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# Trans-resveratrol and antioxidant activity of grape (*vitis vinifera* sp) byproducts

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## ABSTRACT

Polyphenols from agroindustry's byproducts have great potential as a source of antioxidant compounds. The present study evaluated the antioxidant activity and polyphenol content of the husks and seeds of grapes obtained from a winery. The hydroalcoholic extract (HAE) of the husks was obtained through an exhaustive maceration process. Seeds were extracted in a Soxhlet system, mixed with hydroalcoholic solution and the dried extract was partitioned into solvents to yield the chloroform fraction (CLF), ethyl acetate fraction (EAF), and butanol fraction (BUF). The EAF and HAE had phenolic equivalents of 200.76 and 320.64 mg of gallic acid per gram of extract, respectively. The HAE showed higher antioxidant activity than the fractions in the phosphomolybdenum method, the same in DPPH assay, with $IC_{50}$ of 16.82 mg/mL, and *β*-carotene/linoleate system, with antioxidant activity of 82.09%. High performance liquid chromatography (HPLC) method was developed and validated for quantifying *trans*-resveratrol in the HAE. The method showed linearity (2.5-7.5 µg/mL), coefficient of determination of 0.9907, precision and accuracy (resveratrol recovery = 101.02%). The HAE had a *trans*-resveratrol concentration of 17.33 µg/mg. The results of the validation of the HPLC method were satisfactory, which allowed analysis of the extract with precision and accuracy.

**PALAVRAS-CHAVE:** Grape; polyphenols; residue; seeds; husks.

## INTRODUCTION

Interest in discovering natural antioxidants has grown considerably since the 1980s. Many phytochemicals have significant antioxidant capacity and can ameliorate or prevent chronic and degenerative diseases that are caused by the accumulation or increased production of free radicals (Aruoma, 1998; Vinson *et al.*, 2001; Zheng; Wang, 2001). Isolated phenolic compounds have shown high antioxidant activity in various species (Cho *et al.*, 2003; Kikuzaki *et al.*, 2002; Oliveira-Júnior *et al.*, 2013a; Oliveira-Júnior *et al.*, 2013b).

Brazil has expanded its wine production through improvements in grape cultivar conditions and cultivation equipment. Knowledge of the health benefits of wine has resulted in increased consumption. The expansion of trade in the wine industry has increased demand for raw materials and generated a large amount of byproducts, such as seeds and peels, after the fermentation process (Santos *et al.*, 2011).

A study of *Vitis vinifera* grape byproducts in Brazil that was conducted over two consecutive years reported that high amounts of bioactive and functional compounds were produced by the action of enzymatic (yeast mediated) and chemical reactions during winemaking (Barcia *et al.*, 2014). Seeds and peels have high concentrations of bioactive substances, including phenolic compounds, especially *trans*-resveratrol (SANTOS *et al.*, 2011). *trans*-Resveratrol (*trans*-3,5,4'-trihydroxy-trans-stilbene) is a phytoalexin that is found in grape skin of fresh fruit. Its content is highly affected by grape varieties (ROCKENBACH *et al.*, 2011b).

The search for uses for such residues and byproducts is important and beneficial to the economy, society, and the environment (DEVESA-REY *et al.*, 2011; Lavelli *et al.*, 2014; PEDROZA *et al.*, 2012). Residues that are produced in wineries are rich in polyphenols, substances that demonstrate health benefits with potential use as antioxidants in food preparations or in the production of phytochemicals (BULLA *et al.*, 2015; JAYAPRAKASHA; SELVI; SAKARIAH, 2003; ROCKENBACH *et al.*, 2011a; ROCKENBACH *et al.*, 2011b; SHINAGAWA *et al.*, 2015). The development of cookies containing grape pomace flour as a replacement for conventional wheat flour, showed significant increase of the content of fibers, antioxidant activity and total phenolic content of the cookies (KARNOPP *et al.*, 2015).

Chromatographic methods for the determination of phenolic compounds that employ reverse-phase high-performance liquid chromatography (HPLC) combined with a diode array detector (DAD) or ultraviolet (UV) light detection have been shown to be robust methods for separating phenolic compounds (ZHANG *et al.*, 2013).

The objective of the present study was to evaluate grape seed and husk byproducts with regard to their phenolic content and antioxidant activity. We developed and validated an analytical HPLC/DAD method for *trans*-resveratrol quantification in extracts of the seeds and husks.

## MATERIAL AND METHODS

### MATERIALS

Vegetal material (i.e., seeds and husks of *Vitis vinifera* sp grapes) were obtained from the Miolo winery located at Vale dos Vinhedos, Rio Grande do Sul, Brazil, in April 2009.

Standards (*trans*-resveratrol, gallic acid, ascorbic acid, butylated hydroxytoluene [BHT], quercetin, and rutin) and reagents (linoleic acid, β-carotene, 2,2-diphenyl-1-picrylhy-drazyl [DPPH], polyoxyethylenesorbitanmonooleate [Tween 80], and Folin-Ciocalteu's phenol reagent were purchased from Sigma (St. Louis, MO, USA). Methanol (Carlo Erba) and acetic acid (J.T. Baker) were HPLC-grade. All of the other chemicals and solvents were of analytical grade.

### SAMPLE PREPARATION

Samples from industrial winery waste were dried in an circulating air oven at 60°C followed by separation of the seeds and peels and these samples were triturated in a knife mill (Use-ram). They were stored in a dry place that was protected from light.

### SEEDS

Grape seed powder (440 g) was extracted using the Soxhlet system (PRISTA; CORREIA; MORGADO, 1991) with 700 mL hexane as the solvent at 70°C for 4 h. The solvent was evaporated under reduced pressure at 40°C, and the precipitate was referred to as the hexane extract of the grape seeds, kept in a dark glass flask at -4°C.

The defatted precipitate was extracted by exhaustive maceration in ethanol/water (9:1 v/v) in an amber flask at 25°C. The solution was filtered daily, and a new aliquot of the solvent was added to the flask with occasional stirring until obtained a limpid supernatant. The obtained filtrate was mixed, concentrated under vacuum at 40°C and lyophilized to obtain the dried extract of the seeds (SILVA *et al.*, 2008; SONAGLIO *et al.* 2007). The dried extract was partitioned into solvents with increasing polarity to yield the chloroform fraction (CLF), ethyl acetate fraction (EAF), and butanol fraction (BUF) of the grape seeds. Experiments of extraction and partitioned were carried out in three repetitions.

The defatted precipitate was mixed with a hydroalcoholic solution (90% v/v, ethanol: water) at 1:10 (m/v) (SILVA *et al.*, 2008; SONAGLIO *et al.* 2007). Extraction occurred in an amber flask at 25°C. The solution was filtered daily, and a new aliquot of the solvent was added to the flask with occasional stirring. The obtained filtrate was mixed, and the solvent was eliminated by reduced pressure at 40°C and lyophilized. The dried extract was partitioned into solvents with increasing polarity to yield the chloroform fraction (CLF), ethyl acetate fraction (EAF), and butanol fraction (BUF) of the grape seeds. Experiments of extraction and partitioned were carried out in three repetitions.

## HUSKS

Grape husk powder was extracted at 1:10 (m/v) with a hydroalcoholic solution (90% v/v) by on exhaustive maceration process. The obtained filtrate was mixed, and the solvent was eliminated by reduced pressure at 40°C and lyophilized. The hydroalcoholic extract of the grape husks (HAE) was stored in polyethylene film bags and frozen. Experiments of extraction were carried out in three repetitions.

## PHENOLIC CONTENT

The amount of total phenolic compounds in the samples was determined according to the Folin-Ciocalteu procedure, with modifications (IKAWA *et al.*, 2003). Gallic acid was used as a standard, and the calibration curve was generated with gallic acid concentrations of 10, 50, 100, 150, 250, and 350 µg/mL in methanol. The solutions of the HAE, CLF, EAF, and BUF were diluted in methanol at 1000 µg/mL.

A 100 µl aliquot of the samples was introduced into a volumetric flask (10 mL), and 500 µl of Folin-Ciocalteu reagent and 6 mL of distilled water were added and mixed for 1 min. Afterward, 2 mL of sodium carbonate (15%) was added and mixed for 30 s. Water was then added to the flasks to a final volume of 10 mL, and the samples were allowed to stand for 2 h. Absorption was read at 750 nm with a spectrophotometer (Spectro Vision Model UV-Vis SB-1810S 46 BioSystems). Total phenolic content is expressed as gallic acid equivalents (GAE) in milligrams per gram of extract. All of the measurements were performed in triplicate.

## ANTIOXIDANT ACTIVITY

### Phosphomolybdenium method

The total antioxidant capacity of the grape byproducts was evaluated according to the method of Prieto, Pineda and Aguiar (1999). The phosphomolybdenum complex was prepared by reacting 28 mL of sodium phosphate solution ($Na_3PO_4$; 0.1 mol/L), 12 mL of ammonium molybdate solution ($[NH_4]_6Mo_7O_{24}\bullet 4H_2O$; 0.03 mol/L), 20 mL of sulfuric acid solution ($H_2SO_4$; 3 mol/L), and 40 mL of distilled water.

To the vials was added 600 µl of the test samples, HAE, CLF, AEF, and BUF solutions (200 µg/mL), and 2 mL of phosphomolybdenum complex reagent solution. The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples cooled to room temperature, the absorbance of the mixture was read at 695 nm against a blank using a spectrophotometer (Spectro Vision Model UV-Vis SB-1810S 46 BioSystems).

The antioxidant capacity of the extract and each fraction is expressed as relative antioxidant activity (RAA) to ascorbic acid, which was used as a standard (200 µg/mL), and RAA to rutin prepared in methanol (200 µg/mL). The reference

antioxidant activity was considered as 1. The assays were performed in triplicate and the results are expressed according to equations (1) and (2):

$$RAA\% \text{ (ascorbic acid)} = [(Abs_{sample} - Abs_{blank}) / (Abs_{ascorbic\ acid} - Abs_{blank})] \times 100 \quad (1)$$

$$RAA\% \text{ (rutin)} = [(Abs_{sample} - Abs_{blank}) / (Abs_{rutin} - Abs_{blank})] \times 100 \quad (2)$$

## DPPH Method

This method is based on DPPH (2,2-diphenyl-1-picryl-hidrazil) radical scavenging activity (RSA) of the extract and fractions (BLOIS, 1958; BRAND-WILLIAMS; CUVELIER; BERSET and 1995). The extract and fractions were diluted in methanol. From each stock solution (1000 µg/mL), dilutions in methanol were made to obtain concentrations in the linear range of each sample. The linear ranges of the HAE, CLF, AEF, and BUF were 5-30, 62.5-250, 7.5-32, and 15-62.5 µg/mL, respectively.

To evaluate antioxidant activity, 375 µl of 1 mM DPPH solution was added to 3 mL of each solution of HAE, CLF, AEF, and BUF. As the positive control, 3 mL of a methanolic solution of butylated hydroxytoluene (BHT) (0.5 mg/mL) was added to 375 µl of DPPH solution. A solution that contained 3 mL of methanol and 375 µl of 1 mM DPPH solution was used as the negative control. After 30 min, the absorbance of the solutions was read at 517 nm using a spectrophotometer (Spectro Vision Model UV-Vis SB-1810S 46 BioSystems). All of the measurements were performed in triplicate. The RSA was calculated as the following (3):

$$\%RSA = [(Abs_0 - Abs_1) / Abs_0] \times 100 \quad (3)$$

$Abs_0$ is the absorbance of the negative control. $Abs_1$ is absorbance in the presence of the test sample at different concentrations. The $IC_{50}$ (i.e., the concentration that provided 50% inhibition) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration *vs*. the corresponding scavenging activity.

## β-carotene/linoleate model system

The *β*-carotene/linoleic acid system was designed according to Barros *et al.* (2007). Antioxidant activity (AA%) was determined based on the oxidation of *β*-carotene induced by the oxidative degradation of linoleic acid. To prepare the emulsion, 0.4 mg of *β*-carotene was dissolved in 2.0 mL of chloroform. The chloroform was evaporated at 40°C under vacuum, and 40 mg linoleic acid and 400 mg Tween 80 were added. To this solution was added 100 mL of hydrogen peroxide (distilled water treated with $O_2$), with vigorous stirring.

A 4.8 mL emulsion of *β*-carotene/linoleate was added to 0.2 mL of the samples, extract and fractions, at concentrations of 500 and 1000 µg/mL in methanol. The same procedure was performed for the rutin, quercetin, and ascorbic acid standards at concentrations of 500 and 1000 µg/mL in methanol. The BHT standard was used at 1000 µg/mL in methanol. The samples were then placed in a water bath at 50°C for 2 h, and absorbance was read at 470 nm using

a spectrophotometer (Spectro Vision Model UV-Vis SB-1810S 46 BioSystems). Analyses were carried out in triplicate.

The percentage of inhibition of oxidation was calculated using the following equation (4):

AA% = (content of *β*-carotene after 2 h / initial content of *β*-carotene) x 100 (4)

## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

### Trans-Resveratrol analysis

HPLC analysis was carried out according to Chafer *et al.* (2005) with modifications described as follows.

HPLC was performed using a Shimadzu system (SCL-20A) with a UV DAD (SPD-M20AVP; 220-600 nm), automatic sampler (SIL-20ADVP), and quaternary pump (LC-20ADvp) coupled to LC solution software for data acquisition. Components were separated using a C18 column (Phenomenex Luna C18, 250 x 4.6 mm inner diameter, 5 µm particle size) held at 40°C. The samples were filtered using a Millex filter, and a 20 µl aliquot was injected.

The mobile phase consisted of 0.30% glacial acetic acid and methanol at a flow rate of 1.0 mL/min. The gradient elution mode was prepared as the following: 0.30% acid water and methanol at proportions of 80:20 v/v (0-15 min), 55:45 v/v (15-30 min), and 80:20 v/v (30-35 min). *trans*-Resveratrol was detected at 306 nm, and the identification was based on the comparison of the retention time and UV-visible light spectral data of the detected peak with the reference standard compound. Quantification was performed using external calibration curves with a pure standard, and the results are expressed as mg/g. The retention time of *trans*-resveratrol in the samples was 24 min, with a total run time of 35 min. Analyses were carried out in triplicate.

### Validation study

To study the HPLC system's suitability, the theoretical plate number, peak asymmetry factor, and peak resolution were analyzed before starting the sampling injection according to Cass e Degani (2001).

A validation study was performed with the HAE of the husks (200 µg/mL) and the *trans*-resveratrol standard (5 µg/mL), both prepared in methanol. The following parameters were determined: linearity, precision, limit of detection (LOD), limit of quantification (LOQ), and accuracy, according to Brazilian legislation requirements (BRASIL, 2003) and the Center for Drug Evaluation and Research (1994).

Linearity was evaluated according to an analytical curve using the external *trans*-resveratrol standard from three replicates. In addition to the parameters evaluated by linear regression, the linearity of the residues was determined using the F test.

Precision was determined by considering repeatability and intermediate precision. Three solutions at distinct concentrations (2.50, 5.00, and 7.50 mg/mL) were analyzed in three replicates on the same day. A similar procedure was applied on two different days and on two separate occasions to evaluate intermediate precision. The results are expressed as relative standard deviation (RSD %).

The LOD was evaluated using diluted solutions of the standard at decreasing concentrations, until a signal-to-noise ratio of 3:1 was reached. The LOQ was calculated according to analytical curve data, based on a concentration that produced a signal-to-noise ratio greater than 10:1 relative to baseline. The areas were calculated using LC Solution software based on the analytical curves.

After the linearity parameters were set, accuracy was evaluated at three levels for the HAE (80%, 100%, and 120% of the label claim). The analysis was performed in triplicate at each level, and the recovery mean indicates the accuracy of the method.

Stability assays were performed using a *trans*-resveratrol standard solution and HAE, which were stored at 4°C and protected from light. The samples were stored at 25°C for 72 h and analyzed every 24 h.

### STATISTICAL ANALYSIS

The results were analyzed using analysis of variance (ANOVA), and mean values were compared using Tukey's test. Values of $p \leq 0.05$ were considered statistically significant. The data were analyzed using Statistica 5.1 software (StatSoft, 1996).

## RESULTS AND DISCUSSION

### ANTIOXIDANT ACTIVITY

Table 1 shows the antioxidant activity, and phenolic content using the phosphomolybdenum method of the HAE of the husks, CLF, EAF, and BUF of the seeds. Significant differences ($p \leq 0.05$) were observed between samples in all of the analyses. The highest phenolic content was 320.64 mg GAE/g in the EAF. Santos *et al.* (2011) also found higher amounts of phenolic compounds in seeds, compared with peels.

Antioxidant activity, showed that the HAE had a higher RAA, relative to both rutin and ascorbic acid, followed by the EAF, BUF, and CLF (Table 1). A similar antioxidant activity profile was observed in the DPPH analysis. The HAE had a smaller $IC_{50}$ value than the other fractions. The EAF of seeds presented the best results in the DPPH test, similar to Santos *et al.* (2011). The $IC_{50}$ values of grape byproducts using the DPPH method reported by Ruberto *et al.* (2007) ranged from 14 to 39 µg/mL in samples provided by different wineries in Italy.

Table 1 – Antioxidant activity and phenolic content using the phosphomolybdenum method of extracts produced from grape seeds and husks.

| Sample[1] | Phenolic compounds (mg GAE/g)[2] | RAA%[3] (ascorbic acid) | RAA%[3] (rutin) | DPPH ($IC_{50}$) |
|---|---|---|---|---|
| HAE | 200.76c ± 8.99 | 29.19d ± 1,65 | 86.15d ± 4.88 | 16.82a ± 0.67 |
| CLF | 25.17a ± 1.09 | 7.36a ± 0.64 | 21.71a ± 1.88 | 242.62c ± 7.57 |
| EAF | 320.64d ± 2.86 | 19.94c ± 1.26 | 58.85c ± 3.73 | 25.58a ± 0.95 |
| BUF | 46.36b ± 1.43 | 8.79b ± 0.61 | 25.95b ± 1.79 | 110.71b± 3.13 |
| HAE | 200.76c ± 8.99 | 29.19d ± 1,65 | 86.15d ± 4.88 | 16.82a ± 0.67 |
| CLF | 25.17a ± 1.09 | 7.36a ± 0.64 | 21.71a ± 1.88 | 242.62c ± 7.57 |

[1]HAE, hydroalcoholic extract of the husks of the seeds of grapes; CLF, chloroform fraction of the seeds of grapes; EAF, ethyl acetate fraction of the seeds of grapes; BUF, butanol fraction of the seeds of grapes.
[2]Milligrams of gallic acid equivalents per gram of extract (mg GAE/g).
[3]Relative antioxidant activity to ascorbic acid and rutin.
The results are expressed as the mean ± standard deviation of three replicates. Means followed by different letters in the same column are significantly different by Tukey's test ($p \leq 0.05$).

The HAE sample had a smaller phenolic content than EAF but higher antioxidant activity based on all of the evaluation methods (Table 1). Phenolic compounds are described as being responsible for the antioxidant properties of grapes, as well as other species (Ferreira *et al.*, 2010; Gris *et al.*, 2013; Oliveira-Júnior *et al.*, 2013a).

To study antioxidant properties in an oil model, the samples were evaluated using the *β*-carotene/linoleate model system. The extracts were evaluated at two concentrations (500 and 1000 µg/mL; Table 2). This method considers the quenching power of the extract to avoid free radical formation that occurs during lipid oxidation, which may attack the *β*-carotene structure and cause a loss of color at 415 nm (JAYAPRAKASHA; SINGH; SAKARIAH, 2001). The rutin (RTN), quercetin (QRC), and ascorbic acid (ASC) standards were also evaluated. The HAE had higher values of inhibition of linoleate peroxide radical-induced *β*-carotene degradation, similar to quercetin. All of the fractions studied exhibited powerful antioxidant ability against the emulsion system compared with ascorbic acid, indicating that these chemicals are prone to act as radical scavengers in oil/water systems.

Table 2 – Antioxidant activity (%AA) of extracts produced from grape seeds and husks in the *β*-carotene/linoleate model system.

| Sample[1] | 500 µg/mL | 1000 µg/mL |
|---|---|---|
| ASC | 51.39$^{a}$ ± 1.53 | 55.35$^{a}$ ± 1.91 |
| RTN | 75.88$^{e}$ ± 1.76 | 79.25$^{b}$ ± 3.28 |
| QRC | 89.43$^{g}$ ± 2.61 | 92.38$^{g}$ ± 3.04 |
| HAE | 82.09$^{f}$ ± 3.01 | 88.94$^{f}$ ± 2.90 |
| CLF | 60.04$^{b}$ ± 1.39 | 83.05$^{c}$ ± 1.31 |
| EAF | 69.00$^{d}$ ± 3.08 | 88.18$^{e}$ ± 2.47 |
| BUF | 62.32$^{c}$ ± 2.08 | 84.38$^{d}$ ± 0.71 |

[1]HAE, hydroalcoholic extract of the husks of the seeds of grapes; CLF, chloroform fraction of the seeds of grapes; EAF, ethyl acetate fraction of the seeds of grapes; BUF, butanol fraction of the seeds of grapes. The results are expressed as the mean ± standard deviation of three replicates. Means followed by different letters in the same column are significantly different by Tukey's test ($p \leq 0.05$).

## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS

### Trans-Resveratrol analysis

A method for determining resveratrol (*trans*-3,5,4'-trihydroxy-trans-stilbene) was performed using the HAE of the husks, which presented the highest antioxidant properties among the tested samples.

The proposed method was developed to detect and quantify *trans*-resveratrol, it has been shown beneficial to health, with antioxidant, anticancer and anticoagulant properties (OLAS and Wachowicz, 2005; Olas *et al.*, 2001; Surh *et al.*, 1999). Rockenbach *et al.* (2011b) identified individual phenolic compounds in grape pomace, including anthocyanins, quercetin, rutin, kaempferol derivatives, catechin, epicatechin, gallic acid, and *trans*-resveratrol in *Vitis vinifera* L. residue.

### Validation study

The analytical method for quantifying *trans*-resveratrol in grape byproducts was validated using standards by considering linearity, precision, LOD, LOQ, specificity, accuracy, and robustness (data not shown). The linearity range of 2.5-7.5 µg/mL was obtained using the *trans*-resveratrol standard, and the coefficient of determination achieved was of 0.9907. Other parameters that are related to the analytical curve are presented in Table 3. The linearity evaluated using the F test compared the F value (-49.67) with the critical F value (3.7083), with 3 and 10 degrees of freedom and a 95% confidence level. Because the condition F < critical F was met, linearity was confirmed.

Table 3 – Validation parameters for HPLC method using trans-resveratrol as a standard.

| Parameter | Value |
|---|---|
| Intercept (a) | 214126 |
| Slope (b) | -79948 |
| Linear regression coefficient (R) | 0.9907 |
| Standard error | 57448.7 |
| Repeatability (RSD %) | 0.83 |
| Intermediate precision (RSD %) | 0.24 |
| LOD (µg/mL) | 0.000364 |
| LOQ (µg/mL) | 0.001214 |
| Accuracy (%) | 101.02 |

Precision was evaluated at three concentrations (2.5, 5.0, and 7.5 µg/mL), with RSD values < 5% (Table 3) when considering both repeatability and intermediate precision. These were acceptable values according to validation guidance (BRASIL, 2003). The limit of detection (LOD) and limit of quantification (LOQ) for *trans*-resveratrol were 0.000364 and 0.001214 µg/mL, respectively. Accuracy was evaluated by recovering the *trans*-resveratrol standard at three concentrations (160, 200, and 240 µg/mL), delimited by the linear range of the HAE (Table 3). The mean value of 99.88% ± 1.01% (RSD 1.01%) (Table 4) indicates that the proposed method presents accuracy at acceptable levels.

Table 4 – Validation parameters for HPLC method using trans-resveratrol as a standard.

| Concentration (µg/mL) | Accuracy (%) mean ± SD |
|---|---|
| 160 | 98.78 ± 0.38 |
| 200 | 99.89 ± 0.30 |
| 240 | 100.98 ± 0.47 |

Stability assays were performed during solution preparation to ensure that *trans*-resveratrol remained stable and monitor possible side reactions. The standard solution and HAE presented variations in the *trans*-resveratrol concentration of 4.08% and 1.33%, respectively, over 24 h. Higher trans-resveratrol losses in standard solutions compared to phenolic extract (HAE) allowed to infer that other compounds are present and can act as antioxidant. The results are in agreement with Sautter *et al*. (2005), which showed *trans*-resveratrol might be converted in *cis*-resveratrol in presence of light.

## Trans-Resveratrol in extracts

*trans*-Resveratrol was quantified in the HAE of the husks, with 17.33 mg of resveratrol/g of extract. Santos *et al.* (2011) reported the largest amounts of *trans*-resveratrol in peels compared with seeds, whereas no *trans*-resveratrol was detected in grape pulp. Iacopini *et al.* (2008) detected *trans*-resveratrol in small quantities in Sangiovese and Foglia Tonda skin (0.8 ± 0.18 mg/100 g) and higher values in Cabernet Sauvignon (25.5 ± 1.16 mg/ 100 g) and Merlot (10.5 ± 1.84

mg/100 g). Studies appoint that the number of stilbenes present in grapes is dependent on the amount of resveratrol, considered the precursor of these phenolic compounds (Coutos-Thévenot *et al.*, 2001; FERNÁNDEZ-MARÍN *et al.*, 2013).

## CONCLUSIONS

The EAF of the seeds and the HAE of the husks of grapes showed the greatest antioxidant activity. The HAE sample had a smaller phenolic content than EAF but higher antioxidant activity based on all of the evaluation methods. The stronger antioxidant activity might be associated with its ability to scavenge free radicals and participate in reactions that involve hydrogen donation and electron transfer. The proposed method was fully validated and allowed the detection of *trans*-resveratrol and quantification in real samples. Identifying vegetal extracts that are rich in bioactive *trans*-resveratrol and have potential antioxidant properties using this method may be useful in the pharmaceutical industry. This method may also bring value to winery byproducts.

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