 method[6]. Phenolics were extracted using water as a solvent, as described in BR102024004616-1 (INPI, Brazil). Spectrophotometry quantified the Anthocyanin amounts[7], total phenolic content[8], and antioxidant activity by the $DPPH^{\bullet}$ method[9].

UPLC-MS/MS Analysis

Phenolic compounds were identified using an ABSciex triple Quad 4500 mass spectrometer equipped with an electrospray (TurboV) interface coupled to an Eksigent Ekspert Ultra LC100 with an Ekspert Ultra LC100-XL autosampler system (AB/Sciex Concord, ON, Canada). Calibration curves for quantification were constructed using commercially available standards.

Statistical Analysis

Analyzes were performed in triplicate, and results were expressed as mean ± standard deviation. Data were analyzed by variance (One-way ANOVA) and Tukey's post-test or Student's *t*-test, considering a probability level of less than 5% ($p<0.05$), using the Statistical Package for the Social Sciences (SPSS®) versão 21.0.

3 RESULTS & DISCUSSION

Artisanal pomace had a composition of 68% skin, 24% seed, and 8% stalk. Industrial consisted of 83% skin, 16% seed, and 1% stalk. Significant differences between skin and stalk ($p=0.037$ and $p=0.004$, respectively; **Table 1**) content were found. It was also observed that industrial pomace (**Figure 1**) had a lighter color than the artisanal one. This can be attributed to the use of specific enzymes that improve color extraction in red wines[10] and the use of pressurized presses in the industrial process.

Figure 1 Fresh samples from industrial (A) and artisanal (B) wine production. Aqueous extracts (C) and their composition (D).

Table 1 Composition (%) of artisanal *versus* industrial grape pomace

Origin	Skin	Seed	Stalk	Total
Artisanal	67.73 ± 6.67*	23.66 ± 8.37	8.30 ± 1.86*	99.69 ± 0.53
Industrial	82.92 ± 5.38	15.65 ± 4.73	1.41 ± 0.78	99.98 ± 0.01

Data expressed as mean ± standard deviation. *Statistical difference by *t*-test for independent samples ($p<0.05$).

Table 2 presents the nutritional composition and energy value of the samples. Artisanal pomace showed a moisture content about 1.2 times higher when compared to industrial grape pomace ($p=0.001$) and an increase of about 1.5 times in ash ($p=0.018$). Industrial was higher in lipids ($p=0.042$), fiber ($p=0.001$), carbohydrates ($p=0.003$), and energy value ($p=0.001$).

Table 2 Nutritional composition (g/100g) and energy value (kCal/100g) of artisanal *versus* industrial grape pomace

Origin	Moisture	Ash	Lipid	Fiber	Protein	Carbohydrate	Energy value
Artisanal	69.23±0.48*	2.29±0,23*	1.10±0.24*	17.45±0.23*	7.95±0.31	2.47±0.24*	50.60±1.18*
Industrial	57.88 ± 0.82	1.38±0.34	2.17±0.59	23.70±0.34	8.66±0.53	6.19±0.96	79.04±6.00

Data expressed as mean ± standard deviation. *Statistical difference by *t*-test for independent samples ($p<0.05$).

Nutritional composition may vary depending on soil and climate conditions, viticulture practices, and the winemaking process. The type and duration of maceration can also affect the content of minerals, fibers, and lipids in the by-products obtained[11]. The antioxidant activity of the pomace and its phenolic composition was also evaluated in the extracts made using water as a solvent under high-temperature conditions. No significant difference ($p=0.740$) between the aqueous extracts was found, both showed a radical reduction capacity above 75% (**Figure 2 A**). The industrial extract showed an increase in the content of total phenolic content (TPC) by around 40% ($p=0.001$; **Figure 2 B**). However, it was found to contain a lower anthocyanin content (52%; $p=0.001$) compared to the artisanal (**Figure 2 C**).

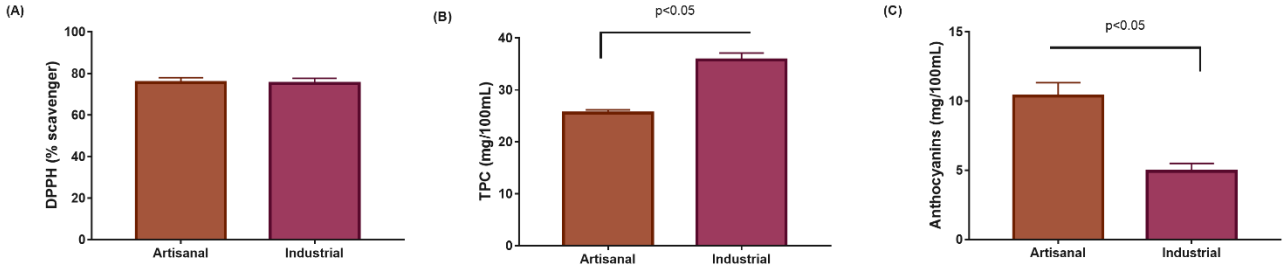
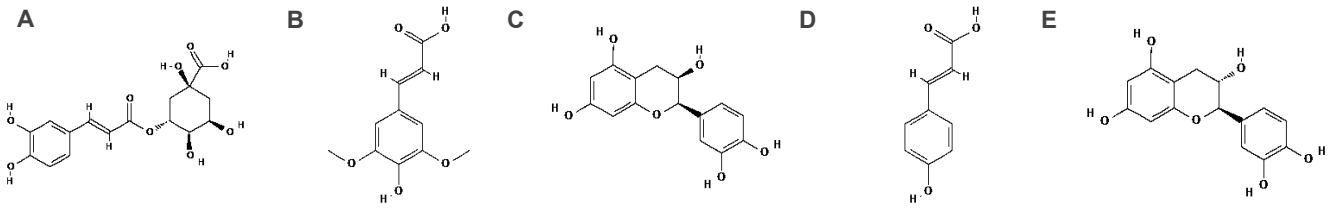


Figure 2 Phenolic composition and antioxidant activity of extracts from *Bordô* grape pomace

Considering the high levels of anthocyanins in the artisanal extract, it was selected to be freeze-dried, and its composition was further evaluated by UPLC-MS/MS. Chlorogenic acid (474.74 ± 13.47 mg/100g; **Figure 3A**), sinapic acid (325.52 ± 3.98 mg/100g; **Figure 3B**), epicatechin (309.52 ± 2.84 mg/100g; **Figure 3C**), *p*-coumaric acid (82.43 ± 9.38 mg/100g; **Figure 3D**), and catechin (74.46 ± 2.82 mg/100g; **Figure 3E**) were the five major compounds in the lyophilized extract.



4 CONCLUSION

Moisture and ashes were higher in the grape artisanal pomace. Industrial exhibited high amounts of lipids, fiber, carbohydrates, and energy value. The extractive method was satisfactory for extracting diverse secondary metabolites from “Bordô” grape pomace. Both extracts displayed antioxidant capacity higher than 75%. Artisanal was rich in anthocyanins, despite presenting low total phenolic content. The employment of water as a solvent could increase the pharmaceutical and nutraceutical industries' potential use of the extract, corroborating a strong global trend regarding the circular economy and the precepts of sustainability and green products.

REFERENCES

1 MELO PS, MASSARIOLI AP, DENNY C, DOS SANTOS LF, FRANCHIN M, PEREIRA GE, VIEIRA TM, ROSALEN PL, DE ALENCAR SM. 2015. Winery by-products: extraction optimization, phenolic composition, and cytotoxic evaluation to act as a new source of scavenging reactive oxygen species. Food Chem. 181:160-9.

2 BRASIL. Lei nº 12.959, de 19 de março de 2014. Diário Oficial da União, Brasília, DF, 20 mar. 2014.

3 DA SILVA MJR, PAIVA APM, SOUZA JF, PADILHA CVDS, BASÍLIO LSP, LIMA MDS, PEREIRA GE, CORRÊA LC, VIANELLO F, LIMA GPP, MOURA MF, TECCHIO MA. 2022. Phytochemical profile of Brazilian grapes (Vitis labrusca and hybrids) grown on different rootstocks. PLoS One. 17(10):e0275489.

4 LI D, WANG P, LUO Y, ZHAO M, CHEN F. 2017. Health benefits of anthocyanins and molecular mechanisms: Update from recent decade. Crit Rev Food Sci Nutr. 57(8):1729-1741.

5 Official Methods of Analysis: 22nd Edition (2023)', in Dr. George W Latimer, Jr. (ed.), Official Methods of Analysis of AOAC INTERNATIONAL, 22.

6 BLIGH, E. G; DYER, W. J. 1959. A Rapid Method of Total Lipid Extraction and Purification. Canadian Journal of Biochemistry and Physiology, 37, 8, p. 911-917.

7 GIUSTI, M. M; WROLSTAD, R. E. 2001. Characterization and Measurement of Anthocyanins by UV-visible Spectroscopy. Handbook of Food Analytical Chemistry, 2, 2, p. 19-31.

8 SINGLETON, L. V; ORTHOFER, R; LAMUELA-RAVENTÓS, R. M. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin Ciocalteu Reagen. Methods in Enzymology, 299, p. 152-178.

9 YAMAGUCHI T, TAKAMURA H, MATOBA T, TERAO J. 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. Biosci Biotechnol Biochem. 62(6):1201-4.

10 ESPEJO F. 2021. Role of commercial enzymes in wine production: a critical review of recent research. Journal of Food Science and Technology, 58, 1, p. 9-21.

11 GARCIA-LOMILLO, J., & GONZÁLEZ-SANJOSÉ, M.L. 2017. Applications of wine pomace in the food industry: approaches and functions. Comprehensive reviews in food science and food safety, 16, 1, p. 3–22.

ACKNOWLEDGEMENTS

The authors would to thank the University of Caxias do Sul and the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq). Dr Banco is the recipient of a CNPq Research Fellowship (302314/2023-7).