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Phenolic compounds of Brazilian beers from different types and styles and application of chemometrics for modeling antioxidant capacity

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ABSTRACT

In the present study we aimed at investigating, for the first time, phenolic compounds in Brazilian beers of different types and styles. We also aimed at applying chemometrics for modeling beer's antioxidant capacity as a function of their physicochemical attributes (density, refractive index, bitterness and ethanol content). Samples ($n = 29$) were analyzed by PCA originating five groups, especially according to ethanol contents and bitterness. In general, Group V (alcoholic beers with very high bitterness) presented higher refractive index, bitterness, ethanol and phenolics contents than Groups I (non-alcoholic beers) and II (alcoholic beers with low bitterness). Brazilian beers phenolics profile was distinct from that of European beers, with high contents of gallic acid (0.5–14.7 mg/L) and low contents of ferulic acid (0.2–1.8 mg/L). Using PLS, beer's antioxidant capacity measured by FRAP assay could be predicted with acceptable precision by data of ethanol content and density, bitterness and refractive index values.

1. Introduction

Beer is the most consumed alcoholic beverage worldwide (Colen & Swinnen, 2011) and Brazil was the 3rd country in the world trade in 2013. At this time, Brazil produced 13.5 billion liters and consumed 1.25 billion liters, which represented 7.0% and 6.6%, respectively, of global beer market (Kirin Beer University Report, 2014). Beer is obtained after yeast alcoholic fermentation of

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brewer's wort, which is composed of barley malt, water and hops. The different combinations of ingredients and brewing processes yield a chemically complex product, which present numerous types and styles (Wunderlich & Back, 2009). Beers are primarily classified according to the fermentation process as top or high, and bottom or low fermentation beers. Lagers, the most consumed type of beer, are produced by "low" fermentation, which is carried out under refrigeration (usually between 6 and 15 °C). After fermentation, yeast cells deposit at the bottom of the fermenter and are usually removed. In contrast, ale type beers are produced by "high" fermentation, occurring between 16 and 24 °C, after which yeast cells rise to the surface of fermentation media, forming a thick film that is generally not completely removed. Different beer styles, such as pilsen (standard American lager), bock, weizen, pale and brown ales, rauchbier and many others originate from variations in processing, formulations and ingredients composition. Additional classification of beers are based on changes in brewing processes, such as for production of draft beers, which are non-pasteurized, and non-alcoholic beers, often produced by limited fermentation. Beer styles may also vary among producing regions, according to cultural aspects and ingredients' availability (Bamforth, 2003).

In the production process, the addition of hops, cereals and malt leads to an increased content of naturally occurring antioxidant compounds in beer, mainly phenolic compounds, and also Maillard reaction products, and sulfites (Zhao, Li, Sun, Yang, & Zhao, 2013). The structural classes of polyphenols in beer include simple phenols, benzoic and cinnamic acids derivatives, coumarins, catechins, di- and tri-oligomeric proanthocyanidins, prenylated chalcones and α- and iso-α acids. It is worth mentioning that phenolic compounds influence beer flavor and are associated with beer chemical stability and shelf life enhancement (Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006; Zhao et al., 2013). Because of the influence on beer sensory quality and stability, there is interest in determining phenolic compounds contents across beer types and styles.

Therefore, the aim of the present study was to characterize the profile of phenolic compounds in Brazilian beers of different types and styles. Additionally, we used Principal Component Analysis (PCA) for discriminating beer samples and Partial Least Squares (PLS) for modeling its antioxidant capacity as a function of their physicochemical attributes (density, refractive index, bitterness and ethanol content). Applying the PLS model would be of practical interest for breweries that wish to predict antioxidant capacity from routine physicochemical analyses, especially in the context of product development aiming at beers with improved flavor, physicochemical stability and shelf life.

2. Materials and methods

2.1. Solvents, reagents and standards

Acetonitrile, formic acid, glacial acetic acid, isooctane, methanol, 1-octanol and hydrochloric acid (fuming 37%) were HPLC grade from Tedia (Fairfield, OH). Ultrapure Milli-Q water (Millipore, Bedford, MA) was used throughout the experiments. Folin–Ciocalteau reagent, 2,2′-azino-bis (2-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), potassium persulfate and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Sodium carbonate, sodium chloride, sodium acetate, zinc acetate and potassium hexacyanoferrate were purchased from Spectrum Chemical Manufacturing Corp. (Gardena, CA). Iron (II) sulfate was purchased from Merck KGaA (Darmstadt, Germany). Gallic, 3,4-dihydroxybenzoic, 3,4-dihydroxyphenylacetic, 5-caffeoylquinic, 4-hydroxyphenylacetic, vanillic, syringic, *p*-coumaric, ferulic and benzoic acids standards were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Beer samples

Twenty-nine Brazilian beers of 14 different commercial brands were purchased at local markets of the metropolitan area of Rio de Janeiro, Brazil. Brand names were omitted and represented by letters (A to N). Two bottles (355 mL) of the same production batch were acquired for each sample. Beers were classified according to the Guidelines of the Beer Judge Certification Program (BJCP, 2008) in two types, ale ($n = 4$) and lager ($n = 25$), and nine styles, American brown ale ($n = 1$), American pale ale ($n = 1$), bock ($n = 1$), rauchbier ($n = 1$), schwarzbier ($n = 1$), German weizen ($n = 2$), premium American lager ($n = 8$), standard American lager ($n = 8$) and non-alcoholic ($n = 5$). The contents of the two bottles were homogenized and degassed by sonication. Samples were stored in amber tubes at −20 °C until analysis.

2.3. Physicochemical attributes

Density at 25 °C (g/mL) was determined by weighing up 1.0 mL of beer in an analytical balance (Sartorius AG Germany, CP224S) with temperature correction.

Bitterness was determined according to Philpott, Taylor, and Williams (1997) with adaptations. In a centrifuge tube, 100 μL of 3 mol/L HCl and 2 mL of isooctane were added to 1 mL of sample. Tubes were then vortexed for 5 min and centrifuged (2000×*g*, 30 min, 25 °C). The absorbance at 275 nm of the supernatant was determined on a UV-spectrophotometer (UV-1800, Shimadzu, Japan) against a blank of isooctane containing a drop of 1-octanol. Results were expressed as Bitterness Units ($BU = Abs_{275nm} \times 50$).

Refractive index was determined using a manual refractometer (Bunker Comercial, model 103, São Paulo, Brazil) previously calibrated with water. Beer samples (100 μL) were added in the equipment and reading was performed against a natural light source. Results were expressed as °Brix. All analyses were performed in triplicate.

Color description and ethanol content were reported as described on the beer samples' labels.

2.4. Phenolic compounds

Sample cleanup was performed as described by Perrone, Farah, and Donangelo (2012) with adaptations. Briefly, 2 mL of sample and 200 μL of each Carrez's solutions were added in a 5 mL volumetric flask and the volume was completed with water. The mixture was homogenized, allowed to stand for 15 min and filtered through filter paper (Whatman No. 1). Prior to HPLC injection, samples were filtered through a cellulose ester membrane (0.22 μm).

HPLC analysis was performed in a Shimadzu system (Kyoto, Japan) equipped with a quaternary pump (LC-10AD), a degasser (DGV-14A), a manual sample injector (7125 Rheodyne valve equipped with a 20 μL loop) and an UV–Vis detector (SPD-10A*vp*). Chromatographic separation was achieved using a Kromasil® C18 column (250 × 4.6 mm, 5 μm) and gradient elution with 0.3% aqueous formic acid (eluent A), methanol (eluent B) and acetonitrile (eluent C, kept constant at 1% throughout the analysis), at flow rate of 1.0 mL/min (Wijeratne, Abou-Zaid, & Shahidi, 2006). The gradient was as follows: 0 min, 24% B; 16 min, 28% B; 30 min, 33% B; 50 min, 65% B. UV detection was performed at 280 nm. Phenolic compounds were identified by comparison of their retention times with those of commercial standards.

Quantification was performed by external standardization. Data were acquired by ClassVp software (Shimadzu Corp., version 6.13, 2003). Results were expressed as mg/L. Analysis was carried out in triplicate.

2.5. Antioxidant capacity

The antioxidant capacity of the extracts was determined by Folin–Ciocalteau Reagent assay, FRAP (Ferric Reducing Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity) assays.

Folin–Ciocalteau reagent assay was performed as described by Floridi, Montanari, Marconi, and Fantozzi (2003), with adaptations. In a test tube, 2 mL of 7.5% Na_2CO_3 solution, 2.5 mL of Folin–Ciocalteau reagent and 500 μL of water were added over 50 μL of sample. After homogenization, the mixture was allowed to stand at 45 °C for 15 min and taken to an ultrasound bath to remove the remaining CO_2. Absorbance was measured at 765 nm using an UV-spectrophotometer (UV-1800, Shimadzu, Japan). Results were expressed as mg of gallic acid equivalents (GAE) per liter.

FRAP assay was performed according to Moreira, Monteiro, Ribeiro-Alves, Donangelo, and Trugo (2005). FRAP reagent was prepared by mixing 2 mL of 10 mM TPTZ solution in 6 N HCl with 2 mL of 20 mM $FeCl_3$ solution and 20 mL of 300 mM acetate buffer (pH 3.6). Twenty microliters of properly diluted sample were pipetted into a 96-well microplate, which was placed in a multilabel counter (Victor^3 1420 PerkinElmer, Turku, Finland) with automatic injector. 180 μL of FRAP reagent were automatically dispensed into each well, the plate was shaken and allowed to stand at 37 °C for 6 min. The absorbance was then read at 595 nm. Quantification was performed using a calibration curve prepared with $FeSO_4$. Results were expressed as mmol of Fe^{+2} equivalents per liter.

TEAC assay was performed according to Re et al. (1999) with slight modifications. The ABTS radical cation stock solution was prepared by reacting $K_2S_2O_8$ and ABTS for 12 h to 16 h prior to use. Then, this solution was diluted (at 1:50) to obtain an absorbance of 0.70 ± 0.02 at 720 nm. Ten microliters of sample were pipetted into a 96-well microplate, which was placed in a multilabel counter with automatic injector. One-hundred and ninety microliters of ABTS radical cation solution were automatically dispensed into each well, the plate was shaken and allowed to stand at 37 °C for 6 min. Sample absorbance was read at 720 nm and subtracted from solvent blank absorbance. Quantification was performed using a calibration curve prepared with Trolox. Results were expressed as mmol of Trolox equivalents per liter. All analyses were performed in triplicate.

2.6. Chemometric techniques

PCA was performed in order to discriminate beer samples (n = 29) according to their values of ethanol content, bitterness and refractive index. The goal of this analysis was to set a pattern for recognition of homogeneous sub-groups between the main groups of samples through evaluation of the principal components responsible for greatest variation in dataset. PCA discriminated samples into five groups (I–V). In a next step, the PLS method was applied to correlate physicochemical attributes (density, ethanol content, bitterness and refractive index) and antioxidant capacity of beers and therefore establish models to calculate FRAP values using these routinely obtained data. Model validation was performed by cross-validation in order to study the predictive power of the PLS-model, via comparison of predicted and measured FRAP values entries. During cross-validation, one sample at a time (of n samples) was left out, and the prediction ability was tested on the sample omitted (leave-one-out validation). This was repeated n times, resulting in n models, from which the one leading to the best prediction was selected. PCA and PLS analyses were performed with The Unscrambler Software (version 8.0, CAMO Process AS, Oslo, Norway).

Table 1
Physicochemical attributes of Brazilian beers of different types and styles analyzed in this study (n = 29).

Sample no.	Brand letter code, beer style	Type	Color description[a]	Ethanol[a] (%, v/v)	Density (g/mL)[b]	Bitterness (BU)[c]	Refraction index (°Brix)
1	**F**, American Brown Ale	Ale	Brown	4.8	1.0145	18.3	6.0
2	**F**, American Pale Ale	Ale	Red	4.8	1.0218	22.1	5.6
3	**G**, German Weizen	Ale	Yellow	4.8	1.0192	21.6	5.8
4	**L**, German Weizen	Ale	Yellow	5.2	1.0146	28.7	5.8
5	**L**, Bock	Lager	Red	6.2	1.0298	35.7	9.0
6	**B**, Non-alcoholic	Lager	Yellow	<0.5	1.0248	22.9	5.2
7	**E**, Non-alcoholic	Lager	Yellow	<0.5	1.0328	22.2	7.0
8	**I**, Non-alcoholic	Lager	Yellow	<0.5	1.0249	21.1	6.6
9	**J**, Non-alcoholic	Lager	Yellow	0.3	1.0311	24.3	6.4
10	**K**, Non-alcoholic	Lager	Yellow	0	1.0283	10.3	6.4
11	**A**, Premium American Lager	Lager	Yellow	4.9	1.0283	12.6	5.8
12	**B**, Premium American Lager	Lager	Yellow	4.8	1.0232	19.0	6.0
13	**D**, Premium American Lager	Lager	Yellow	5.5	1.0116	18.4	6.6
14	**G**, Premium American Lager	Lager	Yellow	4.8	1.0103	14.6	5.0
15	**H**, Premium American Lager	Lager	Yellow	5.0	1.0091	22.9	5.6
16	**I**, Premium American Lager	Lager	Yellow	4.5	1.0208	15.8	5.8
17	**L**, Premium American Lager	Lager	Yellow	6.2	1.0258	19.1	7.8
18	**N**, Premium American Lager	Lager	Yellow	5.2	1.0185	23.5	5.4
19	**G**, Rauchbier, G	Lager	Red	6.5	1.0109	34.8	8.2
20	**G**, Schwarzbier	Lager	Brown	4.8	1.0148	21.0	6.0
21	**L**, Schwarzbier	Lager	Brown	6.2	1.0285	30.3	8.0
22	**A**, Standard American Lager	Lager	Yellow	4.0	1.0127	18.7	5.8
23	**B**, Standard American Lager	Lager	Yellow	4.6	1.0148	15.8	5.6
24	**C**, Standard American Lager	Lager	Yellow	4.8	1.0177	19.8	5.6
25	**D**, Standard American Lager	Lager	Yellow	5.0	1.0106	13.3	5.8
26	**E**, Standard American Lager	Lager	Yellow	4.5	1.0155	18.1	5.6
27	**F**, Standard American Lager	Lager	Yellow	4.8	1.0140	13.6	5.4
28	**I**, Standard American Lager	Lager	Yellow	4.5	1.0226	14.2	5.4
29	**M**, Standard American Lager	Lager	Yellow	4.0	1.0160	19.1	5.4

[a] As reported on the label.
[b] Mean coefficient of variation = 0.05%.
[c] BU: Bitterness Units, mean coefficient of variation = 3.9%.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation. Normality was verified through Kolmogorov–Smirnov test. Analysis of variance (one-way ANOVA) or Kruskal–Wallis test followed by post-tests (Tukey or Dunn multiple comparison tests, respectively) were used to compare physicochemical attributes, contents of phenolic compounds and antioxidant capacity between samples grouped by PCA (Granato, Calado, & Jarvis, 2014; Nunes, Alvarenga, Sant'Ana, Santos, & Granato, 2015). These statistical analyses were performed using GraphPad Prism software for Windows (version 6.01, GraphPad Software, San Diego, CA). Pearson's correlation coefficients between physicochemical attributes and antioxidant capacity were calculated with Statistica software (version 7.0, StatSoft Inc., Tulsa, OK). Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Physicochemical attributes of beer samples

The physicochemical attributes of Brazilian beers analyzed in this study are presented in Table 1. Differences in physicochemical profiles of beers were investigated by PCA (Fig. 1). Chemometric approaches have been successfully used previously to group various foods, including beers, according to quality parameters (Lachenmeier et al., 2005; Lachenmeier, 2007; Granato, Branco, Faria, & Cruz, 2011) and electronic tongue data (Blanco, De la Fuente, Caballero, & Rodríguez-Méndez, 2015). The first PCA analysis matrix (Fig. 1A) included the attributes ethanol content, bitterness and refractive index. Two principal components (PCs) were extracted and after analysis of PC1 *versus* PC2 in a Bi-plot of samples and the selected variables, two groups of samples were discernible according to ethanol content. In this first PCA, PC1 explained 90% of total variance and PC2 explained another 8%. The non-alcoholic beers were then removed to run a second PCA (Fig. 1B) in order to improve grouping of the other samples. In this second analysis, PC1 and PC2 explained 98% and 1%, respectively, of total variance. Beer samples were grouped in four discernible groups, especially in terms of differences in bitterness values.

Based on results of PCA and considering all the samples studied, beers were grouped as follows: Group I ($n = 5$, samples No. 6, 7, 8, 9, 10) consisted of non-alcoholic beers; Group II ($n = 7$, samples No. 11, 14, 16, 23, 25, 27, 28) comprised alcoholic beers with low bitterness ($12.6 < BU < 15.8$); Group III ($n = 8$, samples No. 1, 12, 13, 17, 22, 24, 26, 29) included alcoholic beers with medium bitterness ($18.1 < BU < 19.8$); Group IV ($n = 5$, samples No. 2, 3, 15, 18, 20) and

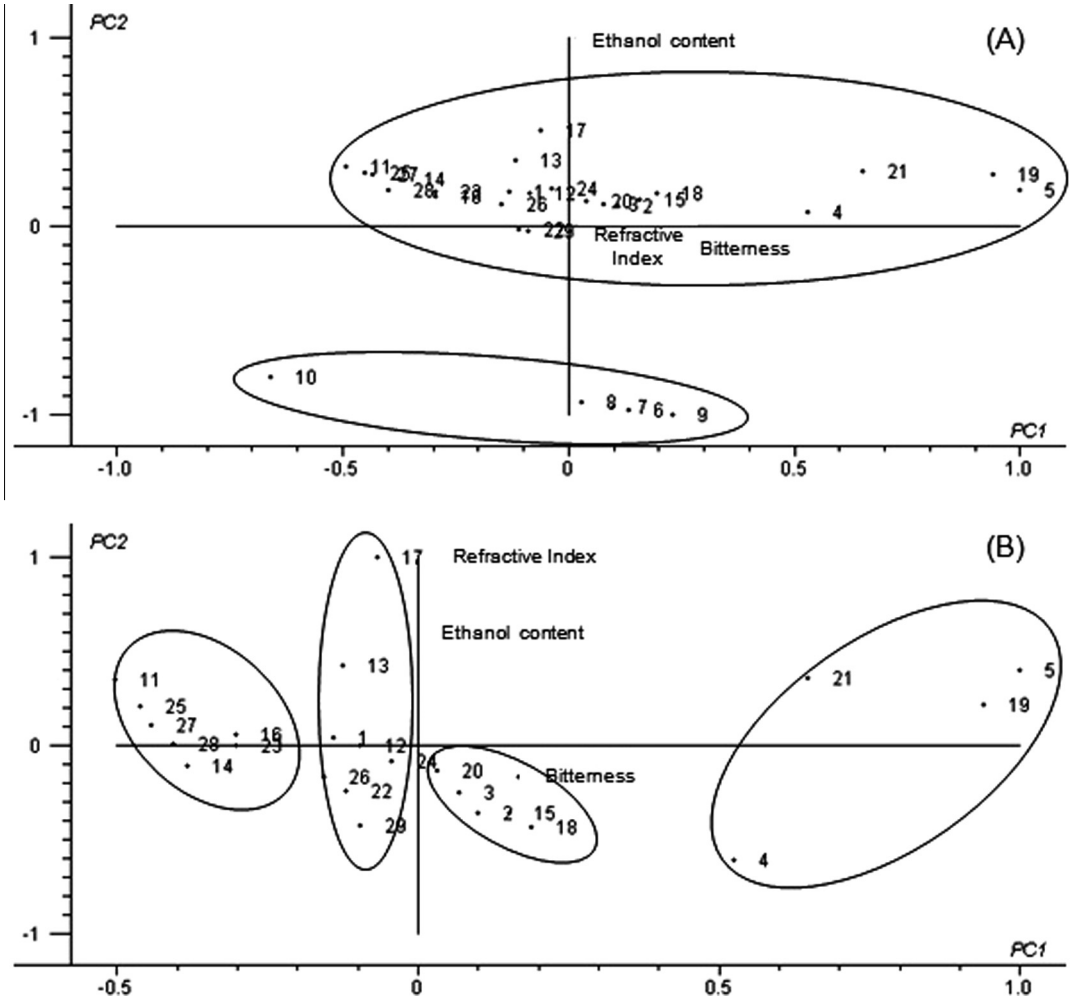

Fig. 1. Scatter plots of PCA scores for specific physicochemical attributes (ethanol content, bitterness and refraction index) of Brazilian beers analyzed in the present study. A. Alcoholic and non-alcoholic beers (PC1 + PC2 explain 98% of total matrix variance). B. Alcoholic beers (PC1 + PC2 explain 99% of total matrix variance). Please refer to Table 1 for samples number identification.

Group V (n = 4, samples No. 4, 5, 19, 21) were formed, respectively, by alcoholic beers with high (21.0 < BU < 23.5) and very high bitterness (28.7 < BU < 35.7). After PCA grouping, beers physicochemical attributes were compared between groups (Fig. 2).

Groups IV and V presented higher ethanol content (4.9% and 6%, respectively) than Group I (0.24%). There was no significant difference between Groups I, II and III, even considering that Group I represented non-alcoholic samples while Groups II and III included part of the alcoholic samples (Fig. 2A).

Variations in bitterness between groups can be observed in Fig. 2B. BU values continuously increased from Group II to Group V of alcoholic samples. Bitterness range observed in this study (10.6–35.7 BU) was similar to that reported in a previous study (6–30 BU) (Schönberger & Kostelecky, 2011) in which samples of ale and lager beers were also analyzed. According to Collin, Derdelinckx, and Dufour (1994), beer sensory analysis by trained sensory panelists showed that bitterness range between 17.5 BU and 25 BU was that of highest preference. These values are similar to those found in the present study, which included the most consumed beers in the Brazilian market.

Beer refractive index showed a similar trend to those of ethanol content and bitterness, being significantly different among alcoholic samples of Group II (5.5 °Brix) and Group V (7.8 °Brix) (Fig. 2C). Beer samples with the highest values of refractive index were those of very high bitterness (Group V) and presented red and brown colors. It is worth mentioning that the sample with the highest refractive index was No. 5 (Bock from brand L), which might be explained by the presence of caramel colorant in the formulation of this beer (Supplementary Table 1).

Density values were similar between beer groups, except for Group I (non-alcoholic beers), which presented higher values than Groups II, III and IV (Fig. 2D). In alcoholic samples, ethanol in concentrations varying from 4.0% to 6.5% reduced density values in comparison to non-alcoholic samples (ethanol content < 0.5%).

3.2. Phenolic compounds

For the first time, phenolic compounds were determined by HPLC in commercial Brazilian beers (Supplementary Fig. 1). Ten phenolic compounds, all of them phenolic acids, were found in the samples analyzed in this study: 3,4-dihydroxybenzoic, 3,4-dihydroxyphenylacetic, 4-hydroxyphenylacetic, 5-caffeoylquinic, benzoic, *p*-coumaric, ferulic, gallic, syringic and vanillic acids (Table 2). This profile of phenolic compounds is in accordance with other studies (Achilli, Cellerino, & Gamache, 1993; Nardini & Ghiselli, 2004; Piazzon, Forte, & Nardini, 2010; Zhao, Chen, Lu, & Zhao, 2010; Quifer-Rada et al., 2015). Six other phenolic compounds (rutin and 2,4-dihydroxybenzoic, 4-hydroxybenzoic, caffeic, *m*-coumaric and salicylic acids) were investigated but not found in the samples. Total phenolic compounds contents ranged from 4.6 mg/L to 28.3 mg/L, with an average of 13.0 mg/L, in agreement with published data for beers (2.9–37.0 mg/L) (Achilli et al., 1993; Nardini & Ghiselli, 2004; Piazzon et al., 2010; Zhao et al., 2010). Phenolic compounds contents may vary depending on the quantity and quality of raw materials and on the industrial brewing process itself (Rodrigues & Gil, 2011).

On average, gallic acid was the most abundant phenolic compound (5.5 mg/L) in Brazilian beers, followed by 5-caffeoylquinic

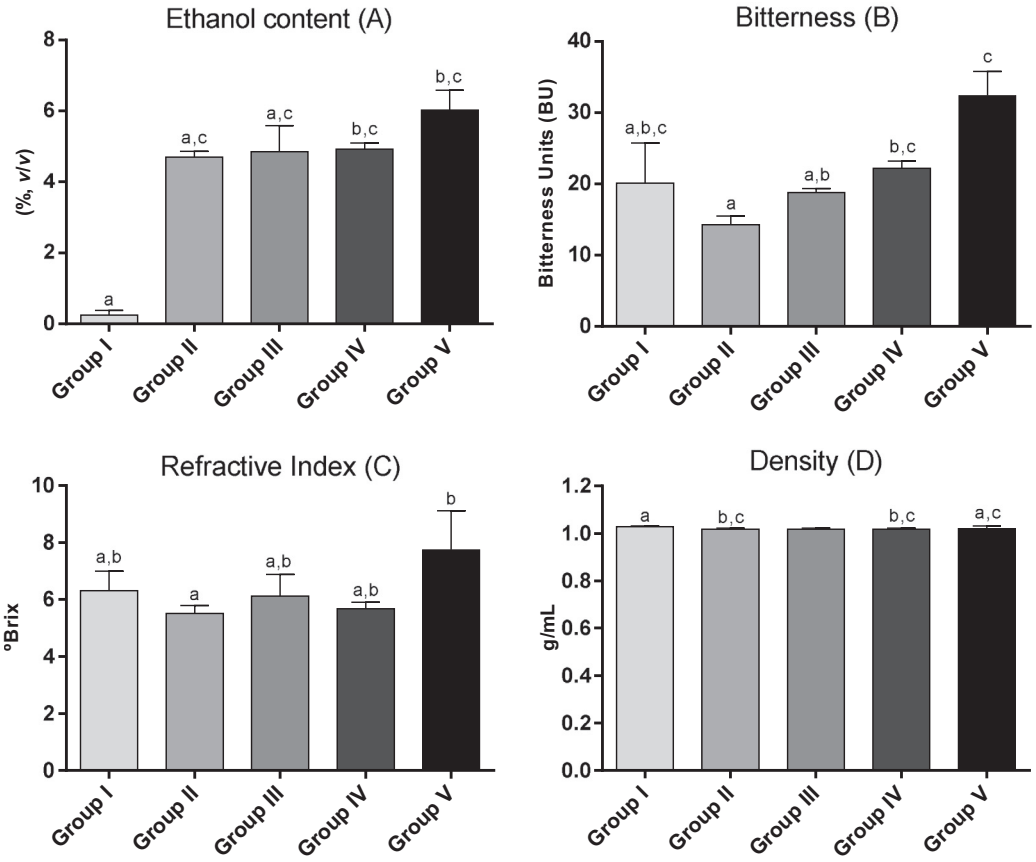

Fig. 2. Physicochemical attributes of Brazilian beers discriminated according to PCA. Data are presented as mean ± standard deviation (n = 29). Different letters indicate significant differences (p < 0.05). Ethanol content, refractive index and bitterness were analyzed by Kruskal–Wallis and Dunn's multiple comparison tests. Density was analyzed by one-way ANOVA and Tukey's multiple comparison tests. Groups: I, non-alcoholic samples (n = 5); II, low bitterness alcoholic samples (n = 7); III, medium bitterness alcoholic samples (n = 8); IV, high bitterness alcoholic samples (n = 5); V, very high bitterness alcoholic samples (n = 4).

Table 2
Contents of phenolic compounds (mg/L) in Brazilian beers ($n = 29$), grouped according to PCA.[1]

Sample No.	5-CQA	GA	*p*-CoA	FA	SA	VA	BzA	3,4-di-OH BzA	3,4-di-OH PhAcA	4-OHPhAcA	Total
Group I (n = 5)	0.23 ± 0.18[a]	3.36 ± 1.73[a]	0.33 ± 0.26[a]	0.33 ± 0.45[a]	0.25 ± 0.34[a]	0.23 ± 0.21[a]	0.04 ± 0.10[a]	0.98 ± 0.69[a]	2.12 ± 2.55[a]	0.53 ± 0.82[a]	8.4 ± 2.7[a]
6	0.50	3.56	0.64	1.05	0.06	0.36	0.22	1.19	3.74	0.40	11.7
7	0.28	4.01	0.26	Nd[2]	Nd	Nd	Nd	0.42	Nd	1.97	6.9
8	0.15	0.51	Nd	Nd	0.41	0.36	Nd	2.08	5.77	Nd	9.3
9	Nd	3.57	0.21	0.13	Nd	Nd	Nd	0.43	Nd	0.29	4.6
10	0.24	5.17	0.55	0.49	0.77	0.43	Nd	0.77	1.08	Nd	9.5
Group II (n = 7)	1.37 ± 0.88[a]	3.48 ± 1.24[a]	0.32 ± 0.36[a]	0.51 ± 0.58[a]	0.18 ± 0.21[a]	0.47 ± 0.86[a]	0.12 ± 0.22[a]	0.90 ± 0.79[a]	1.35 ± 1.04[a]	0.91 ± 0.79[a]	9.6 ± 2.3[a]
11	1.53	3.17	0.33	0.21	Nd	0.19	0.55	0.10	1.01	1.27	8.4
14	1.29	3.73	0.28	0.30	Nd	0.35	Nd	0.54	0.41	0.20	7.1
16	1.54	3.69	0.37	0.46	Nd	2.39	Nd	0.90	0.99	0.34	10.7
23	0.37	5.41	0.08	0.30	0.44	0.16	Nd	1.43	2.66	2.18	13.0
25	2.65	4.11	0.13	0.52	0.09	0.19	0.30	0.33	0.49	0.84	9.6
27	0.15	1.32	1.08	1.76	0.31	Nd	Nd	2.41	2.99	1.52	11.5
28	2.03	2.94	Nd	Nd	0.43	Nd	Nd	0.56	0.93	Nd	6.9
Group III (n = 8)	1.76 ± 1.13[a]	5.43 ± 3.06[a,b]	0.41 ± 0.30[a]	0.64 ± 0.51[a]	0.07 ± 0.09[a]	0.19 ± 0.18[a]	0.17 ± 0.23[a]	0.54 ± 0.58[a]	0.71 ± 0.31[a]	0.35 ± 0.46[a]	10.3 ± 5.3[a]
1	2.31	6.60	0.42	0.63	0.09	Nd	Nd	0.21	0.95	0.27	11.5
12	1.42	4.57	0.18	0.18	0.06	0.53	0.12	0.14	0.60	Nd	7.8
13	1.75	4.83	0.19	0.72	Nd	0.18	0.58	0.41	0.45	0.94	10.0
17	2.83	12.43	1.10	1.83	Nd	0.16	Nd	1.88	0.57	1.16	22.0
22	3.19	4.36	0.33	0.51	Nd	0.08	Nd	0.21	0.79	Nd	9.5
24	0.09	2.82	0.30	0.40	0.13	0.18	0.22	0.39	0.21	Nd	4.7
26	2.23	4.76	0.26	0.30	0.25	0.37	0.45	0.85	1.19	Nd	10.7
29	0.25	3.08	0.46	0.56	Nd	Nd	Nd	0.25	0.89	0.39	5.9
Group IV (n = 5)	2.50 ± 1.93[a]	7.38 ± 3.98[a,b]	0.50 ± 0.20[a]	0.64 ± 0.37[a]	0.23 ± 0.35[a]	1.35 ± 0.98[a]	0.19 ± 0.27[a]	2.40 ± 2.60[a]	1.10 ± 0.53[a]	0.92 ± 1.12[a]	17.2 ± 7.4[a,b]
2	5.36	7.02	0.50	0.69	0.18	2.91	0.55	1.10	1.80	0.63	20.7
3	0.23	5.90	0.27	0.90	0.83	1.28	Nd	0.24	1.09	0.94	11.7
15	3.28	14.29	0.82	Nd	Nd	1.45	Nd	4.85	0.76	2.82	28.3
18	1.69	5.36	0.45	0.79	Nd	0.27	Nd	0.25	1.42	Nd	10.2
20	1.92	4.33	0.46	0.84	0.12	0.86	0.40	5.58	0.45	0.20	15.2
Group V (n = 4)	6.98 ± 3.47[b]	9.63 ± 3.70[b]	0.59 ± 0.18[a]	0.60 ± 0.36[a]	0.07 ± 0.06[a]	2.91 ± 3.33[a]	0.11 ± 0.22[a]	2.64 ± 2.15 [a]	0.74 ± 0.73[a]	0.52 ± 0.37[a]	24.8 ± 9.8[b]
4	6.53	7.49	0.41	0.13	0.10	0.32	Nd	0.42	0.24	Nd	15.6
5	10.96	14.67	0.83	0.82	0.14	7.17	Nd	2.15	0.14	0.81	23.0
19	2.59	6.33	0.52	0.94	0.05	0.20	0.43	5.59	1.73	0.75	19.1
21	7.85	10.02	0.59	0.52	Nd	3.94	Nd	2.41	0.85	0.52	26.7

[1] Values reported for each group are mean ± SD. Different letters in the same column indicate significant differences between groups of beers ($p < 0.05$, Analysis of variance followed by post-tests). 5-CQA: 5-caffeoylquinic acid; GA: gallic acid; *p*-CoA: *p*-coumaric acid; FA: ferulic acid; SA: syringic acid; VA: vanillic acid; BzA: benzoic acid; 3,4-di-OH BzA: 3,4-dihydroxybenzoic acid; 3,4-di-OH PhAcA: 3,4-dihydroxyphenylacetic acid; 4-OH PhAcA: 4-hydroxyphenylacetic acid. [2]Not detected.

(2.2 mg/L), 3,4-dihydroxybenzoic (1.3 mg/L) and 3,4-dihydroxyphenylacetic acids (1.2 mg/L) (Table 2). These compounds accounted together for 78% of the total phenolic compounds in the studied beers. Gallic and 3,4-dihydroxybenzoic acids were found in all analyzed samples ranging from 0.5 to 14.7 mg/L and 0.1 to 5.6 mg/L, respectively. 5-Caffeoylquinic acid was detected in all samples, with the exception of sample No. 9, ranging from 0.1 to 11.0 mg/L. On average, ferulic (0.6 mg/L), *p*-coumaric (0.4 mg/L), syringic (0.2 mg/L) and benzoic (0.1 mg/L) acids were found at the lowest concentrations in Brazilian beers (Table 2).

Similarly to our results, gallic acid was the major phenolic compound identified in commercial beers, most of them from Chinese origin (Zhao et al., 2010). On the other hand, gallic acid was not reported in European beers from Austria, Belgium, Czech Republic, England, France, Germany, Ireland and Netherlands (Nardini & Ghiselli, 2004; Piazzon et al., 2010) and 5-caffeoylquinic acid was only observed at low concentrations (0.24 mg/L) in samples from Austria, Italy and Germany (Montanari, Perretti, Natella, Guidi, & Fantozzi, 1999; Nardini & Ghiselli, 2004). Differently from the observed in the present study, ferulic acid was reported to be one of the most abundant phenolic compounds in European and Chinese beers (Montanari et al., 1999; Nardini & Ghiselli, 2004; Piazzon et al., 2010; Zhao et al., 2010). Furthermore, sinapic acid was observed in European beers at high concentrations (Piazzon et al., 2010) and rutin was observed in Indian beers (Pai et al., 2015), but these compounds were not found in Brazilian beers (Table 2). Rutin was also not detected in four representative Italian brands of lager beers (Montanari et al., 1999), in contrast to other European beers (Piazzon et al., 2010). It is known that raw materials used for beer production, as well as malting and brewing processing parameters affect the chemical composition of the final product, which might explain the divergences in phenolic compounds profile between Brazilian beers analyzed in the present study and those reported elsewhere. However, independently of their profile, it is known that high contents of phenolic compounds improve beer flavor and stability (Zhao et al., 2013).

Ethanol content, bitterness and refractive index were correlated with the contents of total phenolic compounds and with individual phenolics ($r > 0.42$, $p < 0.025$), especially 5-caffeoylquinic and gallic acids. On average, Group V (alcoholic samples with very high bitterness) presented 3 and 30 times higher contents of gallic and 5-caffeoylquinic acids, respectively, when compared to Group I (non-alcoholic samples). A similar trend was observed for total phenolic compounds (Table 2). Piazzon et al. (2010) reported that total phenolic compounds contents in bock beers were 3 times as high as in non-alcoholic beers. Among our samples, the only bock beer (sample No. 5) was classified in Group V, exactly the group with higher phenolic content.

The effect of ethanol content might be explained by the higher solubility of phenolic compounds in this solvent in comparison to water, increasing their extraction from raw materials during brewing. Non-alcoholic beers not only showed lower contents of phenolic compounds, but are known to be less effective vehicles for their transference into body fluids (Ghiselli et al., 2000), and therefore might be considered as less bioactive than alcoholic beers. The positive correlation observed between bitterness and phenolic compounds is probably explained by the amount of hops used in the formulations, as this ingredient is responsible for up to 30% of beer phenolic compounds (De Keukeleire, 2000). Hops contain about 14.4% of polyphenols, mainly phenolic acids, prenylated chalcones, flavonoids, catechins and proanthocyanidins (Magalhães et al., 2010). Refractive index and phenolic compounds were positively correlated probably due to the higher amount of phenolic-rich raw materials (barley and wheat malts, non-malted cereals and hops) used for beer production.

3.3. Modeling of antioxidant capacity using PLS

The antioxidant capacity of Brazilian beers was measured by FRAP, TEAC and Folin–Ciocalteau assays (Fig. 3). Antioxidant capacity of Brazilian beers varied between types and styles, as well as between PCA groups. FRAP, TEAC and Folin–Ciocalteau values ranged from 0.81 mmol Fe^{+2}/L (sample No. 6) to 6.37 mmol Fe^{+2}/L (sample No. 19), 0.40 mmol Trolox/L (sample No. 25) to 3.02 mmol Trolox/L (sample No. 19) and 164 mg GAE/L (sample No. 26) to 572 mg GAE/L (sample No. 3), respectively. Piazzon et al. (2010) also observed that the antioxidant capacity of commercial beers measured by FRAP assay was remarkably different depending on beer type, ranging from 1.5 mmol Fe^{+2}/L in non-alcoholic beers to 4.7 mmol Fe^{+2}/L in bock beers. TEAC values observed in the present study are in agreement with other studies, which evaluated beer samples from different countries (Tafulo, Queirós, Delerue-Matos, & Sales, 2010; Zhao et al., 2010; Polak, Bartoszek, & Stanimirova, 2013). It is worth noting that beer antioxidant capacity is similar to that of other widely consumed alcoholic beverages, such as white and rosé wines, whisky, and cognac and higher than grappa and rum (Pellegrini et al., 2003).

Similarly to phenolic compounds, beer antioxidant capacity was correlated with ethanol content, bitterness and refractive index ($r > 0.39$, $p < 0.02$). On average, Groups IV (alcoholic samples with high bitterness) and V (alcoholic samples with very high bitterness) presented, respectively, 2.3- and 3.4-times as high FRAP values as Group I (non-alcoholic samples) (Fig. 3). In a previous study in which the antioxidant capacity of commercial brands of beer was evaluated by TRAP, TEAC, DPPH, FRAP, CUPRAC and ORAC, against three different standards (ascorbic acid, gallic acid and Trolox), statistical differences were found between some samples only when FRAP assay was used (Tafulo et al., 2010). In this study, no significant differences were found when comparing the results obtained by other antioxidant capacity assays used besides FRAP. Nevertheless, it was observed that data generated by TEAC and Folin–Ciocalteau assays followed a trend similar to those generated by FRAP. Overall, these differences would be expected since antioxidant assays were strongly correlated ($r > 0.73$, $p < 0.001$).

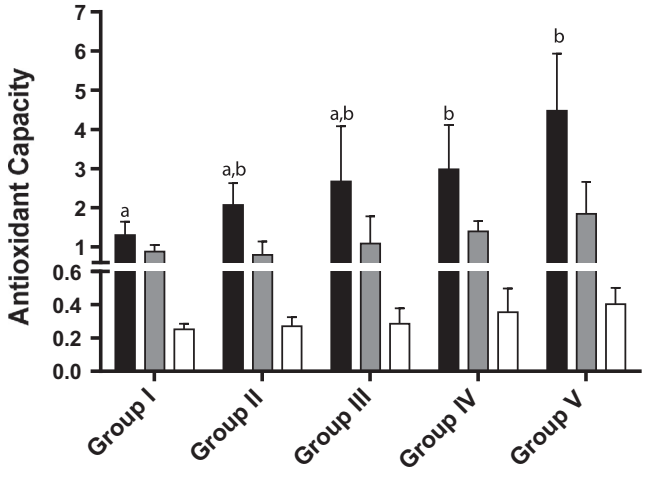

Fig. 3. Antioxidant capacity of Brazilian beers grouped according to PCA measured by FRAP (■) (mmol Fe^{+2}/L), TEAC (▒) (mmol Trolox/L) and Folin–Ciocalteau assays (□) (mg GAE/L). Data are presented as mean ± standard deviation ($n = 29$). Different letters indicate significant differences for FRAP values ($p < 0.05$, Kruskal–Wallis followed by Dunn's post-test). Groups: I, non-alcoholic samples ($n = 5$); II, low bitterness alcoholic samples ($n = 7$); III, medium bitterness alcoholic samples ($n = 8$); IV, high bitterness alcoholic samples ($n = 5$); V, very high bitterness alcoholic samples ($n = 4$).

Table 3
Proposed FRAP (mmol Fe^{2+}/L) prediction models for Brazilian beers classified by PCA as a function of density (*d*, mg/L), refractive index (*RI*, °Brix), bitterness (BU) and ethanol contents (ABV, % *v*/*v*).

Group	Linear regression equation	Average cross-validation error (%)[a]
I (non-alcoholic, n = 5)	FRAP = 0.855 *d* + 0.290 RI − 43.920 BU + 1.166 ABV	7.1
II (alcoholic, low bitterness, *n* = 7)	FRAP = −0.632 *d* − 50.090 RI + 0.170 BU − 4.657 ABV	21.1
III (alcoholic, medium bitterness, *n* = 8)	FRAP = 2.741 *d* + 0.510 RI − 41.720 BU + 0.479 ABV	29.1
IV(alcoholic, high bitterness, *n* = 5)	FRAP = 0.954 *d* + 0.184 RI − 0.851 BU − 0.118 ABV	20.0
V (alcoholic, very high bitterness, *n* = 4)	FRAP = −0.279 *d* + 93.980 RI + 0.243 BU + 56.320 ABV	19.8

[a] Calculated as the average of samples' absolute prediction errors after leave-on-out model validation.

As expected, the contents of total phenolic compounds from HPLC were correlated with FRAP ($r = 0.52$, $p = 0.004$), TEAC ($r = 0.53$, $p = 0.004$) and Folin–Ciocalteau assays ($r = 0.38$, $p = 0.047$). Gallic and 3,4-dihydroxybenzoic acids were the only phenolic compounds that were individually correlated with FRAP ($r = 0.42$, $p = 0.027$ and $r = 0.52$, $p = 0.004$, respectively) and TEAC assays ($r = 0.45$, $p = 0.016$ and $r = 0.57$, $p = 0.001$, respectively), suggesting that these major compounds are important contributors to Brazilian beers antioxidant capacity. Although food additives, such as antioxidants, stabilizers and coloring, may influence antioxidant capacity, in the samples evaluated in the present study they were not the major contributors as the samples that showed the highest FRAP and TEAC values (samples No. 1, 18, 19 and 20) did not contain the aforementioned additives.

By applying PLS analysis, FRAP values were modeled as a function of density, refractive index, bitterness and ethanol contents for each group of samples arranged by PCA. The proposed models after leave-one-out validation presented good predictability. Twenty samples (69% of the sample set) presented an average relative prediction error of 11%. In addition, acceptable mean errors of FRAP values were also observed for groups of beers, which ranged from 7.1% to 29.1% (Table 3). Possibly, these errors in FRAP prediction occurred due to differences in raw materials used for beer production as well as brewing processing parameters. The samples with prediction errors higher than 25% within each group probably behaved as outliers for they presented unique ingredients or characteristics, as further detailed. In Group II, samples No. 14 and 23 showed prediction errors of −27% and +42%, respectively. Sample No. 14 was the only sample in this group with no added antioxidant, while sample No. 23 was the only one that contained processed carbohydrates (Supplementary Table 1). In Group III, sample No. 1 showed a prediction error of -51%, which might be explained as this was the only ale type beer, brown and unfiltered in this group (Supplementary Table 1). In this same group, samples No. 13, 22 and 26 showed prediction errors ranging from +35% to +74% and it is worth mentioning that these samples were the less bitter beers in this group (Table 1). In Group IV, sample No. 3 showed a prediction error of +36%, possibly because this was the only ale type and wheat beer in this group (Supplementary Table 1). In Group V, sample No. 5 showed a prediction error of +30%, probably because it was the only bock style and the only with added colorant (caramel) among the whole set of beers investigated (Supplementary Table 1). In addition, this sample presented the highest bitterness and refractive index of all analyzed beer samples (Table 1).

One can argue that lower prediction errors would be obtained by increasing sample size (Lachenmeier et al., 2005; Lachenmeier, 2007; Mignani et al., 2013), which would in turn allow formation of more homogeneous groups in terms of bitterness. However, similar sample sizes of beers have been successfully classified by PCA (Granato et al., 2011).

4. Conclusions

Commercial Brazilian beers analyzed in the present study showed physicochemical attributes in accordance with international regulations. Contents of phenolic compounds, which were investigated for the first time in Brazilian beers, as well as antioxidant capacity, were similar to those of commercial beers produced elsewhere in the world. The phenolic profile of Brazilian beers was characterized by high contents of gallic acid and low contents of ferulic acid. Using chemometrics, it was possible to classify Brazilian beers according to their ethanol content and bitterness and to successfully model FRAP as a function of density, refractive index, bitterness and ethanol content. This approach may be of valuable use for developing beers with higher antioxidant capacity and therefore potentially improved bioactivity, as well as enhanced sensory quality and chemical stability.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.11.133.

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