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# Malbec variety grape seeds. A waste product from the wine industry containing fatty acids, resveratrol, anthocyanins, and antioxidant compounds

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***Abstract:*** ***Waste from the wine industry is produced in large quantities worldwide. This waste is grape pomace, composed of stems, skins, and seeds. This study aimed to determine fatty acids, resveratrol, anthocyanins, and antioxidant capacity in Malbec grape seeds. First, the oil was extracted from the previously pulverized grape seeds and then the fatty acids were quantified by gas chromatography and resveratrol by spectrophotometry. The results showed that the oil presented a concentration of palmitoleic, stearic, oleic, and linoleic acids of 13.54, 7.06, 32.43, and 51.74 %, respectively, and a resveratrol concentration of 2.89 ± 0.15 mg/kg. Subsequently, the extract of defatted seeds was obtained using ethanol: $H_2O$: HCl and 70 % ethanol, finding a concentration of total anthocyanins of 258.05 ± 5.89 and 121.55 ± 2.42 mg C3G/ 100 g, respectively, and an antioxidant capacity of 15.12 ± 0.12 mmol TE/L and 13.75 ± 0.17 mmol TE/L, respectively. The present study is of crucial importance for developing research in companies dedicated to the production of wine to valorize the components found and take advantage of these wastes economically since their composition can be of pharmaceutical and biotechnological interest.***

***Keywords:*** ***Malbec grape seeds, fatty acids, resveratrol, anthocyanins, antioxidant capacity.***

## I. Introduction

Grapes are produced all over the world for direct consumption and the production of industrial products [1] as the international wine production [2]. Grape production in Peru includes aromatic grapes such as Italia, Moscatel, Albilla, and Torontel and non-aromatic grapes such as Quebranta, Negra Criolla, Mollar and Uvina [3] mainly in departments such as Lima, Ica, Moquegua, Arequipa and Tacna [4]. In the Arequipa region of Perú, wine or pisco is produced from grapes of different varieties, including the Malbec grape variety, which is used for the production of wine, the main producing areas being the valleys of Caravelí, Majes, and Vítor [3].

Although wine production is an important economic source in Peru and the world, it has been demonstrated that this activity is related to the generation of organic and inorganic waste, which represents an environmental problem [5]. Waste from the wine industry corresponds to grape pomace that is not used and is otherwise discarded [6]. Disposing of waste without using it is not in line with sustainable development, which involves three basic pillars: environmental, economic, and social [7]. Based on this, the concept of circular economy proposes a model of society that seeks to convert waste into raw materials [8], taking into account the "reduce, reuse, and recycle" principles of the environmentalist school of thought [9]. For companies to adopt the circular economy model, it is necessary to implement environmental policies [10].

In consideration of the above, the wine industry produces waste with a high potential for reuse [11], this residue is called grape pomace, which consists of the seeds, skins, and stems [12], and represents a source of renewable energies and high value-added compounds [13, 14]. Grape seed is one of the by-products of greatest interest [15] for its oil content with polyunsaturated fatty acids content [16], and resveratrol [17], The seeds also contain phenolic compounds such as anthocyanins, flavonoids, flavanols, tannins and flavan-3-ols [18] that give this residue antioxidant properties [19].

Therefore, this research aimed to determine the concentration of fatty acids, resveratrol, anthocyanins, and antioxidant capacity in Malbec grape seeds to substantiate the potential added value of this residue. Considering the above, the present research was developed according to the process outlined in Fig. 1, which began with obtaining the grape seeds, followed by extraction of the oil, then the fatty acids and resveratrol present in the oil were quantified, and then extracts were obtained from the defatted seeds, from which the concentration of anthocyanins and antioxidant capacity were determined.

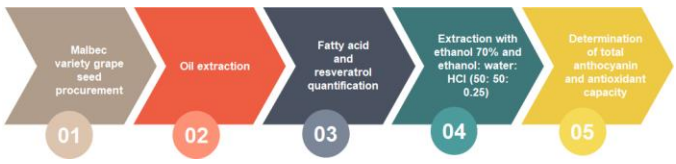


**Fig. 1.** Diagram of the study process of Malbec grape seeds.

**Fig. 2.** Grape seed Malbec variety a) before powdering, and b) after powdering.

## II. Materials and Methods

### A. Collection and characterization of Malbec grape variety seeds

The seed residues of the Malbec variety were obtained from a pisco and wine production company in the district of Vitor, located in Arequipa, Peru. The procedure consisted of collecting the grape seeds, which were taken to the laboratories of the Universidad Católica de Santa María, and then washed with abundant distilled water to eliminate the remains of skins and stems until a quantity of 0.5 kg of seeds was obtained. The seeds were then dried at 40 °Cin a Memmert oven until constant weight. Once the grape seeds were dried (Fig. 2a), they were powdered in a blade mill until a uniform powder was obtained (Fig. 2b). Subsequently, an adequate amount of seeds was analyzed by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) on the Agilent Cary 630 ATR-FTIR Spectrometer to identify the functional groups present in the grape seeds [20].

### B. Oil extraction

Oil extraction was carried out in Soxhlet extraction equipment [21]. For this purpose, 20 g powdered grape seeds were weighed and 150 mL of petroleum ether was used as solvent. Once the oil extraction was finished, the solvent was recovered in a rotary evaporator. The oil obtained was stored in an amber flask under refrigeration at 4 to 8 °C until further analysis.

### C. Determination of the fatty acid profile in oil

The fatty acid profile of the oil was determined by the Gas Chromatography analysis service at the Quality Control Laboratory (LECC) of the Catholic University of Santa Maria (UCSM).

### D. Determination of resveratrol in oil

For the determination of resveratrol, a calibration curve was prepared using the analytical standard resveratrol obtained from Sigma Aldrich. The concentrations of the calibration graph were 0.4, 2, 4, 6, 8, and 10 mg/L in dimethyl sulfoxide (DMSO) which were prepared from a stock solution of 100 mg/L in DMSO. The calibration solutions were prepared in a 10 mL volumetric flask. The oil sample was analyzed using one gram of oil in a 5 mL volumetric flask where it was diluted with DMSO. The absorbances of the standards and the diluted oil sample were analyzed on the Thermo Scientific Genesys 150 Uv-vis Spectrophotometer at 307 nm [22].

### E. Preparation of extracts

Two extracts were prepared by weighing 0.5 g of defatted grape seed powder, then 10 mL of a mixture of absolute ethanol: water: concentrated HCl in a ratio of 50: 50: 0.25 was added, respectively, and then it was taken to ultrasound at 40 kHz for 30 minutes. Another extraction was performed using 70 % ethanol as solvent with the same procedure. The extracts obtained were filtered and made up to 10 mL in a volumetric flask, then stored in the dark under refrigeration until further use.

### F. Determination of total anthocyanins in extracts

Total anthocyanins were quantified using the differential pH assay [23]. The procedure consisted of two assays:

- Assay 1: 0.1 mL of the fluid extract was measured in a 5 mL volumetric flask and made up to volume with Buffer pH=1 and analyzed in a spectrophotometer at 510 nm and 700 nm.

- Assay 2: 0.1 mL of the fluid extract was measured in a 5 mL volumetric flask and made up to volume with Buffer buffer pH= 4.5 and analyzed in a spectrophotometer for readings at 510 nm and 700 nm.

The calculation of anthocyanins was performed by first determining the net Absorbance "Ab" with Equation 1:

$$Ab = (A_{510nm} - A_{700nm})_{pH=1} - (A_{510nm} - A_{700nm})_{pH=4.5} \quad (1)$$

For the calculation of total anthocyanins, the value of Ab was replaced in Equation 2.

$$TA_{(mgC3G/100g)} = \frac{Ab}{\varepsilon \times l} \times MW \times D \times \frac{V}{G} \times 100 \quad (2)$$

Where "$\varepsilon$" is the molar extinction coefficient of cyanidin-3-glucoside (26900 L $mol^{-1}cm^{-1}$), "$l$" is the cell path length (1 cm), "$MW$" is the molecular weight of anthocyanins (449. 2 g/mol), "$D$" is a dilution factor (5 mL/ 0.1 mL), "$V$" is the final volume of extract obtained (10 mL) and "$G$" is the seed weight (0.5 g). The results were expressed as milligrams of cyanidin-3-glucoside equivalents per 100 grams of powdered grape seed weight (mg C3G/100 g).

### G. Determination of antioxidant capacity in extracts

Antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [24, 25, 26]. For this, a calibration curve was prepared by reacting solutions of Trolox calibration solutions at concentrations of 2, 4, 10, 20, 30, and 40 mmol/L. Of these solutions, 0.01 mL was measured in test tubes where 3 mL of DPPH was added at a concentration of 0.5 mg/mL. The analysis of the extract was done in the same way by measuring 0.01 mL of extract in test tube where 3 mL of DPPH solution was added. It was left to stand for 30 minutes in the dark and then the absorbance of DPPH was measured at 517 nm which decreases according to the concentration of Trolox or antioxidant compounds present in the extracts.

## III. Results and Discussion

### A. FTIR characterization

Fig. 3 shows the spectra of the FTIR analysis of Malbec grape seeds. It is observed that at 3320 $cm^{-1}$ there is a characteristic stretching vibration of the -OH groups, likewise, at 2919 $cm^{-1}$ and 2847 $cm^{-1}$ stretching vibrations of $-CH_3$ are observed, at 1739 $cm^{-1}$ stretching vibrations of C=O groups are observed, at 1608 $cm^{-1}$, and 1516 $cm^{-1}$ stretching vibrations of C=C groups are observed, at 1437 $cm^{-1}$ and 1241 $cm^{-1}$ C-H deformation vibrations are observed, finally, at 1025 $cm^{-1}$ C-O stretching vibrations are observed.

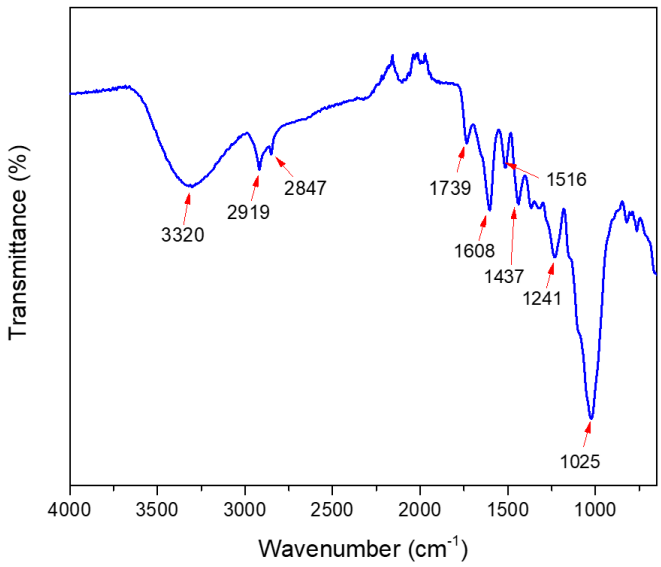


**Fig. 3.** FTIR spectra of Malbec grape seeds.

### B. Fatty acids in grapeseed oil

The Malbec grape seed oil obtained has a greenish-yellow color, as shown in Fig. 4, and 4 mL of oil was obtained.

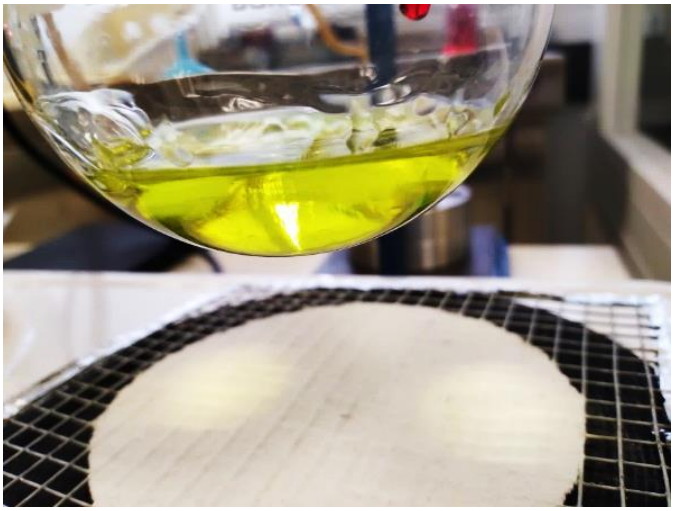

**Fig. 4.** Grape seed oil Malbec variety

Fig. 5 shows the chromatogram of fatty acids present in Malbec grape seed oil obtained by gas chromatography.

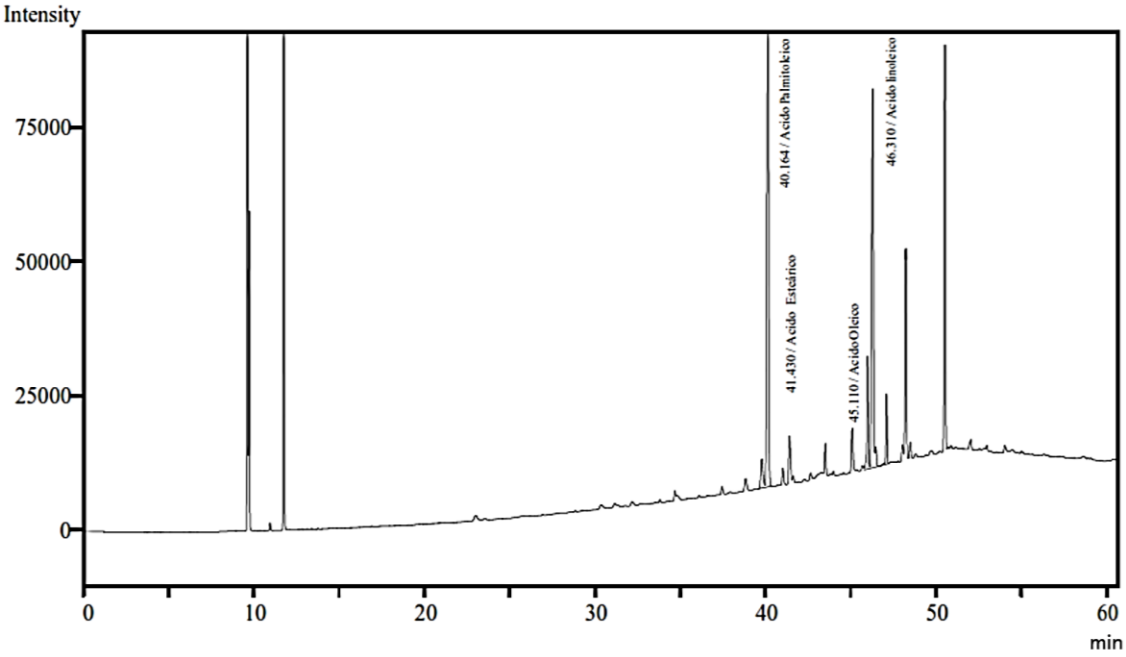


**Fig. 5.** Chromatogram of fatty acids present in Malbec grape seed oil obtained by gas chromatography.

Table I shows the fatty acid profile of Malbec grape seed oil, where it is observed that the oil contains a higher proportion of linoleic acid and oleic acid. A similar study determined a linoleic acid concentration of 71 % in the same grape seed variety [27]. On the other hand, in other grape seed varieties, linoleic acid 71.5 %, oleic acid 17.2 %, palmitic acid 6.6% and stearic acid 4.3 % were found [28], in the Quebranta variety 66.45 % linoleic acid and 20.05 % oleic acid [29] and in the Negra Criolla variety oleic acid concentrations of 17.36 % and linoleic acid of 68.75 % [30].

**TABLE I**
FATTY ACID PROFILE OF MUSCATEL VARIETY GRAPE SEED OIL

| Fatty acids | Percentage (%) |
|---|---|
| Palmitoleic acid | 13.54 |
| Stearic acid | 7.06 |
| Oleic acid | 32.43 |
| Linoleic acid | 51.74 |

## C. Resveratrol in grape seed oil

Fig. 6 shows the calibration graph relating to resveratrol concentration versus absorbance at 307 nm. The coefficient of determination $R^2$ is 0.9972 which indicates a linear correlation between resveratrol concentration and absorbance making quantification possible at this wavelength. In comparison to the study developed by Davidov-Pardo and McClements [22] who developed this spectrophotometric process obtained an $R^2$ of 0.9999 at concentrations between 0.2 to 6 mg/L using DMSO.

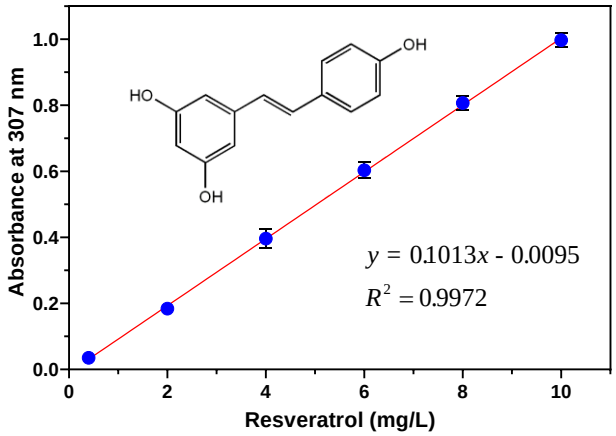


**Fig. 6.** Calibration graph for resveratrol quantification

For the quantification of resveratrol in oil, the equation of the line presented in Fig. 6 and Equation 3 was used:

$$y = 0.1013x - 0.0095 \quad (3)$$

Considering that "$x$" is the concentration of resveratrol in mg/L, and "$y$" the absorbance at 307 nm, Equation 4 is obtained. In this last equation "$V$" corresponds to the volume in liters of the vial where the oil dilution was prepared (0.005 L) and "$m$" is the mass of oil in kilograms diluted in the test tube (0.001 kg). This equation was used to quantify resveratrol in oils.

$$\text{Resveratrol } (mg/kg) = \frac{\text{Absorbance at } 307\,nm + 0.0095}{0.1013} \times \frac{V}{m} \quad (4)$$

Table II shows the concentration of resveratrol present in Malbec grape seed oil, with an average of 2.89 ± 0.15 mg/kg. Other studies found resveratrol in other grape seed varieties from 1.98-2.09 mg/kg [17] and 6.90 mg/kg in grape seed oil Muscatel variety [31].

**TABLE II**
GRAPE SEED OIL FATTY ACIDS

| N | Absorbance at 307 nm | Resveratrol (mg/L) | Resveratrol (mg/kg) | |
|---|---|---|---|---|
| | | | Individual | Average ± s |
| 1 | 0.046 | 0.55 | 2.74 | 2.89 ± 0.15 |
| 2 | 0.049 | 0.58 | 2.89 | |
| 3 | 0.052 | 0.61 | 3.04 | |

s: standard deviation

## D. Anthocyanins in grape seed extract

Table III shows the results of the concentration of total anthocyanins present in the extract of Malbec grape seeds obtained with ethanol: $H_2O$: HCl (50: 50: 0.25) and 70 % ethanol, finding total anthocyanin concentrations of 258.05 ± 5.89 mg C3G/100 g and 121.55 ± 2.42 mg C3G/100 g, respectively. Similar studies in grapes found total anthocyanin concentrations ranging from 50 to 499 mg C3G/100 g [32], in another study carried out on the skin, they found 233.15 mg C3G/ 100 g using methanol: water (50:50) as solvent [33]. They also found concentrations of 11.89 mg C3G/100 g, 219.38 mg C3G/100 g, and 3.41 mg C3G/100 g for pulp, peel, and seed, respectively [34].

**TABLE III**
CONCENTRATION OF TOTAL ANTHOCYANINS IN GRAPE SEED EXTRACTS.

| N | Total anthocyanins (mg C3G/100 g) | |
|---|---|---|
| | Ethanol: $H_2O$: HCl (50: 50: 0.25) | Ethanol 70 % |
| 1 | 253.47 | 124.13 |
| 2 | 255.98 | 121.19 |
| 3 | 264.69 | 119.33 |
| Average ± s | 258.05 ± 5.89 | 121.55 ± 2.42 |

s: standard deviation

## E. Antioxidant capacity of grape seed extracts

Fig. 7 shows the calibration graph obtained by plotting the concentration of Trolox versus the absorbance of DPPH at 517 nm. This figure shows that the response of the method is linear with an $R^2$= 0.9997.

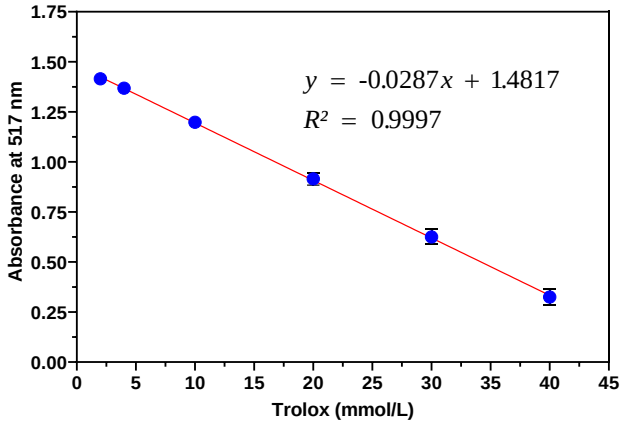


**Fig. 7.** Calibration graph for the quantification of antioxidant capacity by the DPPH method.

Table IV shows the antioxidant capacity expressed in millimoles of Trolox equivalents per liter of fluid extract (mmol TE/L). The antioxidant capacity for the extracts obtained with ethanol: $H_2O$: HCl (50:50:0.25) and 70 % Ethanol was 15.12 ± 0.12 mmol TE/L and 13.75 ± 0.17 mmol TE/L, respectively. Among the group of anthocyanins **present** in grape seed that could provide this residue with antioxidant properties are cyanidin, peonidin, delphinidin, petunidin, malvidin, and pelargonidin [35, 36].

**TABLE IV**
ANTIOXIDANT CAPACITY IN GRAPE SEED EXTRACTS

| N | Absorbance of DPPH at 517 nm | | Antioxidant capacity (mmol TE/L) | |
|---|---|---|---|---|
| | Ethanol: $H_2O$: HCl (50: 50: 0.25) | Ethanol 70 % | Ethanol: $H_2O$: HCl (50: 50: 0.25) | Ethanol 70 % |
| 1 | 1.048 | 1.082 | 15.11 | 13.93 |
| 2 | 1.044 | 1.092 | 15.25 | 13.58 |
| 3 | 1.051 | 1.087 | 15.01 | 13.75 |
| Average ± s | | | 15.12 ± 0.12 | 13.75 ± 0.17 |

**s:** standard deviation

## III. CONCLUSIONS

It was found that grape seeds of the Malbec variety present oil with palmitoleic, stearic, oleic, and linoleic acid content with 13.54, 7.06, 32.43 and 51.74 %, respectively. Also, it was found that the oil presents resveratrol with a concentration of 2.89 ± 0.15 mg/kg. On the other hand, the extract presents a concentration of 258.05 ± 5.89 and 121.55 ± 2.42 mg C3G/ 100 g for the extracts obtained with ethanol: $H_2O$: HCl and 70 % ethanol, respectively. It was demonstrated that acidified ethanol extracts better anthocyanins from Malbec grape seeds. An antioxidant capacity of 15.12 ± 0.12 and 13.75 ± 0.17 mmol TE/L was found for the extracts obtained with ethanol: $H_2O$: HCl and 70 % ethanol,

respectively. With the present research, further studies related to the valorization of anthocyanins and oil present in Malbec grape seed wastes can be initiated, since they can potentially be used in the pharmaceutical and biotechnological industries.

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