Determination of phenolic compounds and evaluation of antioxidant capacity of two grapes residues (*Vitis vinifera*) of varieties dried: Quebranta (red) and Torontel (white)

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Determination of phenolic compounds and evaluation of antioxidant capacity of two grapes residues (Vitis vinifera) of varieties dried: Quebranta (red) and Torontel (white)

A. Solari-Godiño1*, I. Lindo-Rojas2 and S. Pandia-Estrada1

Abstract: The determination of phenolic compounds and antioxidant activity of grape pomace (Vitis vinifera) of two varieties: Quebranta (red) and Torontel (white) using two drying methods: freeze drying and cool air-drying was evaluated, using methods to preserve and maintain bioactive compounds like polyphenols and their antioxidant capacity, these are DPPH and FRAP assays. In general, according to solvent extraction for both grape pomace varieties using the two drying methods, there were obtained high contents of extractable, hydrolysable and condensed tannins polyphenols and therefore exhibited high antioxidants capacities. However, Torontel grape pomace variety obtained by cool air-drying was better in extractable polyphenols, DPPH· and FRAP assays, than those obtained by freeze drying, being 6,095.36 ± 0.58 mg GAE/100 g pomace, 4,042.57 ± 27.80 mg pomace/g DPPH·, 390.23 ± 7.14 μM Trolox/g pomace, respectively. For Quebranta grape pomace variety obtained by cool air-drying and freeze drying; the extractable polyphenols, DPPH· and FRAP assays did not show differences (p < 0.05). On the other hand, there were no differences (p < 0.05) between contents of hydrolyzable and condensed tannins polyphenols and their antioxidant activities of grape pomace (Vitis vinifera) of two varieties: var. Quebranta and var. Torontel.

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PUBLIC INTEREST STATEMENT

Grape pomace from wine industry, specially with emphasis in Pisco processing in Peru, contains important quantities of biological compounds like polyphenols that can serve for human intake and promote well-being in health and nutrition also in foods and meals for feeding acting like powerful antioxidants. On the other side, actually, methods to dry, obtain and concentrate these compounds with biological activity are expensive, for example: freeze drying. However in recent years, some strategies for improve yields have been successful and scalable. We present here, a new proposal through cool air-drying, which is a method more efficient, fast and cheaper to dry natural products, without affectively functional properties and antioxidant activities of grape pomace (Vitis vinifera) of two varieties: var. Quebranta and var. Torontel.

These findings offer to Winery industry other perspective about the use of grape pomace rich in biological compounds and improve opportunities in novel business deal.
tannins in polyphenols for both drying methods, however the antioxidant capacity by DPPH· and FRAP assays showed differences ($p < 0.05$). Finally, this study demonstrated that cool air-drying method preserves the components of grape pomace, being a favorable advantage to optimize costs and drying times, in comparison to freeze drying, and the first one also takes advantage to formulate and elaborate new products to be incorporated in several foods as a functional ingredient.

Subjects: Agriculture and Food; Nutrition; Food Additives & Ingredients; Food Chemistry

Keywords: Torontel; Quebranta; cool air-drying; freeze drying; Pisco; functional ingredient

1. Introduction

The waste from the winery industry consist of seeds and peels mainly, and weighs about 20% of the harvested grapes (Laufenberg, Kunz, & Nystroem, 2003), which constitute an important volume of waste generated and has a strong impact on the environmental, particularly in some parts of Peru where exist industries dedicated to Pisco processing, a spirit drink, obtained by the distillation of the must after grape fermentation.

In 2014 the grape harvest from the most demanded varieties Quebranta and Torontel, destined to Pisco processing, was 21,929 MT nationally (personal communication of CITEAGROINDUSTRIAL-PERÚ) that constitutes 4,835 MT of waste that later was discarded to the environment or used as biofertilizer with low benefit.

In general, wine industry waste is a rich source of phenolic compounds, containing a variety of monomeric phenolic acids, oligomeric proanthocyanidins and glycosylated anthocyanins, which have antioxidant and antimicrobial activity (Saura-Calixto, Pérez-Jiménez, & Goñi, 2010).

Actually, the world market in innovative value-added products with natural phenolic compounds is increasing, such as dietary supplements, beverages and nutraceuticals to promote health in humans, as well as dietary fiber for incorporation into foods of meat products (Moreno-Conde, Solari-Godiño, Pérez-Jiménez, Saura-Calixto, & Borderías Juárez, 2015; Sánchez-Alonso et al., 2007).

Grape dietary fiber predominates in waste of wine industry and is considered as a functional component with benefits related to the risk reduction of cardiovascular diseases, cancer and diabetes (Lizarraaga et al., 2011) and is defined as the edible part of plants or as carbohydrates analogous that are digestion resistant and absorption resistant through small intestinal cells with partial or complete fermentation in the large intestine (AACC, 2001).

Is little known, if dietary fiber is linked to antioxidant compounds of phenolic nature, but it was postulated that dietary fiber has attributes associated with these compounds, which have no action at the level of the small intestine, however at the level of large intestine, it can exert effects in health when it reach the colon (Corrêa et al., 2017; Del Pino-García, González-SanJosé, Rivero-Pérez, Garcia-Lomillo, & Muñiz, 2016).

With the support of chemical analyzes and literature information, phenolic compounds extracted from wine grape pomace can be estimated and quantified using organic solvents of different polarities (methanol, ethanol, acetone, chloroform, etc.). However, there are no solvents that can fully satisfy the extraction of antioxidants present in the food, especially those associated with complex carbohydrates and proteins (Bravo, Abia, & Saura-Calixto, 1994; Pérez-Jiménez & Saura-Calixto, 2005; Saura-Calixto, Serrano, & Goñi, 2007).
Other aspect very important, is how to reduce moisture content, this would be the first step to obtain antioxidant compounds from grape residues drying, which can result in the retention of bioactive compounds or significant and irreversible loss of them, due to the susceptibility to heat and oxygen.

Due the importance of this stage, there are numerous studies related to the evaluation of different drying methods and the biochemical changes of agroindustrial residues (Khanal, Howard, & Prior, 2010). In this sense, Raghavan and Orsat (2007) reported that minimum loss of bioactive compounds, during heat drying in fruit residues, is reached at temperatures no higher than 50°C.

Another very effective drying method for preservation of bioactive compounds, is freeze-drying, however, it involves previous freezing and long times of water sublimation, which leads to high costs of process and energy. Therefore, is necessary to find novel and inexpensive techniques that allow an innovative and functional method, with which it can be obtained these compounds with low cost.

The aim of this study was to determinate phenolic compounds and to evaluate antioxidant capacity of grape pomace (*Vitis vinifera*) of two varieties: var. Quebranta and var. Torontel using two drying methods.

### 2. Materials and methods

#### 2.1. Sample preparation

Two varieties of grape pomace *V. vinifera* cv. Quebranta (red) and cv. Torontel (white) were acquired from Cite Agroindustrial Wine cellar (Guadalupe, ICA, PERÚ). Peels and seeds were obtained after pressing of must fermentation during Pisco processing. Two grapes pomace varieties were separated Quebranta and Torontel pomace and placed in black polyethylene bags to prevent light and those were stored at −18°C until analysis.

Both samples were subjected to two different drying methods: 18°C cool air-drying (ASAHI Air condition Industrial Co., Ltd Hiroshimasi-shi, Japan) for 24 h and freeze-drying (Labconco Co., Kansas, Mi, U.S.A) during 28 h at −84°C collector temperature, vacuum at 0.021 mbar with 4 drying ramps (including freezing).

Dried samples of two varieties were ground (Retsch ZM 200 Haan, Germany) with particle size of 0.25 mm. The powders were then vacuum-packaged (Multivac C100, Haggenmüller, Germany) and stored at −18°C, in order to extract and analyze polyphenols contents and antioxidant capacity in both powders of grape pomace varieties Quebranta and Torontel.

#### 2.2. Sample extraction of polyphenols of powder dried

The procedure followed for extraction of antioxidants is shown in Figure 1 and was according to Saura-Calixto et al. (2007) with slight modifications. The proposal of this extraction was to obtain extractable antioxidants using aqueous-organic solvents and non-extractable antioxidant using acidic hydrolysis. 0.5 g of sample was placed in a capped centrifuge tube; 20 mL of acidic methanol/water (50:50, v/v; pH 2) was added and the tube was thorughly shaken (MX-RL-Pro DLAB Ltd, Beijing, China) at room temperature for 1 h. The tube was centrifuged (IEC Centra CL2, Mildford MA, U.S.A), at 3,000 rpm and the supernatant was recovered. Twenty milliters of acetone/water (70:30, v/v) was added to the residue, and shaking and the centrifugation was repeated. Methanolic and acetonic extracts (Figure 2) were combined and used to determine extractable polyphenols and the antioxidant capacity associated

The residues of these extractions were subjected to two different acidic treatments according to Pérez-Jiménez and Saura-Calixto (2008) with slight modifications in order to release non-extractable antioxidants, which make up a quantitatively important fraction of the dietary intake of antioxidants:
• The residues were mixed with 20 mL of methanol and 2 mL of concentrated sulphuric acid. Samples were placed in a water bath with constant shaking (Incubator IB200, Rika, Tokyo, Japan) at 85°C for 20 h then centrifuged (3,000 rpm for 10 min) and supernatants were recovered. After two washings with distilled water, the final volume was taken up to 50 mL (Hartzfeld, Forkner, Hunter, & Hagerman, 2002 cited by Pérez-Jiménez & Saura-Calixto, 2008). The antioxidant capacity of this residue refers to hydrolysable tannins and other phenolic linked to carbohydrates and proteins.

• The residues were treated with HCl/butanol/FeCl₃ (5:95, v/v) at 100°C for 3 h. Samples were then centrifuged (3,000 rpm for 10 min) and supernatant was recovered. After two washings with HCl/butanol (5:95, v/v), the final volume was taken up to 25 mL (Pérez-Jiménez & Saura-Calixto, 2008). The antioxidant capacity of this residue refers to condensed tannins (proanthocyanidins) not extracted by the previous aqueous-organic procedure (Figure 1).

2.3. Determination of polyphenols content according to extraction procedure
Extractable polyphenols obtained from powders of grape pomace varieties Quebranta and Torontel were analyzed by Folin-Ciocalteau procedure (Singleton, Orthofer, & Lamuela-Raventós, 1999) with slight modifications. 0.5 mL of sample was mixed with 0.5 mL Folin-Ciocalteau reagent and swirled. After 3 min, 14 mL of distilled water was added and mixed. Additionally, 10 mL of sodium carbonate solution (75 g/L) was added and mixed vigorously. After 1 h, the absorbance at 750 nm was recorded and calibration curve was plotted using gallic acid as a standard and it was expressed in mg acid gallic equivalents (GAE)/100 g of dry powder of grape pomace according to variety. Tannins hydrolysables were also analyzed with the same procedure.
Condensed tannins-proanthocyanidins polyphenols were calculated according to Saura-Calixto et al. (2007). The absorbance at 550 nm of the anthocyanidin solutions and the calibration curve was plotted using Mediterranean carob pod (*Ceratonia siliqua* L.) treated under the same conditions.

2.4. Determination of antioxidant capacity according to extraction procedure of samples

2.4.1. DPPH assay
DPPH assay, involves the reducing in absorbance of free radical DPPH· (2,2-Diphenyl-1-picrylhydrazyl) for antioxidant compounds action, scavenge DPPH· radical according by Brand-Williams, Cuvelier, and Berset (1995) with slight modifications. After adjusting the blank with methanol, samples was mixed with DPPH· methanolic solution. The absorbance at 515 nm was measured until the reaction has reached the plateau. A calibration curve was plotted at the wavelength to calculate the remaining DPPH·. The parameter EC_{50}, which reflects 50% depletion of the free radical, is expressed in terms of mg of dry weight/g of DPPH·.

2.4.2. FRAP assay
FRAP assay, involves measuring the ferric cation reducing ability according to Benzie and Strain (1996); Pulido, Bravo, and Saura-Calixto (2000) cited by Pérez-Jiménez and Saura-Calixto (2008). FRAP reagent, containing TPTZ, FeCl₃, and acetate buffer pH 3, was mixed with distilled water and test sample (aqueous-organic extract) or the blank (solvent). Maximum absorbance values at 595 nm are taken at 37°C after 30 min, using a spectrophotometer Perkin Elmer Lambda 950 (Perkin Elmer, Shelton, and U.S.A). Trolox solutions of different concentrations are used for calibration and expressed in terms of μM Trolox/g pomace.
2.5. Statistical analysis
Results were expressed by means ± standard deviation of three more separate determinations. Comparisons of means were performed by one way analysis of variance (ANOVA). Tukey test was used to determine the differences in the mean value (p < 0.05). Data were analyzed using MINITAB V 18 software.

3. Results and discussion

3.1. Polyphenols content according to extraction procedure
A higher amount of extractable polyphenols is observed in both grape pomace through the two drying methods. However, there were no significant differences (p < 0.05) for Quebranta variety pomace between the two drying methods, but it was the opposite in Torontel variety pomace, it was observed higher extractable polyphenol content in cool air drying method and there was significant different (p < 0.05) such is indicated in Table 1.

These results are corroborated with studies by Fuhrman and Aviram (2001), where is argued that polyphenol content in white grape peels would be almost as high as in red varieties.

In the case of hydrolyzable tannins polyphenols contents, there were no significant differences for both varieties in the two methods of drying, also for condensed tannins, very similar results were obtained in comparison to hydrolyzable tannins. Xia et al. (2014) indicated that the peels and seeds are which contribute to the phenolic fractions that have the greatest antioxidant effect, this is mainly because of the amount of peels that can be found in a bunch of grapes, which in fresh weight would be four to eight times greater than the weight of the seeds (Flancy, 2000), and therefore would present greater amount of phenolic compounds, which would result in a greater antioxidant capacity delivered from the must during maceration.

The good amount of extractable polyphenols, present in the samples compared to the values of other fruits and vegetables, may be very related to their antioxidant capacity, because these types of polyphenols are the ones that can be extracted more easily (Saura-Calixto et al., 2010).

| Polyphenol       | Variety   | Drying method | Dry base (mg GA/100 g of pomace) |
|------------------|-----------|---------------|----------------------------------|
| Extractable      | Quebranta | Freeze-drying | 5,580.07 ± 0.23\textsuperscript{a} |
|                   |           | Cool air-drying | 5,349.96 ± 0.39\textsuperscript{a} |
|                   | Torontel  | Freeze-drying | 4,967.98 ± 0.27\textsuperscript{a} |
|                   |           | Cool air-drying | 6,095.36 ± 0.58\textsuperscript{a} |
| Hydrolyzable tannins | Quebranta | Freeze-drying | 3,097.17 ± 0.23\textsuperscript{a} |
|                   |           | Cool air-drying | 3,529.59 ± 0.55\textsuperscript{a} |
|                   | Torontel  | Freeze-drying | 3,415.54 ± 0.28\textsuperscript{a} |
|                   |           | Cool air-drying | 3,194.96 ± 0.43\textsuperscript{a} |
| Condensed tannins | Quebranta | Freeze-drying | 30,014.82 ± 3.5\textsuperscript{a} |
|                   |           | Cool air-drying | 31,549.72 ± 4.4\textsuperscript{a} |
|                   | Torontel  | Freeze-drying | 31,314.72 ± 8.3\textsuperscript{a} |
|                   |           | Cool air-drying | 26,029.96 ± 4.1\textsuperscript{a} |

Note: Different letters between methods of drying for each variety according to polyphenol indicate significative differences (p < 0.05).
These results can be verified by the studies carried out by Pinelo, Rubilar, Jerez, Sineiro, and Núñez (2005), where they indicate that the grape seeds contain polyphenols, representing a high content of tannic compounds, which alone represent 30% of the total phenolic compounds (Fuhrman & Aviram, 2001).

Another research work indicates that the concentrations of polyphenols in seed are higher than in peel, and most are tannins (Flancy, 2000). Tannins are abundant in pips, because they have between 50 and 90% of the total proanthocyanidins (Flancy, 2000).

According to Kennedy et al. (2000), the function of phenols in seed is related to the dormancy stage and its viability, since the content of phenols makes the seed permeable to water making germination possible.

At the same time, it can be said that hydrolyzable tannins are in small amounts compared to the extractable and condensed tannins.

3.2. Determination of antioxidant capacity according to extraction procedure

3.2.1. Antioxidant capacity by DPPH· assay

DPPH· assay for extractable polyphenols, obtained from the cool air-drying method used for Quebranta variety, did not showed significative differences ($p < 0.05$) with those from the freeze drying method; but for Torontel variety, cool air-drying presented significatives higher values than those from the freeze drying method, being 4,042.57 and 2,786.82 mg dry weight pomace/g DPPH· respectively (Table 2).

Hydrolyzable tannins polyphenols antioxidant capacity, obtained from Cool air-drying method used for Quebranta variety presented higher significative ($p < 0.05$) values than those from the freeze drying method, being 2,289.72 and 2,069.48 mg dry weight pomace/g DPPH· respectively.

Antioxidant capacity of condensed tannins polyphenols presented significative differences ($p < 0.05$) in both methods for each variety. But higher values were observed in cool air-drying method for Torontel variety.

| Polyphenol       | Variety  | Drying method   | Dry base (mg pomace/g DPPH·)  |
|------------------|----------|-----------------|-------------------------------|
| Extractable      | Quebranta| Freeze-drying   | 3,329.89 ± 30.50$^a$          |
|                  |          | Cool air-drying | 3,265.00 ± 30.25$^a$          |
|                  | Torontel | Freeze-drying   | 2,786.82 ± 31.44$^a$          |
|                  |          | Cool air-drying | 4,042.57 ± 27.80$^b$          |
| Hydrolyzable tannins | Quebranta| Freeze-drying   | 2,069.48 ± 44,69$^a$          |
|                  |          | Cool air-drying | 2,289.72 ± 48.09$^b$          |
|                  | Torontel | Freeze-drying   | 3,391.70 ± 18.02$^a$          |
|                  |          | Cool air-drying | 1,928.48 ± 68.02$^a$          |
| Condensed tannins | Quebranta| Freeze-drying   | 2,534.42 ± 39.84$^a$          |
|                  |          | Cool air-drying | 2,406.60 ± 26.84$^a$          |
|                  | Torontel | Freeze-drying   | 2,697.48 ± 26.11$^a$          |
|                  |          | Cool air-drying | 3,381.58 ± 14.44$^b$          |

Note: Different letters between methods of drying for each variety according to DPPH· antioxidant capacity indicate significative differences ($p < 0.05$).
According to the obtained results, the antioxidant capacity for both varieties are similar. However, the greatest antioxidant capacity for extractable polyphenols is founded in Torontel variety using cool air-drying method; which has a direct relationship with the amount of polyphenols by natural characteristic of the variety, which has a thicker peel in comparison to Quebranta variety; so the results give a better visibility, because in the peel, there is the greater amount of extractable polyphenols and less amount of hydrolysable tannins.

Bartolomé et al. (2004) found values of antioxidant capacity in extract of dry grapes peel between 3.2 and 11.1 mg/mL DPPH, being these values much lower than those found in the present study. Thus, Moreno and Orlando (2004) obtained values of 2.0 mg/mL DPPH in Merlot and Chardonnay varieties grape seeds. The same author mentioned that antioxidant activity can be influenced by the variety, stage of development and year of grape harvest, so the samples of the varieties studied here represent a greater antiradical power.

The variety with the highest antioxidant capacity in grape seed was the variety Merlot from Maipo Valley, with a value of 0.337 g/mL DPPH, unlike Chardonnay from Casa Rivas (lower part of the valley) which obtained the lowest antioxidant capacity.

3.2.2. Antioxidant capacity by FRAP assay

Extractable polyphenols by FRAP assay using cool air-drying method for both varieties showed higher values than those using freeze drying method. However, antioxidant capacity was only significative (\( p < 0.05 \)) for Torontel variety being 390.23 μM Trolox/g (Table 3).

Deng, Penner, and Zhao (2011) showed higher antioxidant capacities in varieties of red grapes (Pinot noir, Merlot and Cabernet Sauvignon) than varieties of white grapes (Morio Muscat, Muller, Thurgau), nevertheless this study showed that Quebranta (red) and Torontel (white) had similar values. Xia et al. (2014) and Pérez-Jiménez and Saura-Calixto (2008) indicated the content of antioxidants in grape or wine has a direct relation with the content of different polyphenols in them. The content of polyphenolic material varies considerably according to the type of grape, variety, environmental factors and winemaking techniques.

| Table 3. FRAP antioxidant capacity in dry base (μMTrolox/g of pomace) extractable, hydrolyzable tannins and condensed tannins from var. Quebranta and var. Torontel grape pomace through two drying methods |
| --- |
| **Polyphenol** | **Variety** | **Drying method** | **Dry base (μMTrolox/g of pomace)** |
| Extractable | Quebranta | Freeze-drying | 335.19 ± 5.24\(^a\) |
| | | Cool air-drying | 352.76 ± 10.89\(^a\) |
| | Torontel | Freeze-drying | 293.22 ± 6.56\(^a\) |
| | | Cool air-drying | 390.23 ± 7.14\(^b\) |
| Hydrolyzable tannins | Quebranta | Freeze-drying | 18.98 ± 0.30\(^a\) |
| | | Cool air-drying | 21.39 ± 0.38\(^b\) |
| | Torontel | Freeze-drying | 22.23 ± 0.44\(^a\) |
| | | Cool air-drying | 18.96 ± 0.32\(^b\) |
| Condensed tannins | Quebranta | Freeze-drying | 346.95 ± 13.88\(^a\) |
| | | Cool air-drying | 383.43 ± 13.61\(^b\) |
| | Torontel | Freeze-drying | 340.43 ± 9.41\(^a\) |
| | | Cool air-drying | 308.13 ± 21.10\(^b\) |

Note: Different letters between methods of drying for each variety according to FRAP antioxidant capacity indicate significative differences (\( p < 0.05 \)).
Hydrolyzable tannins polyphenols presented lowest values by FRAP assay respect to other polyphenols, probably due to strong hydrolysis process (sulfuric acid and heat) during extraction procedure, which decrease reductor power. Cool air-drying presented higher values than those from freeze drying method in Quebranta variety and otherwise occurred with Torontel variety, being significative in both cases (p < 0.05).

Antioxidant capacity of condensed tannins polyphenols presented high values by FRAP assay, similar to extractable polyphenols. Condensed tannins belong to NEPP Class II, characterized by structures strongly polymerized with high molecular weight, which is abundant in the cell wall (Arranz, Silván, & Saura-Calixto, 2010).

Cool air-drying presented higher antioxidant capacity values than those from freeze drying method in Quebranta variety being 383.43 and 346.95 μM Trolox/g respectively and otherwise occurred with Torontel variety being significative in both cases (p < 0.05).

4. Conclusions
Grape pomace (Quebranta y Torontel) employing two dried methods, exhibited high contents of extractable, hydrolyzable and condensed tannins polyphenols and as consequence great antioxidant capacity. Cool air-drying method showed to be a very efficient method to preserve high contents of polyphenols, antioxidant capacity, how it was checked by the DPPH and FRAP assays.

Freeze drying method is actually the best alternative to preserve natural products with high value added, however cool air drying emerged as a new method to low cost with the same benefits.

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Competing Interests
The authors declare no competing interest.

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