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Original Article

Characterization of polyunsaturated fatty acids and antioxidant activity of *Vitis vinifera* L. (grape) seeds from the Ica Valley, Peru

[Caracterización de ácidos grasos poliinsaturados y actividad antioxidante de las semillas de *Vitis vinifera* L. (uva) del valle de Ica, Perú]

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Abstract

Context: In the Valley of Ica-Peru, the seeds of the fruit of *Vitis vinifera* L. (grape) are residues from the Pisco Industry, and due to their bioactive compounds, they can represent a new economic and sustainable activity for the population of the mentioned Valley.

Aims: To characterize polyunsaturated fatty acids from the seeds of the fruit of *Vitis vinifera* L. (grape) from the Ica Valley, Peru and determine the antioxidant activity *in vitro*.

Methods: The oil from five grape varieties (Quebranta, Moscatel, Torontel, Mollar, and Italia) was obtained by ethereal extraction with ultrasound. The profile of fatty acids was characterized by gas chromatography with a flame ionization detector (GC-FID). The total polyphenolic content was determined by the Folin-Ciocalteu method and antioxidant activity by two *in vitro* methods.

Results: The values obtained from the 5 grape varieties are presented in a range: total polyphenolic content (TPC: 0.59-154 mg GAE/g); linoleic acid content (69.90-71.87%), oleic acid (18.35-19.90%) and others in a lower percentage ($p>0.05$). Antioxidant activity: DPPH IC_{50} 38.30-48.43 µL; FRAP 0.49-0.77 µg TEAC/g. TPC/DPPH and TPC/FRAP were significantly different ($p<0.05$), except for the Mollar TPC/FRAP variety ($p>0.05$).

Conclusions: The oils from the seeds of the *Vitis vinifera* fruit (grapes) that are residues from the production of Pisco present physicochemical characteristics of oils of high nutritional value with a predominance of polyunsaturated fatty acids and potential antioxidant activity.

Keywords: *in vitro* antioxidant activity; Ica; phenolic compounds; polyunsaturated fatty acids; *Vitis vinifera*.

Resumen

Contexto: En el valle de Ica, Perú, las semillas del fruto de *Vitis vinífera* L. (uva) son residuos provenientes de la Industria Pisquera, y debido a sus compuestos bioactivos pueden representar una nueva actividad económica y sostenible para la población del mencionado Valle.

Objetivos: Caracterizar ácidos grasos poliinsaturados de las semillas del fruto de *Vitis vinífera* L. (uva) procedentes del valle de Ica, Perú y determinar la actividad antioxidante *in vitro*.

Métodos: El aceite de cinco variedades de uvas (Quebranta, Moscatel, Torontel, Mollar e Italia) se obtuvo por extracción etérea con ultrasonido. Por cromatografía de gases con detector de ionización de llama (CG-FID) se caracterizó el perfil de los ácidos grasos. El contenido polifenólico total se determinó por el método Folin-Ciocalteu; actividad antioxidante por dos métodos *in vitro*.

Resultados: Los valores obtenidos de las 5 variedades de uvas se presentan en un rango: contenido polifenólico total (CPT: 0.59-154 mg GAE/g); contenido de ácido linoleico (69.90-71.87 %), oleico (18.35-19.90 %) y otros en menor porcentaje ($p>0.05$). Actividad antioxidante: DPPH IC_{50} 38.30-48.43 µL; FRAP 0.49-0.77 µg TEAC/g. CPT/DPPH y CPT/FRAP significativamente diferentes ($p<0.05$), excepto para la variedad Mollar CPT/FRAP ($p>0.05$).

Conclusiones: Los aceites de las semillas del fruto *Vitis vinifera* (uvas) que son residuos de la producción de Pisco presentan características fisicoquímicas de aceites de alto valor nutricional con predominio de ácidos grasos poliinsaturados y una potencial actividad antioxidante.

Palabras Clave: actividad antioxidante *in vitro*; ácidos grasos poliinsaturados; compuestos fenólicos; Ica; *Vitis vinifera*.

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INTRODUCTION

Plant *Vitis vinifera* L. (vine) is a perennial plant that lives for decades, whose leaves and fruits (berry or grape) are according to the variety of the vine, and belongs to the *Vitaceae* family (Cáceres et al., 2017). They are cultivated worldwide for their appreciated fruit used for wine production (Chowdahary et al., 2021; Tita et al., 2021). In Peru, two groups of varieties of the species of *V. vinifera* are cultivated for the production of Peruvian Pisco; among the aromatic varieties are Albilla (adult pentagonal-shaped leaf, spherical and yellow-green berry), Italia (adult wedge-shaped leaf, inverse ovoid berry and yellow-green), Moscatel (pentagonal adult leaf, spherical and red berry) and Torontel (adult wedge-shaped leaf, spherical and yellow-green berry); and among the non-aromatic ones Negra Criolla (pentagonal-shaped adult leaf, flattened spherical red berry), Mollar (orbicular-shaped adult leaf, short elliptical berry, and pink color), Quebranta (wedge-shaped adult leaf, short elliptical berry and red in color) and the Uvina (adult leaf in pentagonal shape, spherical berry and blue-black in color) (Cáceres et al., 2017). The Coastal Valley of Ica, Peru, has a total area of 7662 ha for the cultivation of *V. vinifera,* of which 3164 ha are used by industrial and artisanal wineries for the production of wines and Piscos, representing between 60%-65% of the national production of Pisco, this shows that it is one of its main economic activities in the Ica region (Cáceres and Julca, 2018). After the extraction of grape juice by pressing, large amounts of a by-product called pomace (skin and seeds) are obtained, which are discarded without commercial value (Bordiga et al., 2019; Kapcsandi et al., 2021).

The seeds represent between 20-26 % of the pomace, which contain various phenolic compounds and mono- and polyunsaturated fatty acids (Garavaglia et al., 2016; Kaseke et al., 2020; Konuskan et al., 2019), according to the seed variety, cultivation environment (land, climate and cultivation work), degree of maturity and a lesser extent by the seed extraction method (Kapcsandi et al., 2021; Martín et al., 2020; Tita et al., 2021). Said bioactive components can be used in the cosmetic industry and functional medicine as antioxidants to prevent and treat diseases with an oxidative stress component (Baroi et al., 2022). Studies indicate that oxidative stress is an imbalance in the production of reactive oxygen species (ROS) and physiological antioxidants (Sánchez-Valle and Méndez-Sánchez, 2013). This imbalance is associated with chronic diseases and various types of cancer, especially those that have environmental and genetic components (Sung et al., 2021; Wongpratate et al., 2020). The *CYP1A1, CYP2E1, CYP2C19, CYP2D6,* and *CYP17* genes encode their respective isoenzymes that biotransform procarcinogens (benzopyrene) into reactive metabolites that bind to DNA guanine (Alvarado et al., 2021; Sung et al., 2021; Wongpratate et al., 2020). Fig. 1 shows a summary of the production of hydrogen peroxide (H_2O_2) when superoxides ($^{\cdot}O_2$) react with hydrogen ions (H^+) by the action of superoxide dismutase 2 (SOD2). When H_2O_2 increases, deficient neutralization by antioxidant enzymes is generated. This activates the Fenton reaction pathway to form superhydroxyls ($^{\cdot}OH$) that generate lipid peroxidation of the inner membrane of the mitochondria, forming malondialdehyde, which is responsible for the oxidation of guanine (8-hydroxyguanosine) from DNA (Alvarado et al., 2021; Wongpratate et al., 2020).

The use of polyunsaturated oils (PUFA) and phenolic compounds from seeds, fruits, leaves, stems, or roots of vegetable plants could sequester free radicals, stimulate the synthesis of the antioxidant system or induce the catalytic activity of catalase (CAT), superoxide dismutase (cytosolic SOD1; mitochondrial SOD2; extracellular SOD3), and glutathione peroxidase (GPX) (Shetty, 2004). Fig. 2 shows the metabolic pathway of oleic and linoleic oils to form docosahexaenoic acid (DHA), which is part of cell membranes, especially neuronal and retinal membranes. Subsequently, said acid is metabolized by the action of 15-lipoxygenase into resolvins (RvD1 and RvD4) and neuroprotectin D1 (NPD1), which suppress reactive oxygen species (ROS) (Yang et al., 2018), prevent apoptosis induced by oxidative stress, by inhibiting caspase-3, inducing an increase in antiapoptotic proteins Bcl-2 and BclxL, and decreasing the expression of proapoptotic proteins such as Bax and Bad (Bazan, 2005; Sun et al., 2018).

The largest amount of grapeseed oil sold worldwide is from French winemakers (Kapcsandi et al., 2021). Therefore, a study is warranted and justified for two reasons: firstly, to characterize the types of fatty acids in oils obtained from five varieties of grape seeds and evaluate their potential antioxidant activity, and based on the results, encourage their consumption as a nutraceutical; and secondly, since it is a by-product of the production of Pisco that is discarded without commercial value, it represents a new business opportunity in the functional food and cosmetics industry for the inhabitants of the Ica region; at the same time, the reuse of this waste reduces the environmental risk. Therefore, the objective was to characterize polyunsaturated fatty acids from the seeds of the fruit of *V. vinifera*, which are residues of Pisco production in the Ica Valley, Peru, and to determine the antioxidant activity *in vitro*.

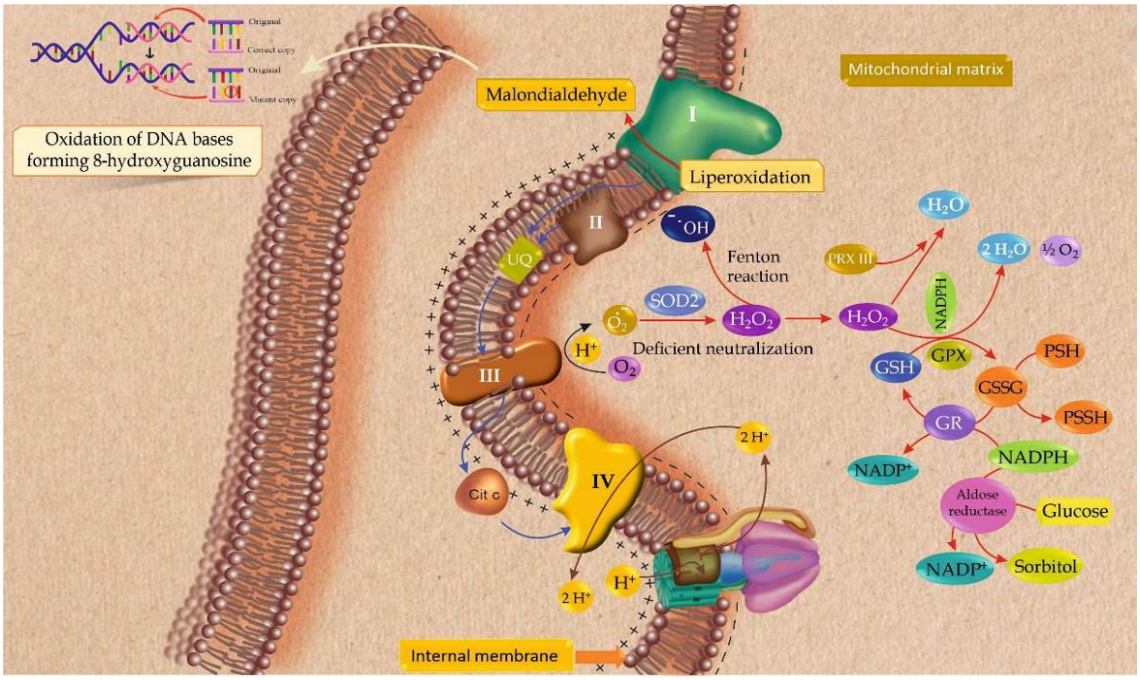

Figure 1. Formation of superhydroxyl by Fenton reaction and lipid peroxidation of the inner membrane of mitochondria.

It is proposed that antioxidant enzymes lose their activity, so the neutralization of hydrogen peroxide (H_2O_2) is deficient: peroxiredoxin III (PRX III) biotransforms H_2O_2 into water molecules (H_2O); reduced glutathione (GSH) is converted to oxidized glutathione (GSSG) by activating glutathione peroxidase (GPX) with the cofactor NADPH to metabolize H_2O_2 into two H_2O molecules; at the same time, GSSG is reduced to GSH by the action of glutathione reductase (GR) with the participation of the NADPH cofactor, which is also oxidized to $NADP^+$; NADPH is consumed by activating aldose reductase that converts glucose into sorbitol (Carvajal, 2019). Figure made by the authors.

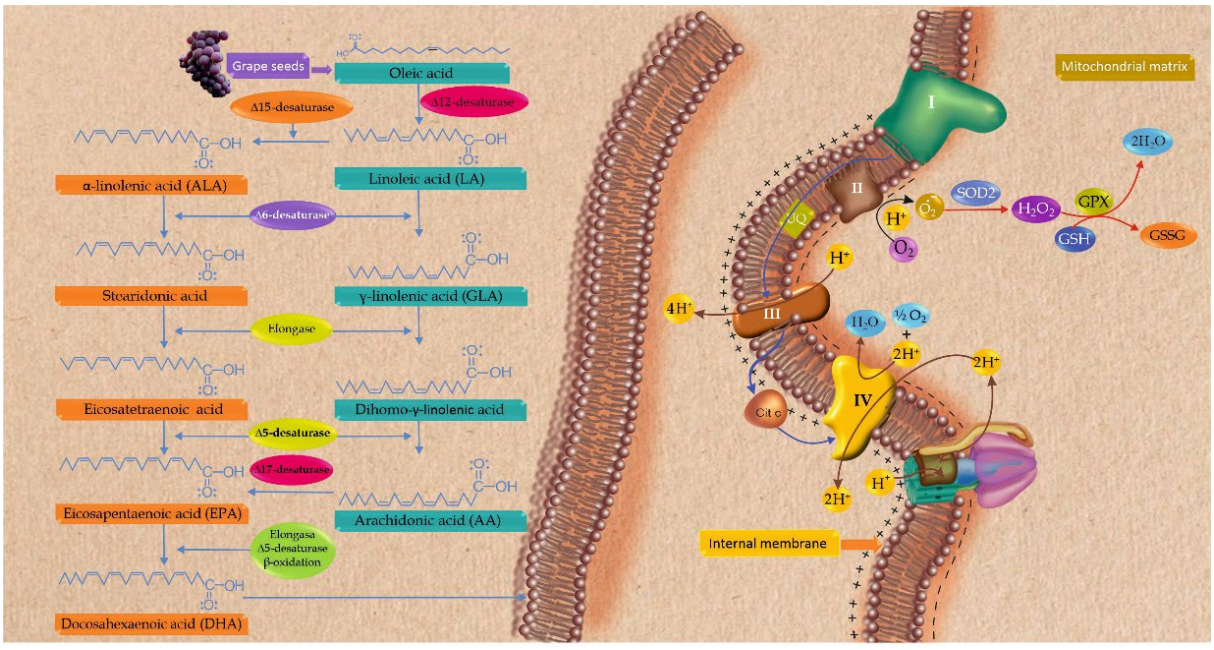

Figure 2. Polyunsaturated oil from the seeds of *V. vinifera* fruits and possible incorporation into cell membranes.

It is proposed that the oleic and linoleic acid of grape seeds, when consumed by humans, are metabolized to form docosahexaenoic acid (DHA) and its insertion into cell membranes. The restoration of the function of the inner membrane of the mitochondria and the electron transport chain is also indicated. It is observed that superoxide dismutase 2 (SOD2) generates hydrogen peroxide (H_2O_2) from superoxide ($^{\cdot}O2$) and hydrogen ion (H^+); immediately, H_2O_2 is broken down by glutathione peroxidase (GPX) into two molecules of water (2 H_2O). Reduced glutathione (GSH) is converted to oxidized glutathione (GSSG) upon activation of GPX. Figure made by the authors.

MATERIAL AND METHODS

Chemical and reagents

All chemicals and solvents used were reagent grade: chloroform, petroleum ether, ethanol, methanol, and carbon tetrachloride (Brand Beaker, USA); sodium acetate, acetic acid, hydrochloric acid, starch, dimethyl sulfoxide, phenolphthalein, sodium hydroxide, potassium hydroxide, sodium thiosulfate, ferric trichloride, and Folin Ciocalteu reagent from Merck (Germany); gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ [2,4,6-Tris(2-pyridyl)-s-triazine] brand Sigma-Aldrich (USA); and the Supelco brand fatty acid methyl ester mixture standard (Chávez et al., 2021).

Obtaining samples and their treatment

The pomace samples obtained after the extraction of the grape juice through the pressing process were collected during the months of February-March 2020 from the Center for Productive Innovation and Agroindustrial Technology Transfer of Ica (CITE Agroindustrial) (13°59'10" S, 75°46'23"W at 430 m.a.s.l), Salas district, Ica province, Ica region, Peru. The collected samples were transported to the Instrumental Analysis laboratory of the Faculty of Pharmacy, San Luis Gonzaga National University, maintaining a cold chain and under quality criteria to avoid pomace failure (Carrasco et al., 2013; Huaccho et al., 2012). In the laboratory, it was dried under shade for 15 days, then they were manually pulped. The seeds were placed in a sieve and washed under running water. Then, it was conditioned on Kraft paper and allowed to dry at room temperature for 24 hours; the dry seeds were stored in an amber glass bottle and at room temperature until processing (Garavaglia et al., 2016; Konuskan et al., 2019).

Oil extraction process

The grape seeds were ground in a manual mill (Ivymen JP Selecta, YCW-010E, Spain). The finely ground was incorporated into a 500 mL flask, and petroleum ether was immediately added as extraction solvent (sample: solvent ratio 1:3). Then extraction was carried out using an ultrasonic bath (MH series of 5.7 L Model M3800H-E/Branson Ultrasonics, USA) for 30 min. The process was carried out during three periods, and between periods a 10 min rest was carried out (this extraction was carried out in duplicate). The obtained liquid extract was filtered and dried with a rotary evaporator (Heidolph model Laborota 4000, Germany) at reduced pressure. Then it was taken to a water bath (Gemmyco, YCW-010E, Taiwan) at 40°C for one hour to eliminate the solvent residue. The oil obtained was stored in an amber bottle at 4°C until analysis (Hanganu et al., 2012; Kaseke et al., 2020).

Physicochemical characterization of the oil

Refractive Index: It was measured directly with a refractometer (Abbe Refractometer, Atago, model DR-A1/NAR, Japanese) at 25°C (Franco-Mora et al., 2015).

Humidity: The sample was dried in an oven at 105°C (Binder Model ED-23, Germany) until constant weight, determining the weight loss as humidity and volatile matter (Franco-Mora et al., 2015).

Density: The mass of an empty pycnometer (10 mL) was determined, then with water and later with each of the oil samples on an analytical balance (Sartorius model ED224S, Germany), expressing the data in mass per conventional volume at 25°C (Franco-Mora et al., 2015).

Acidity index: 5 g of sample was weighed and dissolved with 100 mL of a solution (alcohol/ether), and 0.5 mL of phenolphthalein was added as an indicator and titrated with 0.1 N sodium hydroxide. The results are reported in the percentage of oleic acid (Franco-Mora et al., 2015).

Peroxide index: It was determined by volumetric titration of the iodine (I_2) released with sodium thiosulfate ($Na_2S_2O_3$) 0.002 N in a glacial acetic-chloroform medium. The results were expressed in mEq of active oxygen per kg of fat (Franco-Mora et al., 2015).

Saponification index: It was determined by volumetric titration with 0.5 N HCl after saponifying the sample with 0.5 N KOH in ethanol. The results are expressed in mg of potassium hydroxide required to saponify one g of oil (Franco-Mora et al., 2015).

Iodine index: 0.2 g of sample was weighed in an Erlenmeyer flask, immediately, added 20 mL of carbon tetrachloride (CCl_4) and 25 mL of Wijs reagent. The mixture was left to rest in the dark for 30 min, then 20 mL of potassium iodide (KI 15%) and 100 mL of distilled water were added. It was titrated with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) using starch as an indicator (Franco-Mora et al., 2015).

Characterization of fatty acids: It was determined using a high-performance Agilent 6890 (USA) gas chromatograph by using advanced electronic pneumatic control (EPC) and temperature control modules; The equipment consists of a flame ionization detector (FID), injector and automatic sampler, oven, injection tower, a tray for 100 samples and connected to a computer. The conditions of use were: split injec-

tor at 250ºC, ratio 50:1, Oven 130ºC at 215ºC at 2.5ºC/min at 215°C for 12 min, 215ºC at 230ºC for 3 min. Detector Fit at 280ºC, carrier hydrogen at 41 cm/sec, and injection volume 1 mL. Aldrich-Sigma brand fatty acid methyl ester mixture standards were used to characterize fatty acids (Hanganu et al., 2012; Konuskan et al., 2019).

In each physicochemical characterization test, each sample was measured in triplicate.

Total polyphenol content using the Folin-Ciocalteu method

To determine the total polyphenolic content and the antioxidant activity, dilutions of the seed oils in dimethyl sulfoxide (DMSO) (Merck Brand) were previously made.

Previously, a calibration curve of the gallic acid standard (Sigma-Aldrich) in 70% ($^{v}/_{v}$) alcohol in the concentration range of 1-5 mg/L was performed. 0.1 mL of oil sample was reacted with 0.25 mL of Folin-Ciocalteu solution (Merck, ratio 1 reagent: 2 HPLC water) and was sonicated for 5 min. After that time, 1.25 mL of 20% sodium carbonate (Na_2CO_3) and 0.4 mL of ultrapure water (HPLC grade) were added and stirred to homogenize. It was left to react for 90 min in the dark and at laboratory temperature. The absorbances of the samples and the blank were read in triplicate at a wavelength of 760 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). The total polyphenolic content was expressed in mg of gallic acid (GAE)/mL of oil (Bouyahya et al., 2018; Fruehwirth et al., 2020; Surco-Laos et al., 2022).

Antioxidant activity

DPPH radical scavenging assay

The reactive solution was prepared by diluting 3.1 mg of the DPPH radical (Sigma) in 100 mL of 80% methanol (Analytical Grade, Beaker) and using the spectrophotometer, the absorbance was determined, which was 0.9-1.1 at a length waveform of 517 nm. To 0.1 mL of the various oil dilutions, 2.9 mL of DPPH solution was added, obtaining a final volume of 3 mL, then it was shaken vigorously. The mixture (oil + DPPH) reacted for 60 min protected from light; after that time, both the reaction sample and the blank (methanol) were measured in triplicate at a wavelength of 517 nm (Spectrophotometer Peak Instrumental, model C-7100 USA). The percentage inhibition was determined by the formula [1].

$$\text{Inhibition (\%)} = \frac{\text{Blank abs.} - \text{Sample abs.}}{\text{Blank abs.}} \times 100 \quad [1]$$

From a curve of inhibition% *vs*. µL of oil, the IC_{50} was determined (Ramos-Escudero et al., 2012; Surco-Laos et al., 2022).

Ferric Reducing Antioxidant Power (FRAP)

The FRAP solution was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 10 mM diluted HCl (2,4,6-tripyridyl-s-triazine) TPTZ solution, and 40 mM trichloride ferric solution ($FeCl_3 \cdot 6H_2O$) 20 mM. 1.5 mL of FRAP solution is added to 0.10 mL of oil samples, homogenized in a vortex for 30 s, then incubated at laboratory temperature for 6 min. The study sample (oil + FRAP solution) and the FRAP solution were measured in triplicate at a wavelength of 593 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). Trolox was used as a reference compound at 0.0312-1.0 mM concentrations. The final absorbance was obtained by subtracting the absorbance value from the initial FRAP solution. A quantification curve of mM Trolox/mL of oil was made (Ramos-Escudero et al., 2012; Szabó et al., 2021).

Statistical analysis

The results were transcribed into an Excel spreadsheet, from where they were exported for statistical analysis. Data were expressed as mean ± standard deviation (SD). A 95% confidence interval (CI) and one-way variance analysis (ANOVA) were calculated. A $p<0.05$ was considered statistically significant. The GraphPad Prism 9 Statistical Software, version 9.1.2, was used.

RESULTS

In the production of Peruvian Pisco, eight varieties of *V. vinifera* are used, of which five varieties of grapes have been evaluated, which are typical of the Ica Valley. The total oil yield obtained was expressed in mL of oil/100g of seeds, finding a higher volume in the Moscatel and Quebranta varieties, but in the overall analysis, there are no major differences between the five grape varieties (Table 1). A previous study conducted by Fernandes et al. (2013) reported that the Portuguese varieties of *V. vinifera* contain between 3.95-12.4 g/100 g. Subsequently, Vieira et al. (2015) found 12-14 g/100 g in a *V. labrusca* species. Kapcsándi et al. (2021) recently obtained values between 9.9-12.6 g/100 g of the *V. vinifera* varieties from northeastern Hungary. Based on these studies, we can observe that the oil yield in the seeds of the Ica Valley is in the range of the reported percentages, even though it has been obtained from seeds that are residues of a productive process.

Table 1. Total oil yield obtained from five varieties of seeds of the fruit *V. vinifera.*

Grape seed variety	Seed weight (g)	Oil volume (mL)	Oil color
Mollar	100 ± 2.3	10.3 ± 0.7	Light yellow
Moscatel	100 ± 1.7	12.7 ± 0.4	Yellow-orange
Torontel	100 ± 2.4	11.8 ± 0.3	Yellow
Quebranta	100 ± 1.3	12.3 ± 0.2	Deep yellow
Albilla	100 ± 0.9	11.3 ± 0.5	Transparent yellow

Values in each variety are expressed as mean ± SD (n = 3).

Table 2. Physicochemical parameters of the oils obtained from five varieties of seeds of the fruit *V. vinifera*.

Parameter	Oil according to seed variety					p-value
	Mollar	Moscatel	Torontel	Quebranta	Albilla	
Refractive Index	1.460 ± 0.01	1.483 ± 0.02	1.473 ± 0.02	1.473 ± 0.02	1.473 ± 0.01	0.354
Humidity g/100g	0.130 ± 0.02	0,153 ± 0.03	0.133 ± 0.02	0.143 ± 0.03	0.130 ± 0.02	0.625
Density g/mL	0.917 ± 0.01	0.928 ± 0.01	0.927 ± 0.01	0.924 ± 0.01	0.928 ± 0.01	0.453
Acidity index	2.47 ± 0.06	2.23 ± 0.06	3.13 ± 0.06	2.06 ± 0.01	2.56 ± 0.01	5.84×10^{-10}
Peroxide index	19.47 ± 0.06	15.17 ± 0.12	20.47 ± 0.06	13.33 ± 0.12	16.77 ± 0.06	9.99×10^{-16}
Saponification index	196.37 ± 0.12	177.17 ± 0.06	172.83 ± 0.12	187.43 ± 0.12	197.33 ± 0.12	1.64×10^{-20}
Iodine Index	122.67 ± 0.06	124.03 ± 0.12	121.17 ± 0.06	120.33 ± 0.12	125.67 ± 0.06	2.39×10^{-14}

Values in each variety are expressed as mean ± SD (n = 3).

Table 2 shows the physicochemical parameters that characterize the oils according to their origin. The analysis of variance (ANOVA) shows that the means of the values of the acidity index, peroxide index, saponification index, and iodine index are statistically significant (p-value < α = 0.05);. In contrast, the means of the values of the refractive index, humidity, and density are not different in the varieties of seeds of the fruit *Vitis vinifera* (grape) (p-value > α = 0.05) between the varieties of grapes studied. The specific values found are consistent with what has been reported in previous studies and for oils for human consumption (Hasan et al., 2016; Negash et al., 2019; Paucar-Menacho et al., 2015; Vujasinović et al., 2021).

Various fatty acids obtained from the seeds of the *V. vinifera* fruit were characterized. Among them, a higher concentration of linoleic acid (Cis-9,12-octadecadienoic) was found, which is an essential polyunsaturated fatty acid (PUFA) of the omega(ω) 6 series, followed by a monounsaturated acid from the omega (ω) 9 series, called oleic acid (Cis-9-octadecenoic). To a lesser extent, another polyunsaturated fatty acid was identified as ɣ-linolenic acid (Cis-6,9,12-octadecatrienoic). These fatty acids vary according to the variety of *V. vinifera* fruit seeds (Table 3, Fig. 3A). The ANOVA shows that the means of the values (g/100 g) among the 5 varieties of grape seeds are statistically significant (p-value < α = 0.05), except for cis-10-heptadecanoic acid (p-value 0.871 > α = 0.05). Fig. 3B shows the superimposed chromatograms of the five types of oil analyzed by gas chromatography with a flame ionization detector (CG-FID). The identification was made based on the retention times of the mixtures composed of 37 methyl esters fatty acids according to the standard used. No residues of the solvent used in the extraction (ether) of the oils are observed, which elutes between approximately 1.5 to 2 min. Human beings cannot synthesize oleic acid and linoleic acid (LA), so consumption through the diet is required (they are essential fatty acids). It is known that oleic acid is a precursor of linoleic acid. The latter originates through two different metabolic pathways to arachidonic acid (AA) and eicosapentaenoic acid (EPA), then EPA is converted into docosahexaenoic acid (DHA) (Zavala-Naranjo, 2020). In a study conducted by Bazan (2005), it is indicated that DHA participates in memory, membrane excitability, neuronal signaling, and in neuroprotection; photoreceptor cell biogenesis and the functional integrity of retinal pigment epithelium (RPE) cells; through the metabolite neuroprotectin D1 (NPD1). It protects cells from apoptosis induced by oxidative stress. This is possible by inhibiting caspase-3, inducing an increase in the antiapoptotic proteins Bcl-2 and BclxL, and decreasing the proapoptotic expression of Bax and Bad. Subsequently, Sun et al. (2018) mentioned that DHA from the synaptic membranes of the brain and retina are metabolized by 15-lipoxygenase,

Table 3. Fatty acids of the oils from five varieties of seeds of the fruit *V. vinifera* piscoras.

Fatty acid (g/100g)	Gape variety					p-value
	Mollar	Moscatel	Torontel	Quebranta	Albilla	
Unsaturated	89.31	90.05	90.13	89.58	90.53	1.90×10^{-25}
Monounsaturated	19.13	19.82	20.03	18.97	18.47	2.89×10^{-26}
Oleic acid (18:1 ω-9)	18.99	19.70	19.90	18.35	18.85	1.64×10^{-19}
Polyunsaturated	70.18	70.23	70.10	70.61	72.06	4.80×10^{-21}
Linoleic acid (18:2 ω-6)	70.01	70.07	69.90	71.87	70.42	2.81×10^{-15}
ɣ-linolenic acid (18:3 ω-6)	0.17	0.16	0.20	0.19	0.19	5.34×10^{-5}
Palmitoleic acid (16:1Δ9)	0.09	0.07	0.09	0.07	0.07	8.49×10^{-11}
Cis-10-pentadecanoic acid (C15:1)	0.02	0.02	0.01	0.02	0.02	1.25×10^{-6}
Cis-10-heptadecanoic acid (C17:1)	0.03	0.03	0.03	0.03	0.03	0.871
Saturated	10.70	9.95	9.87	10.42	9.47	7.82×10^{-19}
Myristic acid (C 14:0)	0.08	0.03	0.04	0.03	0.04	6.96×10^{-16}
Palmitic acid (C16:0)	6.85	6.26	7.02	6.56	6.56	1.91×10^{-18}
Stearic acid (C18:0)	3.72	3.66	2.80	2.88	3.81	1.45×10^{-20}
Trans fatty acids	0.00	0.00	0.00	0.00	0.00	-

Values in each variety are expressed as mean.

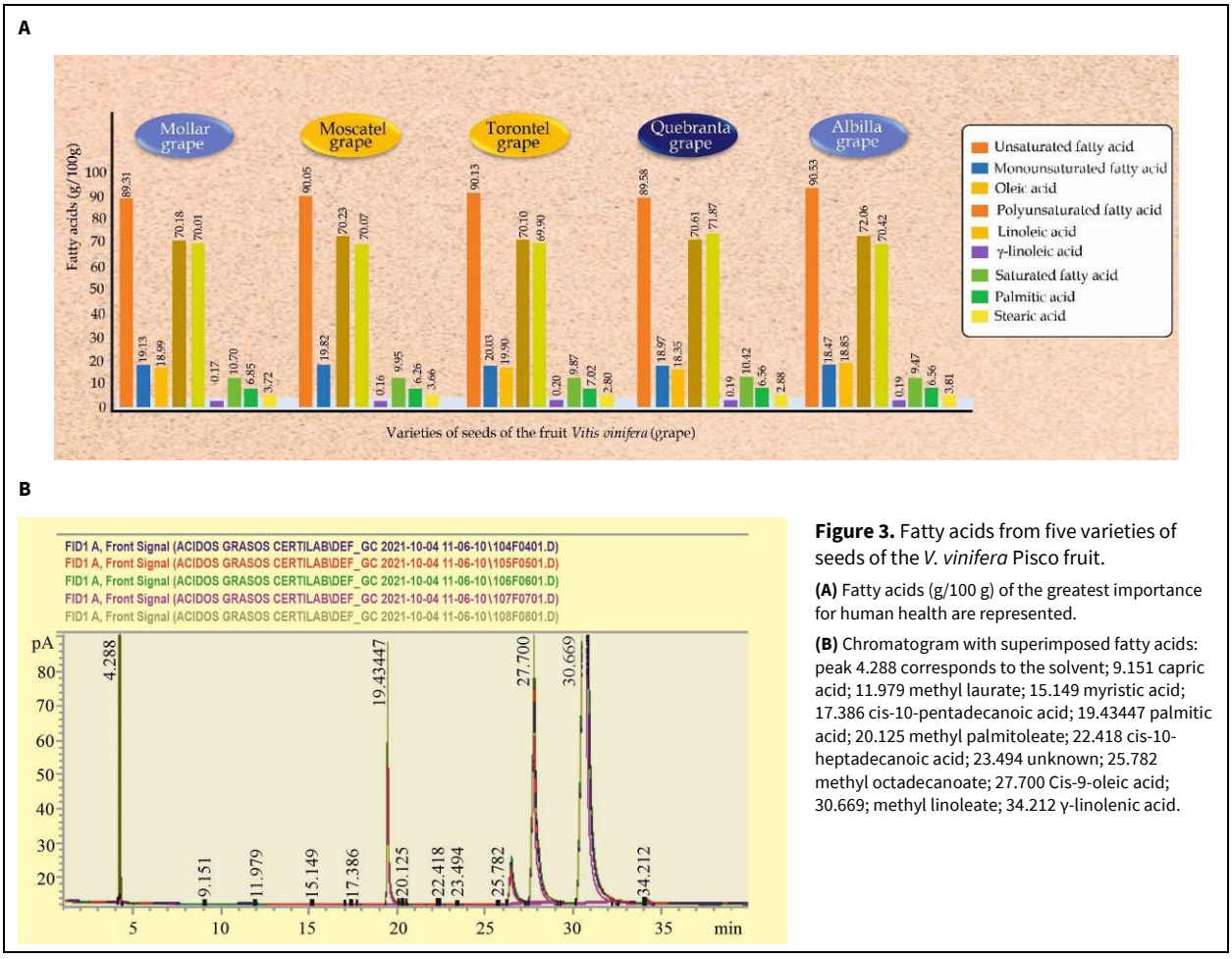

Figure 3. Fatty acids from five varieties of seeds of the *V. vinifera* Pisco fruit.

(A) Fatty acids (g/100 g) of the greatest importance for human health are represented.

(B) Chromatogram with superimposed fatty acids: peak 4.288 corresponds to the solvent; 9.151 capric acid; 11.979 methyl laurate; 15.149 myristic acid; 17.386 cis-10-pentadecanoic acid; 19.43447 palmitic acid; 20.125 methyl palmitoleate; 22.418 cis-10-heptadecanoic acid; 23.494 unknown; 25.782 methyl octadecanoate; 27.700 Cis-9-oleic acid; 30.669; methyl linoleate; 34.212 ɣ-linolenic acid.

Table 4. Estimation of the content of total polyphenols and antioxidant activity of the seed oils of five varieties of seeds of the fruit *Vitis vinifera* (grape) piscoras.

Seed variety	Assay		
	TPC (mg GAE/g)	DPPH (IC_{50}) µL/mL	FRAP (µg TEAC/g)
Mollar	0.72 ± 0.03	44.60 ± 0.30	0.66 ± 0.04
CI	0.0285	0.3395	0.0471
Moscatel	1.54 ± 0.04	38.30 ± 0.80	0.77 ± 0.02
CI	0.0397	0.9053	0.0226
Torontel	0.59 ± 0.04	48.43 ± 0.35	0.49 ± 0.03
CI	0.0397	0.3974	0.0285
Quebranta	1.39 ± 0.04	42.70 ± 0.40	0.58 ± 0.03
CI	0.0471	0.4526	0.0285
Albilla	1.22 ± 0.03	42.50 ± 0.53	0.58 ± 0.03
CI	0.0346	0.5988	0.0285
Reference compound*	Gallic acid 33.5-700 µg/mL	Trolox 0.032-0.5 mM	Trolox 0.032-0.5 mM
p-value	3.017×10^{-11}	4.153×10^{-9}	3.531×10^{-6}

Values in each variety are expressed as mean ± SD (n = 3). *Values of the calibration curve. The one-factor analysis of variance (ANOVA) for CPT/DPPH and CPT/FRAP shows that the mean difference is statistically significant ($p<0.05$) except for the Mollar CPT/FRAP variety ($p>0.05$). TPC: total polyphenol content (mg GAE/g); TEAC: mg equivalent to 1 mM Trolox/g. CI: 95% Confidence Interval.

forming resolvins (RvD1, RvD4) and neuroprotectin D1 (NPD1), which are related to learning and intelligence level of newborns and infants. At the same time, Yang et al. (2018) demonstrated that DHA and its metabolites suppress the production of reactive oxygen species (ROS) in BV-2 microglial cells. Di Stefano et al. (2022) mention that grape seeds are a source of oil with great food, cosmetic and industrial value. In this sense, finding DHA precursor fatty acids in grape seeds becomes a potential nutraceutical with neurofunctional activity.

The total polyphenolic content (TPC) was determined in mg gallic acid equivalents (GAE)/g of the *V. vinifera* fruit seed oil. The potential antioxidant activity was carried out with the DPPH method (by transfer of a hydrogen atom to the free radical, HAT), for which the percentages of inhibition were calculated, and with these data, the concentration in which 50% of the antioxidant is neutralized was determined DPPH free radicals (IC_{50}); and by the FRAP method (transfer of an electron from the antioxidant compound, SET) a calibration curve was made with the trolox standard, to establish the antioxidant activity as Trolox equivalent. It is observed that the oil obtained from the five varieties of seeds of the *V. vinifera* fruit present antioxidant activity by in vitro methods, the oil of the Moscatel variety being more active (Table 4); such activity may be due to the presence of phenols in the grape seeds. The ANOVA shows that the means of the TPC, DPPH, and FRAP values among the five seed varieties of the *Vitis vinifera* fruit (grape) are statistically significant (p-value < $\alpha = 0.05$). Di Stefano et al. (2022) found various phenolic compunds in Sicily grape seeds, such as p-coumaric acid, gallic acid, caffeic acid, myricetin, epicatechin, quercetin, resveratrol, flavonoids and anthocyanins. In another study carried out by Ferreira and Santos (2022) reported that grape seeds contain 17.4 ± 0.4% TPC; and the antioxidant capacity measured by the DPPH method showed an IC_{50} of 55.9 ± 0.7 µg of extract/mL DPPH.

The limitation of this study is the number of samples studied. Five of the eight varieties of grapes from the Ica Valley used in the elaboration of Pisco have been studied. Another limitation is not having quantified or elucidated the chemical structure of phenolic compounds, which our research group is considering to be evaluated in future studies. Notwithstanding the foregoing, we believe that this study is relevant since it identifies essential mono and polyunsaturated fatty acids in grape seeds that are residues of the production process of the wine industry, especially Pisco, which has characteristics typical of the Region. This can represent a new economic and sustainable activity for the inhabitants of the Ica Valley.

CONCLUSION

Oils from the seeds of the *Vitis vinifera* fruit (grapes), which are residues from the production of Pisco, present physicochemical characteristics of oils of high nutritional value with a predominance of polyunsaturated fatty acids and potential antioxidant activity.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Surco F	García JA	Bendezú MR	Alvarado AT	Laos D	Valle M	Panay J	Palomino JJ	Yarasca PE	Muñoz AM	Bolarte M	Pineda M	Loja B
Concepts or ideas	x	x	x	x	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x							x		
Literature search					x	x	x	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x	x	x	x				
Data acquisition	x	x	x		x	x							
Data analysis	x			x				x		x	x	x	x
Statistical analysis			x					x				x	
Manuscript prepara-tion	x	x	x	x									
Manuscript editing	x	x	x	x	x					x			
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x	x

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