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RESEARCH ARTICLE

# Characterization of Cabernet, Grenache, and Syrah grape marc powders produced in northwestern Mexico

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## ABSTRACT

Grape marc powders from winemaking using the cultivars Cabernet Sauvignon (Cabernet), Grenache noir (Grenache), and Petite Syrah (Syrah) produced in northwestern Mexico were evaluated for potential incorporation into food formulations. The powders showed water activity of ≤ 0.4, the microbiological load was four log CFU/g without the presence of Gram-negative bacteria. The physical properties of all powders revealed intermediate flow properties. Grenache showed higher values in color than Cabernet and Syrah. The main chemical compositions present were as follows: fat, 7-9%; protein, 12-13%; and dietary fiber, 56-66%. The total phenols were 2.50, 1.47, and 2.30 g GAE/100 g powder for Cabernet, Grenache, and Syrah, respectively. Antioxidant capacity (DPPH) was 19.25, 19.32, and 17.52, μM ET/g for Cabernet, Grenache, and Syrah, respectively. Trace of soluble sugars and monosaccharides were present in all powders. The powders contained minerals Cu and Mn to such a concentration that they could provide ≈ 100% of the recommended daily intake, while Ca, Fe and Mg were present to provide ≈ 50%; Na concentration was low (≈ 2%). Taken together, we found that the grape marc powders of northwestern Mexico are suitable to be incorporated into food formulations.

**Keywords:** Nutritional; Antioxidant; Grape pomace

## INTRODUCTION

In Mexico, winemaking is the primary use for industrially produced common grapevine (*Vitis vinifera* L.). In 2019, the country harvested 65,576 tons of grapevine, with northwestern Mexico contributing 56,574 tons, or 86%, of the total production (SIAP, 2020). Cultivars produced in northwestern Mexico include Cabernet Sauvignon (Cabernet), Grenache noir (Grenache), and Petite Syrah (Syrah). In recent years, wine production in Mexico has increased, with an annual per capita consumption of 1.02 liters. In response to this industry growth, a collective called Vino Mexicano, consisting of Mexico's wine producers (Consejo Mexicano Vitivinícola, 2021), was founded. However, such growth in the wine industry has led to the increased generation of agro-industrial waste. The waste produced in grape winemaking is equivalent to more than 25% of total production (Gómez-Brando et al., 2019) and can be an environmental pollution problem. Grape marc (waste) includes stalks, seeds, and skin discarded during the pressing of the grapes in wine production (Zhang et al., 2017). These grape byproducts, though, may have value in the food sector as a source of nutritional and functional compounds with potential health benefits (Moncalvo et al., 2016; Sette et al., 2020). Slight variations in the elemental make-up of grape marc biomasses have been found (Zhang et al., 2017). Macronutrients, micronutrients, and functional compounds content varying depending on the cultivar and the region in which the grapes were harvested (Apolinar-Valiente et al., 2015). These variations can guide the application of the byproducts in raw material, compost, feedstock, energy, or other products (Zhang et al., 2017). This research aimed to evaluate the use of grape marc powders from Cabernet, Grenache, and Syrah cultivars harvested and processed in Mexico as ingredients in food products.

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## MATERIALS AND METHODS

### Grape marc powders

For the present study, grape marcs from northwestern Mexico were obtained in 2020. The cultivars Cabernet, Grenache, and Syrah, were used. The residence time of the grape marcs at room temperature was 24 hours after pressing. Then, the grape marcs were transferred onto the ice and stored at -20 °C. Next, 250-gram lots of grape marc powder were obtained by lyophilization at 0.133 mBar, -40 °C by 48 hours (FreeZone 7751020, LABCONCO), and milled at 280 rpm (Grinder, Huangchueng, China) for 60 seconds. The powders were stored at -20 °C until being analyzed.

#### *Microbial load*

Ten grams of powder were mixed with 90 mL of sterile peptone water (0.1%), and serial dilutions were prepared. Next, 1 ml of each dilution were homogenized with Plate Count Agar and incubated at 37 °C for 48 hours. The microbial load was expressed as colony formed unit log per g of powder (log CFU/g) (Hawashin et al., 2016). In addition, to quantify the Gram-negative bacteria, plates with MacConkey agar were incubated at 37 °C for 48 hours (Rostami et al., 2018).

#### *Chemical composition*

The moisture (gravimetric), fat (Soxhlet), ash (muffle furnace), and proteins (micro Kjeldahl) of the grape marc powders were calculated using the Association of Official Analytical Chemists (AOAC, 2000) methods. Content fiber (insoluble and soluble) was calculated based on method 991.43 (AOAC), using the Total Dietary Fiber Assay Kit Megazyme (NEOGEN, USA) according to the manufacturer's instructions.

#### *Total phenol and antioxidant capacity*

Grape marc powder (2.5 g) and ethanol at 80% (25 ml) were homogenized for 30 seconds (Ultra-Turrax, T25); then ethanol at 80% was added up to 50 ml, and the mixture was sonicated (Ultrasonic 1520-BranSon) for 30 minutes and centrifugated (Allegra 64R-Centrifuge) at 16300 x g for 5 minutes to 5 °C. The supernatants (ethanolic extracts) were stocked at -20 °C until use. The ethanolic extracts were diluted in ethanol (1:10). Total phenols were quantified according to Jang et al. (2018), with some modifications. Briefly, the reaction was carried out on a 96-well plate, where 20 microliters of ethanol extract were mixed with 120 microliters of Folin-Ciocalteu reagent (2 N) and 120 microliters of $NaCO_3$ (7.5%). The mixture was incubated for 30 minutes at room temperature and in the absence of light; the absorbance was measured using the Microplate Reader (LMPR-101 LABOCON, Leicester, United Kingdom) at 630 nm. The results were expressed grams of gallic acid equivalents per 100 grams of powder (GAE/100 g), using gallic acid as the standard. The antioxidant capacity was obtained by dissolving 2.5 mg of radical DPPH (2,2-Diphenyl-1-picryhydrazyl) in 100 ml of ethanol, and the absorbance of the solution was adjusted to 0.7 by adding ethanol or traces of DPPH. Subsequently, on the 96-well plate, 280 microliters of DPPH solution were mixed with 20 microliters of extract and incubated at room temperature for 30 minutes in the absence of light; the absorbance was obtained at 492 nm, and the result was expressed in μM Equivalents Trolox per 100 grams of powder (μM ET/100 g).

#### *Content of sugars*

Soluble available sugars were measured as glucose, fructose, and saccharose. Briefly, grape marc powder (0.1 g) was sonicated with 15 ml of ethanol-water (80:20, v/v) for 20 minutes and centrifuged at 3000 x g for 10 minutes. Three supernatant extractions were pooled, and 0.5 ml was evaporated and resuspended with 1 ml of water. Sugars were determined using the Sucrose/D-Fructose/D-Glucose Megazyme Assay Kit (Bray, Business Park, Bray, Co., Wicklow, Ireland) according to the manufacturer's instructions.

Furthermore, the quantification of the monosaccharides in the powders was performed using the method described in Espino-Díaz et al. (2010), with minor modifications. Briefly, powder (10 mg) was hydrolyzed with 5 mL of chlorohydric acid (HCl; 1 M) at 100 °C for 150 minutes. After this, the mixture was filtered using Sep Pak C-18 (Waters, Massachusetts, USA), dried, and washed-dried two times with HPLC-grade water. Finally, the residue was resuspended with water and filtered through a membrane (0.45 μm) before being injected into a DIONEX DX 600 high-performance liquid chromatography (HPLC) system (Dionex Corp., New York, USA) equipped with an ED50 electrochemical detector and a CarboPac PA1 column (4 X 250 mm; Dionex Corp.). The mobile phase was HPLC-grade water (1 mL/min) and 300 mM NaOH post column (0.6 mL/min). Arabinose, galactose, glucose, trehalose, and xylose were quantified by comparing their retention times and areas of the corresponding peaks obtained for the external standard solution.

#### *Minerals*

One gram of grape marc powder was incinerated in a calcination muffle at 550 °C for 8 hours. The ash obtained was resuspended with 5 mL of HCl, and 5 ml of bi-distilled water was added. The acid solution was then filtered using ash-free quantitative filter paper (Whatman No. 41) and gauged to 50 ml in a volumetric flask. After this, the acid solution was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES; Agilent® 725-

ES, Agilent Technologies Inc., Mulgrave, Australia) to determine concentrations of calcium (Ca), copper (Cu), phosphorus (P), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), and zinc (Zn). The instrument was calibrated with standard solutions and operated according to the recommended procedures in the instrument manual.

### *Analysis physic*

The grape marc powders' water activity (aw) was measured using a Water Activity Indicator (Rotronic HygroPalm AW1, Rotronic Instruments, Huntington, NY, USA). The angle of repose was calculated using the "fixed and free-standing cone" method (Train, 1958; Tan et al., 2015). Absolute density was calculated according to an adapted version of the method outlined by Franco et al. (2016) and Caparino et al. (2012). Briefly, grape marc powder (1g) was placed in an empty Gay–Lussac Pycnometer (Class A, Glassco, 25 mL), and the total volume was filled to 50% with toluene and let to stand for 5 minutes at room temperature; next, the total volume was filled to 100% with toluene. The absolute density ($\varrho_{abs}$) was obtained using the equation shown below (1), where $m_s$ and $v_t$ are the solid's mass and volume of toluene ($cm^3$), respectively.

Equation (1): $\varrho_{abs} = \dfrac{m_s}{v_t}$

Carr's Compressibility Index was obtained for the samples using the values for bulk and tapped densities according to the report by Chinwan and Castell-Pérez (2019).

The surface color, lightness (L), chroma (c), and hue (h) of the samples were measured using a Konica Minolta color reader (Chroma Meter CR 400, Sensing, Inc., Japan).

### Statistical analysis

The experimental study design was completely randomized. The one-way classification factor of the study included industrial wine residue in three different grape cultivars (Cabernet, Grenache, and Syrah). The experimental unit was 250 grams of freeze-dried grape marc powder with three replicates. Analyses of variance (ANOVA) and Tukey Test ($p < 0.05$) were carried out using NCSS 2021 Statistical Software (NCSS, LLC., Kaysville, Utah, USA). Data for each sample was reported as a mean of the three replicates, except when reporting on dietary fiber, soluble fiber, insoluble fiber, sugars, and monosaccharides ($n = 2$).

## RESULTS AND DISCUSSION

The water activity (aw) of the powders was less than 0.4. It is known that aw values <0.6 do not allow the growth and physiological activity for cell division of most microorganisms (Rifna et al., 2019). Additionally, the microbiological loads of the three batches were 4 log CFU/g. In the MacConkey agar culture medium were not developed Gram-negative bacteria. Santana et al. (2013) reported a microbial load of 2 log for grape marc powder from the juice industry obtained via dehydration by forced-air convention at 70 °C. The marc used in this study was obtained from the wine industry and dehydrated via lyophilization, favoring a greater microbial load. However, Googoolee et al. (2020) mentioned the upper limit of 7 log CFU/g in foods. These results show that grape marc powders can be incorporated as ingredients in food development. Non-pathogenic microbial loads can be reduced by using a temperature-increase drying method. The powders' low microbial load, low moisture content, and low water activity (Table 1) reduce the risk of deterioration due to microbial growth during storage. In relation to the physical tests, the low hygroscopic index obtained in the powders (≈ 0.20) could increase the shelf life at room temperature. However, the porosity index (> 0.6) indicates the spaces between particles and consequently the area of contact with oxygen and favor oxidation processes (Tonon et al., 2010) due to the fat content of the powders (Table 1).

About the main nutrient groups, fat and protein contents were 7–9% and 12–13%, respectively (Table 1), which were percentages similar to those of 10 varieties of grape-pomace byproducts reported by Mohamed Ahmed et al. (2020), albeit with higher moisture levels (pomace is residue created when pressing fruit, while marc is residue created when grapes are pressed during winemaking).

It is crucial to mention by the content of fat found in grape marc powders from northwestern Mexico, the nutritional and functional benefits of grape-seed oil that have been, including grape-seed oil consumption as an antioxidant and anti-inflammatory, as well as for other therapeutics properties (Martin et al., 2020). In addition, grape marc powders have higher dietary fiber content (Table 1), adding functional properties to powders. Today, there is an increasing demand for products with high dietary fiber content, as high-fiber diets have been shown to promote digestive and systemic health (Bordenave et al., 2020). In terms of mineral analysis(Table 2), our results were similar to those reported by Corbin et al. (2015) in powders of Cabernet for Ca, Fe, Mg, P, K, and Na concentrations. The Cu and Mn in the powders can provide ≈ 100% of the recommended daily intake, while the Ca, Fe, and Mg powders can provide ≈ 50% (Quintaes and Diez-García, 2015); in contrast, the Na concentration is low (≈ 2%) (Table 2). Regarding sugars and monosaccharides (Table 1), Corbin et al. (2015) reported Cabernet powders with higher concentrations than those in our study for Cabernet,

**Table 1: Chemical composition, antioxidant capacity, and physical analysis of grape marc powders from northwestern Mexico.**

| Response variable | Cabernet | Grenache | Syrah | HSD | P-value | CV (%) |
|---|---|---|---|---|---|---|
| Moisture (%) | 4.44 A | 4.94 A | 2.04 B | 1.11 | 0.0004 | 11.66 |
| Fat (%) | 7.66 B | 7.09 B | 9.38 A | 1.14 | 0.0021 | 5.68 |
| Ash (%) | 6.63 B | 7.62 A | 6.67 B | 0.13 | <.0001 | 0.74 |
| Protein (%) | 12.90 A | 12.51A | 13.97 A | 1.49 | 0.0538 | 3.93 |
| Dietary fiber (%) | 66.28 A | 56.41 B | 65.25 A | 5.73 | 0.0098 | 2.19 |
| Soluble fiber (%) | 6.83 A | 6.31 A | 6.66 A | 1.10 | 0.2736 | 3.99 |
| Insoluble fiber (%) | 59.44 A | 50.1 B | 58.59 A | 5.40 | 0.0095 | 2.30 |
| Total phenols (g GAE/100 g powder) | 2.50 A | 1.47 C | 2.30 B | 0.16 | <.0001 | 3.09 |
| Antioxidant capacity-DPPH (mM ET/g powder) | 19.25 A | 19.32 A | 17.52 B | 0.77 | 0.0006 | 1.63 |
| Sugars by enzymatic method (%,) | | | | | | |
| Glucose | 0.74 B | 2.66 A | 0.58 B | 0.30 | <.0001 | 9.01 |
| Fructose | 1.16 B | 4.48 A | 0.90 B | 0.25 | <.0001 | 4.66 |
| Saccharose | 0.07 B | 0.07 B | 0.39 A | 0.09 | 0.0011 | 12.21 |
| Monosaccharides by HPLC (ppm) | | | | | | |
| Arabinose | 2.44 B | 1.92 C | 3.06 A | 0.56 | 0.0063 | 5.01 |
| Galactose | 0.78 B | 0.81 B | 1.46 A | 0.17 | 0.0008 | 4.07 |
| Glucose | 0.69 B | 1.67 A | 1.94 A | 0.30 | 0.0008 | 4.96 |
| Trehalose | 2.36 B | 1.18 C | 2.64 A | 0.14 | <.0001 | 1.64 |
| Xylose | 3.36 A | 1.37 B | 3.47 A | 0.70 | 0.0018 | 6.15 |
| Angle of repose | 53.44 A | 51.92 A | 44.85 B | 1.61 | <.0001 | 1.28 |
| Carr's Index | 29.49 A | 27.36 A | 20.35 B | 5.63 | 0.0059 | 8.74 |
| Absolute density | 1.34 A | 1.21 A | 1.29 A | 0.17 | 0.1647 | 5.22 |
| Porosity | 0.68 A | 0.67 A | 0.69 A | 0.04 | 0.1895 | 2.18 |
| Hygroscopicity | 0.21 A | 0.24 A | 0.21 A | 0.03 | 0.0463 | 6.25 |
| Color | | | | | | |
| Lightness | 5.69 B | 15.71 A | 3.94 B | 3.52 | 0.0001 | 16.66 |
| Chroma | 10.59 B | 12.73 A | 9.72 B | 1.97 | 0.0086 | 7.14 |
| Hue | 41.48 B | 55.97 A | 32.78 C | 3.42 | <.0001 | 3.14 |

Values expressed in dry matter. Means with different letters in rows show statistical differences (Tukey, P ≤ 0.05). HSD: honestly significant difference CV: coefficient of variation.

**Table 2: Mineral content in grape marc powders from northwestern Mexico and recommended daily allowance in human nutrition.**

| Mineral | Cabernet | Grenache | Syrah | | | | DRI* |
|---|---|---|---|---|---|---|---|
| | | mg/100 g powder | | HSD | P-value | CV (%) | mg/day |
| Calcium (Ca) | 303.00 B | 346.00 B | 412.00 A | 64.02 | 0.0055 | 7.22 | 800-1000 |
| Copper (Cu) | 0.49 B | 1.10 A | 0.70 B | 0.32 | 0.0033 | 16.92 | 0.54-1 |
| Phosphorus (P) | 175 B | 177 AB | 201 A | 24.90 | 0.0309 | 5.39 | 580-1055 |
| Iron (Fe) | 13.02 A | 13.44 A | 9.11 B | 2.59 | 0.0069 | 7.39 | 5.9-23 |
| Magnesium (Mg) | 90.42 A | 95.32 A | 98.77 A | 2.59 | 0.2764 | 5.43 | 200-300 |
| Manganese (Mn) | 1.94 A | 2.04 AB | 2.24 B | 0.28 | 0.0414 | 5.43 | 1.9-2.6 |
| Potassium (K) | 2319 B | 2662 A | 2501 AB | 326 | 0.0491 | 5.22 | 4500-5100 |
| Sodium (Na) | 29.36 A | 26.93 A | 31.93 A | 12.05 | 0.4768 | 14.43 | 1200-1500 |
| Zinc (Zn) | 1.18 A | 1.01 A | 2.57 A | 1.93 | 0.4709 | 37.56 | 7-10.9 |

Values expressed in dry matter. Means with different letters in rows show statistical differences (Tukey, P ≤ 0.05). HSD: honestly significant difference. CV: coefficient of variation.
* Reference intakes dietary nutrients essential to human life in variation accordance with sex, age, pregnancy, or lactation in Quintaes and Diez-García (2015).

Grenache, and Syrah powders. These differences may be due to the fermentation of sugars and monosaccharides during the residence time of the grape marcs at room temperature after pressing (24 hours in our study). The low concentration of sugars in the powders, such that they could be considered trace concentrations, could allow for the powders' use in low-calorie food formulations. Finally, about the phenolic compounds and antioxidant capacities (Table 1), in our study, we found ≈ 50% less in phenolic content than had been previously reported for the marc variety "Negro amaro" by Negro et al. (2003); however, we found a higher concentration than reported for ten other grape pomace varieties by Mohamed Ahmed et al. (2020). While in antioxidant capacity ≈ 50% less Trolox equivalents than grape byproducts reported in Merlot variety by Gülcü et al. (2019). These differences may be due to the variety, the byproduct origin (juice or wine industry), the gaining dry mass method, and method quantification of antioxidant activity. It is recommended in future investigations to evaluate the antioxidant capacity with other analytical methods and the quantification of specific bioactive compounds. The phenolic compounds have biological activity with health benefits (antioxidant, anticancer, antiviral), and industrial applications like preservative, bioactive, UV protection or colorants (Albuquerque et al., 2020).

Due to the protein content, fat, dietary fiber, minerals, and phenolic compounds present in grape marc powders, can be considered for use in food development as ingredients that have potential health benefits.

The possible incorporation of powders in mixtures for food development has been evaluated using the classification of powders in terms of Carr's index and the angle of repose (Chinwan and Castell-Perez, 2019). Accordingly, Cabernet and Grenache grape marc powders have been reported as having "poor flow" with "true cohesiveness" and have shown significant differences against Syrah, which have been reported as having "flair flow" with "some cohesiveness" (Table 1). Cabernet and Grenache showed lower flow capacity than Syrah because the moisture in Cabernet and Grenache varieties is higher than in Syrah (Table 1). Koç et al. (2020) have noted that an increase in humidity leads to increases in the cohesion and adhesion forces of the powders, decreasing their flow properties. In contrast, no significant differences were found in the absolute densities among the powders; this physical property indicated the interstitial space between particles (Santana et al., 2013) and is intrinsic in the composition and molecular weight of the powder. Therefore, the lower flow property of Cabernet and Grenache is due to the moisture content and not to the molecular weights of the powder components. To match the flow properties of Cabernet and Grenache, the moisture content must be reduced to 50%, which can be achieved by increasing the dehydration time. All three powders have intermediate flow properties that may allow for their incorporation as ingredients in food products. Finally, the cultivars were shown to affect color parameters (Table 1). Grenache had statistically higher values for lightness, chroma, and hue, while Syrah showed the lowest color indices. Grape marc residue meals can be reddish, purple, or dark blue, depending on the cultivar (Antonić et al., 2020), which was observed in this study. Among the cultivars, a predominantly reddish color indicates a higher proportion of anthocyanins (peonidin-3-glycoside, malvidin-3-O-glycoside) are present in the grape skin (Pedroza et al., 2012). In Grenache, the highest hue saturation was observed, associated with the anthocyanins present in these grape skins (Han and Xu, 2015).

## CONCLUSIONS

Grape marc powders obtained from the Mexican wine industry for their content of protein, fat, minerals, and phenolic compounds and flux are byproducts with potential added value as ingredients for the development of food products with functional properties for health benefits (for example, low fiber and baked food products). Being powdered ingredients, their transport, shelf life, and incorporation in food formulations easy handled. However, future studies are needed to evaluate the functionality of grape marc powders as an ingredient in a food mix and consumer acceptance.

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### Author's contributions

Cesar San Martín-Hernández participated in the development of the experiment and writing the original draft. Miguel Angel Martínez-Téllez and Josefa Adriana Sañudo-Barajas verified the analytical methods and discussed the results. Rosabel Vélez de la Rocha participated in the development of the experiment, and Eber Addí Quintana-Obregón participated in the development of the experiment, writing the review, and supervising the findings of this work.

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