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Characterization of Black Borgoña (*Vitis labrusca*) and Quebranta (*Vitis vinifera*) grapes pomace, seeds and oil extract

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Abstract

Proximal composition, dietary minerals, total phenolic compounds (TPC) and antioxidant capacity (AC) were quantified in Black Borgoña (BBGP) and Quebranta grapes pomace (QGP) from Chincha and Ica (Peru). TPC and AC of grapes seeds and oil were also investigated, as quality and fatty acids of the oil. The highest amount of protein was for QGP (Chincha) (13.29 ± 0.01 g/100 g dw) and lipids in BBGP (Ica) (13.67 ± 0.01 g/100 g dw). Fe in QGP (Chincha) (61.46 ± 2.76 mg/100 g dw) and Ca in BBGP (Ica) (424.24 ± 13.35 mg/100 g dw) were higher than in the other samples. The most abundant fatty acid was linoleic with 71.70 ± 0.01% in Black Borgoña grape seed oil (BBGSO) (Chincha). Highest TPC corresponded to Quebranta grape seeds (QGS) (Ica) (14.43 ± 0.75 mg GAE/100 g dw). The highest AC by DPPH and FRAP was achieved by QGS (Chincha) (1255.39 ± 61.45 μmol TE/g dw) and QGS (Ica) (960.77 ± 56.68 μmol TE/g dw), respectively. Differences in AC by ABTS for grape seed oil were not significant ($p > 0.05$). In conclusion, BBGP and QGP, seeds and oil showed an enormous potential for their application as functional additives in the food industry.

Keywords: Borgoña; Quebranta; pomace; fiber; antioxidant capacity; seed oil.

Practical Application: Black Borgoña and Quebranta grapes pomace, by-products of the Peruvian wine and pisco industries, are good sources of nutritious compounds, oil and phenolics that are mostly discarded. This oil has potential to be used in the food industry due to its high amount of linoleic acid. Pressurized ethanol extraction method was applied to maximize the total phenolics and antioxidant capacity of grapes pomace and seeds. As a result, the potential for utilizing grapes pomace, seeds and oil was stated as the cultivar areas and variety that provide the greatest extraction of bioactive compounds and other important nutrients.

1 Introduction

Wine production generates a great amount of waste. The disposal fee and fines for unauthorized discharges have increased considerably in some countries such as Spain, and sometimes a prison sentence is also imposed (Devesa-Rey et al., 2011). Similarly, the pisco industry in Peru also generates large quantities of grape pomace (skin and seeds) as cited by Allcca-Alca et al. (2021). Wine production in Peru was 17,986.4 and 21,841.2 thousand liters in 2020 and 2021, respectively, while Pisco production reached 4,446.5 and 6,349.5 thousand liters in the same years (Instituto Nacional de Estadística e Informática, 2022). Peruvian wine exports vary based on the wine variety. Among the largest wine Peruvian exporters, Burgundy Tabernero exported 49.5, 56.8 and 50.6 thousand liters of Tavern burgundy red wine in 2013, 2014 and 2015, respectively (Centro de Inteligencia de Negocios de Mercado, 2016). It is estimated that 25% of grapes results in pomace (Dwyer et al., 2014), thus it is critical to characterize it and provide alternatives for its use in the food industry and give added value to this by-product.

In recent years, agro-industrial by-products, mainly derived from fruits processing and consumption, have become an important source of bioactive products, such as fiber with or without prebiotic potential, and polyphenols with antioxidant activity against free radicals (Pérez-Chabela et al., 2022). Studies on grape pomace and seeds indicate that they are rich in phenolic compounds and have antioxidant properties. Therefore, companies must invest in new technologies to reduce the negative impact of agro-industrial waste on the environment and establish new processes that provide an additional income (Devesa-Rey et al., 2011), and new food sources for human consumption. As an example, previous studies reported the utilization of grape pomace in cookies that resulted in a product with a high content of dietary fiber and great acceptability by consumers (Canett Romero et al., 2004), as well as a significant increase in polyphenols as determined also in cookies by Lou et al. (2022). Guldiken et al. (2021) mentioned that food processing by-products have been used in some functional food innovations because they provide functional macro and micronutrients in a profitable way. Balli et al. (2021) concluded that by-products of wine and olive oil processes are suitable for new healthy staple foods because of their great content of bioactive compounds.

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As determined by Jagadiswaran et al. (2021), waste and by-products of the food industry are often overlooked and used or underused. Zhan et al. (2022) concluded that proanthocyanidins, extracted from grape seeds, have a protective effect on spinal cord injury in rats and that this may be due to the role of those compounds on the reduction of oxidative stress and inflammation in the spinal cord tissues.

Ferri et al. (2020) carried out research to optimize and validate possible routes towards the full recovery of grape residues to produce bioactive molecules and new materials. Within the identification of the 100 richest dietary sources of polyphenols, grapes and their by-products are included, leading clove and pepper, as well as cocoa, oregano, coffee among others (Pérez-Jiménez et al., 2010). Extraction methods for the recovery of different by-products such as oil and bioactive compounds, from the residues of several varieties of grapes, have been extensively reported. In the attempt to promote environmental-friendly methods, non-conventional methods are emerging options (Chowdhary et al., 2021). Some techniques that stand out are microwaves, ultrasound, supercritical CO_2 and pressurized liquid extraction (PLE) that requires less amount of solvent and lower extraction times in comparison with conventional methods (Ilyas et al., 2021). When using PLE, the elevated temperature accelerates the extraction and increases diffusion rate and solubility of the analytes. In addition, higher pressure facilitates extraction of the compounds contained in the pores of the matrix (Tomaz et al., 2019).

Information about bioactive compounds in grape pomace and seeds, by-products of the wine industry, and their extraction techniques is abundant. On the contrary, more research is needed about the influence of the non-conventional extraction methods on those compounds in Borgoña (*Vitis labrusca*) and Quebranta (*Vitis vinifera*) grape pomace, seeds and oil. The objectives of this study were to compare the proximal composition, fiber, mineral nutrients, phenolics and antioxidant capacity of Black Borgoña and Quebranta grapes pomace from Chincha and Ica (Ica-Peru), as well as to evaluate the bioactive compounds of pomace, seeds and oil, and functional quality of grape seed oil. Thus, this comparative analysis will help to determine the future application of Black Borgoña and Quebranta grapes by-products in the food industry or other areas of interest.

2 Materials and methods

2.1 Grape pomace and seeds

Twenty-five kg of Black Borgoña and Quebranta grapes pomace was obtained from Chincha and Ica (Ica-Peru) cultivar zones as shown in Table 1. The fresh pomace was brought to the Agroindustrial Guadalupe (Ica-Peru) and dried in a dryer (Vulcano, EQ-03SW, Peru) at 45 °C and a maximum air relative humidity of 80% for 18 hours. Then, 1 kg of dried pomace was passed through a 7 mm sieve (KM Testing sieve, Japan) to separate the seeds, which were placed in a forced air convection oven (Venticell, model LSIS-B2V / VC 222, USA) at 40 °C for 6 hours until they reached 7% as the maximum moisture content. The dried seeds and the remaining dried pomace were ground in an analytical mill (A 11 Basic, IKA, USA). Finally, dried and ground Black Borgoña and Quebranta grape pomace (BBGP and QGP, respectively) (0.35-0.71 mm) and Black Borgoña and Quebranta grape seeds (BBGS and QGS, respectively) (0.35-0.71 mm) were passed through 25 mesh (0.707 mm) and 35 mesh (0.500 mm) sieves (Retsch, Lima, Peru). The particles retained by both sieves were vacuum packed in polyethylene bags, protected from light and refrigerated at 5 ± 1 °C until later use.

2.2 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), magnesium standard ICP-32N-5 (1000 ug/mL, Accustandard, USA), potassium phosphate monobasic standard crystal ACS (J.T. Baker, USA), sodium standard ICP-54N-5 (1000 ug/mL, Accustandard, USA), potassium standard ICP-43N-5 (1000 ug/mL, Accustandard, USA). Methanol HPLC grade (JT Baker, USA), 99.9% absolute ethanol (Scharlau, Spain), acetone (Merck, USA), a 37-component fatty acid methyl ester (FAME) mixture (C4-C24) (Sigma-Aldrich, USA), gallic acid monohydrate ≥ 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) (95%) (Alfa Aesar, Germany), Trolox (6-hydroxy-2,5,7,8-tetramethylchrome-2-carboxylic acid) (97%) (Sigma-Aldrich, China), TPTZ (Alfa Aesar, UK), hydrochloric acid (JT Baker, Canada), iron trichloride hexahydrate (Merck, Germany), deionized water supplied by the Barnstead

Table 1. Black Borgoña and Quebranta grape pomace.

Cultivar zones	Black Borgoña (*Vitis labrusca*)	Quebranta (*Vitis vinifera*)
Chincha	Viñedo Maskay Pacha de Lomo Largo. Sunampe 13° 25' 19.056" S 76° 10' 9.912" W	Viñedo Maskay Pacha de Lomo Largo. Sunampe 13° 25' 19.056" S 76° 10' 9.912" W
Ica	Santiago Vineyard 14° 11' 2.256" S 75° 42' 51.840" W	Salas Guadalupe Vineyard 13° 59´57.07" S 75° 46´12.62" W

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 grape pomace 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 the 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 duplicate.

2.4 Determination of fiber

The fiber content determination was performed according to AOAC method 985.29 (Association of Official Analytical Chemists, 2005). All analyses were performed in duplicate.

2.5 Determination of dietary minerals

Ca, Fe, Zn, Na, K, Ca, Mg and P determinations were performed with an atomic absorption spectrophotometer (PerkinElmer, Analyst 800, USA) according to AOAC method 968.08 (Association of Official Analytical Chemists, 1990). All analyses were performed in duplicate.

2.6 Grape seed oil extraction by Supercritical CO_2

The Black Borgoña grape seed oil (BBGSO) and Quebranta grapes seed oil (QGSO) extractions with supercritical CO_2 were carried out with a multi-solvent extractor equipment Model 2802.000 (Top Industrie, France) as described by Barriga-Sánchez & Rosales-Hartshorn (2022) with a CO_2 flow of 40 g/min for 180 min. Carbon dioxide (99.5% v/v liquefied gas) was used for the extraction process and oil was stored in a nitrogen atmosphere at 5-8 °C. Oil samples were kept at room temperature (20-21 °C) for 2 hours before analysis. Analyses were performed in duplicate.

The global grape seed oil yield was calculated as the mathematical relationship between the extracted oil and mass of the sample (dry basis) according to Equation 1 (Pinto et al., 2020).

$$Y_{(\%db)} = \left(\frac{m_o}{m_a\left(1-\frac{U_a}{100}\right)} \right) \times 100 \quad (1)$$

Where: $Y_{(\%db)}$ is global grape seed oil yield (%), m_0 is oil mass (dry basis), m_a is sample mass, and U_a is sample moisture content (6%)

2.7 Peroxide value (POV)

Oil sample was dissolved in a mixture of acetic acid and chloroform (3:2) Then, a potassium iodide solution was added to liberate iodine. The titration was carried out with a standardized solution of sodium thiosulfate until starch turned from a light blue color to a transparent hue (Association of Official Analytical Chemists, 2012).

2.8 α-tocopherol

Tocopherol extraction was carried out as explained by Shiozaki & Murakami (2016) and α-tocopherol was quantified as described by Martínez-Rojano et al. (2016). Two mL of ethanol was added to grape seed oil extract, passed through 0.2 µm pore nylon filters and injected into the HPLC equipment (Series 200, Perkin Elmer, USA) with a UV detector at 290 nm, equipped with a Kromasil C18 reversed phase column of 5 µm and dimensions 4.6 x 150 mm. The isocratic mobile phase consisted of methanol: water (96: 4), injection volume: 50 µL, temperature: 40 °C at a flow rate of 2 mL/min. The α-tocopherol content was expressed as mg of α-tocopherol per 100 g of sample, using a curve generated with standard solutions of 3, 6, 9, 12 and 15 mg of α-tocopherol (≥ 96%) per liter in ethanol.

2.9 Fatty acids profile

The fatty acid profile was determined as described by Prevot & Mordret (1976) as cited by

Barriga-Sánchez et al. (2022a). Peak areas were calculated using the TotalChrom Navigator software (Version: 6.2.0.0.0:B27, 2001, USA), and the percentage of each fatty acid was determined by comparing each peak area with the fatty acids total area.

2.10 Preparation of extracts for phenolic compounds and antioxidant capacity assays

Pressurized ethanol extraction (PEE) system, previously described by Barriga-Sánchez & Rosales-Hartshorn (2022), was selected for phenolics extraction of grapes pomace and seeds.

Pomace

Dried pomace (40 g) (0.35-0.71 mm) and five alternating layers of 3 mm glass beads (700 g) were filled into the extraction cell (internal diameter: 8 cm, internal height: 14.8 cm). The solvent used was ethanol: water (50/50).

Seeds

Approximately, 15 g of dried defatted grape seeds and three alternating layers of 3 mm glass beads (630 g) were filled into the extraction cell (internal diameter: 8 cm, internal height: 14.8 cm). The solvent used was ethanol: water (70/30).

Each extraction, for seeds and pomace respectively, was performed at 120 °C according to Duba et al. (2015) and 170 bar. The desired pressure was achieved by the corresponding ethanol solution cited above, absorption with a co-solvent pump at 20 mL/min and kept under these conditions for 2 h. 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 duplicate.

2.11 Total phenolic content (TPC)

The phenolic compounds of grape seed oil were extracted with a methanol: water mixture in a 90:10 ratio (Bail et al., 2008). TPC of grape pomace, seeds and oil was determined according to Singleton et al. (1999). A gallic acid standard curve was prepared with concentrations of 50, 100, 150, 200 and 400 mg/L. The readings were carried out at 750 nm with a UV-VIS spectrophotometer (Perkin Elmer, Perkin Elmer®, LAMBDA 950, USA). The results were expressed in g of gallic acid equivalent (GAE) per 100 g of pomace or seeds (dw) and mg GAE per 100 g of oil (dw).

2.12 Antioxidant capacity (AC)

ABTS

The 2-2′-azino-bis (3-ethyl- benzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging capacity test of grape seed oil was performed according to Prior et al. (2005). Previously, grape seed oil samples were extracted with a methanol: water mixture in a 90:10 ratio (Bail et al., 2008). The results were expressed as µmol of Trolox equivalent (TE) per gram of oil using a reference curve with concentrations of 0.1, 0.5, 1.0, 1.5 and 2.0 mM Trolox in methanol.

DPPH

AC of grape pomace and seeds was determined according to Kim et al. (2002). A calibration curve was generated with Trolox standard solutions of 50, 100, 250, 500, 750 and 1000 µM. Absorbance was measured in a UV-VIS spectrophotometer at 518 nm and the percentage inhibition of different concentrations of grape pomace or seeds extracts against DPPH radical was calculated. The concentration necessary to inhibit 50% of DPPH radicals was expressed in µg extract/mL (IC_{50}). Also, IC_{50} for Trolox was determined to express the AC for Trolox with respect to AC of the extract which was expressed in µmol Trolox Equivalent (TE) per g sample (dw).

FRAP

AC of grape pomace and seeds was determined according to the methodology of 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). Absorbance was measured with UV-VIS spectrophotometer (Perkin Elmer, Lambda 850, 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 of Trolox Equivalent (TE) per g sample (dw).

2.13 Statistical analysis

A complete randomized design (CRD) of 1 x 4 with 2 repetitions was used to evaluate the effects of grape variety and cultivar zones on the proximal composition, fiber content, dietary minerals, TPC and AC of grape pomace. Also, the effects of grape variety and cultivar zones on grape seed oil yield, POV, α-tocopherol, fatty acids, TPC and AC were evaluated. Experimental results were analyzed for significant differences using a one-way analysis of variance (ANOVA). Tukey's HSD multiple comparisons of means were determined at the 0.05 confidence level. All analyzes were carried out with the SPSS statistic software v. 20 (IBM, Peru). Data were calculated with Excel 2016 (Microsoft, USA) and reported as mean ± standard deviation (SD).

3 Results and discussion

3.1 Proximal compositional analysis, fiber content and dietary minerals of grape pomace

Proximal composition of dried grape pomace is shown in Table 2. Significant differences were found between Black Borgoña and Quebranta grapes pomace. Protein content found in this study varied from 9.83 to 13.29% with QGP from Chincha showing the greatest amount. This protein value was lower than the found by Canett Romero et al. (2004) in Carignan noir grape pomace (skin) (14.72%). No significant differences were observed between the protein content of BBGP from Chincha and QGP from Ica. While studying BBGP from Chincha, Barriga-Sánchez et al. (2022b) reported protein and lipids content of 11.92 ± 0.43% and 11.90 ± 0.25%, respectively that fell within the ranges found in the present study. The lowest ash content was showed by QGP from Ica (5.73 ± 0.02%). Regarding lipids, they were found in the range of 8.76 to 13.67% which were higher than the value reported by Canett Romero et al. (2004) of 1.07% in Carignan noir grape pomace (skin). BBGP from Ica had the greatest lipid content (13.67 ± 0.01%). No significant differences in lipids were observed in QGP, regardless of location. Mohamed Ahmed et al.

Table 2.- Proximal composition of Black Borgoña (*Vitis labrusca*) and Quebranta (*Vitis vinifera*) grapes pomace (g/100 g pomace).

Cultivar zone	Pomace	Moisture[1]	Protein[2]	Lipids[2]	Ash[2]	Fiber[2]
Chincha	BBGP	9.77 ± 0.01[a]	9.83 ± 0.21[c]	10.31 ± 0.08[b]	6.46 ± 0.11[c]	55.07 ± 0.11[c]
	QGP	5.58 ± 0.01[c]	13.29 ± 0.01[a]	8.76 ± 0.04[c]	9.73 ± 0.07[a]	57.76 ± 0.06[a]
Ica	BBGP	5.18 ± 0.02[d]	11.40 ± 0.06[b]	13.67 ± 0.01[a]	7.78 ± 0.05[b]	55.43 ± 0.06[b]
	QGP	8.22 ± 0.06b	10.17 ± 0.22[c]	8.80 ± 0.12[c]	5.73 ± 0.02[d]	52.46 ± 0.08[d]

Data are expressed as the mean ± standard deviation from two independent extracts (n = 2). Means containing different superscript letters within the same column represent significant differences ($p < 0.05$). [1]Results were calculated on wet weight basis. [2]Results were calculated on a dry weight basis.

(2020) reported moisture (20.64 - 28.47%), protein (9.28 - 14.41%), lipids (6.44 - 10.47) and ash content (3.21 - 6.86) in ten varieties of grape pomace from Mersin and Konya provinces in Turkey. QGP from Chincha showed the greatest amount of fiber (57.76 ± 0.06%). As mentioned by Zhu et al. (2015), grape pomace is rich in phenolic compounds and dietary fiber. Therefore, it can be used as a dietary supplement that combines fibers and antioxidants with several benefits on human health such as prevention of cancer and cardiovascular diseases.

Some mineral elements such as cooper (Cu), iron (Fe), zinc (Zn), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and phosphorous (P) were detected in BBGP and QGP as shown in Table 3. The mineral analysis showed that QGP from Chincha had the greatest amount of Cu, Fe, Zn, Na, K and P. No significant differences in calcium were observed between BBGP from Chincha and Ica, 359.41 ± 43.20 and 424.24 ± 13.35 mg/100 g, respectively, while the latter had the greatest amount of magnesium (107.12 ± 0.27).

No significant differences were found between the Zn and P content of pomace samples from Ica and BBGP from Chincha. Similarly, no significant differences were observed in K and Mg content between BBGP from Chincha and QGP from Ica. Sousa et al. (2014) found a lower value of calcium (0.44 mg/100 g) and zinc (0.98 mg/100 g) in *Vitis vinifera* grape pomace from Brazil. The aforementioned authors also reported an iron content of 18.08 mg/100 g which was lower than values determined in this study for BBGP and QGP from Chincha and QGP from Ica (24.54 ± 0.84, 61.46 ± 2.76 and 42.56 ± 2.12 mg/100 g, respectively). When studying Pinotage (*Vitis vinifera*) grape pomace, Chikwanha et al. (2018) determined a Ca content of 3.62 g/kg DM similar to the values found for BBGP from Chincha and Ica. They also reported a Cu value of 9.08 mg/kg DM that was higher than those reported for BBGP but lower than values found for QGP from Chincha and Ica. A similar trend was observed when comparing Fe content of Pinotage grape pomace (166 mg/kg DM) that was lower than the determined for QGP from Chincha and Ica (614.6 and 425.6 mg/kg, respectively).

Dietary minerals such as iron and calcium are 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 and dietary minerals between this study and the literature could also be attributed to the grape cultivars, geographic location, climate, seasonal influences, soil composition, irrigation system, maturity stage, viticulture techniques and efficiency in pressing during winemaking as stated by Ribeiro et al. (2015).

3.2 Yield, POV, α-tocopherol and fatty acids of grape seed oil

Extraction yield

Oil extraction yields of Black Borgoña (*Vitis labrusca*) grape seeds from Chincha and Ica on a dry basis (db%) were significantly different ($p<0.05$) with values of 10.85 ± 0.15% (w/w) and 13.16 ± 0.52% (w/w), respectively. On the other hand, no significant differences ($p>0.05$) were found between Quebranta (*Vitis vinifera*) from Chincha and Ica with oil yield values of 12.57 ± 0.37% (w/w) and 11.97 ± 0.41% (w/w) (Ica), respectively. The greatest amount of oil yield was obtained from BBGSO from Ica; even though no significant differences were observed between BBGSO (Ica) and QGSO from Chincha and Ica. Rodriguez & Ruiz (2016) pointed out that grape seeds are essentially (w/w) fiber (40%), oil (16%), protein (11%), phenolics (7%) like tannins and other compounds such as sugars and minerals. Also, grape seeds contain non- phenolic antioxidants such as tocopherols and β- carotene that are mainly concentrated in grape seed oil.

3.3 POV

BBGSO and QGSO from Chinch and Ica, complied with the quality limit of 15 milliequivalents of active oxygen/kg oil established by Codex Alimentarius (2019) for cold pressed and virgin oils. In this study, the highest POV (Table 4) was determined for QGSO from Ica and Chincha, 1.91 ± 0.18 and 1.54 ± 0.14 milliequivalents of peroxide/kg oil, respectively, although no significant differences were found between the BBGSO and QGSO from Chincha. Barriga-Sánchez et al. (2018) found a POV of 38.44 ± 0.44 milliequivalent/kg for Quebranta grape seed oil (*Vitis vinifera*) extracted with hexane. Our lower number of POV indicates a good quality of oil and a good preservation status, possibly due to the supercritical CO_2 oil extraction technique used. In addition, as stated by Coelho et al.

Table 3. Dietary minerals of Black Borgoña (*Vitis labrusca*) and Quebranta (*Vitis vinifera*) grapes pomace (mg/100 g).

	Chincha		Ica	
	BBGP	QGP	BBGP	QGP
Cu	0.80 ± 0.07[b]	1.92 ± 0.06[a]	0.11 ± 0.00[c]	1.14 ± 0.20[b]
Fe	24.54 ± 0.84[c]	61.46 ± 2.76[a]	6.71 ± 0.11[d]	42.56 ± 2.12[b]
Zn	1.22 ± 0.04[b]	2.00 ± 0.10[a]	1.12 ± 0.01[b]	1.28 ± 0.02[b]
Na	20.09 ± 0.40[c]	34.01 ± 0.98[a]	2.90 ± 0.23[d]	26.12 ± 2.17[b]
K	1085.58 ± 26.85[c]	1655.94 ± 16.10[a]	1254.57 ± 60.18[b]	1022.95 ± 25.66[c]
Ca	359.41 ± 43.20[ab]	272.22 ± 16.22[bc]	424.24 ± 13.35[a]	255.84 ± 13.50[c]
Mg	73.09 ± 2.75[c]	86.12 ± 0.35[b]	107.12 ± 0.27[a]	79.29 ± 1.89[bc]
P	183.01 ± 19.30[b]	306.27 ± 15.29[a]	214.72 ± 20.91[b]	195.68 ± 12.38[b]

Data are expressed as the mean ± standard deviation from two independent samples (n = 2). Means containing different superscript letters within the same row represent significant differences ($p < 0.05$). Results were calculated on a dry weight basis.

(2020), besides CO_2 generating cleaner extracts than other conventional extraction techniques, it is a cheap, safe, non-toxic and easy to recycle solvent.

3.4 α-tocopherol

The highest concentration of α-tocopherol was found in QGSO from Chincha (21.85 ± 0.50 mg α-tocopherol/100 g oil) (Table 4). This value was higher than the values determined by Mohamed et al. (2016) in grape seed oils, from Northern Italy, extracted by supercritical CO_2 (8.7 to 17.4 mg α-tocopherol/100 g oil). Converting 21.85 ± 0.50 mg α-tocopherol/100 g oil to 2.75 mg α-tocopherol/100 g grape seed to allow for a comparison, we observed that our concentration was higher than the found by Agostini et al. (2012) for Isabel (*Vitis labrusca*) (0.39 mg α-tocopherol/100 g grape seed). BBGSO from Chincha and QGSO from Ica had α-tocopherol values (6.13 ± 0.19 and 7.46 ± 0.02 mg α-tocopherol/100 g oil, respectively), which fell within the range of 3.63 to 7.84 mg α-tocopherol/100 g oil reported by Dimić et al. (2020) in red grape (*Vitis vinifera L.*) seeds oil extracted with supercritical CO_2.

Table 4. Valor peroxide and α-tocopherol of Black Borgoña and Quebranta grapes seed oil.

Cultivar zones		POV (meq peroxide/kg oil)	α-tocopherol (mg α-tocopherol /100 g oil)
Chincha	BBGSO	1.22 ± 0.14^{bc}	6.13 ± 0.19^{d}
	QGSO	1.54 ± 0.14^{ab}	21.85 ± 0.50^{a}
Ica	BBGSO	1.06 ± 0.18^{c}	9.18 ± 0.16^{b}
	QGSO	1.91 ± 0.18^{a}	7.46 ± 0.02^{c}

Data are expressed as the mean ± standard deviation from two independent extracts (n = 2). Means containing different superscript letters within the same column represent significant differences ($p < 0.05$).

Concentrations of α-tocopherol reported in this study (Table 4) were higher than levels of α-tocopherol established by Codex Alimentarius (2019) for grape seed oils (1.6 - 3.8 mg/100 g oil). This contribution is extremely beneficial in the diet considering that vitamin E, also called α-tocopherol, acts as an antioxidant when protects the cells from damage caused by free radicals and boosts the immune system. Also, the recommended daily intake of vitamin E as specified by NIH (2020) is 15 mg for adults and the amount for children 1-13 years old varies from 6-11 mg. Therefore, these grapes under study may have an important role at fulfilling this daily recommended amount.

3.5 Fatty acid profile of grape seed oil

BBGSO from Chincha showed the highest concentration of unsaturated fatty acids (89.65 g/100 g oil) followed by QGSO from Ica with 89.22 g/100 g oil (Table 5). Among the unsaturated fatty acids, the highest concentration was found for linoleic acid (C18: 2ω-6) in all grape samples with 71.70 ± 0.01 g/100 g oil as the highest value for BBGSO from Chincha, concentration that falls within the range specified by FAO (Codex Alimentarius, 2019) for grape seed oil and which is also similar to the value reported by Dalposso et al. (2022) (74.49 ± 3.78 g/100 g of grape seed oil). No significant differences were found between linoleic acid concentrations of BBGSO from Ica and QGSO from Chincha, 66.94 ± 0.46 and 67.74 ± 0.17 g/100 g oil, respectively. Oleic acid followed with the highest value found for BBGSO from Ica, 19.22 ± 0.39 g/100 g oil, as shown in Table 5. No significant differences were found between the oleic acid concentrations of BBGSO from Ica and QGSO from Ica and Chincha.

Linoleic acid, a polyunsaturated fatty acid (PUFA), is associated with human health because of its role at promoting cardiovascular health in animal models as cited by Martin et al.

Table 5. Fatty acid profile of Black Borgoña (*Vitis labrusca*) and Quebranta (*Vitis vinifera*) grape seed oil from Chincha and Ica extracted by supercritical CO_2.

Fatty Acids	Oil extraction yield (g/100 g seed oil)			
	Quebranta		Black Borgoña	
	Chincha	Ica	Chincha	Ica
C 14:0 (Myristic)	0.04 ± 0.0^{c}	0.04 ± 0.0^{c}	0.07 ± 0.01^{b}	0.12 ± 0.01^{a}
C 16:0 (Palmitic)	7.03 ± 0.11^{b}	6.96 ± 0.04^{b}	6.99 ± 0.02^{b}	7.91 ± 0.04^{a}
C 16:1 (Palmitoleic)	0.09 ± 0.01^{c}	0.10 ± 0.0^{c}	0.13 ± 0.01^{b}	0.22 ± 0.01^{a}
C 18:0 (Stearic)	4.72 ± 0.08^{a}	3.61 ± 0.01^{c}	3.14 ± 0.01^{d}	3.90 ± 0.04^{b}
C 18:1 ω-9 (Oleic)	18.92 ± 0.06^{a}	19.14 ± 0.09^{a}	16.23 ± 0.01^{b}	19.22 ± 0.39^{a}
C 18:1 ω-7 (Vaccenic)	0.64 ± 0.04^{a}	0.69 ± 0.09^{a}	0.85 ± 0.01^{a}	0.84 ± 0.05^{a}
C 18:2 ω-6 (Linoleic)	67.74 ± 0.17^{c}	68.80 ± 0.06^{b}	71.70 ± 0.01^{a}	66.94 ± 0.46^{c}
C 18:3 ω-6 (γ-Linolenic)	0.11 ± 0.00^{a}	0.05 ± 0.00^{b}	0.10 ± 0.01^{a}	0.06 ± 0.01^{b}
C 18:3 ω-3 (α-Linolenic)	0.31 ± 0.01^{b}	0.30 ± 0.00^{b}	0.50 ± 0.01^{a}	0.47 ± 0.01^{a}
C 20:4 ω-6 (Arachidonic)	0.21 ± 0.00^{a}	0.17 ± 0.00^{b}	0.14 ± 0.01^{c}	0.16 ± 0.01^{b}
C 20:1 ω-9 (Eicosenoic)	0.18 ± 0.00^{a}	0.14 ± 0.00^{a}	0.14 ± 0.01^{a}	0.16 ± 0.01^{a}
Saturated Fatty Acids (SFA)	12	10.78	10.35	12.09
Monounsaturated Fatty Acids (MUFA)	19.84	20.07	17.35	20.44
Polyunsaturated Fatty Acids (PUFA)	68.16	69.15	72.3	67.47

Data are expressed as the mean ± standard deviation from two independent extracts (n = 2). Means containing different superscript letters within the same row represent significant differences ($p < 0.05$).

(2020). Orsavova et al. (2015) determined PUFA as the predominant part of fatty acid composition in grapes (*Vitis vinifera*) (74%) that was higher than the values found in this study. Among MUFA, oleic acid was found in great quantities too, while SFA were present in small amounts. As concluded by Garavaglia et al. (2016), several studies suggest cardioprotective and anticancer properties of grape seed oil, but great amounts of oil should be consumed to achieve those beneficial effects.

3.6 Total phenolic content of grape pomace, seeds and oil

Grape pomace, seeds and oil were analyzed for TPC as shown in Tables 6 and 7. No significant differences ($p > 0.05$) were found between QGP from Chincha and Ica, 7.24 ± 0.05 and 7.05 ± 0.14 g GAE/100 g, respectively (Table 6). BBGP from Ica showed the lowest TPC 5.80 ± 0.04 g GAE/100 g. Values reported in this study were higher than values found by Da Porto et al. (2014) in defatted grape pomace extracts from several *Vitis vinifera* L. varieties (3.493 g GAE/100 g) obtained with ultrasound and supercritical CO_2. Similarly, no significant differences ($p > 0.05$) were found between QGS from Chincha and Ica, 13.35 ± 0.71 and 14.43 ± 0.75 g GAE/100 g, respectively. Regarding grape seed oil, QGSO from Chincha and Ica showed the highest TPC but significant differences were found between those values (13.59 ± 0.40 and 12.97± 0.09 mg GAE/100 g, respectively) (Table 7).

When studying muscadine grape seed oils from 48 cultivars (*Vitis vinifera*), Zhao et al. (2017) reported phenolic compounds ranging from 124.79 to 358.04 mg GAE/kg oil. As cited by Yang et al. (2021), TPC in grape seed oils is usually low and phenols contribution to oxygen radical absorbance capacity can be as high as 20.00 ± 5.65 µmol TE/g oil as determined by Dalposso et al. (2022) in *Vitis labrusca* grape seed oil. On the other hand, as mentioned by Chakka & Babu (2022) on health benefits of grape seed extracts, various studies were conducted to demonstrate their role in controlling postprandial hyperlipidemia, which refers to elevated levels of cholesterol in the blood. Hence, they concluded that long term consumption of those compounds may have health benefits as antihyperlipidemic effect among others.

3.7 Antoxidant capacity of grape pomace, seeds and oil

Table 6 shows AC of grape pomace and seeds determined by DPPH and FRAP. When using the DPPH method, QGP from Chincha showed the highest AC (771.84 ± 48.56 µmol TE/g). BBGP from Chincha and Ica showed the lowest AC values, 266.35 ± 2.36 and 269.12 ± 9.78 µmol TE/g, respectively, but no significant differences were found between them.

Similar to AC by DPPH, QGP from Chincha showed the highest AC by FRAP but no significant differences were found between AC of QGP from Chincha and Ica, 551.22 ± 7.48 and 519.54 ± 7.71 µmol TE/g, respectively. AC in seeds by DPPH and FRAP followed a similar trend. When using DPPH, QGS from Chincha showed the highest AC (1255.39 ± 61.45 µmol TE/g) but no significant differences were found between AC by DPPH of QGS from Chincha and Ica. BBGS from Chincha and Ica showed the lowest AC values, 579.78 ± 29.61 and 551.77 ± 16.66 µmol TE/g, respectively, but no significant differences were found between them. A similar scenario was determined by FRAP, where QGS from Ica showed the highest AC but no significant differences were found between AC of QGS from Chincha and Ica, 912.84 ± 6.39 and 960.77 ± 56.68 µmol TE/g, respectively. BBGS from Chincha and Ica showed the lowest AC values, 518.29 ± 6.31 and 359.55 ± 25.69 µmol TE/g, respectively, and significant differences were found between those values.

Regarding grape seed oil, AC was determined by ABTS and no significant differences were found in AC among the varieties and locations under study (Table 7). As cited by Xia et al. (2010), dietary consumption of grapes and their products is associated with a lower incidence of degenerative diseases such as cardiovascular diseases and certain types of cancer. More recent interest has focused on bioactive grape phenolic compounds. Anthocyanins, flavanols, flavonols, and resveratrol are the most important grape phenolics because they possess many biological activities, including antioxidant, cardioprotective, anticancer, anti-inflammatory, antiaging, and antimicrobial properties. Thus, optimization of the extraction process will maximize the utilization of bioactive compounds towards human health.

Grape pomace and seeds, as by-products of wine and pisco production, represent a valuable source of important nutrients

Table 6. Total phenolic content and antioxidant capacity of Black Borgoña (*Vitis labrusca*) and Quebranta (*Vitis vinifera*) pomace and defatted grape seeds.

Sample type	Cultivar zones		TPC* (g GAE/100 g)	IC 50 (µg extract/mL)	DPPH* (µmol TE/g)	FRAP* (µmol TE/g)
Pomace	Chincha	BBGP	6.31 ± 0.14^{b}	31.71 ± 0.28^{a}	266.35 ± 2.36^{c}	448.84 ± 10.57^{b}
		QGP	7.24 ± 0.05^{a}	14.75 ± 2.28^{b}	771.84 ± 48.56^{a}	551.22 ± 7.48^{a}
	Ica	BBGP	5.80 ± 0.04^{c}	16.56 ± 0.6^{b}	269.12 ± 9.78^{c}	425.67 ± 3.88^{b}
		QGP	7.05 ± 0.14^{a}	18.97 ± 0.38^{b}	538.27 ± 10.9^{b}	519.54 ± 7.71^{a}
Seeds	Chincha	BBGS	8.11 ± 0.41^{b}	5.48 ± 0.28^{a}	579.78 ± 29.61^{b}	518.29 ± 6.31^{b}
		QGS	13.35 ± 0.71^{a}	3.02 ± 0.15^{c}	1255.39 ± 61.45^{a}	912.84 ± 6.39^{a}
	Ica	BBGS	6.76 ± 0.16^{b}	4.16 ± 0.13^{b}	551.77 ± 16.66^{b}	359.55 ± 25.69^{c}
		QGS	14.43 ± 0.75^{a}	3.8 ± 0.25^{b}	1245.21 ± 81.78^{a}	960.77 ± 56.68^{a}

Data are expressed as the mean ± standard deviation from two independent extracts (n = 2). Means for pomace containing different superscript letters within the same column represent significant differences ($p < 0.05$). Means for seeds containing different superscript letters within the same column represent significant differences ($p < 0.05$). *Results were expressed on a dry weight basis.

Table 7. Total phenolic content and antioxidant capacity of Black Borgoña (*Vitis labrusca*) and Quebranta (*Vitis vinifera*) grapes seed oil (GSO).

Cultivar zones		TPC* (mg GAE/100 g)	ABTS* (µmol TE/g)
Chincha	BBGSO	5.51 ± 0.03[c]	1.71 ± 0.69[a]
	QGSO	13.59 ± 0.40[a]	1.99 ± 0.20[a]
Ica	BBGSO	6.04 ± 0.40[c]	1.73 ± 0.02[a]
	QGSO	12.97 ± 0.09[b]	1.97 ± 0.04[a]

Data are expressed as the mean ± standard deviation from two independent extracts (n = 2). Means containing different superscript letters within the same column represent significant differences ($p < 0.05$). *Results were expressed on a dry weight basis.

that can have a great impact on prevention and onset of diseases (Antonić et al., 2020; Martin et al., 2020). As also determined in this study, dietary fiber and polyphenols are the main bioactive compounds in grape pomace. Those compounds can be used as fortification elements, resulting in increased levels of total phenolics in fortified products (Antonić et al., 2020). In addition, grape pomace is a great alternative source for fiber extraction, mainly pectin, different from the conventional ones such as apple pomace and citrus peels (Spinei & Oroian, 2021).

Regarding grape seed oil, there are three important groups of molecules in its lipophilic part: Essential fatty acids such as linoleic acid as determined in this study, vitamin E isomers and phytosterol. As cited by Martin et al. (2020), those elements appear to be promising not only as nutritional but also as therapeutic compounds because grape seed oil may prevent or improve physiological disorders related to chronic diseases. Therefore, grapes pomace and seeds contain high amounts of health promoting compounds with great nutritional quality.

4 Conclusions

QGP from Chincha showed the highest content of TPC and AC by DPPH and FRAP methods.

The results showed that Black Borgoña and Quebranta grapes pomace and seeds are important sources of compounds with functional properties. Grape pomace, mainly QGP from Chincha showed the highest amount of protein, while BBGP from Ica had the highest concentration of lipids. The amount of total dietary fiber is quantitatively greater compared to that of proteins, and lipids, indicating that this residue could be included in the daily diet as a source of fiber and food supplement. As for minerals, iron and potassium were present in higher concentrations in QGP from Chincha while calcium and magnesium were found in higher concentrations in BBGP from Ica. Regarding grape seed oil functional quality, the greatest concentration of linoleic acid was found in BBGSO from Chincha. Black Borgoña and Quebranta grape pomace and seeds are potential sources of bioactive compounds, especially Quebranta which showed higher concentration of TPC and AC irrespective of the cultivar zone. Therefore, the results of this study suggest that different by-products of the wine and pisco industries in Peru, may be potential food ingredients in the daily diet or as nutritional supplements. However, considering that scale-up of the PLE method for bioactive compounds is currently at an early stage, further studies should work on the challenges that this process involves while maximizing its efficiency. Furthermore, the investment and processing costs should be estimated to ensure technical and economic viability of this green method, PLE.

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