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Chemical composition and mineral content of Black Borgoña (*Vitis labrusca L.*) grapes, pomace and seeds, and effects of conventional and non-conventional extraction methods on their antioxidant properties

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Abstract

Grape pomace and seeds, good source of functional compounds such as polyphenols, are waste by-products generated during wine making. Extraction is a very important step for later use of polyphenols. Herein we investigated the effects of conventional (CSE) and non-conventional extraction methods (subcritical water extraction (SWE) and pressurized ethanol extraction (PEE) on extraction yield, total phenolic content (TPC) and antioxidant capacity (AC) of Black Borgoña (*Vitis labrusca L.*) grape, pomace and seeds. Chemical composition showed the greatest amount of protein and lipids in pomace and seeds. Calcium was present in a high concentration in seeds (137.24 mg/100 g DM). PEE samples retained the highest TPC among all the extraction methods for grape, pomace and seeds with 21.42 ± 0.43, 54.36 ± 3.37 and 56.5 ± 3.81 mg GAE/g DM, respectively. The highest AC was determined by FRAP in seeds, 431.16 ± 37.71 μmol TE/g DM, followed by 329.04 ± 13.3 μmol TE/g DM in pomace when PEE was used. Significant correlations between antioxidant capacity and total phenolics were observed for all samples. This study demonstrated that non-conventional green method PEE is an efficient method to achieve the highest TPC and AC of Black Borgoña grape, pomace and seeds.

Keywords: *Vitis labrusca*; phenolics; antioxidants; pomace; green technology; pressurized liquids.

Practical Application: Black Borgoña grape pomace and seeds are good sources of nutrients and compounds with antioxidant capacity; therefore, they are great alternatives as functional ingredients to develop healthy foods. Extraction with pressurized liquids such as water and ethanol maximized total phenolics and antioxidant capacity of pomace and seeds. As a result, the extraction method was stated as the potential for utilizing grape pomace and seeds in food and pharmaceutical industries.

1 Introduction

Seventy-seven million tons of grapes were produced worldwide in 2019 while 645,545 tons were produced in Peru in the same year (Food and Agriculture Organization of the United Nations, 2021). Similar to other countries, grape utilizing Peruvian industry, such as wine, generates every year millions of tons of waste such as vine shoots, grape pomace (skins, seeds, stems), and wine lees that may be about 20% of weight of the processed grapes (Demirkol & Tarakci, 2018). In consequence, disposal of those by-products without treatment generates health and environmental concerns. Thus, waste management strategies have been stated in the industries around the world (Maroun et al., 2017). In addition, the use of these residues may represent significant economic gains (Rockenbach et al., 2011a). This is not the case in Peru where grape pomace that represents around 25% of the grape total weight (Beres et al., 2017) causes pollution, disposal difficulties, economic losses (Ilyas et al., 2021) and also health problems. For example, shoots are burnt in the field and release carcinogenic polycyclic aromatic hydrocarbons (catechin, naphthalene, etc.) and greenhouse gases (Maroun et al., 2017).

As cited by Rosales Soto et al. (2012), these waste by-products have various bioactive compounds, such as antioxidant polyphenols, which have potential health-promoting and disease-protective characteristics because of their high antioxidant activity. Moreover, foods with high content of antioxidants have been related to reduced risk of chronic health disorders including coronary heart diseases and cancer. Considering the growing interest of consumers in health-promoting molecules and functional foods with antioxidant characteristics, the recovery of bioactive molecules from grape processing by-products could be an alternative management solution. This is because these molecules may find applications as additives in foods, as well as valuable components in agriculture, pharmaceutical, and cosmetic industries (Maroun et al., 2017). Among them, phenolics are usually used for fortification of many food products, including fish and seafood, meat, juices as well as bakery and dairy products (Carullo et al., 2020). For example, grape pomace has been added to noodles, pancakes, and cereal bars (Rosales Soto et al., 2012) and muffins (Bender et al., 2017).

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On this regard, the extraction process plays a key role in the recovery of biomolecules as it affects both their quantity and quality. Solid-liquid extraction that is traditionally used and usually demands the consumption of large quantities of organic solvents and energy is not a good option due to the negative impact of those solvents on humans and the environment (Maroun et al., 2017). For this reason, environmental- friendly procedures known as "green chemistry" or non-conventional methods such as ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, solid-liquid extraction, pressurized liquid extraction, supercritical fluid extraction, and matrix solid phase dispersion (Tomaz et al., 2019) have been conceived as emerging options to mainly reduce the exposure to toxic solvents. In addition, green methods also require less amount of solvent and lower extraction times in comparison with conventional methods (Ilyas et al., 2021).

Pressurized liquid extraction (PLE), as affirmed by Belwal et al. (2020), has become a popular green extraction method for different compounds found in environmental, food and botanical samples. The achieved results clearly show the effectiveness of PLE and its applications on the industrial level. Nevertheless, the scale-up of this process is currently at an early stage so there is a great necessity to work on the challenges that this process involves. PLE is based on the use of solvents at high pressure (< 20 MPa), short extraction time (< 30 min), elevated temperature in the liquid state (25-200 °C), automatization of process and low solvent consumption (Petersson et al., 2010; Petkova et al., 2020). The required equipment is relatively simple as stated by Tena (2019), but expensive, and includes a solvent container, an oven that holds the extraction cell, a pump, blocking valves, and a collecting vial (Ebrahimi & Lante, 2022). If the liquid is water, it is called sub-critical water extraction (SWE) (Herrero et al., 2015) that allows extraction of organic solutes from different matrices (Abdelmoez & Abdelfatah, 2017). The water heating process reduces its surface tension, viscosity, and permittivity, increasing thus its diffusivity attributes. Water is maintained in its liquid form at high temperatures under an adequate pressure that allows to dissolve various molecules with low polarity (Maroun et al., 2017). Therefore, determination of phenolics and antioxidant capacity of grape, pomace and seeds extracts will help to assess how efficient these non-conventional methods are and compare them with some conventional methods.

The objectives of this study were to determine the proximal composition and presence of some dietary minerals in Black Borgoña (*Vitis labrusca*) grape, pomace and seeds. Also, the effects of non-conventional extraction methods such as pressurized alcohol and subcritical water extractions, on their total phenolic content and antioxidant capacity were evaluated.

2 Materials and methods

2.1 Grapes, pomace and seeds

Fifteen kg of Black Borgoña (*Vitis labrusca*) grapes and 50 kg of grape pomace were donated by the winery Maskay Pacha in Lomo Largo from the district of Sunampe, Chincha (Ica-Peru) (13° 25' 19.056" S, 76° 10' 9.912" W) at 64 m.a.s.l in March 2020. Grapes and fresh pomace were brought to the CITEagroindustrial Ica facilities and dried in a dryer (Vulcano, EQ-03SW, Peru) for 26 h and 18 h, respectively at 45 °C and a maximum air relative humidity of 80%. The dried pomace was sent to the Laboratorio de Compuestos Bioactivos del Instituto Tecnologico de la Produccion, where seeds were separated from a portion of the grape pomace. Dried material (grapes, pomace and seeds) was vacuum packed, protected from light and refrigerated at 5 °C. Before each analysis, samples were ground and passed through a 0.71 mm sieve. Ground grape seeds were previously defatted by supercritical carbon dioxide (Sánchez et al., 2018) before extraction.

2.2 Chemical reagents

Sulfuric acid (95-97%) for analysis (Merck, Germany), hydrochloric acid ultrapure reagent (32-35%) (J.T.Baker, Canada), calcium standard (1000 mg/L, Merck, Germany), iron standard ICP-27N-5 1000 ug/mL (Accustandard, USA), cooper standard ICP-15N-5 1000 ug/mL (Accustandard, USA), zinc standard (1000 mg/L, Sigma-Aldrich, Canada), monohydrated gallic acid (≥ 98.5%, ACS) (Sigma-Aldrich, China), sodium carbonate (≥ 99.9%) (Merck, USA), Folin Ciocalteu's phenol reagent (2N) (Sigma-Aldrich, USA), DPPH 2,2-diphenyl-1-picrylhydrazyl ≥ 96% (Alfa Aesar, Germany), ethanol 99.5% (Scharlau, Spain), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) ≥ 96% (Sigma Aldrich, China), TPTZ (Alfa Aesar, UK), ethyl acetate (Merck, HPLC, Germany), iron trichloride hexahydrate (Merck, Germany), deionized water supplied by the Barnstead water purification system (Barnstead, Model D11911, Germany), carbon dioxide 99.5% v/v liquefied gas (Linde, Peru), nitrogen atmosphere Ultrapuro (Linde, Peru).

2.3 Proximal compositional analysis

The proximal composition analysis of grapes, pomace and seeds was done according to Food and Agriculture Organization of the United Nations (1986). Moisture content was estimated using the gravimetric method by drying the sample in an oven (Venticell Ecoline, CzechRepublic). Ash content was determined by incinerating the dried sample overnight in a muffle furnace (Barnstead, Thermolyne, Model 48000, USA). Total nitrogen content was determined by Kjeldahl method using an automated Kjeldatherm TZ block digester (Germany) and a distillation unit Buchi K-350 (Spain). The fat content was determined by the Soxhlet method using a Universal Extractor Buchi E-800 (Switzerland). All analyses were performed in duplicates.

2.4 Determination of dietary minerals

Ca, Fe, Cu and Zn determinations were performed with an atomic absorption spectrophotometer (PerkinElmer, Analyst 800, USA) according to AOAC methods 968.08 (Association of Official Analytical Chemists, 1998). All analyses were performed in duplicates.

2.5 Preparation of extracts for phenolic and antioxidant capacity assays

Three extraction methods were selected for phenolics extraction of grapes, pomace and seeds: Conventional solvent

extraction (CSE), subcritical water extraction (SWE) and pressurized ethanol extraction (PEE).

Conventional Solvent Extraction (CSE)

The extraction of phenolics from grapes, pomace and seeds was done with ethanol-water mixture at 70% (v/v) in a ratio sample: solvent (1 : 12 w/v) according to Sanchez-Gonzales et al. (2019). Four g of dry grapes, pomace or seeds was weighed into a 100 mL glass bottle and extracted with 48 mL of either 15, 32.5, 50 or 75% (v/v) ethanol/water solution. The bottles were vortexed in a magnetic stirrer (IKA, RT 10, Germany) for 2 h at 60 rpm at 25 °C. The mixture was then filtered through Whatman No. 4 (20-30 µm) filter paper and stored at 5°C in airtight glass bottles until analysis. Extracts were analyzed in triplicate.

Pressurized fluid extraction

The pressurized fluid extraction system has been described previously by Sanchez-Gonzales et al. (2019). Briefly, the multi-solvent extractor equipment (Top Industrie, series 2802.0000, Vaux le Pénil, France) consisted of a CO_2 pump (HP Flow Pump 50-100), a co-solvent pump (90-2491 REV L, SSI, USA), a cooling system (PCPR 13.02-NED, National Lab, Germany) and a reactor (ø 163 x 353 mm).

Dried grapes, pomace or seeds (30 g) and five alternating layers of 3 mm glass beads (700 g) were filled into the extraction cell (709 mL) (internal diameter: 8 cm, internal height: 14.8 cm). Extractions were performed at 120 °C, and 100 bar according to Duba et al. (2015). The desired pressure was achieved by water or 50% ethanol solution absorption with a co-solvent pump at 20 mL/min and kept under these conditions for 2 h. The solvents used were deionized water for subcritical water extraction (SWE) and 50% ethanol for pressurized ethanol extraction (PEE). Solvents were previously degasified for 60 min at 25 °C in a sonicator (VWR International, SymphonyTM, 97043-942, China). Finally, extracts were cooled in an ice bath until full recovery of the extract from the system (60 min). Collected extracts were stored under refrigeration (4 ± 1 °C) until analysis. Extracts were analyzed in triplicate.

2.6 Extraction yield

The extraction yield of each method was expressed as weight percentage of the extract relative to the dried matter (DM) of the sample used for extraction, as described in Equation 1:

$$Extraction\ yield\,(\%) = \frac{\text{weight of extract}}{\text{weight of dry matter}} \times 100 \quad (1)$$

2.7 Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu procedure described by Singleton et al. (1999) with modifications. Briefly, 71 µL of extract were combined with 71 µL of Folin Ciocalteu, 1430 uL of 6% sodium carbonate (w/v) and 2000 uL of deioinized water. The mixture was allowed to stay in the darkness at room temperature for 1 h. The reading was carried out at 750 nm using a spectrophotometer model Genesys 180 (Thermo Scientific, USA) and the results were reported as mg gallic acid equivalent (GAE) per g of dry matter (DM) using a curve generated with standard solutions of 50, 100, 150, 200 and 400 mg/L.

2.8 Determination of Antioxidant Capacity (AC)

The AC of the grape, pomace and seeds extracts was measured using single electron transfer methods: DPPH and FRAP.

DPPH assay

The antioxidant capacity of grape, seeds and pomace extracts was determined as described by Brand-Williams et al. (1995) and modified by Kim et al. (2002). A Genesys 180 spectrophotometer (Thermo Scientific, USA) was used to determine the concentration of DPPH at 518 nm. A calibration curve was generated with Trolox standard solutions of 50, 100, 250, 500, 750 and 1000 µM. The decrease in absorbance of DPPH in methanol solution was measured against grape extracts with Trolox. The percentage of DPPH inhibition (Equation 2) was used to determine the minimal concentration of grape extract needed to inhibit 50% of free radicals (expressed as mg extract/mL) (IC50). The antioxidant capacity was expressed in µmol TE per g dry matter (DM).

$$\%\ \text{DPPH Inhibition} = \frac{\left(100 - \left[\text{DPPH}\right]\text{extract}\right)}{\left[\text{DPPH}\right]\text{control}} \text{x}100 \quad (2)$$

FRAP assay

The antioxidant capacity of grape extracts was determined using FRAP assay as described by Benzie & Strain (1996). Appropriate diluted samples were added to the FRAP reagent (acetate buffer pH 3.6, TPTZ (2,4,6-tripyridyl-s-triazine) and $FeCl_3\ 6H_2O$ in a ratio 25 : 2.5 : 2.5). The mixture was incubated at 20 °C for 30 min and the absorbance was measured with a Genesys 180 UV-VIS spectrophotometer (Thermo Scientific, USA) at 595 nm. A calibration curve was prepared using Trolox standard solutions of 50, 150, 300, 400, 500 and 600 µM. FRAP values were expressed as µmol TE per g dry matter (DM).

2.9 Statistical analysis

The experiment was performed in a completely randomized design with a 3 x 6 factorial scheme in 3 replicates. Factor A represented by sample type: grapes, pomace, and seeds, and factor B represented by extraction methods: 15, 32.5, 50 and 70% ethanol (v/v), subcritical water and pressurized ethanol. Data are reported as mean ± standard deviation (SD) and analyzed for significant differences using a one-way analysis of variance (ANOVA). Tukey's HSD multiple comparisons of means were determined with Minitab 17 (Minitab, USA) at the 0.05 confidence level and used to analyze both TPC and AC of Black Borgoña (*Vitis labrusca*) grape, pomace and seeds extracts. The Pearson test was performed to obtain correlation (r) values between TPC and AC (DPPH and FRAP) using SPSS statistic version 26 (IBM, Peru).

3 Results and discussion

3.1 Proximal compositional analysis and dietary minerals

Proximal composition of oven dried grape samples is shown in Table 1. Significant differences were found among samples with pomace and seeds showing the highest content of protein (11.92 and 12.13%, respectively) and lipids (11.90 and 12.05%, respectively). Moisture content varied between 8.19% and 17.05% for grape, pomace and seeds. Drying temperature and method affect moisture content in samples. Tseng & Zhao (2012) reported moisture content in the range of 4.40%-7.65% in red wine grapes (Pinot Noir and Merlot) pomace after using different drying methods, which were lower than 10.77% found in this study for Black Borgoña grape pomace. Ribeiro et al. (2015), determined moisture content of 2.85% and 8.52% for two *Vitis labrusca* grape pomace samples from Brazil. The protein and ash content of Black Borgoña grape pomace obtained in this study, 11.92 ± 0.43% and 5.55 ± 0.12%, respectively, agrees with the ranges reported by Bordiga et al. (2019) in pomace except for the lipids content that were higher in our study (11.9%).

Black Borgoña grape pomace and seeds are similar to cereals such as wheat in their content of macro and micronutrients. As specified by the Codex Alimentarius Commission (1995), wheat flour must contain a minimum of 7% protein. In this study, the protein content of Black Borgoña grape pomace and seeds was 11.92% and 12.13%, respectively, and no significant differences were found between them. Therefore, pomace and seeds can be used as partial substitutes for wheat flour. When comparing the protein content of some cereal flours such as corn (8.7%), quinoa (12.4%), wheat (10.5%) and kiwicha (12.2%) (García et al., 2017) with the results obtained in this study, Black Borgoña grape pomace and seeds had relatively higher or similar protein content.

Some mineral elements such as calcium (Ca), iron (Fe), cooper, (Cu), and zinc (Zn) were detected in the grape samples (Table 1). The results of the mineral analysis showed that seeds had the greatest amount of Ca (137.24 ± 1.54 mg/100 g) while pomace had the greatest amount of Fe (12.16 ± 0.04 mg/100 g) and Cu (1.18 ± 0.01). Black Borgoña grape seed flour has a higher calcium content than some cereal flours like corn (64 mg/100 g), quinoa (104 mg/100 g), wheat (36 mg/100 g) but lower than kiwicha (214 mg/100 g). Grape pomace has a higher amount of iron when compared to corn flour (2 mg/100 g), quinoa (9.65 mg/100 g), iron-fortified wheat flour (5.5 mg/100 g) and kiwicha (5.3 mg/100 g) (García et al., 2017).

No significant differences were found between the Zn content of pomace and seeds, 0.86 ± 0.03 and 0.81 ± 0.07 mg/100 g, respectively. According to the Tukey's test, there were significant differences in the content of Ca, Fe and Cu among grape, pomace and seeds ($p < 0.05$). Sousa et al. (2014), when studying grape pomace (*Vitis vinifera L.*), Benitaka variety from Brazil, found a lower value of calcium (0.44 mg/100 g), a similar value for zinc (0.98 mg/100 g) and a higher value of iron (18.08 mg/100 g) in comparison with the values determined in our study for Black Borgoña grape pomace.

These minerals such as iron are considered essential for the human body. As pointed out by Sousa et al. (2014), iron is associated with the production of blood cells, calcium helps with bone and teeth building and regulation of certain body processes, and zinc is crucial for the immune system. Differences in the proximate compositional analysis between this study and the literature could also be attributed to the variety of grape cultivar, geographic location, climate, seasonal influences, chemical composition including soil composition, irrigation system, maturity stage, viticulture techniques and efficiency in pressing during winemaking as mentioned by Ribeiro et al. (2015).

3.2 Extraction yield

TPC and AC are strongly dependent on the sample type, the extraction method, and the nature of the solvent. The presence of different compounds with varied chemical characteristics and polarities requires the selection of a suitable solvent (Drosou et al., 2015). Therefore, two solvents with different polarities were selected (water and ethanol). In addition, two different extraction systems were selected: A conventional solvent extraction (CSE) with 4 different concentrations of ethanol in water (15, 32.5, 50 and 70%), and two non-conventional methods: a pressurized ethanol extraction (PEE) and a pressurized water extraction (SWE).

Furthermore, those extractions methods were selected to study their effects on the extraction yield. The three grape samples under study exhibited the highest extraction yield with PEE as shown in Table 2. Nevertheless, no significant differences

Table 1. Proximate composition and dietary minerals of Black Borgoña (*Vitis labrusca*) grapes, pomace and seeds.

	Grapes	Pomace	Seeds
Moisture[1]*	$17.05^{a} \pm 0.21$	$10.77^{b} \pm 0.13$	$8.19^{c} \pm 0.23$
Protein[2]*	$2.82^{b} \pm 0.10$	$11.92^{a} \pm 0.43$	$12.13^{a} \pm 0.13$
Lipids[2]*	$1.23^{b} \pm 0.10$	$11.90^{a} \pm 0.25$	$12.05^{a} \pm 0.10$
Ash[2]*	$4.74^{c} \pm 0.19$	$5.55^{b} \pm 0.12$	$8.71^{a} \pm 0.11$
Ca[2]**	$25.96^{c} \pm 0.76$	$45.89^{b} \pm 0.23$	$137.24^{a} \pm 1.54$
Fe[2]**	$1.57^{c} \pm 0.07$	$12.16^{a} \pm 0.04$	$2.80^{b} \pm 0.02$
Cu[2]**	$0.37^{c} \pm 0.00$	$1.18^{a} \pm 0.01$	$0.92^{b} \pm 0.01$
Zn[2]**	$0.25^{b} \pm 0.01$	$0.86^{a} \pm 0.03$	$0.81^{a} \pm 0.07$

Means containing different superscript letters within the same row represent significant differences ($p < 0.05$). [1]Results were calculated based on a wet weight basis. [2]Results were calculated based on a dry weight basis. *Results were expressed as g/100 g. **Results were expressed as mg/100 g.

Table 2. Extraction yield of dried grape, pomace and seeds (g dried residue/100 g sample).

Extraction treatments	Grapes	Pomace	Seeds
Et15%	$79.27^{Ab} \pm 2.42$	$35.23^{Bbc} \pm 3.10$	$20.37^{Cab} \pm 3.41$
Et32.5%	$76.99^{Ab} \pm 2.64$	$32.26^{Bc} \pm 0.88$	$18.60^{Cb} \pm 0.82$
Et50%	$74.93^{Ab} \pm 0.83$	$30.79^{Bc} \pm 1.72$	$18.17^{Cb} \pm 1.21$
Et70%	$76.76^{Ab} \pm 2.71$	$30.72^{Bc} \pm 1.55$	$14.11^{Cb} \pm 0.86$
SWE	$77.18^{Ab} \pm 4.92$	$42.59^{Bb} \pm 1.95$	$14.45^{Cb} \pm 1.36$
PEE	$88.29^{Aa} \pm 3.20$	$54.46^{Ba} \pm 4.96$	$26.16^{Ca} \pm 4.66$

Means containing different upper case (row) or lower case (column) letters are significantly different ($p < 0.05$).

were observed between 15% ethanol (20.37 g/100 g) and PEE (26.16 g/100 g) for seeds. The extraction yields of grapes ranged from 74.93 to 88.29 g/100 g with the highest yield for PEE and lowest for 50% ethanol extraction. For pomace and seeds, extraction yield ranges varied from 30.72 to 54.46 g/100 g and 14.11 to 26.16 g/100 g, respectively, being highest for PEE and lowest for 70% ethanol extraction. When 70% ethanol and SWE methods were used, our extraction yields were similar to 15.31% as reported by Baydar et al. (2004) for the Narince variety when using an acetone:water:acetic acid (90:9.5:0.5) mixture.

3.3 Effect of different extraction methods on the total phenolic content and antioxidant capacity

As shown in the ANOVA table (Table 3), TPC and AC (DPPH and FRAP) were significantly affected by extraction methods and sample type ($p < 0.05$). There were interaction effects between extraction method and sample type on TPC, DPPH and FRAP.

3.4 Total phenolic content

Several factors such as drying and extraction methods may influence TPC. Grapes, pomace and seeds were analyzed for TPC and significant differences ($p < 0.05$) were found between their TPC as shown in Figure 1. This was to be expected since the phenolic concentration in grapes is dependent on the sample type and the extraction method as the main factors. Thus, seeds showed the greatest TPC when compared with TPC of grapes and pomace for each extraction method. Yilmaz et al. (2015) also determined the highest total phenolic content in seeds of 18 grape varieties analyzed.

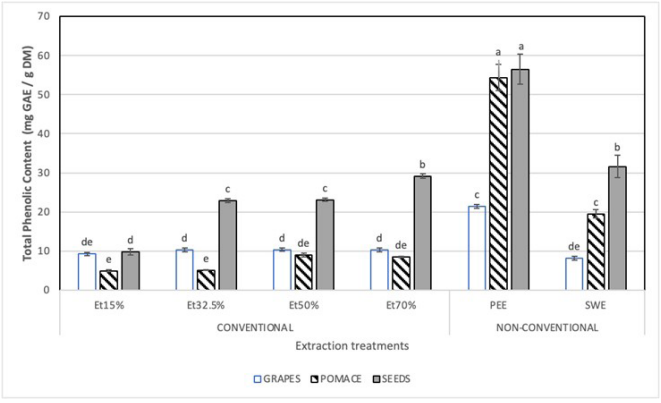

Figure 1. Total phenolic content of grapes, pomace and seeds using conventional ethanol extraction at different concentrations (15, 32.5, 50 and 75%), pressurized ethanol (PEE) and pressurized water (SWE). Results reported are mean of three determinations ± SD. Within treatments, bars with different letters at each sample type are significantly different according to Tukey's HSD comparison.

According to Chen et al. (2020), grape seeds extract mainly contains phenolic compounds with pharmacological properties such as anti-inflammatory, anticancer and antioxidant activities such as catechin, epicatechin, flavonols and others. Also as cited by Rockenbach et al. (2011a), grape extracts consist of anthocyanins from the skin and procyanidins from the seeds. Thus, phenolic compounds profile of each sample type may help us to elucidate the differences in TPC between samples considering that phenolics in grape are found roughly 10% in pulp, about 60% in seeds and 30% in the skins as cited by Bordiga et al. (2019). In addition, variation in TPC may be also due to the number of seeds present in the grape pomace. Then, extraction methods showed a significant effect on TPC of all grape samples (Figure 1). Statistically significant influence of the PEE method was observed in TPC found in grapes, pomace and seeds with pomace (54.36 ± 3.37 mg GAE/g DM) and seeds (56.5 ± 3.81 mg GAE/g DM) showing around twice the content observed in grapes (21.42 ± 0.43 mg GAE/g DM). Thus, the whole fractions of grape pomace, as well as seeds, can be considered important sources of polyphenols and, depending on the end use, separation of fractions in preliminary steps are not always necessary as cited by Rockenbach et al. (2011a). Furthermore, non-conventional methods, especially PEE, showed to be more effective than the conventional solvent extraction (CSE), regardless of the ethanol concentration, at extracting phenolic compounds from grape, pomace and seeds according to Tukey's HSD comparison ($p < 0.05$). Huaman-Castilla et al. (2019) evaluated TPC extracted from Carménère grape pomace with 15, 32.5 and 50% ethanol at high pressure and temperature, and determined the greatest TPC with 32.5% ethanol at 150 °C (~ 54 mg GAE/g DM) that was similar to 54.36 mg GAE/g DM obtained in our study with PEE (50% ethanol at 120 °C). Compared to our CSE, a greater amount of phenolics (13.96 mg GAE/g) was observed by Pertuzatti et al. (2020) in cultivar Bordo (*Vitis labrusca*) grape pomace while using a mixture of methyl alcohol, water, and formic acid (50:48.5:1.5 v/v/v). With similar conditions, Pereira et al. (2019) also obtained the highest TPC in grape (*Vitis vinifera* L. CV. Syrah) pomace while using pressurized liquid extraction with ethanol-water (50% w/w) at 100 °C and 10 Mpa.

As cited by Saldaña et al. (2021), pressurized fluid extraction has several advantages over CSE methods when conducted at atmospheric pressure. Thus, pressurized solvents remain in a liquid state above their boiling points which allows a high-temperature extraction and enhances diffusion of the solvent through the matrix and solubility of the components. Consequently,

Table 3. ANOVA table for bioactive compounds of grape samples.

Source	df	TPC		DPPH		FRAP	
		F-value	p-value	F-value	p-value	F-value	p-value
Sample type	2	672.84	0	2110	0	848.62	0
Extraction method	5	702.86	0	202.29	0	551.98	0
Extraction method x Sample type	10	95.97	0	39.46	0	92.71	0

extraction solvents that are inefficient in extracting phenolics, like anthocyanins at low temperatures, become efficient at elevated temperatures under pressurized fluid conditions. This was also confirmed by Ju & Howard (2003) who determined that high-temperature pressurized liquid extraction (80-100 °C) using acidified water, an environmentally friendly solvent, was as effective as acidified 60% methanol in extracting anthocyanins from grape skins.

Concerning PEE and SWE, there were significant difference between TPC from seeds, 56.5 ± 3.81 and 31.58 ± 2.87 mg GAE/g DM, respectively. This behavior was also observed for grapes and pomace. This significant decrease in TPC is due to solvent characteristics such as lower density of aqueous ethanol compared to the density of water, hence less mass of solvent used (Saldaña et al., 2021). Therefore, the use of pressurized ethanol would be favorable for a higher yield on the recovery of phenolics as we observed in this study. In addition, PEE resulted in an increase of TPC extracted from different grape samples due to the low dielectric constant of ethanol (25.02 ± 0.02 at 20 °C) (Saldaña et al., 2021).

Regarding the CSE methods used for seeds, 70% ethanol showed the highest TPC (29.16 ± 0.59 mg GAE/g DM) when compared to 15% ethanol (9.85 ± 0.84 mg GAE/ g DM). No significant differences were found between 32.5 and 50% ethanol. For pomace, no significant differences were found amount the different ethanol concentrations used. A similar scenario was observed for grapes. These results indicated that solvent concentration is an important factor in maximizing phenolic extraction from seeds. Higher ethanol concentrations (70% > 50% > 32.5% > 15%) were needed to maximize phenolic recovery in seeds. Jiménez-Moreno et al. (2019) observed that ethanol concentration was the only factor that had a significant influence on TPC and antioxidant capacity of Spanish grape extracts. Allcca-Alca et al. (2021) also found that an increase in the ethanol concentration allowed a greater recovery of TPC when hot pressurized liquid was applied through an accelerated extraction system with solvent for phenolics extraction in Negra Criolla grape seeds. Additionally, as cited by Ribeiro et al. (2015), the use of organic/alcoholic solvent-water mixture during the extraction can increase the permeability of cell tissue and thus enables better mass transfer by molecular diffusion that increases TPC in grape pomace. On the other hand, when comparing 70% ethanol and SWE, no significant differences in TPC were found for grapes and seeds, while a greater content of TPC was found for pomace when using SWE (19.54 ± 1.09 mg GAE/g DM). When Ahmed et al. (2020) used hexane as extraction solvent for different grape varieties, they found lower TPC (76.48-147.51 mg GAE/100 g DM) than the values achieved in this study.

In relation to the effect of drying conditions, grape pomace was dried for 18 h at 45 °C. Demirkol & Tarakci (2018) determined that different drying methods and temperatures influenced the biochemical changes in *Vitis labrusca L.* grape pomace. They used an acidified methanolic solution for phenolics extraction and found a value of 24.11 ± 1.04 mg GAE/g DM when a forced air oven was used at 40 °C for 72 h and 27.08 ± 0.75 mg GAE/g DM at 80 °C for 24 h. Those values were higher than those found for grape pomace in our study when CSE or SWE were used as shown in Figure 1. Even though, our drying process differed from the study cited above, PEE allowed the highest extraction of TPC in grape pomace (54.36 ± 3.37 mg GAE/g DM). Other drying methods such as an increase of temperature i.e., 80 °C and lyophilization could be coupled with the PEE method to determine if a substantial increase of TPC occurs.

3.5 Effect of extraction method on antioxidant capacity

DPPH

Figure 2 shows the effect of different extraction methods on AC determined by DPPH. Significant differences ($p < 0.05$) were observed among extracts. As it was seen for total phenolics, seeds had the highest AC (351.35 ± 20.75 µmol TE/g DM) when PEE was used. This was followed by 333.54 ± 14.63 µmol TE/g DM in seeds when SWE was used. No significant differences were observed between PEE and SWE methods in seeds. PEE also showed the highest AC in pomace and grapes (192.11 ± 13.89 and 128.17 ± 9.75 µmol TE/g DM, respectively) when compared with other extractions methods for each sample type. Similar results were also achieved by Allcca-Alca et al. (2021) who determined that Negra Criolla grape pomace hot pressurized liquid extracts had higher antioxidant activity than conventional extracts. Likewise, Otero-Pareja et al. (2015) determined that 50% ethanol/water as the pressurized solvent at 90 bar, 120 °C were the best conditions that allowed the highest antioxidant activity by DPPH in a variety of red grape Petit Verdot.

Since other factors also may influence the antioxidant capacity, Benbouguerra et al. (2020) reported the highest total AC with DPPH, in the skin Syrah grape extract, of (853 µmol TE/g DM) in the green stage compared with maturity (557 µmol TE/g DM). They also reported the highest antioxidant capacity at close to veraison in seeds than that found at the green stage and maturity.

About conventional methods, there were not significant differences in AC among grape samples. Also, no significant

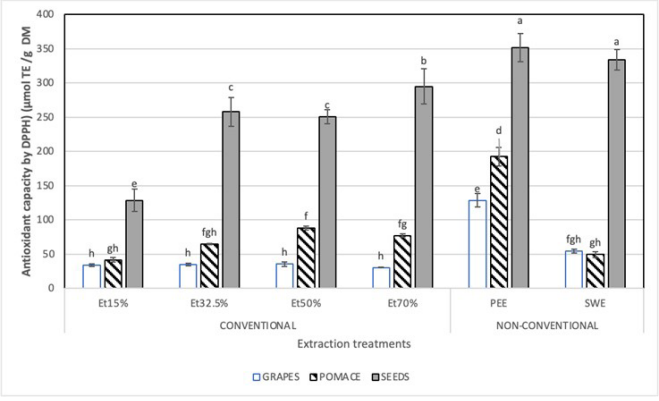

Figure 2. Antioxidant capacity of grapes, pomace and seeds by the DPPH method using the conventional ethanol extraction at different concentrations (15, 32.5, 50 and 75%), pressurized ethanol (PEE) and pressurized water (SWE). Results reported are mean of three determinations ± SD. Within treatments, bars with different letters at each sample type are significantly different according to Tukey's HSD comparison.

differences were observed between SWE and CSE irrespective of the ethanol concentration (Figure 2). No significant differences were found in AC from pomace when 32.5, 50 or 70% ethanol was used but a lower AC was determined with 15% ethanol. For seeds, 70% ethanol showed the highest AC when CSE was used (294.75 ± 25.49 µmol TE/g DM). As concluded by Jiménez-Moreno et al. (2019), generally the ethanol concentration is the most determinant parameter on the final composition of the extracts. Also, selection of extractions conditions will also depend on the desired compounds to be extracted and the source of those compounds. As a result, a higher or lower AC will be obtained.

FRAP

Figure 3 shows the effect of different extraction methods on AC determined by FRAP. Like TPC and AC by DPPH, seeds extracted with PEE, showed the greatest AC (431.16 ± 37.71 µmol TE/g DM) when compared with AC of grapes and pomace for all extraction methods. PEE also provided the highest AC in grapes and pomace, 115.01 ± 9.4 µmol TE/g DM and 329.04 ± 13.3 µmol TE/g DM, respectively, relative to other extraction methods used for each sample.

No significant differences were observed between the AC of grapes when either CSE or SWE was used. A similar scenario was observed for pomace. Contrary to what was observed for TPC, an increase of the ethanol concentration when CSE was used, resulted in a decrease of the AC for grapes. This was 45.07 ± 4.88 µmol TE/g DM with 50% ethanol and 36.9 ± 2.74 µmol TE/g DM with 70% ethanol but no significant differences were found between those values. As observed with AC by DPPH, CSE with 50% ethanol provided the highest AC by FRAP for pomace (67.46 ± 2.84 µmol TE/g DM) but no significant differences were found among the AC values at all ethanol concentrations. Rockenbach et al. (2011b) achieved higher AC by FRAP method when using a conventional acidified (0.1% HCl) methanol extraction of Cabernet Sauvignon (*Vitis vinifera* L.) grape pomace from Brazil (249.46 µMol TEAC/g DM). Also, when using CSE, our values were lower than the AC by FRAP found for Bordeaux and Isabel (*Vitis labrusca* L.) grape pomace, 208.43 and 117.79 µMol TEAC/g DM, respectively.

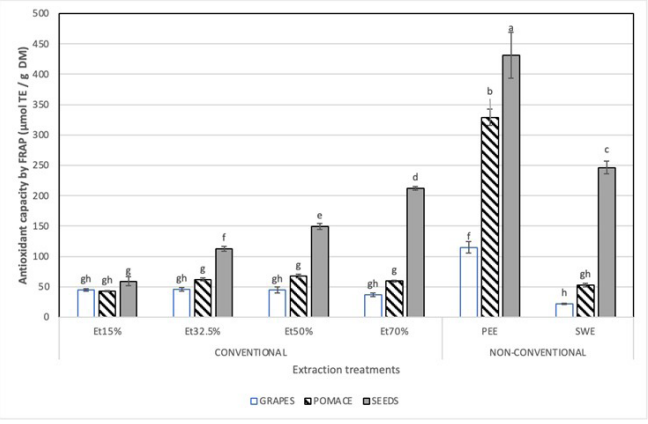

Figure 3. Antioxidant capacity of grapes, pomace and seeds by the FRAP method using the conventional ethanol extraction at different concentrations (15, 32.5, 50 and 75%), pressurized ethanol (PEE) and pressurized water (SWE). Results reported are mean of three determinations ± SD. Within treatments, bars with different letters at each sample type are significantly different according to Tukey's HSD comparison.

In this study, Pearson's coefficient showed that FRAP method led to strong correlation with TPC (r = 0.962) with a level of significance of 95%, indicating that antioxidant capacity of all sample types was related to the presence of phenolic compounds. Similar behaviors were observed for TPC vs DPPH (r = 0.77) and FRAP vs DPPH (r = 0.832). Pereira et al. (2019) also achieved a high and positive correlation coefficient for FRAP while studying Grape (*Vitis vinifera* L. CV. Syrah) pomace.

4 Conclusions

Black Borgoña (*Vitis labrusca*) grape pomace and seeds constitute nutrient-rich materials that can be used as substitutes or for fortifications of several food products due to their significant content of protein, lipids, and dietary minerals such as Fe in grape pomace and Ca in grape seeds. This study also reported the effects of non-conventional and conventional extraction methods on TPC and AC of Black Borgoña (*Vitis labrusca*) grape, pomace and seeds. Apart from the pretreatment process, the extraction procedure and solvents used affect the efficiency of the process. The pressurized ethanol extraction (PEE) at 120 °C and 100 bar presented great potential for the recovery of bioactive compounds from Black Borgoña grape, pomace and seeds, evidenced by high content of phenolics and antioxidant capacity compared to other extraction conditions under study. It is necessary to emphasize that different antioxidant capacity methods yield different results, probably due to the simultaneously present antioxidants in grape samples, which show different mechanisms of action. The results of this work confirm that the total phenolic and antioxidant capacity values of grape, pomace and seeds vary depending mainly on the extraction method used but also on the protocol of the test. Further studies should include alternative drying technologies to protect the bioactive compounds and a rigorous optimization of extraction parameters such as temperature and pressure.

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