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Artificial neural network: a powerful tool in associating phenolic compounds with antioxidant activity of grape juices

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Abstract

In vitro techniques are essential to assess the antioxidant potential of foods, although methods with different action mechanisms make troublesome data analysis. This article describes the use of artificial neural network (ANN) to associate phenolic compounds with antioxidant activity in vitro (AOX) of grape juices. A multilayer perceptron (MLP) ANN was obtained with 28 phenolics quantified, as input layers, and AOX measuring by DPPH, ABTS, FRAP, H_2O_2, and β-carotene/linoleic acid bleaching assay (βCLA) methods, as output layers. To improve discussion in food sciences, the ANN results were compared with Pearson's correlation and principal component analysis (PCA), methods largely used in food studies. Pearson's technique showed correlations between antioxidant methods and some of the phenolic compounds, but with limitations. PCA proved to be a more powerful method than Pearson's correlation, as it positively associated 13 phenolics with four out of five antioxidant methods. The MLP-ANN allowed simultaneous association of 19 individual phenolics, while a single hidden layer predicted 15 phenolics with simultaneous action in all AOX methods. The power of association was: ANN > PCA > Pearson. It was evidenced that ANN is a powerful tool for screening antioxidants in different AOX systems, which is applicable in health interests.

Keywords Antioxidant methods · Bioactivity · Chemometrics · Grape polyphenols

Introduction

Grape juice is one of the most consumed fruit juices in the world, given its sensory quality and worldwide acceptance, along with its functional properties such as beneficial health effects related to antioxidant, anti-inflammatory, anticarcinogenic, and antibacterial activities (Mcgill et al. 2013; Camargo et al. 2014; Toaldo et al. 2016; Granato et al. 2016; Corredor et al. 2016; Toscano et al. 2017). The in vitro antioxidant activity (AOX) is the most studied activity of grape juice and is often associated with broad classes of phenolic compounds, such as flavonoids, phenolic acids, and stilbenes, among others (Camargo et al. 2014; Lima et al. 2014; Toaldo et al. 2015; Granato et al. 2015, 2016; Moreno-Montoro et al. 2015; Margraf et al. 2016; Beara et al. 2017; Dutra et al. 2018; Silva et al. 2019). The potential beneficial effects of non-nutrient substances such as polyphenols are studied using a variety of methods that include in vitro or/and in vivo protocols. Most of those are generally evaluated separately, and their outcomes are assessed in terms of distinct variables. However, the attempt to associate in vitro and in vivo antioxidant methods has generated current debates and controversial opinions among researchers (Granato et al. 2018a).

The strict validation of antioxidant properties of a food or biological matrix when using quantitative literature methods

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is nowadays a challenging task, especially when compounds' heterogeneity and matrix complexity are considered. Measurements to determine the AOX of grape juices have been based extensively on free radical scavenging methods using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), hydrogen peroxide scavenging activity (H2O2), cupric ion reducing antioxidant capacity (CUPRAC), and oxygen radical absorbance capacity (ORAC) assays (Lima et al. 2015; Granato et al. 2015, 2016; Silva et al. 2015; Moreno-Montoro et al. 2015; Lopes et al. 2016; Margraf et al. 2016; Beara et al. 2017; Dutra et al. 2018). For Harnly (2017), methods of measuring AOX using DPPH or ABTS, or by FRAP, CUPRAC, or ORAC, are not suitable for measuring the antioxidant activity of foods because they cannot be compared to each other and besides fail to estimate the real effect in vivo. On the one hand, it has become evident that complex physiological, chemical, and enzymatic mechanisms are involved in the outcome of the antioxidant activity derived from foods or biological systems and that the antioxidant activity is dependent on numerous factors related to the presence of antioxidants, their compound structures, and to food/biological matrix interactions (Pandey and Rizi 2009; Granato et al. 2018a). On the other hand, before setting up study designs and ethical in vivo protocols to study dietary polyphenols, the knowledge of a potential antioxidant source through chemical in vitro techniques has been proven essential as preliminary evidence for further assessment of health interests. In vitro/in vivo studies have positively correlated phenolic compounds with the antioxidant activity of grape juices that showed direct effects on consumers' health such as decreases in oxidative stress biomarkers (Toaldo et al. 2015), decreased levels of biomarkers of DNA damage in renal patients undergoing hemodialysis (Corredor et al. 2016), and improvement of cardiometabolic status in adults who exercise (Toscano et al. 2017).

The main ways of associating individual phenolic compounds from grape juices with antioxidant activities in vitro are essentially through linear mathematical models such as Pearson's correlation, and multivariate statistics, mainly principal component analysis (PCA) (Camargo et al. 2014; Lima et al. 2014; Toaldo et al. 2015; Granato et al. 2015; Margraf et al. 2016; Beara et al. 2017; Silva et al. 2019; Dutra et al. 2018; da Silva et al. 2019; Dutra et al. 2021). In these types of analysis, statistical association between a specific phenolic compound and antioxidant activity is generated from direct mathematical relationships between the variables, which, for a complex antioxidant system such as the human body, where several chemical reactions occur simultaneously, do not always apply. Another approach to associating data from two groups of variables in a non-linear manner is using an artificial neural network (ANN), a technique that simulates data processing in a way similar to a biological neuron. This mathematical algorithm has the ability to relate input and output parameters, learning from examples, and using interactions that do not require prior knowledge about the relationships between process variables (Dębska and Guzowska-Świder 2011).

The most widely used artificial neural network in food data analysis is the multilayer perceptron (MLP) trained by the reverse propagation algorithm. In this ANN, the neuron layers are linked together in a feedforward network (Dębska and Guzowska-Świder 2011; Granato et al. 2018b). The MLP-type ANN has allowed prediction of grape variety, vintage, and wine producer by using parameters such as the total content of flavonoids, anthocyanins, and in vitro antioxidant activity as input (neurons) layers (Hosu et al. 2014). A typical MLP consists of an input layer, one or more hidden layers, and an output layer; these can form, for example, the configuration "phenolic compounds hidden layers grape variety". The number of neurons in the hidden layers as well as the number of hidden layers depends on the complexity of the intended classification and the amount of data (Dębska and Guzowska-Świder 2011).

In an MLP, the hidden and output neurons are connected to all nodes by an associated numerical weight called "connection weight" or "synaptic weight" (Bhotmange and Shastri 2011). Mapping of the input/output ratio of each node in the network is obtained with a mathematical activation function, and each neuron also has a threshold value called "bias". When the network is used, the input variables are placed in the input neurons, and in sequence, hidden and output layer neurons are executed progressively. Each neuron calculates its activation value to produce neuron's output (Dębska and Guzowska-Świder 2011). Reverse propagation is mainly used as a learning algorithm, which through negative synaptic weights (synaptic weight < 0) helps to predict the relationship between input and output variables, with activation values being important for interpreting the relevance of connections (Dębska and Guzowska-Świder 2011; Granato et al. 2018b). Based on the above and considering the constant use of multimethod approaches to research bioactive polyphenols, this work aimed to investigate associations between phenolic compounds and AOX of Brazilian grape juices using a multilayer perceptual artificial neural network. The network was proposed with the individual phenolic compounds quantified by RP-HPLC/DAD as input layers and the AOX as measured by DPPH, ABTS, FRAP, H_2O_2, and β-carotene/linoleic acid bleaching assay (βCLA) as output layers. For comparison purposes, the association between phenolic compounds and antioxidant activity was also obtained by Pearson's correlation and PCA techniques. Notwithstanding, this is the first report using ANN to unveil associations and contributions of grape juice polyphenols, which are one of the most substantially used polyphenols in food, nutrition, biological, and pharmaceutical applications.

The approach presented in this study can contribute substantially to future researches with grape polyphenols in these areas.

Materials and methods

Reagents and HPLC standards

TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine), β-carotene, Tween 40, chloroform, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), linoleic acid, 2,2-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric chloride hexahydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethyl alcohol, potassium persulfate, ferrous sulfate, phosphoric acid, hydrogen peroxide, and potassium phosphate monobasic were purchased from Merck (Darmstadt, Germany). Methanol HPLC grade was obtained from J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water was generated by purification in a Marte Científica System (São Paulo, SP, Brazil). External standards of gallic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, syringic acid, *trans*-caftaric acid, hesperidin, procyanidin B1, catechin, epicatechin, naringenin, procyanidin B2, cyanidin-3,5-diglucoside, malvidin-3,5-diglucoside, and pelargonidin-3,5-diglucoside were from Sigma-Aldrich. Procyanidin A2, epigallocatechin gallate, epicatechin gallate, quercetin 3-rutinoside (rutin), kaempferol 3-glucoside, quercetin 3-glucoside, myricetin, malvidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside, and pelargonidin-3-glucoside were from Extrasynthese (Genay, France). The isomers *cis*-resveratrol and *trans*-resveratrol were obtained from Cayman Chemical Company (Michigan, EUA).

Grape juice samples

Samples of five commercial grape juices (CJ) were obtained directly from five producing industries located in the Northeast Region of Brazil, Petrolina, Pernambuco (09º 09′ S; 40º 22′ W). In addition, varietal grape juices were produced in laboratory scale by hot pressing method (Gomes-Silva et al. 2019) with selected Brazilian grapes from the varieties "Isabel Precoce", "BRS Cora", "BRS Violeta", and "BRS Magna" and used as samples. The commercial and varietal grape juices were produced and analyzed in triplicates.

In vitro *antioxidant capacity methods*

The in vitro antioxidant activity of grape juices was evaluated using the methods of free radical scavenging by DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) following the methodologies described by Kim et al. (2002) and Re et al. (1999), respectively. The ferric reducing antioxidant power (FRAP) was determined as described by Rufino et al. (2006), and the hydrogen peroxide scavenging activity (H_2O_2) was assayed according to Ruch et al. (1989). The β-carotene/linoleic acid bleaching assay (βCLA) (Miller, 1971) was also performed. Trolox was used to construct the calibration curves, except in the FRAP method, where ferrous sulfate was used. The results were expressed as Trolox equivalents per liter of grape juice (mmol TE L^{-1}) and mmol of Fe^{2+} per liter of juice (mmol Fe^{2+} L^{-1}). Absorbance measurements were performed on a UV–Vis 2000A spectrophotometer (Instrutherm, São Paulo, Brazil). The methods procedures are described below.

The $ABTS^{\bullet+}$ radical was formed by the reaction of 7 mmol ABTS solution with 140 mmol potassium persulfate, incubated at 25 °C without light incidence for 16 h. The radical was diluted in ethanol to the absorbance of 0.70 ± 0.05 at 734 nm. Then, a 300 µL aliquot of the sample was mixed with 2700 µL of the radical, kept in dark, and absorbance was taken after 6 min.

In DPPH method, a solution of DPPH radical (1 mmol L^{-1}) was prepared in ethanol and diluted to an absorbance of 0.900 ± 0.050 (100 µmol L^{-1}). An aliquot of 2900 µL of the radical was mixed with 100 µL of sample and kept in the dark for 30 min. Absorbance measurements were taken before and after the addition of grape juice. The antioxidant activity of samples was then assayed through the rate of decay in absorbance at 517 nm.

The FRAP reagent was prepared in 300 mmol L^{-1} acetate buffer (pH 3.6), with 10 mmol L^{-1} TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) in a solution of HCl 40 mmol L^{-1} and 20 mmol L^{-1} $FeCl_3$. Grape juice samples (90 µL) were mixed with 270 µL of distilled water and 2.7 mL of FRAP reagent and incubated at 37°for 30 min in a thermodigester block for tubes (Bioplus IT-2002, Barueri, SP, Brazil). After incubation, absorbance measurements were taken at 595 nm.

A solution of hydrogen peroxide (4 mmol) prepared in phosphate buffer (pH 7.4) was used in the H_2O_2 method. In the procedure, the samples (0.4 mL) were mixed with the hydrogen peroxide solution (0.6 mL), with the final volume adjusted to 3 mL using the phosphate buffer. Absorbances were determined spectrophotometrically at 230 nm after 10 min. Phosphate buffer was taken as blank sample.

In the βCLA method, an emulsion was obtained by mixing 20 µL of linoleic acid, 530 µL of Tween 40, 50 µL of *β*-carotene (20 mg mL^{-1}), and 1000 µL of chloroform. The mixture was rotaevaporated at 40 °C to remove chloroform, and the residue was taken up into ultrapure water saturated with oxygen until absorbance at 450 nm was between 0.600 and 0.700. In a test tube, 5 mL of the emulsion was mixed with 50 µL of grape juice. A control was obtained by mixing

5 mL of the emulsion and 50 µL of ethanol. The mixture was incubated at 45 °C in a thermodigester block, and absorbances were taken at time $t = 0$ min and $t = 120$ min. All analyses were carried out in triplicate.

Chromatographic analysis by RP-HPLC/DAD

Individual phenolic compounds of grape juices were determined using the methodology of Dutra et al. (2018). The analyses were performed on an Agilent 1260 Infinity LC liquid chromatograph system (Agilent Technologies, Santa Clara, CA, USA). The column and pre-column used were a Zorbax Eclipse Plus RP-C18 (100 × 4.6 mm, 3.5 µm) and a Zorbax C18 (12.6 × 4.6 mm, 5 µm), respectively (Agilent Technologies). Before sample injection, grape juices were diluted 1:2 with mobile phase A and filtered through a 0.45 µm membrane (Millex Millipore, Barueri, SP, Brazil). Solvent A was a solution of phosphoric acid 0.1 mol L^{-1} (pH 2.0), and solvent B was methanol acidified with 0.5% H_3PO_4. The elution gradient was as follows: 0 to 5 min: 5% B; 5 to 14 min: 23% B; 14 to 30 min: 50% B; and 30–33 min: 80% B. The injection volume was 20 µL, and detection was set at 220, 280, 320, 360, and 520 nm. Quantification of individual polyphenols was performed by comparison with external standards. Data processing was on OpenLAB CDS ChemStation Edition software (Agilent Technologies). Additionally, peaks spectral purity was verified using the tool threshold to ensure the accuracy of the identification of each compound, according to Padilha et al. (2017).

Statistical analysis

Data was analyzed using the SPSS statistics software version 20.0 (IBM, NY, USA). The data was tabulated and presented as mean ± standard deviation. Pearson's correlation analysis ($p < 0.05$), principal component analysis (PCA), and a multilayer perceptron artificial neural network were used to assess the associations between individual phenolic compounds and AOX of grape juices. For the use of multivariate analysis, a high number of data was required in the experiments, in order to guarantee the minimization of type I errors. To guarantee the robustness of the statistical enquiry used in this study, twenty-nine phenolic variables and five methods of antioxidant activity were analyzed in 9 commercial and varietal grape juices, collected or produced in three repetitions, where each repetition corresponded to an independent batch produced (totalizing 27 grape juice samples), which resulted in 918 data points. The neural network was obtained using the MLP technique using 29 variables such as the phenolic compounds as input layers (input neurons) and 5 methods of measuring antioxidant activity as output layers (output neurons).

Results and discussion

Polyphenols profile in HPLC–DAD and AOX of grape juices

The individual phenolic profile of grape juice samples obtained by HPLC–DAD is presented in Table 1. The detection and quantification limits for all analyzed phenolics were LOD < 0.17 mg L^{-1} and LOQ < 1.41 mg L^{-1}, respectively. All calibration curves show good linear regression ($r^2 > 0.998$), and a typical chromatogram representing the separation and identification of phenolic in the grape juice sample is shown in Fig. 1.

Total polyphenols quantified by HPLC varied from 454.3 to 1651.4 mg L^{-1} in grape juices. Among the samples, the monovarietal juices made from Brazilian hybrid grapes (*V. vinifera x V. labrusca*) BRS Cora (1651.4 mg L^{-1}), BRS Violeta (1615.7 mg L^{-1}), and BRS Magna (1006.7 mg L^{-1}) showed the highest concentrations of bioactive phenolic compounds. The concentration of polyphenols in commercial juices varied from 454.3 to 754.6 mg L^{-1}. Among the polyphenolic substances analyzed in samples, phenolic acids and anthocyanins were the most abundant. The main phenolic acids quantified were *trans*-caftaric and chlorogenic acids at concentrations ranging from 111.8 to 285.6 mg L^{-1} and 15.5 to 35.8 mg L^{-1}, respectively. A diversity of anthocyanin compounds was found at a varying range of concentrations: malvidin 3,5-diglucoside (4.2–139.3 mg L^{-1}), petunidin 3-glucoside (3.0–489.9 mg L^{-1}), malvidin 3-glucoside (0–44.7 mg L^{-1}), cyanidin 3,5-diglucoside (0–84.4 mg L^{-1}), and delphinidin 3-glucoside (0.1–40.8 mg L^{-1}). The mono- and diglucoside forms of malvidin were the most abundant anthocyanins in commercial grape juices, while malvidin 3,5-diglucoside and petunidin 3-glucoside were predominant in juices of hybrid grapes. The latter can be pointed out here as a marker phenolic compound for red grape juices of BRS Cora, BRS Violeta, and BRS Magna grapes, since it was easily distinguishable from the commercial juices. This is corroborated by the fact that petunidin 3-glucoside is not generally abundant or commonly identified in *Vitis labrusca* (Andersen and Markham 2006; Toaldo et al. 2015; Haas et al. 2018). The identification of phenolic markers is important because it can assist to predict botanical and geographic origins, as well as the "terroir", the sensory characteristics and quality of grape products (Margraf et al. 2016; Granato et al. 2016).

Procyanidin B1, catechin, and procyanidin A2 were the main flavanols in samples. Levels of flavanols ranged from 26.1 to 214.6 mg L^{-1} in commercial and varietal grape juices, respectively. Concentrations of procyanidin B1 were up to 112.9 mg L^{-1} in varietal juices, while

Table 1 Chromatographic phenolic profiles of commercial and varietal Brazilian grape juices

Phenolic compounds mg L^{-1}	Grape juices								
	CJ1	CJ2	CJ3	CJ4	CJ5	IP	BC	BV	MG
Phenolic acids									
Gallic acid	7.9 ± 0.2	8.6 ± 0.3	ND	ND	ND	ND	ND	ND	ND
Caffeic acid	1.5 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	2.4 ± 0.2	1.0 ± 0.2	1.5 ± 0.2	ND	ND
Syringic acid	ND	4.56	1.30	4.0 ± 0.2	ND	1.3 ± 0.1	2.3 ± 0.1	ND	5.1 ± 0.3
p-Coumaric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorogenic acid	20.8 ± 0.3	35.8 ± 1.2	15.5 ± 1.4	14.2 ± 0.7	21.5 ± 0.9	19.9 ± 1.0	36.5 ± 1.0	31.5 ± 0.8	27.8 ± 0.9
trans-caftaric acid	255.2 ± 2.5	285.5 ± 1.4	188.9 ± 3.5	138.4 ± 2.1	251.2 ± 1.0	245.6 ± 2.1	428.5 ± 7	237.8 ± 4.2	111.7 ± 5
Σ Phenolic acids	285.4 ± 1.4	336 ± 2.4	206.8 ± 4.2	157.9 ± 1.4	275.2 ± 4	267.8 ± 3.1	468.8 ± 1.4	269.3 ± 6	144.7 ± 2.1
Flavanols									
Catechin	13.2 ± 0.4	9.5 ± 0.8	12.7 ± 1.1	5.6 ± 1.4	27.4 ± 2.4	18.3 ± 1.3	66.3 ± 2.1	18.4 ± 2	16.2 ± 1.2
Epicatechin	ND	1.5 ± 0.4	ND	2.3 ± 0.4	ND	ND	ND	ND	ND
Epicatechin gallate	ND	ND	ND	ND	ND	0.70	11.48	ND	ND
Epigallocatechin gallate	ND	ND	2.68	ND	5.80	ND	9.82	19.73	ND
Procyanidin A2	5.4 ± 1	4.9 ± 0.5	5.9 ± 0.6	5.0 ± 1.1	7.7 ± 1.4	6.3 ± 0.4	11 ± 0.7	7.8 ± 1.0	5.9 ± 0.6
Procyanidin B1	8.2 ± 1.4	10.3 ± 1.5	12.5 ± 0.8	16.7 ± 1.2	25.6 ± 2	7.9 ± 0.8	112.9 ± 2.4	88.5 ± 3.4	29.0 ± 3.4
Procyanidin B2	ND	ND	ND	1.3 ± 0.1	1.8 ± 0.3	ND	3.0 ± 0.4	7.1 ± 0.8	7.4 ± 0.5
Σ Flavanols	26.8	26.1	33.8	30.9	68.4	33.2	214.6	141.5	58.5
Flavonols									
Quercetin 3-glucoside	5.3 ± 1.4	4.4 ± 0.8	0.5 ± 0.8	2.5 ± 1.1	3.0 ± 0.6	2.6 ± 0.5	3.8 ± 0.5	3.2 ± 0.4	1.1 ± 0.4
Rutin	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	2.7 ± 0.3	1.0 ± 0.1	0.5 ± 0.1
Kaempferol 3-glucoside	0.7 ± 0.2	0.8 ± 0.1	0.9 ± 0.3	1.7 ± 0.2	2.6 ± 0.3	0.6 ± 0.1	3.9 ± 0.3	9.5 ± 0.3	9.3 ± 0.5
Myricetin	11.2 ± 0.8	27.4 ± 2	21.4 ± 3.2	30.5 ± 3.4	44.9 ± 1.8	14.9 ± 1.1	90.5 ± 2.1	156 ± 3.1	45.1 ± 1.4
Σ Flavonols	17.42	32.95	23.31	35.04	50.92	18.55	100.88	169.59	56.07
Anthocyanins									
Malvidin 3-glucoside	24.1 ± 2.4	1.6 ± 0.3	32.2 ± 0.9	2.7 ± 0.1	20.8 ± 0.5	44.7 ± 3.2	ND	5.7 ± 1	ND
Cyanidin 3-glucoside	ND	ND	ND	1.0 ± 0.2	ND	ND	19 ± 0.8	2.6 ± 0.4	4.5 ± 0.3
Petunidin 3-glucoside	6.9 ± 0.5	8.8 ± 0.5	31.4 ± 1.2	50.8 ± 1.4	87.1 ± 2.8	3.0 ± 0.4	484.9 ± 2.2	335.1 ± 4.2	418.2 ± 3.4
Delphinidin 3-glucoside	1.8 ± 0.2	0.1 ± 0.1	4.3 ± 0.3	2.5 ± 0.2	6.0 ± 0.8	3.2 ± 0.2	40.9 ± 1.1	16.5 ± 1.2	5.9 ± 0.2
Pelargonidin 3-glucoside	4.4 ± 0.3	0.5 ± 0.1	4.5 ± 0.5	1.2 ± 0.2	2.8 ± 0.2	5.4 ± 0.1	ND	2.5 ± 0.1	ND
Peonidin 3-glucoside	3.5 ± 0.2	0.4 ± 0.1	4.7 ± 0.2	0.7 ± 0.1	2.5 ± 0.3	5.4 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	ND
Malvidin 3,5-diglucoside	20.9 ± 1.8	5.7 ± 0.2	18.5 ± 0.4	55.3 ± 0.4	105.1 ± 2.2	41.4 ± 1.8	4.0 ± 0.2	313.5 ± 9.3	139.3 ± 4.5
Cyanidin 3,5-diglucoside	2.0 ± 0.2	ND	6.4 ± 0.2	12.8 ± 1.2	22.9 ± 2.2	ND	57.3 ± 1.2	84.7 ± 4.2	11.5 ± 0.8
Pelargonidin 3,5-diglucoside	ND	ND	ND	ND	ND	ND	ND	ND	ND
Σ Anthocyanins	63.6	17.0	101.9	126.9	247.2	103.1	599.4	762	579.4
Stilbenes									
trans-Resveratrol	0.88 ± 0.02	0.78 ± 0.1	0.71 ± 0.08	0.46 ± 0.03	0.52 ± 0.06	0.45 ± 0.04	0.40 ± 0.01	0.70 ± 0.03	0.47 ± 0.06
cis-Resveratrol	ND	ND	1.3 ± 0.2	5.4 ± 0.8	9.7 ± 1.1	ND	ND	39.7 ± 1.2	45 ± 2
Σ Stilbenes	0.9	0.8	2.0	5.9	10.2	0.5	0.4	40.4	45.5
Flavanones									
Hesperidin	ND	ND	1.4 ± 0.2	ND	ND	ND	24.3 ± 1.2	3.7 ± 0.5	ND
Naringenin	ND	ND	1.7 ± 0.2	1.0 ± 0.1	3.1 ± 0.3	ND	44.2 ± 1.2	11.6 ± 1.2	2.6 ± 0.2
Σ Flavanones	ND	ND	3.1	1.0	3.1	ND	68.5	15.2	2.6
Total phenolics quantified	502.2	612.8	454.3	448.9	757.6	502.7	1651.8	1615.8	1006.7

Results expressed as mean ± standard deviation ($n = 3$). *CJ* commercial grape juices (1–5). Varietal juices: *IP* Isabel Precoce, *BC* BRS Cora, *BV* BRS Violeta; and *MG* BRS Magna

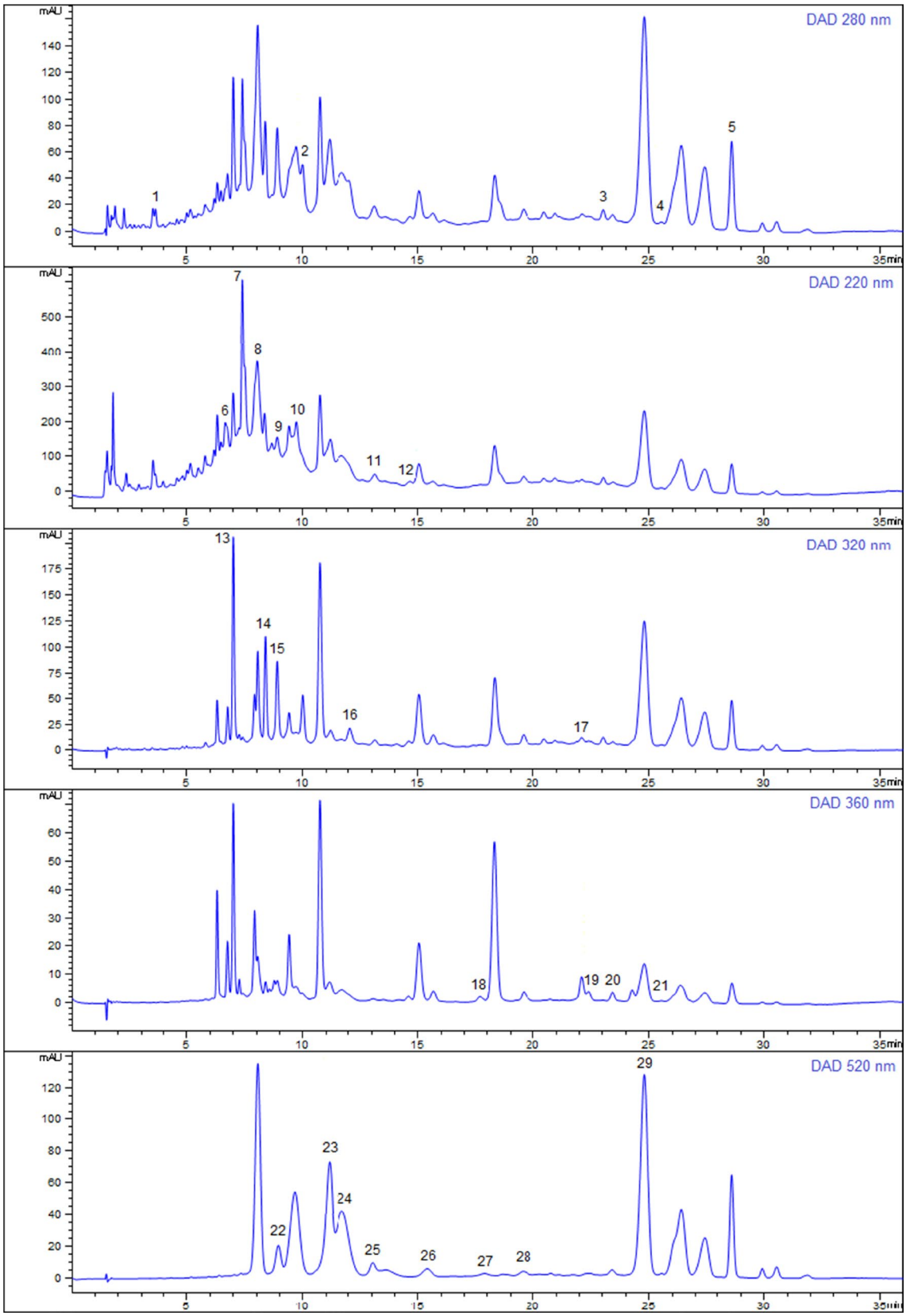

Fig. 1 Chromatogram representing the separation and identification of phenolic in the grape juice samples

concentrations of catechin reached 66.3 mg L^{-1} in the same grape juice (BRS Cora). The flavanol epicatechin was not found in the varietal samples, while its gallate form was not detected in commercial juices. It is important to highlight that although it is not known which grape varieties were used to produce the commercial juices, some particular differences in the polyphenolic profiles of varietal and commercial grape juices can assist in characterizing their origins and phytochemical composition, which can thus allow differentiating their antioxidant activity and bioactive potential. The concentrations of flavonols in grape juices samples varied from 17.42 mg L^{-1} in the commercial juice CJ1 to 169.59 mg L^{-1} in the BRS Violeta juice. The main flavanol found was myricetin that showed concentrations up to 90.5 mg L^{-1}. The stilbene *trans*-resveratrol was quantified in all juice samples, in values ranging from 0.4 to 0.88 mg L^{-1}, while its isomer *cis*-resveratrol showed the highest values in the monovarietal juices BRS Magna (45 mg L^{-1}) and BRS Violeta (39.7 mg L^{-1}), followed by commercial juices CJ5 (9.7 mg L^{-1}) and CJ4 (5.4 mg L^{-1}). The flavanones hesperidin and naringenin were found in some of the grape juices. The polyphenol naringenin was present at higher concentrations, up to 44.2 mg L^{-1}, particularly in the varietal juices. In general, concentrations of individual polyphenols were much higher in the varietal juices. This was observed for phenolic acids, flavanols, flavonols, and flavanones compounds, but most particularly for anthocyanins, which are important and predominant polyphenols in red grape juices. It is known that the presence and the diversity of polyphenols are widely associated with the antioxidant capacity of vegetable matrices (Hosu et al. 2014; Haas et al. 2018) and that phenolic compounds have specific contributions to the antioxidant properties (Petruk et al. 2017; Yang et al. 2018). The AOX of grape juice samples and associations between the phenolic profiles and the AOX of grape juices was then further evaluated in this study.

The AOX of grape juices varies indistinctly in samples and among the antioxidant methods, as shown in Table 2. Taken together, the antioxidant activity measured by the applied methods ranged from 1.07 ± 0.02 to 124.63 ± 4.41 mM TE L^{-1} when using βCLA and H_2O_2 methods, respectively. The AOX values obtained by free radical scavenging methods ranged from 4.7 ± 0.27 to 15.94 ± 0.32 mM TE L^{-1} (DPPH) and from 5.47 ± 0.14 to 23.61 ± 0.89 mM TE L^{-1} (ABTS). In the H_2O_2 inhibition method, AOX ranged from 50.54 ± 2.79 to 124.63 ± 4.41 mM TE L^{-1}. The AOX of grape juice samples varied between 1.07 ± 0.02 to 2.09 ± 0.33 mM TE L^{-1} in the βCLA method and between 12.86 ± 1.30 to 69.21 ± 2.95 mM $FeSO_4^{2+}$ L^{-1} in the FRAP method. The grape juice samples that showed the highest values of polyphenols quantified by HPLC (BRS Violeta, BRS Cora and BRS Magna, in Table 1) were also the ones that showed the highest antioxidant activity when evaluated by the five methods studied. Concentrations of polyphenols and values of antioxidant activity (DPPH, ABTS, and H_2O_2) are in agreement with other previous studies which characterized commercial and monovarietal grape juices from the same region of origin of the studied juice samples (Camargo et al. 2014; Lima et al. 2014; Silva et al. 2015; Padilha et al. 2017; Dutra et al. 2018; Dutra et al. 2021). To the best of our knowledge, studies evaluating the AOX of grape juices from the same region of this study using FRAP or βCLA methods were not performed.

In all methods, the AOX was higher in juices of the hybrid grapes, with the exception of Isabel Precoce juices. The highest AOX was observed for the BRS Magna juice in the H_2O_2 method and the juice had also the highest value of AOX (2.09 ± 0.33 mM TE L^{-1}) when the βCLA method

Table 2 In vitro antioxidant activity of grape juice samples

Grape juices	In vitro antioxidant methods				
	DPPH	ABTS	H_2O_2	FRAP	βCLA
CJ1	5.93 ± 1.06	9.60 ± 0.34	65.42 ± 3.14	29.28 ± 4.39	1.58 ± 0.04
CJ2	9.29 ± 0.06	12.10 ± 0.28	59.42 ± 0.04	37.49 ± 2.26	1.48 ± 0.19
CJ3	4.70 ± 0.27	8.50 ± 1.58	51.24 ± 1.42	15.82 ± 0.06	1.51 ± 0.02
CJ4	5.50 ± 0.10	9.29 ± 1.08	50.54 ± 2.79	24.44 ± 0.90	1.35 ± 0.20
CJ5	6.77 ± 0.24	9.81 ± 0.04	53.88 ± 2.27	30.26 ± 0.50	1.07 ± 0.02
IP	4.95 ± 0.21	5.47 ± 0.14	53.89 ± 0.58	12.86 ± 1.30	1.42 ± 0.14
BC	15.59 ± 0.32	18.55 ± 0.34	116.82 ± 0.92	40.99 ± 0.95	2.00 ± 0.13
BV	15.94 ± 0.32	23.61 ± 0.89	106.99 ± 5.74	69.21 ± 2.95	1.58 ± 0.01
MG	12.08 ± 0.11	13.87 ± 0.36	124.63 ± 4.41	43.97 ± 0.79	2.09 ± 0.33

Results expressed as mean ± standard deviation ($n = 3$). *CJ* commercial grape juices (1–5). Varietal juices: *IP* Isabel Precoce, *BC* BRS Cora, *BV* BRS Violeta, and *MG* BRS Magna. DPPH, ABTS, H_2O_2 e βCLA: antioxidant activity expressed as Trolox equivalents (TE) mM/L. FRAP: antioxidant activity expressed as ferrous sulfate mM/L

was used. When considering this sample, the same was not observed when using FRAP, ABTS, and DPPH methods. This tendency was observed for some of the other juice samples, which suggests discrepancies in terms of effectiveness of measurement, the real antioxidant activity of the sample, and methods capability. Indeed, this is a common issue when using in vitro antioxidant methods and may be explained by different method principles, analytical system, reagent specificity, and sample interferences in a given performance system (Granato et al. 2018a). This is why the complexity of the experimental results obtained in this study warranted a more attentive statistical evaluation in order to determine, with certain reliability, the link between polyphenolic composition and AOX of grape juices.

Statistical evaluation of the association between individual phenolic compounds and AOX

Pearson's correlations

The results of Pearson's correlation analysis are shown in Table 3. For analysis and discussion purposes, only positive values of Pearson's correlation coefficients greater than 0.80 ($r \geq 0.80$) and significant ($p < 0.01$) were considered, following the criteria adopted by Dutra et al. (2018) for strong associations between phenolic compounds and antioxidant activity of grape juices. The methods DPPH and ABTS showed positive correlation with the largest number of individual compounds and were associated with chlorogenic acid, procyanidin B2, myricetin, cyanidin 3,5-diglucoside, and epigallocatechin gallate. Similarly, but to a lesser extent, AOX values measured by the FRAP method showed a positive correlation with myricetin and cyanidin 3,5-diglucoside, and the AOX found in the H_2O_2 method was only correlated with chlorogenic acid, when considering our study criteria. The βCLA method did not show a positive correlation with any individual phenolic. In general, the compounds correlated with the AOX of juices samples were the same, with emphasis on myricetin and cyanidin 3,5-diglucoside, associated with DPPH, ABTS, and FRAP.

To evaluate these results, we can consider that Pearson's correlations show the degree of association between two variables (X and Y), being positive and strong when the values of the two variables increase proportionally in a direct manner (X↑ and Y↑) (Granato et al. 2014). Phenolic compounds that presented $r \geq 0.80$ (chlorogenic acid, procyanidin B2, myricetin, cyanidin 3,5-diglucoside and epigallocatechin gallate) represent here only the compounds that showed the highest concentrations in grape juices with the largest AOX, such as juice samples BC, BV, and MG (Tables 1 and 2). Based on this, it is evident that the use of Pearson's correlations has limitations to demonstrate all the phenolic compounds that were responsible for the AOX of grape juices.

Table 3 Pearson's correlations between phenolic compounds and in vitro antioxidant activity

	DPPH	ABTS	H_2O_2	FRAP	βCLA
Phenolic acids					
Gallic acid	−0.011	−0.071	0.419	0.025	0.215
Syringic acid	0.104	−0.089	0.143	0.165	0.010
trans-Caftaric acid	0.515	0.363	0.691[a]	0.221	0.635
Chlorogenic acid	0.899[a]	0.706[a]	0.917[a]	0.741[a]	0.566
Caffeic acid	−0.325	−0.393	−0.043	−0.437	−0.090
Flavanols					
Catechin	0.521	0.379	0.423	0.194	0.532
Epicatechin	−0.090	−0.106	0.061	0.100	−0.030
Epicatechin gallate	0.513	0.361	0.486	0.142	0.738[a]
Epigallocatechin gallate	0.737[a]	0.870[a]	0.447	0.784[a]	0.287
Procyanidin A2	0.602	0.644	0.156	0.706[a]	0.013
Procyanidin B1	0.596	0.527	0.397	0.350	0.441
Procyanidin B2	0.843[a]	0.840[a]	0.579	0.684[a]	0.617
Flavonols					
Quercetin 3-glucoside	0.289	0.230	0.544	0.259	0.377
Rutin	0.684[a]	0.576	0.580	0.365	0.749[a]
Kaempferol 3-glucoside	0.610	0.667	0.173	0.725[a]	0.012
Myricetin	0.842[a]	0.935[a]	0.508	0.885[a]	0.342
Stilbenes					
trans-Resveratrol	−0.303	−0.125	−0.104	−0.008	−0.333
cis-Resveratrol	0.404	0.487	−0.014	0.612	−0.229
Flavanones					
Hesperidin	0.607	0.500	0.539	0.269	0.776[a]
Naringenin	0.637[a]	0.581	0.551	0.366	0.738
Anthocyanins					
Cyanidin 3,5-diglucoside	0.801[a]	0.904[a]	0.457	0.818[a]	0.360
Malvidin 3,5-diglucoside	0.446	0.617	0.046	0.720[a]	−0.246
Delphinidin 3-glucoside	0.698[a]	0.631	0.519	0.420	0.689
Cyanidin 3-glucoside	0.618	0.484	0.489	0.289	0.724[a]
Pelargonidin 3-glucoside	−0.615	−0.648	−0.511	0.508	0.397
Peonidin 3-glucoside	−0.602	−0.531	−0.470	−0.636	−0.226
Malvidin 3-glucoside	−0.685	−0.639	−0.552	−0.671	−0.446
Petunidin 3-glucoside	0.735[a]	0.681[a]	0.380	0.601	0.423
Total phenolics quantified	0.885[a]	0.908[a]	0.943[a]	0.346	0.705[a]
In vitro *antioxidant activity*					
DPPH	1	0.929[a]	0.857[a]	0.908[a]	0.608
ABTS	0.929[a]	1	0.718[a]	0.943[a]	0.553
H_2O_2	0.857[a]	0.718[a]	1	0.705[a]	0.717

Table 3 (continued)

	DPPH	ABTS	H_2O_2	FRAP	βCLA
FRAP	0.908[a]	0.943[a]	0.705	1	0.346

[a]Significant at 1% probability error

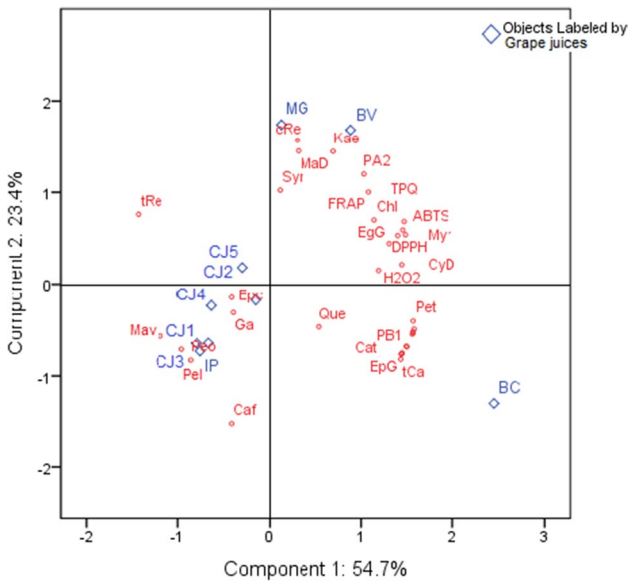

Fig. 2 Principal components analysis (PCA) using the results of phenolic profiles and in vitro antioxidant activity. DPPH, ABTS, FRAP, H_2O_2 e βCLA, antioxidant methods; TPQ, total phenolics quantified by HPLC; Gac, gallic acid; Cfc, caffeic acid; Cfa, *trans* caftaric acid; Sra, syringic acid; Chl, chlorogenic acid; cRe, c*is*-resveratrol; tRe, *trans*-resveratrol; Cat, catechin; Epc, epicatechin; EpG, epicatechin gallate; EgG, epigallocatechin gallate; PA2, procyanidin A2; PB1, procyanidin B1; PB2, procyanidin B2; Que, quercetin 3-glucoside; Rut, rutin; Myr, myricetin; Kae, kaempferol 3-*O*-glucoside; Nar, naringenin; Hes, hesperidin; Mal, malvidin 3-*O*-glucoside; MaD, malvidin 3,5-diglucoside; Del, delphinidin 3-*O*-glucoside; Peo, peonidin 3-*O*-glucoside; CyD, cyanidin 3,5-diglucoside; Pel, pelargonidin 3,5-diglucoside; Pet, petunidin 3-glucoside; CJ, commercial grape juices (1–5); varietal juices: IP-Isabel Precoce; BC-BRS Cora; BV-BRS Violeta; MG-BRS Magna

This can be confirmed by the observation that polyphenols that did not show positive correlations with the AOX of juice samples, such as *trans*-caftaric acid, catechin, procyanidin B1, and malvidin 3,5-diglucoside, or even negative correlation, like malvidin 3-glucoside, are known to be antioxidants in vitro (Muselík et al. 2007; Mudnic et al. 2010; Tabart et al. 2009) and were, in fact, present at high concentrations in the grape juices (Table 1).

Principal component analysis (PCA)

Figure 2 shows the PCA obtained for polyphenolic profile and AOX of grape juices as measured by the different methods. The PCA plot was constructed by plotting the variables and factor loadings. The main components 1 and 2 (PC1 and PC2) explained together 78.1% of the total experimental variance, where PC1 corresponded to most of the variance with 54.7%. The associations between phenolic compounds and antioxidant activity occurred in PC1, where the antioxidant methods were grouped in the positive part of the component (PC1 > 0). The PC2 seemed to associate grape juice samples and phenolic compounds based on their antioxidant activity, as corroborated by the inclusion and association of all antioxidant methods in this component. This may be an indicative of the AOX potential of individual phenolic compounds, particularly those grouped in the positive quadrant of PC2. However, PC2 account for only 23.4% of the data variability. PC2 > 0 separated BV and MG samples due to higher values of procyanidin A2, cis-resveratrol, malvidin 3,5-diglucoside, and kaempferol 3-glucoside. PC1 clearly separated commercial and varietal grape juices by phenolic profile and antioxidant activity. In addition, it can be observed that the majority of individual phenolic compounds are grouped in PC1, and this component accounted for most of the data variability. This is important because it shows that, mostly, grape botanical or varietal factors and a particular phenolic composition contribute to a greater extent to the distinction or association of grape juices, rather than the antioxidant activity alone or the methods used to determine or compare its values.

The analysis factor (component loading > 0.70) directly correlated 13 compounds: *trans*-caftaric acid, catechin, epicatechin gallate, epigallocatechin gallate, procyanidin B1, procyanidin B2, naringenin, hesperidin, cyanidin-3-glucoside, petunidin-3-glucoside, cyanidin-3,5-diglucoside, rutin, myricetin, and the total phenolics quantified, with DPPH, ABTS, H_2O_2, and βCLA methods, as can be seen in the supplementary material (Table S1). By the value of the analysis factor (loading < 0.70), we did not consider the associations between phenolic compounds and the FRAP method in PC1 > 0. The polyphenols malvidin-3-glucoside and *trans*-resveratrol showed inverse correlations (PC1 < 0) with the AOX. Conversely, its isomer *cis*-resveratrol was strongly correlated (0.947) with the AOX of grape juices in the PC2.

The PCA proved to be a more powerful method of association between phenolic compounds and AOX than Pearson's correlations since a greater number of compounds was positively associated ($n = 13$) with four antioxidant methods (DPPH, ABTS, H_2O_2, and βCLA). However, it presented some limitations due to the negative correlations obtained for malvidin 3-glucoside, which was one of the main anthocyanins quantified in the studied samples. The PCA also showed no correlations between AOX and peonidin 3-glucoside and pelargonidin 3-glucoside. The weak positive correlations between individual phenolics and the FRAP method also hinder a possible integrated analysis of

phenolic compounds that exerted simultaneous AOX in the five methods used.

Artificial neural network (ANN)

The summary of data processing showed values of 88.9% for training and 11.1% for testing (100% valid model, SSE = 0.042). Eleven hidden layers were generated, where the function used was the hyperbolic tangent. The output layer was obtained with the activation function being the identity function. The generated artificial neural network model (29–11-5) is shown in supplementary material (Figure S1). The learning algorithm (reverse propagation) represented by the negatives synaptic weight (synaptic weight < 0) predicted the relationships between phenolic compounds and AOX. For interpretation purposes, we considered only the hidden layers that presented the highest negative activation values (synaptic weight < −0.100) of reverse propagation for the largest number of antioxidant methods, considering the bias adjustment. Bias is an extra entry added to neurons that allows a representation of phenomena with thresholds (Faria-Silva et al. 2015).

The ANN parameters obtained are shown in Table 4. The two layers that showed the highest negative synaptic weight values for phenolic compounds associated simultaneously with the largest number of antioxidant methods were the hidden layers H (1:3) (five AOX methods) > H (1:7) (three AOX methods). The H layer (1:3) (bias = −0.465) presented the highest activation values for the phenolic compounds variables ($n = 15$) related simultaneously with the five methods studied (DPPH, ABTS, FRAP, H_2O_2, and βCLA). Layer H (1:3) activated reverse propagation for gallic acid, syringic acid, chlorogenic acid, *trans*-caftaric acid, catechin, epicatechin, procyanidin B1, procyanidin B2, naringenin, hesperidin, cyanidin 3,5-diglucoside, trans-resveratrol, kaempferol 3-glucoside, and rutin, including total phenolics quantified by HPLC (TPQ). Layer H (1: 7) (bias = −0.179) activated the reverse propagation of 10 phenolic compounds with three antioxidant methods (DPPH, ABTS, and H_2O_2), among them epicatechin gallate, epigallocatechin gallate, quercetin 3-glucoside, cyanidin 3-glucoside, and malvidin 3,5-diglucoside.

Considering only the hidden layer H (1:3), a strong association was evidenced of 14 individual phenolics, among the 28 compounds quantified simultaneously, with the five antioxidant methods studied (DPPH, ABTS, FRAP, H_2O_2, and βCLA). When we take together the H (1: 3) and H (1: 7) layers, there is a simultaneous association of 19 individual phenolics with at least three AOX methods (DPPH, ABTS, and H_2O_2). If we consider the 11 hidden layers of ANN in the analysis, 27 out of the 28 individual phenolic compounds would be associated with all the methods of antioxidant activity; however, the threshold of the connections would need to be evaluated by the synaptic weight and

Table 4 Estimation of artificial neural network parameters

		Predicted															
		Hidden Layer 1											[b] *Output Layer*				
Predictor		H(1:1)	H(1:2)	**H(1:3)**	H(1:4)	H(1:5)	H(1:6)	**H(1:7)**	H(1:8)	H(1:9)	H(1:10)	H(1:11)	βCLA	DPPH	ABTS	H2O2	FRAP
Input Layer	(Bias)	0.229	0.196	**-0.465**	0.083	0.366	-0.027	**-0.179**	0.209	0.175	-0.380	0.057					
	Ga	-0.034	-0.099	**-0.279**	0.331	0.371	-0.167	0.232	-0.247	-0.197	0.430	0.149					
	Caf	0.131	0.286	0.273	0.151	0.030	0.350	0.277	0.025	0.401	0.214	-0.372					
	Syr	-0.109	-0.085	**-0.357**	-0.308	-0.142	-0.366	0.072	0.020	0.220	0.360	0.433					
	Chl	0.336	-0.451	**-0.377**	0.291	0.164	0.095	**-0.162**	-0.361	-0.115	-0.095	0.379					
	tCa	-0.508	0.042	**-0.364**	0.056	0.476	0.195	-0.025	0.175	0.131	0.000	-0.001					
	Cat	-0.464	-0.012	**-0.346**	0.402	-0.104	-0.138	0.175	0.186	0.295	0.331	0.025					
	Epc	-0.232	-0.417	**-0.271**	-0.432	0.132	0.409	**-0.449**	0.168	0.201	-0.329	0.390					
	EpG	0.356	0.182	0.018	0.013	-0.449	0.216	**-0.183**	0.411	-0.214	-0.038	-0.305					
	EgG	0.315	0.398	-0.029	-0.403	0.315	-0.424	**-0.214**	-0.189	0.272	0.273	-0.410					
	PB1	0.342	0.047	**-0.272**	-0.438	-0.045	0.170	**-0.411**	0.069	-0.005	0.086	0.219					
	PB2	0.361	-0.294	**-0.383**	-0.510	0.237	0.052	0.090	0.245	0.255	-0.248	0.294					
	PA2	-0.468	-0.003	0.000	-0.186	0.332	-0.094	-0.005	0.237	0.003	0.330	-0.494					
	Nar	-0.031	-0.150	**-0.330**	-0.257	-0.304	0.181	**-0.317**	-0.419	0.160	0.060	0.292					
	Hes	-0.400	-0.007	**-0.395**	0.152	-0.017	0.037	0.131	-0.132	0.365	0.081	0.309					
	Que	0.094	0.244	0.233	0.316	-0.192	0.262	**-0.228**	0.427	-0.062	0.115	0.480					
	Cya	-0.093	0.135	0.369	0.029	-0.415	-0.408	**-0.241**	0.303	-0.249	-0.108	0.136					
	Mav	-0.385	-0.407	0.255	0.446	0.419	0.441	0.309	0.291	0.392	-0.383	-0.236					
	Del	-0.072	0.384	0.134	-0.152	0.418	0.188	0.075	-0.350	-0.056	-0.407	-0.068					
	Pel	0.351	0.446	0.303	0.062	0.478	0.207	0.174	0.318	0.339	0.203	0.502					
	CyD	-0.039	0.233	**-0.238**	-0.440	-0.456	0.282	**-0.408**	-0.429	-0.103	-0.210	-0.198					
	MaD	0.349	0.138	0.083	0.141	0.530	-0.149	**-0.225**	-0.040	-0.032	0.400	0.327					
	cRe	-0.482	0.339	0.206	0.339	0.279	0.462	-0.080	0.344	0.016	-0.429	0.160					

bias of the methods. This analysis would make it possible to screen the selectivity of AOX methods for individual phenolic compounds.

The polyphenols peonidin 3-glucoside and pelargonidin 3-glucoside were the only compounds not associated by ANN with the AOX by any of the antioxidant methods, just like in the PCA analysis. Considering the phenolic profiles of all analyzed juices (Table 1), these two anthocyanins had approximate values in all grape juice samples, which can hinder analysis of correlations regardless of the chosen technique. When analyzing the three statistical tools used in this study, the power of association of individual phenolic compounds with the AOX of grape juices followed the following order: ANN > PCA > Pearson's correlation. Based on the results, it is evident that the use of Pearson's correlations alone is insufficient to associate individual phenolics with AOX. The PCA produced good results, approaching ANN, which was the most powerful technique to demonstrate the association of phenolics with AOX, as well as to study the possible selectivity of these in vitro methods.

Based on ANN and the analysis factor, our study showed that the compounds gallic acid, syringic acid, chlorogenic acid, *trans*-caftaric acid, catechin, epicatechin, procyanidin B1, procyanidin B2, naringenin, hesperidin, cyanidin 3,5-diglucoside, *trans*-resveratrol, kaempferol 3-glucoside, rutin, epicatechin gallate, epigallocatechin gallate, quercetin 3-glucoside, cyanidin 3-glucoside, and malvidin 3,5-diglucoside were the polyphenols associated with the antioxidant activity of grape juices in at least 3 out of 5 antioxidant methods evaluated in the study. Studies that measured the antioxidant activity of several polyphenols standards showed that the compounds that were associated here by the ANN with the AOX of juice samples (hidden layers H (1:3) and H (1:7) have simultaneous antioxidant activity as measured by the methods of DPPH, ABTS, and FRAP (Muselík et al. 2007; Tabart et al. 2009; Mudnic et al. 2010), which corroborates the results predicted by the neural network.

Oxidative stress caused by reactive oxygen species (ROS) plays a crucial role in the pathophysiology associated with diseases such as heart diseases, neoplasia, atherosclerosis, and neurodegenerative diseases. The chemical structure of an antioxidant determines its intrinsic reactivity in the neutralization of free radicals and other ROS and, therefore, its antioxidant potential. Currently, antioxidants are sought with different modes of action on oxidative systems, so that it can increase their effectiveness in the treatment of diseases (Shahidi and Zhong 2015; Shahidi and Ambigaipalan 2015). According to Granato et al. (2018b), in bioactivity studies that take measurements of AOX, it is necessary to make use of methods that simulate different mechanisms of action, such as single electron transfer, transition metal chelating capacity, and hydrogen atom transfer and to associate with those the profile of bioactive compounds quantified by techniques such as HPLC, LC–MS, or other refined instrumental techniques. In the light of these observations, it has become evident the need for approaches that allow to identify or associate individual polyphenols with the ability to act simultaneously in different oxidative processes in vitro, so it can make it possible to screen potential substances or foods with potential bioactivity in vivo. In fact, chemometrics and network statistics seem to represent the state of the art regarding food evaluations and bioactive polyphenols (Camargo et al. 2014; Granato et al. 2014; Dutra et al. 2018; Granato et al. 2018b; Pavlić et al. 2019).

Studies to optimize the extraction of quality components such as essential oils by different methods have used response surface methodology to assess the extraction kinetics (Bahmania et al. 2018a; Shaterabadi et al. 2020). However, ANN with reverse propagation algorithm has obtained good results (Bahmania et al. 2018b). In the study of Dębska and Guzowska-Świder (2011), artificial neural networks were used to classify 28 beer samples using 12 physical–chemical and sensory variables as input variables. In the study of Faria-Silva et al. (2015)(2015, ANN was used to assess the stability of 36 olive oil samples using the variables free fatty acids, peroxide value, specific extinction coefficient, chlorophyll content, tocopherol, total phenolic compounds by Folin-Ciocalteu, color, and dissolved oxygen.

The study findings demonstrated that ANN is a powerful tool in associating phenolic compounds and AOX and can contribute to the characterization of grape juice polyphenols and their respective bioactive potential. This contributes to grape juice typicity and valorization. The ANN was able to predict potential compounds associated with simultaneous antioxidant activity in different systems, as well as to measure the selectivity of in vitro methods in relation to substances of different chemical nature.

Conclusions

The power of association of three statistical tools used in this study to associate individual phenolic compounds with the AOX of grape juices followed the order: ANN > PCA > Pearson's correlation. The phenolic profiles of red grape juices were easily distinguishable between commercial and varietal juices when compound concentrations and AOX values were considered. The monovarietal juices of BRS Cora, BRS Violeta. and BRS Magna showed higher concentrations of most polyphenols found in samples and had the highest AOX. The use of Pearson's correlations had limitations in identifying the phenolics responsible for the AOX of commercial and varietal juices. PCA proved to be a more powerful method than Pearson's correlation, as it positively associated 13 phenolic compounds with four out of five antioxidant methods (DPPH, ABTS, H_2O_2, and βCLA). However, it presented

some limitations due to the negative correlations showed for malvidin 3-glucoside, one of the main anthocyanins quantified in grape juices, and weak correlations with the FRAP method. The ANN generated 11 hidden layers, where a single layer (H (1:3)) predicted a strong simultaneous association of 14 individual phenolics, among the 29 quantified, with five antioxidant methods (DPPH, ABTS, FRAP, H_2O_2, and βCLA), proving to be a powerful tool in the study of the association of polyphenols and AOX. It was evidenced that ANN is a promising tool for screening phenolic antioxidants simultaneously in different in vitro systems with great potential for application in bioactivity studies.

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Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

Conflict of Interest Marcos dos Santos Lima declares that he has no conflict of interest. Emilly Thayná declares that she has no conflict of interest. Januario Ferreira declares that he has no conflict of interest. Marcelo Eduardo Alves Olinda de Souza declares that he has no conflict of interest. Giuliano Elias Pereira declares that he has no conflict of interest. Isabela Maia Toaldo Fedrigo declares that she has no conflict of interest.

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