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Authentication of juices from antioxidant and chemical perspectives: A feasibility quality control study using chemometrics

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ABSTRACT

In this work, Brazilian juices (n = 38) from distinct botanical species were analyzed for the physicochemical properties, major phenolic classes, and antioxidant activity using high-throughput assays. Principal component analysis [PCA] was applied to study the data structure, while classification methods based on partial least squares-discriminant analysis [PLS-DA] and dual data-driven PCA/soft independent modeling of class analogy [DD-SIMCA] were used to predict the class membership of juices. In addition, multiple linear regression [MLR] models were proposed to explain the antioxidant activity of juices. PLS-DA was successfully used to authenticate the class membership of juices, enabling the identification of the main variables responsible for the discrimination. Similarly, DD-SIMCA was shown to be useful for the authentication of juices. Additionally, the main phenolic classes responsible for each of the antioxidant activity were revealed by MLR. Overall, the characterization of juices was reached by the application of relatively simple analytical methods supplemented with modern chemometric tools.

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1. Introduction

In 2013, Brazil produced a total of 43.6 million tons of fruits, in which about 23.8 million tons were used to produce juices, nectars, and pulps. According to the European Fruit Juice Association (AIJN, 2014), the consumption of the fruit juices and nectars in Europe reached 10 billion liters, while fruit juices and nectars accounted for 38.9 billion liters in 2014, generating more than €94 billion. From this amount, Latin America and North America held 9% and 25% of the production, respectively, while EU countries accounted for 26%. The orange and other citric juices (*i.e.,* tangerine/mandarins, lemon/limes) represent about 40% of the worldwide share, while the juice blends (*i.e.,* berries, passion fruit, and peach) and the apple juices gather 33% of the share. Brazil plays an important role in manufacturing of *Citrus*, tropical and apple juices. Development of juice blends is a common practice, but nowadays we observe a new trend in retail stores, the so-called *super juices,* which are juices made from berries and combinations with some vegetables, such as red beet, pomegranate, and cranberry. These products are regarded as healthy because of a high purported nutritive value and high content of bioactive compounds (Medina, 2011).

When the quality of juices is analyzed, many parameters can be determined, such as carotenoids, phenolic compounds, volatile organic compounds, organic acids and sugars, antioxidant activity, physical properties (rheological parameters, color), among others (Jandrić & Cannavan, 2016; Meléndez-Martínez, Vicario, & Heredia, 2007; Spinelli et al., 2016). In a general view, multiple analytical markers can be analyzed and for a large amount of juices, producing a complex data set. Aiming to increase the understanding of the interconnection between intrinsic characteristics of fruits and their technological products, as well as the effects of ripeness, farming system, year of cultivation, and other agronomical factors, multivariate statistical methods are successfully applied in food science and technology (Marseglia et al., 2016; Nyarko, Puzey, & Donnelly, 2014). In addition, these statistical tools are also used to characterize juices the multiple botanical and geographical origin of the juices. Thus, statistical analysis is, *de fato*, an essential tool for quality control purposes (Tassoni, Tango, & Ferri, 2013; Zhao et al., 2016).

Although high-performance liquid chromatography (HPLC coupled or not with mass spectrometry) of individual compounds (*i.e.*, phenolics, pigments, and organic acids) is very reliable and

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well established, it is known that not all food companies perform the quality control based on the use of HPLC (Jandrić & Cannavan, 2016). Therefore, application of alternative methods for the routine analysis of physicochemical parameters, major phenolic classes, and antioxidant activity measured by high-throughput assays can be a low-cost and rapid alternative. Additionally, as Brazilian juices are exported to many countries worldwide, it is important to monitor their authenticity and quality by measuring their chemical composition, physicochemical properties, and antioxidant activity. Therefore, the objectives of this study are to characterize Brazilian juices coming from different species and to classify these juices based on physicochemical and chemical data. The characterization of samples should be reached by the application of relatively simple analytical methods supplemented with modern multivariate data processing.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu's phenol reagent, tripyridyl-2,4,6-*s*-triazine (TPTZ), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, sodium molybdate dihydrate, vanillin, (+)-catechin, chlorogenic acid, 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (ferrozine), quercetin, ferric chloride hexahydrate, and ascorbic acid were obtained from Sigma (St. Louis, MO, USA), whereas $K_3[Fe(CN)_6]$, was obtained from Merck (Germany). All the other reagents used in the experiments were of analytical grade and ultrapure water (Millipore, São Paulo, Brazil) was used.

2.2. Juice samples

In this study, a total of n = 38 samples of juices from different botanical species were either acquired from local shops (Ponta Grossa, PR, Brazil) or squeezed in the laboratory. There are n = 14 orange juices (*Citrus sinensis*), in which n = 8 were commercial 100% juice, n = 1 organic orange juice, and n = 1 orange cv. Bahia, n = 1 orange cv. Pera Rio, n = 2 orange cv. Lime, n = 1 orange cv. Rosada, n = 3 lemon juices (*Citrus limon*) from three different varieties (cv. Cravo, cv. Taiti, and cv. Galego), n = 3 tangerine juices (*Citrus reticulata*), in which two tangerine samples were organic (certified by IBD, Brazil). The commercial *Citrus* samples referred to pasteurized juices (no additives added) marketed in different plastic bottles, while the other *Citrus* juices were squeezed in the laboratory (no thermal process was applied) and centrifuged at 740 *g* for 5 min. The sample set also consisted of n = 1 yellow passion fruit juice (*Passiflora edulis;* pasteurized), n = 6 apple juices (*Pyrus malus;* 2 and 4 freshly-squeezed and centrifuged pasteurized), in which one was organic (certified by IBD; freshly-squeezed and centrifuged), n = 4 pomegranate nectars (*Punica granatum;* sterilized) in which 3 contained 35% pulp and one contained 11%, n = 2 cranberry nectars containing 21.5% fruit pulp (*Vaccinium macrocarpon;* sterilized), n = 2 blackberry juices (*Morus nigra;* 1 pasteurized and 1 freshly-squeezed and centrifuged), n = 2 blueberry (*Vaccinium* sp; pasteurized), and n = 1 red beet juice (*Beta vulgaris;* freshly prepared by cooking 1 kg of roots at 121 °C/15 min and then the content was processed using a juicer machine and centrifuged) was also used for comparison purposes.

2.3. Physicochemical analysis

The pH at 25 °C was measured according to the AOAC (2005) using a pH meter with a previously calibrated electrode. The titratable acidity was determined by potentiometric titration and expressed as g/100 mL (AOAC, 2005) and the total soluble solids content (TSS) was estimated using a refractometer (model Atago N-1α, Japan) according to the AOAC (2005) and the results were expressed as °Brix.

2.4. Chemical composition (phenolic classes)

Total phenolic content of juices was determined using the Prussian Blue assay as described by Margraf, Karnopp, Rosso, and Granato (2015) and results were expressed as mg gallic acid equivalent/L. Total *ortho*-diphenols content was estimated by the colorimetric method that uses sodium molybdate diluted in water and ethyl alcohol (1:1 v/v) (Durán, Padilla, Martín, Fiestas Ros de Ursinos, & Mendoza, 1991) and results were expressed as mg chlorogenic acid equivalent/L. The total flavonoid content was determined using the colorimetric method described by Herald, Gadgil, and Tilley (2012) and results were expressed as mg (+)-catechin equivalent/L. Condensed tannins content was estimated using the method that employs the reaction of vanillin and condensed tannins in acidic medium (Horszwald & Andlauer, 2011) and results were expressed as mg (+)-catechin equivalent/L. The total flavonols content was estimated using the $AlCl_3$ method outlined by Yermakov, Arasimov, and Yarosh (1987) and results were expressed as mg quercetin equivalent/L. The content of monomeric anthocyanins (apple, blackberry, cranberry, pomegranate, and blueberry juices) was determined by UV–Vis spectrophotometry (λ = 520 nm and λ = 700 nm) using the differential pH method as described by Lee, Durst, and Wrolstadt (2005) and expressed as mg/L. Betalains were quantified in red beet juice in aqueous medium using the colorimetric method described by Stintzing, Schieber, and Carle (2003). Absorbance values were recorded at λ = 485 nm (betaxantins) and λ = 536 nm (betacyanins) and total betalains content was expressed as the sum of betaxantins and betacyanins (mg/L). Results of anthocyanins and betalains were expressed as total pigments (mg/L). Details on these spectrophotometric methods are fully explained by Granato, Santos, Maciel, and Nunes (2016).

2.5. In vitro antioxidant activity

The free radical scavenging activity toward DPPH was quantified using the conditions described by Granato, Karnopp, and van Ruth (2015). The assay was conducted at pH 6.0 using 50 mmol/L sodium phosphate and ethyl alcohol at a 1:1 (v/v) proportion as solvents of the DPPH radical (0.10 mmol/L) (Zheng, Lin, Su, Zhao, & Zhao, 2015). Results were expressed as mg ascorbic acid equivalent/L. The scavenging of $ABTS^{\bullet+}$ was determined as described by Re et al. (1999) and the values were expressed as mg ascorbic acid equivalent/L.

The Fe^{2+} chelating ability of juices was assessed using a spectrophotometric assay that employs ferrozine and $FeSO_4$ using a protocol proposed by Santos, Brizola, and Granato (2017). Ultrapure water was used as control and the percentage of Fe^{2+}-ferrozine complex formation was calculated as: Fe^{2+} chelating rate (%) = $[(Abs_{sample} - Abs_{solution\ without\ ferrozine})/Abs_{control}] \times 100$ and an analytical curve using different EDTA-Na_2 concentrations was plotted. Results were expressed as mg EDTA equivalents/L.

The Folin–Ciocalteu's reducing capacity of juices was assessed using the modified Folin–Ciocalteu assay and results were expressed as mg gallic acid equivalent/L (Singleton, Orthofer, & Lamuela-Raventos, 1999). Ferric reducing antioxidant power (FRAP) of juices was determined according to the method described by Benzie and Strain (1996) and results were expressed as mg ascorbic acid equivalent/L. Total reducing capacity (TRC) was used to measure the potential of both water-soluble and lipophilic

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antioxidants in juices, following the protocol and experimental conditions adopted by Margraf, Santos, de Andrade, van Ruth, and Granato (2016), and results were expressed as mg quercetin equivalent/L.

2.6. Statistical analyses

2.6.1. Data set

Data are organized as a ($I \times J$) matrix X that has $I = 38$ rows (juice samples) and $J = 15$ columns (variables). Variables were divided into two subsets, which are: *Bio* subset (all six antioxidant assays), and *PhysChem* subset (all nine physicochemical and chemical analyses), see Table 1. Juices were initially grouped in three categories: *Citrus* (Class 1; ●; n = 20) (orange, tangerine, and lemon), *super juices* (Class 3; ▲; n = 11) (red beet, blackberry, cranberry, pomegranate, and blueberry), and *other juices* (Class 2; ■; n = 7) (yellow passion fruit, red and green apples).

2.6.2. Descriptive analysis and principal components analysis

All analyses were performed in triplicate (except for acidity, pH and TSS, which were analyzed in duplicate and expressed as means) and data were presented as means followed by the standard deviation. Normality of all responses was checked using the Kolmogorov-Smirnov test prior to correlation analysis. Pearson's correlation coefficients were calculated to associate the physicochemical chemical composition and antioxidant activity of juices (Granato, de Araújo Calado, & Jarvis, 2014). For explorative purposes, principal component analysis (PCA) was applied to the whole dataset. The ($I \times J$) data matrix **X** (duly preprocessed, e.g. centered) was decomposed according to Equation (1):

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathbf{t}} + \mathbf{E} \quad (1)$$

where $\mathbf{T} = \{t_{ia}\}$ is the ($I \times A$) scores matrix; $\mathbf{P} = \{p_{ja}\}$ is the ($J \times A$) loadings matrix; $\mathbf{E} = \{e_{ij}\}$ is the ($I \times J$) matrix of residuals; and A is the number of principal components (PC). The samples are projected on the factor-space (PC1 x PC2) to understand the grouping according to the experimental data (Brereton, 2015; Nunes, Alvarenga, Sant'Ana, Santos, & Granato, 2015).

2.6.3. Supervised classification of juices

Partial least squares discriminant analysis (PLS-DA) based on PLS2 algorithm was used using Microsoft Excel using Chemometrics Add-In (Pomerantsev, 2014). PLS-DA is a supervised classification method that is based on the premise that several classes can be separated by rotating the principal components in a way that a maximum separation among classes is obtained (Margraf et al., 2016; Porto-Figueira, Freitas, Cruz, Figueira, & Câmara, 2015). It is a conventional PLS regression method, where the ($I \times J$) matrix **X** is a predictor matrix, and the ($I \times K$) response matrix **Y** comprises categorical (dummy) variables that describe class memberships. K is equal to the number of classes. When PLS regression is developed, the response value $\mathbf{Y}_{\text{pred}}$ is predicted for a new sample. The decision is based on the comparison of $\mathbf{Y}_{\text{pred}}$ with given categorical variables in **Y**. The sample is attributed to the class, which dummy variable **Y** is closest to $\mathbf{Y}_{\text{pred}}$. PLS-DA has been widely used in food research (Melucci et al., 2016). Two PLS-DA models were considered. The first model, which is called full, was developed using the whole block of variables (Bio + PhysChem). The second model, which is termed short, was obtained when only PhysChem variables were employed. In the PLS-DA approach, each model was developed according to the following procedure: the dummy responses **Y** were constructed using patterns (1, 0, 0), (0, 1, 0), and (0, 0, 1), and they were centered. Predictors block **X** (full or short) were column-wise centered and weighted by the standard deviation values. Predicted responses $\mathbf{Y}_{\mathbf{pred}}$ were obtained using PLS2 regression with three latent variables (LV). To develop the decision rules, matrix $\mathbf{Y}_{\mathbf{pred}}$ (which rank is 2) was projected onto the PCA scores space. After that step, linear discriminant analysis was applied (Indah, Martens, & Næs, 2007).

A modification of the well-known soft independent modeling of class analogy (SIMCA) method (Wold & Sjostrom, 1977, pp. 243–282) called data-driven SIMCA or DD-SIMCA (Pomerantsev & Rodionova, 2014a) was used as a one-class classifier (OCC) technique. An ultimate outcome of OCC is the decision rule that answers the key question – whether a new sample belongs to the target class or not. DD-SIMCA consists of two steps. At a first step, PCA (Eq. (1)) is applied to the data from the target class. At the second step, for each object from the training set, two distances are calculated. They are the score distance (SD), h_i, and the orthogonal distance (OD), v_i, according to Equation (2):

$$h_i = \mathbf{t}_i^{\mathrm{t}}(\mathbf{T}^{\mathrm{t}}\mathbf{T})^{-1}\mathbf{t}_i, \quad v_i = \sum_{j=1}^{J} e_{ij}^2 \quad (2)$$

The SD represents the position of a sample within the score space, and the OD characterizes the distance of the sample to the score space. DD-SIMCA adds the possibility of estimation the data-driven distribution parameters, which are the mean values v_0 and h_0, and the numbers of the degrees of freedom N_h and N_v for the SD $\{h_i\}$ and OD $\{v_i\}$, respectively. Thus, we can develop an acceptance area/decision rule for a given value (Pomerantsev & Rodionova, 2014a). Optionally, when an alternative class is available, DD-

Table 1
Summary of variables used in the juice samples analysis.

#	Variable subsets	Name	Units	Maximum value	Minimum value
1	Bio	DPPH	mg ascorbic acid equivalent/L	1388	6
2		ABTS	mg ascorbic acid equivalent/L	10,350	99
3		Fe^{2+} chelating ability	mg EDTA equivalent/L	10,633	20
4		FRAP	mg ascorbic acid equivalent/L	10,724	83
5		Folin-Ciocalteu's reducing capacity	mg gallic acid equivalent/L	8183	145
6		Total reducing capacity	mg quercetin equivalent/L	8018	151
7	PhysChem	pH	adimensional	5.85	2.29
8		Acidity	g/100 mL	5.50	0.04
9		Total soluble solids	°Brix	14.6	1
10		Total phenolic content	mg gallic acid equivalent/L	2485	47
11		Flavonoids	mg (+)-catechin equivalent/L	925	45
12		Flavonols	mg quercetin equivalent/L	239	4
13		Condensed tannins	mg (+)-catechin equivalent/L	1620	0
14		*Ortho*-diphenols	mg chlorogenic acid equivalent/L	1202	28
15		Total pigments	mg/L	854	0

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SIMCA provides the possibility to calculate the type II β error and construct the corresponding extended acceptance area, which guarantees that the risk of accepting a sample from the alternative class is not greater than β (Pomerantsev & Rodionova, 2014b). The results of classification are described in terms of 'sensitivity' and 'specificity' or with traditional statistical terms as the type I error, α, and the type II error, β. Sensitivity denotes a share of correctly identified samples of the target class. Specificity is a portion of objects of an alternative class that was correctly identified as members of that alternative class. Following statistical terminology, sensitivity can be defined as 100 (1−α)% and specificity as 100 (1−β)% (Rodionova, Balyklova, Titova, & Pomerantsev, 2014).

2.6.4. Regression models

Multiple linear regression (MLR) analysis was used to understand quantitatively how each physicochemical parameter and phenolic class affected the antioxidant activity measured by the six different assays. The least squares estimation was used to generate the MLR equations, in which the best-fitting line for the observed data was calculated by minimizing the sum of the squares of the vertical deviations from each data point to the line (Alberti et al., 2016). The generated equations were expressed according to Equation (3):

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots \beta_p x_{i1} + \varepsilon_i \quad \text{for } i = 1, 2 \ldots n. \tag{3}$$

Regression coefficients (β) were analyzed to evaluate key variables for the prediction of the antioxidant activity of juices. The magnitude of β allows comparing the relative contribution of each independent variable (chemical composition and physicochemical properties) in the prediction of the dependent variable (antioxidant activity). For this purpose, the goodness of fit of the generated models was measured by calculating the statistical significance (*p*-value) of the model using analysis of variances, the determination coefficient (R^2) and the adjusted R^2. Additionally, residuals (predicted minus observed values) were checked for normal distribution by the Kolmogorov-Smirnov test and a *t*-test for the regression coefficients was used to assess whether the predictor was significantly ($p < 0.10$) related to the antioxidant activity. MLR was performed using Statistica v.7 software (Statsoft Inc., USA).

3. Results and discussion

3.1. Exploratory data analysis

Table 2 shows the results of all chemical and physicochemical analyses. It is possible to observe that a considerable variability within the same species was found but these data are reasonable if one considers the factors influencing the results: maturity index, geographical origin, chemistry and soil type and other agronomical/environmental factors, dilution factor (in the case of nectars), cultivar, among others (Costa, da Silva, Cosme, & Jordão, 2015; Stinco et al., 2015; Margraf et al., 2016).

The total soluble solids content of juices ranged from 1 (cranberry) to 14.6 °Brix (pomegranate). Titratable acidity ranged from 0.04 g/100 mL (organic orange) to 5.50 g/100 mL (lemon cv. Galego) and the pH values ranged from 2.29 (lemon cv. Galego) to 5.85 (orange cv. Lime). These physicochemical parameters are closely related not only to the maturity degree of the materials but also with the variety. Juices with higher total phenolic content were pomegranate (2485 mg GAE/L), blackberry (1767 mg GAE/L), and blueberry (1596 mg GAE/L), while apple and *Citrus* juices presented the lowest values. The total flavonoids content was higher in blueberry (665 mg CTE/L) and passion fruit juice (558 mg CTE/L), whereas the monomeric anthocyanins content was higher in blackberry (506 mg/L) and blueberry (302 mg/L) juices. Overall, blueberry, blackberry, red beet and pomegranate juices showed the highest antioxidant activity. These results are in agreement other reports on commercial juices (Gardner, White, Mcphail, & Duthie, 2000; Granato et al., 2015; Cardeñosa, Girones-Vilaplana, Muriel, Moreno, & Moreno-Rojas, 2016). Indeed, blueberry and blackberry juices usually have a high content of anthocyanins, compounds that are able to donate electrons and stabilize free radicals (Kim, Perkins-Veazie, Ma, & Fernandez, 2015; Cardeñosa et al., 2016), while pomegranate juices have a considerable amount of punicalagins, ellagic acid, and punicalins, which are flavonoids with numerous phenolic hydroxyls that are responsible to scavenge free-radicals and chelate transition metal ions (Hacke et al., 2016; Mousavinejad, Emam-Djomeh, Rezaei, & Khodaparast, 2009).

One important observation should be made here: although the spectrophotometric assays (phenolic classes) used in the current research are widely employed in food science and technology (including research centers and industries), the specificity of some methods is limited and results should be analyzed carefully. For instance, although red beet does not contain significant amounts of condensed tannins, the vanillin-H_2SO_4 assay provided a high mean value (1620 mg/L). The same happened to the flavonoid/flavonols contents (925/239 mg/L) using the $AlCl_3$ method for red beet juice (i.e., red beet contains a trace level of flavonoids). As betalains (i.e., red beet) and some anthocyanins (i.e., berries) absorb light at 530 nm, interference was obvious in the vanillin-H_2SO_4 assay (all juices containing anthocyanins and betalanins) and in the flavonoid/flavonol assays (red beet juice). Indeed, all spectrophotometric assays used to estimate the content of major phenolic classes in extracts have pros and cons, but they can be employed to characterize commercial samples in routine analysis (Touati, Barba, Louaileche, Frigola, & Esteve, 2016). To overcome this methodological limitation, the use of chromatographic methods, such as HPLC, is recommended when the aim is to quantify individual compounds (targeted method). Additionally, in the food industry, there is a need to establish fast indicators of nutritional and quality loss of juices, so in the near future the shelf life can be established not only based on food safety but also on food quality indices (i.e., phenolic compounds and antioxidant activity).

As juice samples are characterized by many variables, multivariable tools were used for data analysis. The PCA method helps to explore the whole dataset and reveal hidden data patterns. The loading plot of the first two PCs illustrates the main dependences between variables (Fig. 1a). It can be seen that the Fe^{2+}-chelating capacity is connected with condensed tannins and total pigments. The other *Bio* variables form the other group, which can be associated with the *ortho*-diphenols. pH, acidity, and TSS are poorly coupled with the group of *Bio* variable. From the score plot (Fig. 1b and c) it can be seen, that samples from the third class (*super juices*) considerably differ from all other samples. First two classes are located in the center of the score plot (see Fig. 1c) and are well mixed and *Class 3* is rather heterogeneous. Red beet juice differs from all other samples (Fig. 1b) because of high Fe^{2+}-chelating capacity. The other three samples from *Class 3* are located together with the samples from the first two classes. These are *Pomegranate (commercial) 2*, *Cranberry (commercial) 1*, and *Cranberry (commercial) 2* juices. All these samples have rather small values of *Bio-variables* in comparison with other samples from *Class 3*. The above-mentioned samples may be treated as outliers for *Class 3*. This explorative analysis provides possibility to reveal the main dependences between variables and disclose abnormal/suspicious samples inside classes. Two PCs explains up to 72% of all variation in the data, but higher PCs also do not provide possibility for clearer separation of the classes.

Using the correlation analysis, results showed that DPPH, ABTS,

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Table 2
Results for the characterization of Brazilian juices according to the physicochemical properties, antioxidant activity and major phenolic classes.

Genus/species	Cultivar	Total phenolic content (mg GAE/L)	Total flavonoids (mg CTE/L)	Flavonols (mg QE/L)	*Ortho*-diphenols (mg CAE/L)	Condensed taninns (mg CTE/L)	Total pigments (mg/L)	DPPH (mg AAE/L)	ABTS (mg AAE/L)	FRAP (mg AAE/L)	Total reducing capacity (mg QE/L)	Folin-Ciocalteu's reducing capacity (mg GAE/L)	Fe^{2+} chelating ability (mg EDTA/L)	Acidity (g/100 mL)	pH	Soluble solids (°Brix)
Citrus reticulata	Tangerine	208 ± 6	59 ± 2	8 ± 0	109 ± 2	11 ± 2	0	58 ± 4	632 ± 45	983 ± 25	824 ± 39	753 ± 45	29 ± 1	0.71	3.45	11.2
	Organic Tangerine	212 ± 26	56 ± 8	4 ± 0	83 ± 3	0	0	42 ± 4	472 ± 19	612 ± 5	815 ± 59	882 ± 21	56 ± 7	0.38	3.86	11.7
	Organic Tangerine	425 ± 9	153 ± 1	8 ± 1	282 ± 27	0	0	76 ± 4	928 ± 16	1514 ± 26	1500 ± 93	919 ± 66	31 ± 5	0.57	3.91	12.4
Citrus sinensis	Orange cv. Rosada	310 ± 25	50 ± 1	10 ± 0	78 ± 2	20 ± 0	0	81 ± 2	805 ± 76	974 ± 22	961 ± 68	193 ± 7	31 ± 3	0.50	4.10	9.9
	Organic orange	545 ± 13	59 ± 8	14 ± 0	103 ± 6	55 ± 7	0	81 ± 1	1138 ± 100	1482 ± 25	1021 ± 39	741 ± 35	29 ± 1	0.04	3.85	9.0
	Orange cv. Lime	87 ± 5	61 ± 27	21 ± 1	70 ± 1	7 ± 1	0	15 ± 2	789 ± 37	688 ± 23	221 ± 23	408 ± 35	28 ± 4	0.16	5.56	10.2
	Orange cv. Lime	78 ± 6	67 ± 11	17 ± 0	80 ± 7	80 ± 15	0	11 ± 2	591 ± 92	509 ± 19	210 ± 16	370 ± 26	26 ± 2	0.11	5.85	5.9
	Orange cv. Pera Rio	365 ± 4	45 ± 7	5 ± 0	60 ± 1	44 ± 1	0	78 ± 3	537 ± 39	1106 ± 27	1585 ± 90	596 ± 24	32 ± 1	0.91	3.55	10.9
	Orange cv. Baia	556 ± 15	104 ± 0	4 ± 0	83 ± 0	17 ± 1	0	81 ± 2	858 ± 7	1523 ± 13	596 ± 13	783 ± 55	25 ± 3	0.94	3.44	13.1
	Orange (commercial)	90 ± 7	78 ± 1	59 ± 9	114 ± 4	304 ± 27	0	13 ± 1	324 ± 24	556 ± 17	867 ± 30	358 ± 24	20 ± 5	0.70	3.78	10.0
	Orange (commercial)	266 ± 12	81 ± 13	23 ± 3	58 ± 4	201 ± 8	0	58 ± 6	479 ± 36	895 ± 22	204 ± 27	533 ± 35	23 ± 2	0.08	3.55	11.0
	Orange (commercial)	230 ± 2	101 ± 9	27 ± 3	52 ± 4	234 ± 51	0	25 ± 4	265 ± 25	872 ± 10	1149 ± 53	288 ± 45	23 ± 3	0.82	3.70	9.8
	Orange (commercial)	212 ± 6	116 ± 17	40 ± 5	62 ± 8	226 ± 18	0	46 ± 3	279 ± 42	725 ± 60	610 ± 39	247 ± 10	53 ± 6	0.71	3.76	10.8
	Orange (commercial)	529 ± 19	93 ± 10	39 ± 6	59 ± 4	319 ± 24	0	44 ± 8	555 ± 10	1392 ± 8	640 ± 54	353 ± 18	44 ± 1	0.81	3.69	10.2
	Orange (commercial)	216 ± 9	122 ± 3	55 ± 4	103 ± 10	0	0	29 ± 2	386 ± 25	819 ± 18	640 ± 54	530 ± 46	47 ± 2	0.71	3.82	11.0
	Orange (commercial)	661 ± 71	70 ± 0	24 ± 5	48 ± 2	132 ± 31	0	20 ± 1	176 ± 8	603 ± 13	687 ± 53	406 ± 18	56 ± 1	0.65	3.63	9.2
	Orange (commercial)	179 ± 6	100 ± 10	74 ± 0	116 ± 8	155 ± 7	0	19 ± 4	653 ± 6	793 ± 18	961 ± 44	566 ± 37	49 ± 2	0.73	3.78	10.0
Citrus limon	Lemon cv. Cravo	111 ± 1	197 ± 19	13 ± 1	88 ± 10	33 ± 20	0	10 ± 2	153 ± 25	147 ± 1	552 ± 10	221 ± 10	36 ± 3	1.81	2.33	6.2
	Lemon cv. Galego	171 ± 42	176 ± 22	21 ± 2	112 ± 10	49 ± 5	0	36 ± 1	220 ± 5	157 ± 1	629 ± 52	362 ± 10	35 ± 3	5.50	2.29	5.1
	Lemon cv. Taiti	274 ± 5	125 ± 13	4 ± 0	70 ± 4	25 ± 3	0	18 ± 3	217 ± 32	216 ± 4	535 ± 22	377 ± 33	48 ± 6	4.78	2.38	5.2
Passiflora edulis	Yellow passion fruit (commercial)	640 ± 4	558 ± 23	65 ± 10	144 ± 8	327 ± 59	0	38 ± 2	795 ± 29	599 ± 32	1115 ± 44	411 ± 85	52 ± 7	3.43	2.80	10.2
Pyrus malus	Organic apple	160 ± 8	188 ± 7	47 ± 0	60 ± 2	0	6 ± 0	52 ± 5	236 ± 22	339 ± 10	595 ± 36	399 ± 33	25 ± 0	0.14	4.18	13.1
	Apple cv. Fuji	140 ± 1	134 ± 16	12 ± 0	149 ± 10	207 ± 32	3 ± 0	6 ± 0	99 ± 16	326 ± 9	634 ± 24	145 ± 6	25 ± 2	0.26	3.54	11.3
	Apple cv. Gala	47 ± 3	87 ± 11	21 ± 0	41 ± 0	0	18 ± 0	53 ± 1	290 ± 38	83 ± 16	640 ± 7	302 ± 16	25 ± 1	0.20	3.71	11.9
	Green Apple	113 ± 12	70 ± 20	10 ± 0	28 ± 1	0	9 ± 0	42 ± 2	137 ± 19	279 ± 45	1620 ± 39	1048 ± 131	28 ± 2	0.05	3.31	12.0
	Apple (commercial)	218 ± 7	168 ± 7	27 ± 3	110 ± 1	126 ± 11	8 ± 0	76 ± 1	570 ± 24	275 ± 7	1218 ± 112	540 ± 31	58 ± 4	0.30	3.50	9.9
	Apple (commercial)	285 ± 1	129 ± 7	10 ± 0	65 ± 8	161 ± 21	0	79 ± 1	759 ± 26	329 ± 4	843 ± 89	471 ± 20	25 ± 2	0.33	3.31	12.0
Punica granatum	Pomegranate (commercial)	715 ± 1	275 ± 47	236 ± 4	1202 ± 187	68 ± 45	1 ± 0	1251 ± 57	10,350 ± 1086	10,724 ± 57	2645 ± 214	8183 ± 25	1374 ± 129	1.42	3.16	13.0
	Pomegranate (commercial)	102 ± 2	90 ± 10	18 ± 0	102 ± 0	6 ± 1	0	182 ± 19	1851 ± 97	1635 ± 34	236 ± 13	650 ± 48	25 ± 3	0.24	3.02	10.8
	Pomegranate (commercial)	2485 ± 14	500 ± 61	211 ± 40	754 ± 35	28 ± 7	33 ± 0	803 ± 20	7574 ± 518	8901 ± 437	8018 ± 258	3164 ± 44	1318 ± 56	0.55	3.13	14.6
	Pomegranate (commercial)	2247 ± 24	492 ± 73	211 ± 11	994 ± 22	104 ± 16	30 ± 0	1388 ± 42	7948 ± 415	8607 ± 136	4630 ± 214	3014 ± 164	1408 ± 31	0.57	3.14	13.0
Beta vulgaris	Red beet	1169 ± 15	925 ± 81	239 ± 21	49 ± 7	1620 ± 118	854 ± 1	325 ± 51	2388 ± 66	3954 ± 86	1237 ± 129	1263 ± 30	10,633 ± 1343	0.65	5.45	9.0
Morus sp	Blackberry	1170 ± 47	344 ± 25	83 ± 1	215 ± 10	1136 ± 182	506 ± 1	274 ± 38	2404 ± 83	3478 ± 172	2915 ± 64	1000 ± 142	1317 ± 168	0.79	2.88	6.0
	Blackberry (commercial)	1767 ± 4	446 ± 75	126 ± 4	460 ± 19	638 ± 51	58 ± 2	576 ± 13	3822 ± 201	4442 ± 148	569 ± 30	1970 ± 130	1286 ± 161	1.23	3.09	7.2
Vaccinium sp	Blueberry (commercial)	1596 ± 7	665 ± 17	142 ± 6	541 ± 12	1318 ± 118	302 ± 7	612 ± 18	3707 ± 675	4803 ± 202	2383 ± 178	2289 ± 221	26 ± 3	0.52	3.19	10.0
	Blueberry (commercial)	939 ± 17	601 ± 42	135 ± 10	79 ± 1	498 ± 54	13 ± 1	487 ± 9	2519 ± 118	3165 ± 46	1998 ± 109	1523 ± 163	25 ± 0	0.15	3.00	12.0
Vaccinium macrocarpon	Cranberry (commercial)	391 ± 17	157 ± 10	56 ± 1	133 ± 6	128 ± 0	7 ± 0	226 ± 18	1424 ± 181	1333 ± 41	489 ± 52	503 ± 74	24 ± 1	4.85	2.72	11.0
	Cranberry (commercial)	227 ± 39	161 ± 16	33 ± 0	402 ± 11	61 ± 1	13 ± 0	84 ± 11	1044 ± 22	1166 ± 131	151 ± 7	391 ± 75	38 ± 2	0.49	2.59	1.0

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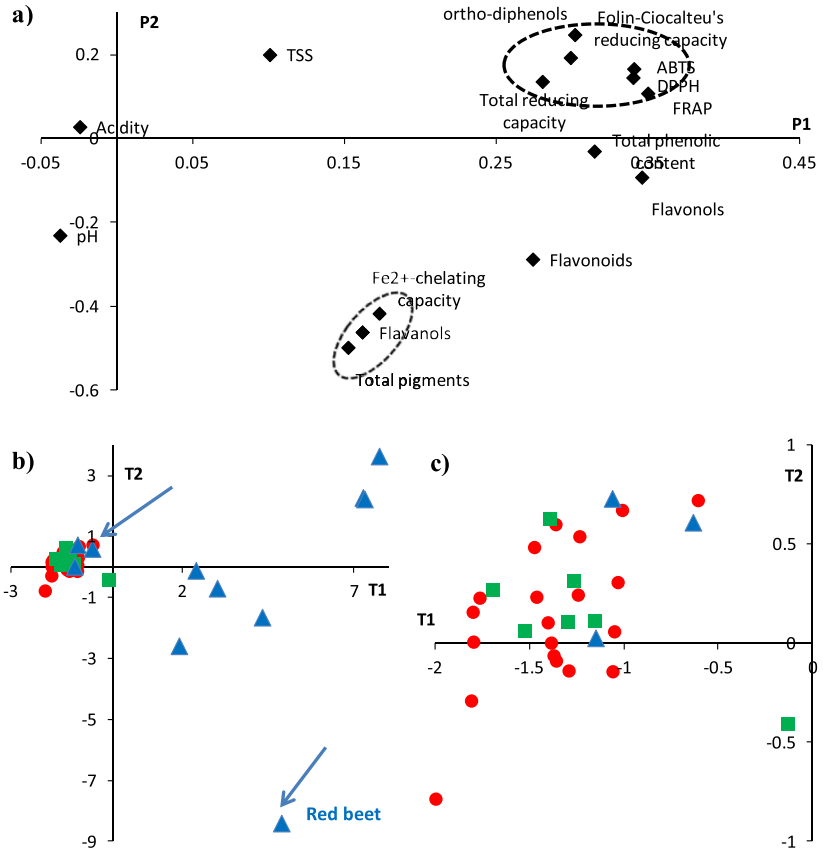

Fig. 1. PCA analysis of the entire dataset. a) Loading plot for PC1 x PC2. b) Score plot for PC1-PC2. c) Score plot for PC1-PC2, only central part enlarged for illustrative purposes. Note: Juice samples from Class 1 ●; Class 2 ■; and Class 3 ▲.

Folin-Ciocalteu's reducing capacity and FRAP were closely associated ($p < 0.001$) with the total phenolic content ($r = 0.786$; $r = 0.755$; $r = 0.519$; $r = 0.811$, respectively) and *o*-diphenols content ($r = 0.918$; $r = 0.942$; $r = 0.877$, $r = 0.881$, respectively), while the TRC was strongly associated with total phenolic content ($r = 0.796$, $p < 0.001$), flavonols ($r = 0.663$, $p < 0.001$), *o*-diphenols ($r = 0.664$, $p < 0.001$), and total flavonoids ($r = 0.476$, $p = 0.003$). The Fe^{2+} chelating ability was demonstrated to be associated with total pigments ($r = 0.851$, $p < 0.001$), total flavonoids ($r = 0.664$, $p < 0.001$), condensed tannins ($r = 0.663$, $p < 0.001$), flavonols ($r = 0.612$, $p < 0.001$), and total phenolic content ($r = 0.367$, $p = 0.024$). This result is justified as flavonoids have a considerable number of hydroxyl groups that chelate transition metals, such as Fe^{2+} and Cu^{2+}.

3.2. Supervised classification of juices

3.2.1. Discriminant analysis

The discriminant analysis allows taking into account information concerning the membership of samples to a particular class explicitly. Therefore, aiming to classify the juice samples according to the class they belong, PLS-DA was used. *Class 1 - Citrus* (100% accuracy) and *Class 3 - Super Juices* (73% accuracy in the external validation) were significantly differentiated using both the full data set (*Bio + PhysChem*) and the short data set (*PhysChem*) (Fig. 2). However, different classification rates were obtained for *Class 2* (0% accuracy), whereas *Class 3* is well separated from other classes (although *Class 3* is rather heterogeneous). Again, as in the PCA analysis, we reveal the same three abnormal samples. In PLS-DA, these samples are not treated as outliers but are wrongly classified as *Class 1* members. The results of PLS-DA analysis show that both application of full or short matrix of predictors does not influence the results of classification.

The main advantage of PLS-DA is the possibility to reveal important variables responsible for detachment of classes. The main variables that aided in the classification of Brazilian juices based on the full data set model were total flavonoids, pH, and acidity, whereas total flavonoids, total phenolic content, and pH were the main responsible for the classification of juices using the *PhysChem* data set. PLS-DA has the ability to analyse highly collinear and noisy data and represents a suitable option for the analysis of large, highly-complex data sets which are common outputs in food chemistry studies and quality control programs (Song, Lee, & Kim, 2016).

Overall, PLS-DA data rendered suitable differentiation between classes, especially for *Citrus* juices, showing that the category models for such a class are sensitive and specific. In quality control programs, the characterization and classification of juices based on spectrophotometric and physicochemical data is of paramount economic importance as large quantities of juices are consumed yearly (Bartoszek & Polak, 2016).

3.2.2. One- class classification (DD-SIMCA)

The drawback of the discriminant methods is the necessity of the exhaustive representation of all classes. The DD-SIMCA models were developed using target class samples only and the results are presented in Table 3.

When *Class 1* (*Citrus*) is the target (Fig. 3a), all objects were

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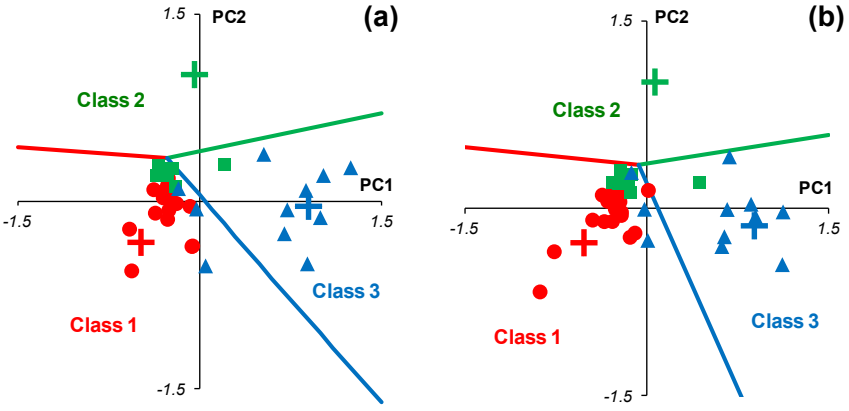

Fig. 2. PLS-DA classification of juices obtained using full (plot a) and short (plot b) datasets.

Table 3
Final results for DD-SIMCA models – sensitivity and specificity.

Classes	*Citrus*	*Others*	*Super juices*
Full data set (*Bio* + *PhysChem*)			
Citrus	100	86	100
Others	100	100	100
Super juices	15	0	100
Short data set (*PhysChem*)			
Citrus	100	86	91
Others	80	100	91
Super juices	10	0	100

correctly classified except for sample *Apple (commercial) 2*. For the correct classification of this object, a model with 5 PCs is needed. When the target is *Class 2* (*Others*), all objects are correctly classified (Fig. 3b), although a training set with 7 objects can hardly be considered as representative. It is better to use objects from *Class 2* only for testing the other classes. When the target is *Class 3* (*Super juices*), the model with 2 PCs correctly classified the training objects Fig. 3c, but failed in recognition of aliens. Only a model with 6 PCs provides perfect classification in case α-value is set to 0.25. However, this modeling is too risky because n = 11 objects are not enough to build a reliable model with 6 PCs. Yet, type I error, $\alpha = 0.25$, demonstrates that the probability of wrong decision is

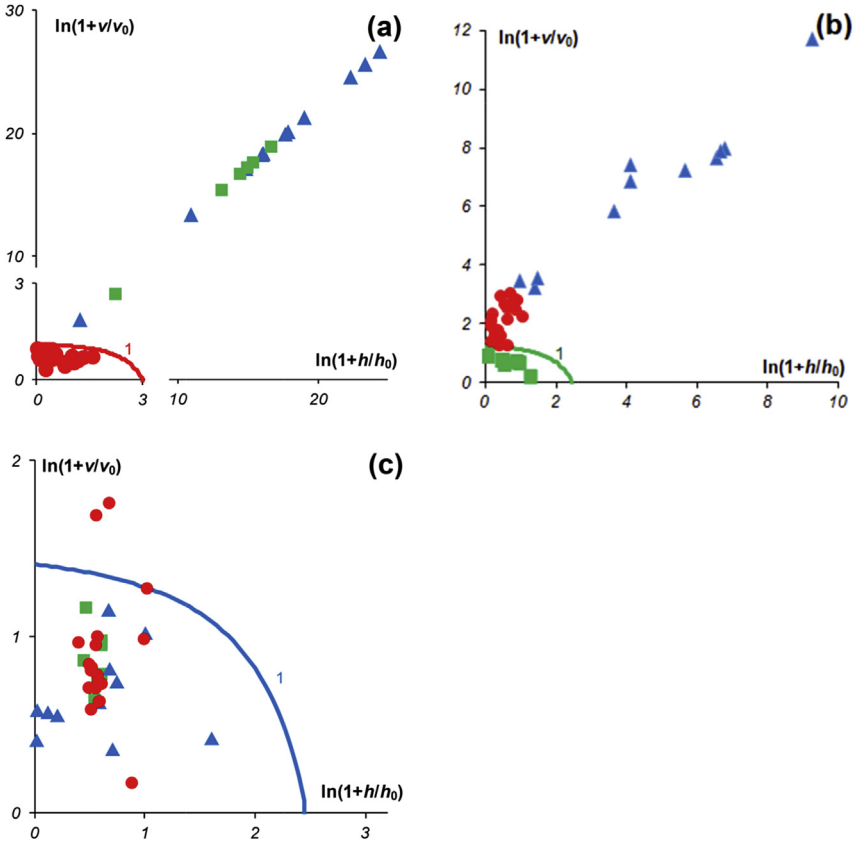

Fig. 3. DD-SIMCA classification of juices using the full dataset (physicochemical properties and antioxidant activity data). Curves (1) represent the border of acceptance area. Plot (a) Class 1 (*Citrus*) is the target class. Plot (b) Class 2 (*Others*) is the target class. Plot(c) Class 3 (*Super juices*) is the target class.

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rather high. The results obtained for full and short datasets are similar (Table 3).

It is interesting to compare the results obtained by PLS-DA and DD-SIMCA. When *Class 1* is modeled using DD-SIMCA, we can see that all alien objects, except one, are located very far from *Class 1*, implying that the properties of *Citrus* juices are very different from non-*Citrus* ones. We cannot see this using PLS-DA. Moreover, using DD-SIMCA, we reliably separated *Class 2* from all other classes. At the same time, *Class 3* is so versatile that its DD-SIMCA model easily accepts all other classes as the members of this class. In general, the results of DD-SIMCA application demonstrate that this OCC method has good prospective to be used for juice classification and that it can be employed for revealing of possible adulteration and for solving authentication issues in future studies (Rodionova, Titova,

Table 4
Multiple linear regression models to explain the antioxidant activity of Brazilian juices based on physicochemical properties and major phenolic classes.

Predictors	Regression coefficient	Standard error of β	t-value	p-value
Free radical scavenging activity toward DPPH radical				
Intercept	−184.62	57.66	−3.20	<0.001
Total phenolic content	0.08	0.04	1.99	0.055
Flavonols	1.31	0.40	3.25	<0.001
Ortho-diphenols	0.74	0.09	8.49	<0.001
Soluble solids	11.80	5.69	2.08	0.046
R^2	0.930			
R^2_{adj}	0.921			
p-value (model)	<0.001			
p-value (residuals)	0.210			
Free radical scavenging activity toward ABTS radical				
Intercept	−1084.76	334.68	−3.24	<0.001
Flavonols	11.05	1.93	5.72	<0.001
Ortho-diphenols	5.99	0.49	12.15	<0.001
Soluble solids	83.40	33.08	2.52	0.017
R^2	0.951			
R^2_{adj}	0.946			
p-value (model)	<0.001			
p-value (residuals)	0.185			
Ferric reducing antioxidant power (FRAP)				
Intercept	−860.72	371.74	−2.32	0.027
Total phenolic content	1.00	0.27	3.77	<0.001
Total flavonoids	−2.77	0.97	−2.87	<0.001
Flavonols	16.27	3.75	4.34	<0.001
Ortho-diphenols	4.87	0.69	7.08	<0.001
Total pigments	1.64	0.85	1.93	0.062
Soluble solids	82.84	35.73	2.32	0.027
R^2	0.964			
R^2_{adj}	0.958			
p-value (model)	<0.001			
p-value (residuals)	0.508			
Total reducing capacity				
Intercept	−486.25	495.39	−0.98	0.333
Total phenolic content	2.11	0.24	8.76	<0.001
Condensed taninns	−2.26	0.76	−2.97	0.005
Total pigments	3.38	1.63	2.07	0.046
Soluble solids	93.54	47.72	1.96	0.058
R^2	0.761			
R^2_{adj}	0.731			
p-value (model)	<0.001			
p-value (residuals)	0.906			
Folin-Ciocalteu's reducing capacity				
Intercept	−596.89	339.65	−1.76	0.088
Total phenolic content	−0.87	0.25	−3.54	0.001
Flavonols	8.58	2.38	3.61	0.001
Ortho-diphenols	4.20	0.51	8.21	<0.001
Soluble solids	73.30	33.49	2.19	0.036
R^2	0.866			
R^2_{adj}	0.849			
p-value (model)	<0.001			
p-value (residuals)	0.126			
Fe^{2+} chelating ability				
Intercept	62.64	155.19	0.40	0.689
Flavonols	17.08	3.47	4.92	<0.001
Ortho-diphenols	−2.40	0.76	−3.16	0.003
Condensed taninns	−2.95	0.66	−4.47	<0.001
Total pigments	11.56	1.50	7.69	<0.001
R^2	0.872			
R^2_{adj}	0.857			
p-value (model)	<0.001			
p-value (residuals)	0.063			

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& Pomerantsev, 2016).

Overall, although we use a very simple analytical approach to characterize juices from various species, some exploratory and classification statistical techniques were used to study the generated data. For instance, chemometric tools have been extensively used in food science and technology to unravel technological problems and to understand complex data sets. Some examples are: studies regarding bioactive components (Chen, Luo, Zhang, & Kong, 2016), classification of geographical origin of foods (Zhao et al., 2016), differentiation of thermal processes used in the dairy sector (Capuano, Gravink, Boerrigter-Eenling, & van Ruth, 2015), discrimination between products containing specific ingredients (Cruz et al., 2013), sensory studies (Seisonen, Vene, & Koppel, 2016), and detection of transgenic foods (Liu, Liu, Hu, Yang, & Zheng, 2016), among others. Future works may be conducted using different variables (i.e., total carotenoids, sugars, instrumental color, and rheology parameters) and other statistical techniques aiming to monitor the quality attributes of juices.

3.3. Regression models

In the current research, MLR was used as a mathematical method to model the antioxidant activity data according to the content of phenolic content and physicochemical properties of juices (Table 4). It is possible to observe that flavonols, *o*-diphenols, and total soluble solids were the main predictors ($p < 0.05$) of DPPH, ABTS, and Folin-Ciocalteu's reducing capacity, while the total phenolic content was also related to Folin-Ciocalteu's reducing capacity and DPPH. Total phenolic content, condensed tannins, pigments and soluble solids were the main predictors of total reducing capacity, while FRAP could be explained by the total soluble solids, total phenolic content, flavonoids, pigments, flavonols, and *o*-diphenols. Similarly, Fe^{2+}-chelating ability of juices can be predicted by the content of flavonols, *o*-diphenols, condensed tannins, and pigments. pH and acidity were not good predictors ($p > 0.10$) of antioxidant activity of the studied juices.

In relation to the goodness of fit of the generated MLR models, the R^2 values were higher than 0.75 and the residuals were normally distributed. These statistical parameters indicate that the MLR models were adequate to predict the antioxidant activity of juices using different phenolic classes and total soluble solids. These models are very useful in quality control programs because they can be used to have an idea of the antioxidant activity without performing further experiments (Navarro-Pascual-Ahuir, Lerma-García, Simó-Alfonso, & Herrero-Martínez, 2015). Additionally, this work reinforces the fact that the antioxidant activity of beverages and other extracts should be assessed by multiple antioxidant assays (based on different mechanisms, principles and chemical reactions).

4. Conclusions

PLS-DA was successfully used to predict the class membership of juices using physicochemical properties and rapid high-throughput determination of phenolic classes and antioxidant activity, enabling the identification of the main variables responsible for the discrimination. Similarly, DD-SIMCA was shown to be useful for the authentication of juices. Although the number of samples was limited, the use of some simple physicochemical characteristics of juices and spectrophotometric measurements allied with the use of chemometrics was a suitable approach to study the quality traits of juices consumed in Brazil and to authenticate their class membership. No deployment of high performance liquid chromatography, nuclear magnetic resonance spectroscopy or other very efficient but expensive and not always available techniques were engaged in the analysis.

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