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Effects of subcritical water extraction and cultivar geographical location on the phenolic compounds and antioxidant capacity of Quebranta (*Vitis vinifera*) grape seeds from the Peruvian pisco industry by-product

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Abstract

The objectives of this study were to evaluate the effects of the grape cultivar geographical location and extraction technique on the total phenolic compounds (TPC), antioxidant capacity (AC), oil yield and quality of Quebranta (*Vitis vinifera*) grape seeds. Seeds were defatted with supercritical CO_2 and the bioactive compounds were extracted with subcritical water and macerations with methanol, ethanol, and acetone. The differences in grape seed oil yield were not significant ($p > 0.05$). The most abundant fatty acid determined was linoleic (66.37-67.37%). The highest TPC corresponded to the extracts from zones A and B obtained with subcritical water, 167.56 ± 10.40 and 161.83 ± 4.95 mg GAE/g dw, respectively. The highest AC by DPPH was also achieved by the extracts from zones A and B (1,479.90 ± 12.86 and 1,628.15 ± 80.32 µmol TE/g dw, respectively) with subcritical water extraction. The highest AC by FRAP was observed in the subcritical water extracts from zones B and C, 1,429.29 ± 29.75 and 1,389.54 ± 7.46 µmol TE/g dw, respectively. Grape seed is a valuable source of nutritionally oil and bioactive compounds, which can be obtained from by-products of pisco production for potential use in the food and pharmaceutical industries.

Keywords: grape seed oil; bagasse; unsaturated fatty acids; supercritical CO_2; subcritical water extraction; antioxidant capacity.

Practical Application: Quebranta grape seed, by-product of the Peruvian pisco industry, is a good source of nutritious oil and phenolic compounds that is mostly discarded. This oil has potential to be used in the food industry due to its high amount of linoleic acid. Different extraction methods were applied to maximize the total phenolics and antioxidant capacity of the grape seeds. As a result, the potential for utilizing the grape seeds was stated as the cultivar areas and methods that provide the greatest extraction of bioactive compounds.

1 Introduction

Pisco, an alcoholic beverage obtained by the distillation of fresh musts from recently fermented pisco grapes, is produced in the regions of Lima, Ica, Arequipa, Moquegua, and Tacna in Peru (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, 2017). Ica is the most important region in the wine industry, which produces all varieties, with the Quebranta grape being the most productive (Mathis et al., 2017). Pisco production in Peru grows annually, reaching 4190, 4220, 5210 and 4964 m^3 in 2016, 2017, 2018 and 2019, respectively (Instituto Nacional de Estadística e Informática, 2021). This growth, simultaneously, generates a large amount of solid residue because 6 to 7 kg of grapes are used to make 1 L of pisco (Programa de las Naciones Unidas para el Desarrollo, 2004). In Ica, solid waste is often burned or discarded in landfills near the food industries, creating environmental and health problems. The solid residue, called pomace, is made up of a mixture of husks, seeds and stems; and they are known to be a source of antioxidants such as phenolic acids and flavonoids (Cheng et al., 2012). Among pomace components, seeds represent 38 to 52% based on the dry matter. In addition, the seeds stand out for being a source of oil, rich in polyunsaturated fatty acids, particularly linoleic acid (Maier et al., 2009). Likewise, seeds have a high content of phenolic compounds such as flavonoids, anthocyanins, catechins, flavanol glycosides and phenolic acids (Porto & Natolino, 2017; Lafka et al., 2007), resveratrol (Tian et al., 2017) that provide beneficial biological effects for human health (Paladino & Zuritz, 2011).

The extraction methods for the recovery of bioactive compounds, from the residues of the pisco industry, have been extensively reported. Some environmentally friendly methods that stand out for grape oil extraction are expanded CO_2 (Li et al., 2020), microwaves, ultrasound and supercritical CO_2 (Dimić et al., 2020). Non-conventional technologies used for the recovery of phenolic compounds from grape seed include subcritical water (Duba et al., 2015; Loarce et al., 2020) and pressurized liquid extraction (Allcca-Alca et al., 2021). Regarding the conventional methods, studies of grape seed extracts obtained by maceration with methanol (Porto et al., 2013), and other solvents such as acetone, ethanol and methanol have been reported (Cheng et

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al., 2012; Paladino & Zuritz, 2011). However, there have been no studies assessing non-conventional extraction methods in Quebranta (*Vitis vinifera*) grape seeds.

Information about bioactive compounds in grape seeds, a by-product of the pisco industry, and their extraction techniques is abundant. On the contrary, more research is needed about the influence of the cultivar different locations and non-conventional extraction methods on those compounds or others of interest. Therefore, the objectives of this study were to evaluate the effects of the cultivar geographical location and extraction method on the TPC and AC of the defatted Quebranta (*Vitis vinifera*) grape seeds. Also, the influence of the cultivar location on the grape seed oil yield and fatty acids profile was investigated.

2 Materials and methods

2.1 Sample preparation

Approximately, 60 kg of Quebranta (*Vitis vinifera*) grape pomace from the production of pisco, were used. The pomace came from 3 geographical areas: San Juan Bautista (14°0'12.01"S 75° 44'21.27"W), Subtanjalla (14°1'17.82"S 75°44'35.43"W) and Los Patos (14°2'56.22"S 75° 43'52.36"W) located in the Ica Valley (Ica-Peru). The pisco production process was similar in the three areas mentioned above. Briefly, the Quebranta grape clusters were placed in the crusher-destemmer machine. Then, the grape was pressed, the juice was obtained, and the pomace (peels and seeds) was separated. The must, fermented grape juice, was later distilled to obtain pisco. The samples, kept at 0 to 5 °C, were brought to the Instituto Tecnologico de la Produccion (ITP) (Callao, Peru). The pomace was dried in a cold air dryer (Asahi, CV-20AN, Japan) at 25 °C for 36 h until they reached 13% as the maximum moisture content. Then, it passed through a 7 mm sieve (KM Testing sieve, Japan) to separate the seeds that were dried in a forced convection oven (Venticell, USA) at 40 °C for 6 h until a maximum content of 7%, and ground in an analytical mill (A 11 Basic, IKA, USA). Finally, the dried and ground Quebranta grape seeds (QGS) 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

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 water purification system (Barnstead, Model D11911, Germany), carbon dioxide 99.5% v/v liquefied gas (Linde, Peru), nitrogen atmosphere Ultrapuro (Linde, Peru).

2.3 Experimental design

A complete randomized design (CRD) of 1 x 3 with 4 repetitions was used to evaluate the effect of three cultivar geographical locations of Quebranta grape: (A) San Juan Bautista, (B) Subtanjalla and (C) Los Patos on yield and fatty acids profile of Quebranta grape seeds (QGS) oil extracted with supercritical CO_2. Subsequently, a 3 x 4 full factorial design with 3 repetitions was used to evaluate the effect of the three cultivar geographical locations and four extraction methods on TPC and AC *in vitro* of QGS. The levels corresponding to the extraction methods factor were: (a) subcritical water, maceration using as solvents (b) 70% ethanol (v/v), (c) methanol and (d) 50% acetone (v/v). The levels corresponding to the location factor were: (A) San Juan Bautista, (B) Subtanjalla and (C) Los Patos.

2.4 Oil extraction with supercritical CO_2

Dried Quebranta grape seeds (QGS) were defatted with supercritical CO_2 using a multi-solvent extractor equipment Model 2802.000 (Figure 1) (Top Industrie, Vaux le Pénil, France) with an extraction cell of 1 L capacity that had a volume reducer device (87 cm^3, internal diameter = 2.8 cm, internal height = 14.1 cm). Approximately, 35 g of QGS and five alternating layers of 5 mm glass beads (5 g) were filled into the extraction cell (Figure 1a). Extractions were performed at 33.5 °C and 188 bar according to Sánchez et al. (2018) with modifications in the CO_2 flow that was 47 g/min in this study. The grape seed oil yield was expressed on a dry basis according to Equation 1 as follows:

$$Grape\ seed\ oil\ yield(\%) = \frac{W_1}{W_2} \times 100 \quad (1)$$

Where: W_1 is the oil mass and W_2 is QGS mass

2.5 Preparation of extracts for phenolic and antioxidant capacity assays

Four extraction methods were selected for phenolics extraction of defatted Quebranta grape seeds

(DQGS): subcritical water extraction (SWE) and by maceration with ethanol, methanol and acetone.

2.6 Subcritical Water Extraction (SWE)

The subcritical water extractions were carried out with a multisolvent extractor equipment (Top Industrie, series 2802.0000, Vaux le Pénil, France) without a volume reducer (Figure 1b), and deionized water as solvent, previously degasified for 30 min at 25 °C in a sonicator (VWR International, SymphonyTM, 97043-942, China). Approximately, 31 g of DQGS and five alternating layers of 5 mm glass beads (700 g) were filled into the extraction cell (709 cm^3, internal diameter: 8 cm, internal height: 14.1 cm) as shown in Figure 1b. As described by Duba et al. (2015), extractions were performed at 120 °C and 100 bar. Then, 500 mL of water were added to the reactor and absorbed with the cosolvent pump at 15 mL/min for 40 min until the desired

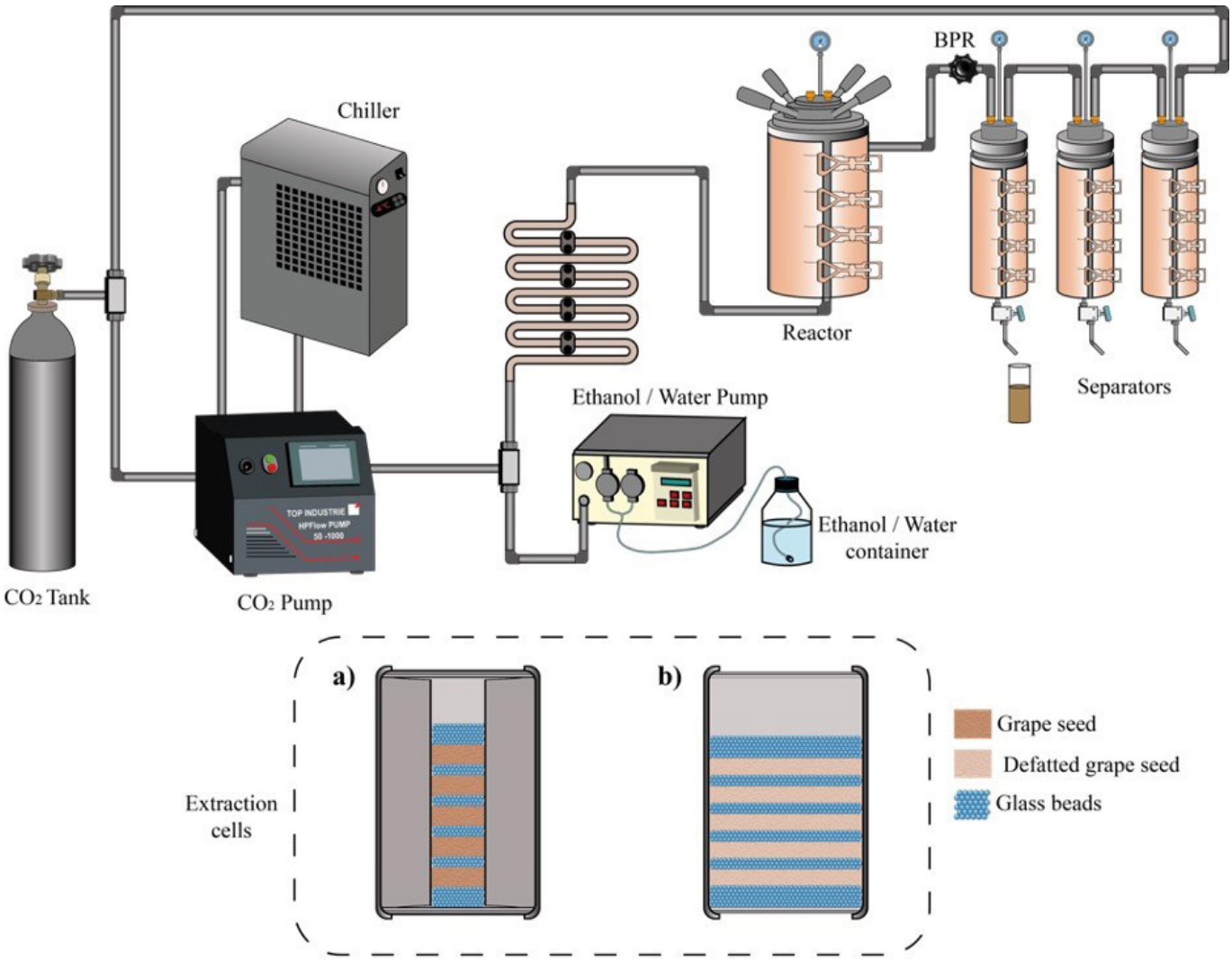

Figure 1. Multisolvent extraction system. a) Cell for extraction with supercritical CO_2. b) Cell for extraction with subcritical water. BPR: Back Pressure Regulator.

pressure was achieved. This condition was kept static for 3 h for a greater extraction of phenolic compounds. Finally, extracts were cooled in an ice bath for 10 min until full recovery of the extract from the system. Collected extracts were stored at 4 ± 1 °C until analysis. Extracts were analyzed in triplicate.

2.7 Extraction by maceration with ethanol, methanol or acetone

The extraction of phenolics from DQGS was done with ethanol, methanol or acetone as solvent.

Approximately, 4 g of DQGS was weighed into a 100 mL glass bottle and extracted with 80 mL of ethanol/water (70 : 30 v/v), methanol or acetone/water (50 : 50 v/v) (Sánchez et al., 2018). The bottles were vortexed with a magnetic stirrer (IKA, RT 10, Germany) at 60 RPM for 3 h at 25 °C. Finally, the extracts were filtered through Whatman No. 4 (20-30 µm) filter paper and stored at 4 ± 1 °C in airtight glass bottles until analysis. Extracts were analyzed in triplicate.

2.8 Chemical analysis

Fatty acid profile of grape seed oil

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

A gas chromatograph with an FID detector (Autosystem XL, Perkin Elmer, USA) equipped with a Supelcowax 10 column (Merck, Germany) (30 m × 0.25 mm id; film thickness: 0.25 µm) was used. Hydrogen was used as the carrier gas at 5 psi. The injector and detector temperatures were 250 °C and 270 °C, respectively. A volume of 2 µL was injected at a split ratio 100 : 1. The fatty acid peaks were identified by comparison with the retention times of the Fatty Acid Methyl Ester Mix C4-C14 (Sigma-Aldrich, USA). The area of the peaks was calculated using the TotalChrom Navigator software (v. 6.2.0) (Perkin Elmer, USA). The percentage of each fatty acid was calculated by comparing the individual area of each peak with the fatty acids total area. Oil samples were analyzed in quadruplicate.

Total Phenolic Content (TPC)

TPC 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 mg of gallic acid equivalent (GAE) per g of DQGS (dw).

2.9 Antioxidant capacity assays

DPPH

The antioxidant capacity of DQGS 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. The absorbance was measured in a UV-VIS spectrophotometer at 518 nm. The percentage of inhibition of different concentrations of DQGS extract against the 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 the AC of DQGS extract which was expressed in mmol Trolox Equivalent (TE) per g sample (dw).

FRAP

The AC of DQGS samples 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 FeCl3 6H2O in a ratio 25 : 2.5 : 2.5). The 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).

2.10 Statistical analysis

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. The Pearson test was performed to obtain correlation (r) values between TPC and AC (DPPH and FRAP). All analyzes were carried out with SPSS statistic software v. 26 (IBM, Peru). Data are reported as mean ± standard deviation (SD) and were calculated with Excel 2016 (Microsoft, USA).

3 Results and discussion

3.1 Oil yield and fatty acid profile of Quebranta grape seed oil

Quebranta grape seed oil yields are shown in Table 1. No significant differences ($p > 0.05$) were observed due to the effect of the cultivar geographical location. The QGS oil yield from areas A, B and C were higher than the values obtained through supercritical CO_2 extraction by Jokić et al. (2016) from Croatian grape seeds (14.49%) and Souza et al. (2020) from *Vitis vinifera* grape seed (Brazil) (12.54%). Lower yields in the range of 12.0 to 12.7% were also reported by Coelho et al. (2018) in grape seeds from Portugal. These differences may be due to different cultivars as determined by Wen et al. (2016), who found significant differences among extraction yields of various grape seed cultivars that ranged from 13.71 to 15.92%.

Table 1. Oil yield and fatty acid composition of Quebranta (*Vitis vinifera*) grape seed oil.

Cultivar Areas	A	B	C
Yield (%) (dw)	16.07 ± 1.47[a]	17.00 ± 0.75[a]	16.17 ± 0.91[a]
FATTY ACIDS			
Palmitic C16:0	7.02 ± 0.11[a]	7.19 ± 0.29[a]	7.12 ± 0.24[a]
Palmitoleic C16:1	ND	ND	0.11 ± 0.01
Stearic C18:0	4.81 ± 0.10[a]	4.89 ± 0.37[a, b]	4.35 ± 0.26[b]
Oleic C18:1 ω9	19.55 ± 0.17[a]	20.01 ± 0.31[a]	19.66 ± 0.29[a]
Vaccenic C18:1 ω7	0.85 ± 0.02[a]	0.80 ± 0.03[a]	0.77 ± 0.07[a]
Linoleic C18:2 ω6	67.19 ± 0.22[a, b]	66.37 ± 0.55[b]	67.37 ± 0.41[a]
α-Linolenic C18:3 ω3	0.28 ± 0.01[c]	0.29 ± 0.01[b]	0.31 ± 0.00[a]
Arachidic C20:0	0.17 ± 0.01[a]	0.28 ± 0.16[a]	0.17 ± 0.03[a]
Eicosenoic C20:1	0.14 ± 0.01[a]	0.17 ± 0.02[a]	0.16 ± 0.03[a]
SFA	11.99 ± 0.06[b]	12.35 ± 0.23[a]	11.64 ± 0.11[c]
MUFA	20.54 ± 0.18[a]	20.98 ± 0.35[a]	20.68 ± 0.33[a]
PUFA	67.47 ± 0.22[a, b]	66.67 ± 0.55[b]	67.68 ± 0.41[a]

Data were obtained in quadruplicate and expressed as mean ± SD. Cultivar areas: A = San Juan Bautista; B = Subtanjalla; C = Los Patos. ND = no detectable. Means containing different superscript letters within the same row represent significant differences ($p < 0.05$). Results were expressed as g/100 g seed oil on a dry weight basis.

The fatty acid composition of QGS oils from areas A, B and C is shown in Table 1. Nine kinds of fatty acids were detected in grape seed oil samples. Results revealed that grape seed oil was mainly composed of polyunsaturated fatty acids (PUFA) which account for 66.67-67.68% of total fatty acids followed by monounsaturated fatty acids (MUFA). No significant differences ($p > 0.05$) were found between PUFA of grape seed oil from areas A and C. Also, no significant differences were found between MUFA of grape seed oil for the 3 areas under study. On the contrary, significant differences were found in saturated fatty acids (SFA) with oil from area B showing the greatest amount (12.35%). The most abundant fatty acid was linoleic acid (C18:2) ranging from 66.37-67.37%, then oleic acid (C18:1) (19.55-20.01%), palmitic acid (C16:0) (7.02-7.19%) and stearic acid (C18:0) (4.35-4.89%) as also reported by Wen et al. (2016) on several grape varieties. No significant differences ($p > 0.05$) were observed between the linoleic acid content of grape seed oil from areas A and C but significant differences ($p < 0.05$) were found between areas B and C with the latter area showing the greatest content (67.37%). These results were in accordance with those reported for *Vitis vinifera* seeds from Italy and Mexico by Fiori et al. (2014) and Franco-Mora et al. (2015), respectively, who used supercritical CO_2 for oil extraction. These results were also verified by Coelho et al. (2018) who reported range values for linoleic, oleic, palmitic, and stearic acids from grape (*Vitis vinifera* L.) seeds from the center of Portugal, similar to the ranges reported in this study. Also, comparable results were obtained by Bada et al. (2015) in grape seed oil from Spain extracted with hexane. Conversely, Souza et al. (2020) reported lower values of linoleic and oleic fatty acids while higher values of stearic and palmitic acids from a Brazilian grape variety. Preharvest and processing parameters

are the main factors that influence the quality of fruit seed oil (Kaseke et al., 2020). Differences in grape seed oil fatty acid composition may be caused by different cultivars of grape seed, cultivation conditions, cultivar geographical location and the oil extraction method used.

3.2 Total Phenolic Content (TPC)

The TPC of DQGS is shown in Table 2. TPC ranged from 27.89 ± 2.24 mg GAE/g dw to 167.56 ± 10.40 mg GAE/g (dw). The cultivar geographical location, the extraction method and their interaction had a significant influence ($p < 0.05$) on the TPC (Table 3). The highest TPC was obtained with subcritical water (SWE), 167.56 and 161.83 mg GAE/g dw, in DQGS from areas A and B, respectively, and no significant differences ($p > 0.05$) were found between the values. Those values were close to those reported by Bozan et al. (2008) in seeds of *Vitis vinifera* variety Papaz Karazi (Turkey). Ordoñez et al. (2019) reported lower TPC in *Vitis vinifera* Black grape seeds extracted with methanol (9.07 g GAE/100 g dry sample). A similar scenario was also described by Orellana et al. (2019) in Quebranta grape seeds where lower TPC values were found (97.26 mg GAE/g dry sample) when using an acidified methanol/water solution as the extraction method.

TPC of DQGS from area A extracted by SWE was higher than the values determined by conventional methods with different solvents (70% ethanol, methanol and 50% acetone). This may be due to the subcritical state of the water that facilitates the diffusion of the analyte, the decrease in viscosity, surface tension and dielectric constant (Turner & Ibañez, 2012; Zhang et al., 2020). In addition, the thermal energy supplied with subcritical water decreases the activation energy required for desorption process which can interrupt cohesive (solute-solute) and adhesive (solute-matrix) interaction (Teo et al., 2010). Duba et

Table 2. Total phenolics content and antioxidant capacity of Quebranta (*Vitis vinifera*) grape seed extracts.

Cultivar area	Extraction method	TPC (mg GAE/g dw)	DPPH (μmol TE/g dw)	FRAP (μmol TE/g dw)
A	a	167.56 ± 10.40^{a}	1,479.90 ± 12.86^{a}	845.13 ± 95.32^{f}
A	b	27.89 ± 2.24^{c}	179.59 ± 46.36^{f}	200.39 ± 19.86^{h}
A	c	32.40 ± 2.15^{c}	174.74 ± 26.18^{f}	253.19 ± 10.89$^{g, h}$
A	d	40.43 ± 3.91^{c}	409.82 ± 81.30^{e}	347.07 ± 36.55^{g}
B	a	161.83 ± 4.95^{a}	1,628.15 ± 80.32^{a}	1,429.29 ± 29.75^{a}
B	b	108.32 ± 7.34^{b}	331.71 ± 39.45$^{e, f}$	1,126.52 ± 84.83$^{c, d, e}$
B	c	107.49 ± 3.98^{b}	253.18 ± 32.76$^{e, f}$	1,110.85 ± 24.05$^{d, e}$
B	d	160.04 ± 11.13^{a}	842.12 ± 93.05^{d}	1,219.17 ± 55.46$^{c, d}$
C	a	147.63 ± 16.18^{a}	1,167.92 ± 106.17$^{b, c}$	1,389.54 ± 7.46$^{a, b}$
C	b	104.52 ± 3.28^{b}	1,004.98 ± 64.00$^{c, d}$	1,019.96 ± 44.50^{e}
C	c	104.62 ± 5.69^{b}	1,234.26 ± 32.66^{b}	1,143.05 ± 59.36$^{c, d, e}$
C	d	152.65 ± 4.97^{a}	1,149.50 ± 18.90$^{b, c}$	1,266.06 ± 21.28$^{b, c}$

Data were obtained in triplicate and expressed as mean ± SD. Cultivar areas: A = San Juan Bautista; B = Subtanjalla; C= Los Patos. Extraction methods: (a) subcritical water and maceration with (b) ethanol 70%, (c) methanol y (d) acetone 50%. Means containing different superscript letters within the same column represent significant differences ($p < 0.05$).

Table 3. Full factorial analysis of variance.

Source of variation	Dependent variable	Sum of squares	df	Mean square	F-statistic	p-value
MAIN EFFECTS						
A: Area	TPC^{a}	32880	2	16440.1	289.87	0.000
	DPPH^{b}	2166259	2	1083130	158.95	0.000
	FRAP^{c}	5142326	2	2571163	1075.72	0.000
B: Extraction methods	TPC	37424	3	12474.8	219.96	0.000
	DPPH	3973538	3	1324513	194.38	0.000
	FRAP	1032946	3	344315	144.05	0.000
INTERACTIONS						
AB	TPC	17924	6	2987.3	52.67	0.000
	DPPH	2144481	6	357414	52.45	0.000
	FRAP	173417	6	28903	12.09	0.000
RESIDUAL	TPC	1361	24	56.7		
	DPPH	149912	24	6814		
	FRAP	57364	24	2390		
TOTAL (Adjusted)	TPC	89589	35			
	DPPH	8508384	35			
	FRAP	6406053	35			

a: $R^2 = 0.9848$ (adjusted $R^2 = 0.9778$). b: $R^2 = 0.9824$ (adjusted $R^2 = 0.9736$). c: $R^2 = 0.9910$ (adjusted $R^2 = 0.9869$).

al. (2015) reported a TPC of 124 mg GAE/g in grape seeds from Italy extracted with subcritical water. Our results were higher than those reported by the aforementioned authors possibly due to the variety of grape and growing area, as well as the solvent/sample ratio in the extraction process and other parameters, as reported by Ravber et al. (2015). Lachman et al. (2009) reported significant differences among vineyard regions and varieties in total polyphenol content in grape skins. Further, they found significant differences in polyphenolic antioxidants of red and white Spanish wines of different geographical origins.

In relation to areas B and C, no significant differences in TPC were observed between SWE and the extraction method with acetone. No significant differences were found in TPC of grape samples from area A extracted by the conventional methods. DQGS from area A extracted with 70% ethanol, methanol and 50% acetone showed the lowest TPC values, 27.89, 32.40 and 40.43 mg GAE/g dw, respectively, when compared with TPC of the grape samples from areas B and C extracted with the same methods. These values were higher than those reported by Paladino & Zuritz (2011) in Cabernet Sauvignon (Argentina) grape seed extracts obtained with water, ethanol, methanol and acetone. They were also higher than the values reported by Laos et al. (2020) in Quebranta grape seed extracts obtained by an ultrasound bath with ethanol: water: acetic acid (90/9.5/0.5). Differences in the results between this study and the literature could be attributed to the extraction method as reported by Rababah et al. (2008), variety of grape cultivar o seasonal influences among others (Yilmaz et al., 2015).

3.3 Antioxidant Capacity (AC)

The AC of DQGS by DPPH and FRAP is shown in Table 2. The cultivation area and the extraction technique had a significant effect on the DPPH and FRAP values, as well as a combined effect on those values (Table 3). Likewise, the Pearson analysis showed a correlation at a level of 0.01 between TPC and DPPH (R = 0.7544), TPC and FRAP (R = 0.8582) and DPPH with FRAP (R = 0.6444). As pointed out by Lachman et al. (2009), TPC is mainly correlated with the antioxidant potency and the antiradical activity. Thus, a positive correlation between TPC and its antioxidant power was confirmed.

The AC values by DPPH ranged from 174.74 ± 26.18 to 1628.15 ± 80.32 μmol TE/g dw and were higher than those reported by Coklar (2017) in *Vitis vinifera* seeds from Turkey. The greatest AC values by DPPH were found in DQGS from areas A and B extracted with SWE, 1,479.90 ± 12.86 and 1,628.15 ± 80.32 μmol TE/g dw, respectively. No significant differences were found between those values. The highest values obtained could be due to the structural and molecular modifications because of SWE treatment which improves the biological activity of antioxidants (Getachew & Chun, 2017). For the DQGS from area C, no significant differences were found on AC by DPPH of extracts obtained with SWE, methanol and 50% acetone. The lowest AC values by DPPH were observed for the extracts from areas A and B when 70% ethanol and methanol extraction methods were applied. DQGS from area B extracted with 50% acetone showed higher AC values by DPPH when compared to 70% ethanol and methanol extractions.

Regarding the AC by FRAP, the values ranged from 200.39 ± 19.86 to 1,429.29 ± 29.75 μmol TE/g dw. The greatest AC values by FRAP of DQGS from areas B and C extracted with SWE were 1,429.29 ± 29.75 and 1,389.54 ± 7.46 μmol TE/g dw, respectively. No significant differences were found between those values. The extract from area A showed a lower AC by FRAP when the SWE was used (845.13 ± 95.32 μmol TE/g dw).

The non-conventional extraction technologies, such as SWE, currently underused because of the lack of data on the profitability of the investment, offer great opportunities and challenges. However, high capital investment, high running cost, training, maintenance cost, etc. increase limitations to scale-up green extraction methods. Thus, a cost assessment analysis could provide an understanding of the cost-benefit related to the utilization of those "green techniques". In addition, there is a great necessity to work on some parameters such as replacement of solvents with emerging green alternatives for efficient extraction (Belwal et al., 2020; Picot-Allain et al., 2021).

In the case of AC by FRAP of DQGS from area B, no significant differences were found among the extracts obtained with the conventional methods. The lowest AC values by FRAP were observed for the extracts from area A when 70% ethanol and methanol extraction methods were applied as was also observed with DPPH. Garrido (2016) analyzed Pedro Ximénez grape variety from Spain and found an AC by FRAP of 249.83 ± 62.69 μmol TE/g dw similar to the value determined in this study when using DQGS methanol and ethanol extracts from area A. On the other hand, our AC by FRAP determined in all extracts from areas B and C were higher than the value obtained by Garrido (2016).

Furthermore, the extracts from area A obtained with 50% acetone presented higher AC values by DPPH and FRAP methods compared to 70% ethanol extracts. No significant differences ($p > 0.05$) were observed between AC by DPPH and FRAP of DQGS from area C extracted with 50% acetone and methanol. Margraf et al. (2016) evaluated purple grape juice and concluded the geographical origin and variety of grapes have an important role in distinguishing Brazilian purple grape juices according to the free-radical scavenging activity (ABTS) and reducing capacity (FRAP).

4 Conclusions

This study reported the effects of the cultivar geographical area and extraction methods on the TPC and AC of DQGS obtained from the pisco industry in Ica-Peru. Also, the influence of the cultivar location on grape seed oil yield and fatty acids profile was reported. The QGS oil, from the three areas under study, showed a high content of PUFA with linoleic acid present in the highest amount. In comparing extraction methods, subcritical water treatment, a suitable environmentally friendly technique, achieved the highest average value of TPC in DQGS (area A), and AC determined by DPPH and FRAP methods in DQGS (area B). These results show the advantages of green technologies such as subcritical water for extraction of bioactive compounds with antioxidant properties from Pisco production waste. Therefore, further studies should explore the application of QGS oil and

extracts obtained with subcritical water as food ingredients and in the pharmaceutical industry. In addition, it could be also concluded that geographical location had a significant effect on TPC and AC of Quebranta grapes. Thus, as future perspective, study of conditions such as temperature, soil type, availability of nutrients and other environmental factors may help to maximize health benefits of bioactive compounds.

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