Food Chemistry 199 (2016) 105–113

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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

Nathália Moura-Nunes [a], Thárcila Cazaroti Brito [b], Nívea Dias da Fonseca [b], Paula Fernandes de Aguiar [c], Mariana Monteiro [d], Daniel Perrone [b,*], Alexandre Guedes Torres [b]

[a] Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, R. São Francisco Xavier, 524 Pavilhão João Lyra Filho, 12° andar, Bloco D, sala 12002, 20559-900 Rio de Janeiro, Brazil
[b] Laboratório de Bioquímica Nutricional e de Alimentos, Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Av. Athos da Silveira Ramos 149 CT, Bloco A, sala 528A, 21941-909 Rio de Janeiro, Brazil
[c] Laboratório de Quimiometria, Departamento de Química Analítica, Instituto de Química, Universidade Federal do Rio de Janeiro, Av. Athos da Silveira Ramos 149, CT, Bloco A, sala 517, 21949-909 Rio de Janeiro, Brazil
[d] Laboratório de Alimentos Funcionais, Departamento de Nutrição Básica e Experimental, Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373, CCS, Bloco J, sala J2-16, 21941-902 Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 23 November 2015
Accepted 28 November 2015
Available online 30 November 2015

Chemical compounds studied in this article:
3,4-Dihydroxyphenylacetic acid (PubChem CID-547)
4-Hydroxyphenylacetic acid (PubChem CID-127)
Ferulic acid (PubChem CID-445858)
Gallic acid (PubChem CID-370)
3,4-Dihydroxybenzoic acid (PubChem CID-72)
5-Caffeoylquinic acid (PubChem CID-1794427)
Syringic acid (PubChem CID-10742)
Vanillic acid (PubChem CID-8468)
Benzoic acid (PubChem CID-243)
p-Coumaric acid (PubChem CID-637542)

Keywords:
Principal component analysis (PCA)
Partial least squares (PLS)
FRAP
Brewing
Multivariate data analysis

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

* Corresponding author.
E-mail addresses: nunesnathalia@yahoo.com.br (N. Moura-Nunes), tharcilacazaroti@hotmail.com (T.C. Brito), niveadiasdafonseca@gmail.com (N.D.d. Fonseca), paula.fdeaguiar@gmail.com (P.F. de Aguiar), mariana@nutricao.ufrj.br (M. Monteiro), danielperrone@iq.ufrj.br (D. Perrone), torres@iq.ufrj.br (A.G. Torres).

http://dx.doi.org/10.1016/j.foodchem.2015.11.133
0308-8146/

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.