International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

Evaluation of hot and cold extraction of bioactive compounds in teas

Vanessa de Carvalho Rodrigues,[1] Marcos V. da Silva,[2] Adriele R. dos Santos,[2] Acácio A. F. Zielinski[3] & Charles W. I. Haminiuk[1]*

1 Programa de Pós-Graduação em Tecnologia de Alimentos (PPGTA), Universidade Tecnológica Federal do Paraná, Campo Mourão, CEP (87301-899), Brasil
2 Departamento Acadêmico de Alimentos (DALIM), Universidade Tecnológica Federal do Paraná, Campo Mourão, CEP (87301-899), Brasil
3 Programa de Pós-Graduação em Engenharia de Alimentos (PPGEAL), Universidade Federal do Paraná, Curitiba, CEP (81531-980), Brasil

(Received 8 March 2015; Accepted in revised form 30 April 2015)

Summary This study evaluated the bioactive compounds of different types of tea by comparing hot and cold infusions. A multivariate data analysis was carried out, where the principal components analysis (PCA) and hierarchical cluster analysis (HCA) were used. Phenolic compounds varied between 267.27–2896.00 mg GAE L^{-1} and 215.10–7351.33 mg GAE L^{-1} for hot (80 °C) and cold extraction (20–25 °C), respectively. In the case of their antioxidant activity, results with DPPH were 43.10–73.67% for hot extraction and 46.80–77.13% for cold extraction. The average values for the $ABTS^{\cdot+}$ method ranged between 2535.43 and 33 300.17 μmol TE L^{-1} and between 1110.34 and 38 300.67 μmol TE L^{-1}, respectively, for hot and cold extraction. Different compounds were identified by liquid chromatography in the samples evaluated, where caffeine presented the higher concentrations in the teas. Samples of green and black tea (hot extraction) and white tea (cold extraction) showed bacteriostatic activity for *S. aureus* and *E. coli*. No extract had any bactericide activity. The current study revealed that cold infusion was more efficient in the extraction of bioactive compounds.

Keywords antimicrobial activity, antioxidant activity, bioactive compounds, hierarchical cluster analysis, principal components analysis, tea.

Introduction

Several researchers have recently focused their attention on biologically active ingredients, especially alkaloids and phenolic compounds, in food and beverages, due to their positive effects on human health (Damiani *et al*., 2014). Teas are one of the most consumed beverages worldwide. Due to the attractive aroma and specific flavour, its popularity is also a result of their potentially health-promoting properties (Horžić *et al*., 2009; Bae *et al*., 2015).

White, green, oolong and black teas, among other types, are produced from leaves and sprouts of the *Camellia sinensis* (L.) (family *Theaceae*) and are categorised according to variations in harvests, processing and oxidation degrees of their phenolic compounds (Sharangi, 2009; Unachukwu *et al*., 2010).

Peumus boldus Molina, commonly known as boldo, is an endemic shrub native of Chile and used in the preparation of infusions with several health benefits associated with essential oils, alkaloids and phenolic compounds (Soto *et al*., 2014). *M. recutita* L. is an herbaceous plant native from Europe and western Asia (Mckay & Blumberg, 2006). Several products, especially infusions, obtained from chamomile are commercially available (Mckay & Blumberg, 2006). The intake of the chamomile infusion amounts is estimated to more than a million cups per day (Petronilho *et al*., 2012).

Baccharis trimera (Less) DC, commonly known as 'carqueja' in Brazil, is a very popular plant in South America, and 'carqueja' infusions are used in Brazilian folk medicine. In fact, the species has been characterised by diterpene lactone, sesquiterpenes, flavonoids, saponins, tannins, phenolic compounds and essential oils (Grance *et al*., 2008). *Cymbopogon citratus* (DC.) Stapf (Cy), *Poaceae-Gramineae*, commonly known as 'capim-limão' or lemon grass, is a spontaneous perennial grass widely distributed in tropical and subtropical countries. Water extracts of its dry leaves are used in folk medicine for the treatment of several inflammatory pathologies (Francisco *et al*., 2011).

*Correspondent: E-mail: haminiuk@utfpr.edu.br

doi:10.1111/ijfs.12858

The *Ilex paraguariensis* St. Hil., commonly known as yerba mate or mate, is a plant native to the subtropical regions of South America and widely produced and consumed in southern Brazil, Argentina, Uruguay and Paraguay. Mate is rich in different biologically active compounds such as phenols, methylxanthines, triterpenes saponins, flavonoids, minerals and others (Murakami *et al*., 2011).

The anti-inflammatory, antioxidant, anti-allergic and anti-obesity functions of several types of tea have been reported (Hu *et al*., 2009; Sharangi, 2009). The above biological activities are partially associated with the antioxidant activity of the chemical compounds in teas, especially flavonoids and phenolic acids (Damiani *et al*., 2014; Zielinski *et al*., 2014a). The chemical components of the tea leaves include phenolic compounds (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, polysaccharides, amino acids, lipids, vitamins, inorganic elements and others. However, the phenolic compounds are mainly responsible for the beneficial properties of tea (Sharangi, 2009).

These compounds have an aromatic ring within their structure, with one or more hydroxyl groups, and may vary from one simple phenolic molecule to a complex molecular mass polymer (Balasundram *et al*., 2006).

The antioxidant compounds provide the free radical with an electron and transform it into an inert molecule (Haminiuk *et al*., 2012a). Structurally they differ as from simple molecules and are mainly classified according to the number of phenolic rings (Haminiuk *et al*., 2011).

The antioxidant capacity of phenolic compounds mainly occurs by a redox mechanism where they function as reduction agents, hydrogen providers, deactivators of singlet oxygen or metal chelates (Rice-Evans *et al*., 1997). Therefore, phenolic compounds may hinder the formation of free radicals, such as superoxide anions (radical O_2), nitric oxide (radical NO) and also as nonfree radicals, such as hydrogen peroxide (H_2O_2) and nitrous acid (HNO_2) (Zhu *et al*., 2002). The evaluation of total and individual quantification of phenolic compounds is essential for the corelationship with their biological activity (Zielinski *et al*., 2014b).

In this article, the bioactive compounds of different types of teas were evaluated by comparing hot and cold infusions. Using different colorimetric assays and high-performance liquid chromatography analysis, it was clearly demonstrated that the temperature plays an important role on the extraction of bioactive compounds in tea samples.

Materials and methods

Reagents

Reagents comprised 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), (+)- hydrated catechin and Folin–Ciocalteau reagent, from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetic acid, gallic acid and the other reagents for the assays were of the purest grade. Water solutions were prepared with ultra-pure water (Milli-Q; Millipore, São Paulo, Brazil). Eosin–methylene blue agar (EMB) was manufactured by Himedia, Mumbai, India; Baird–Parker (BP) agar was manufactured by Acumedia, Lansing, MI, USA; Mueller–Hinton broth (MHB) and Mueller–Hinton agar (MHA) was manufactured Himedia.

Tea samples

Eight different commercial types of teas were obtained on the supermarkets in Campo Mourão city, Paraná state, Brazil: *Camellia sinensis* ($n = 3$) (green tea; white tea; black tea), *Peumus boldus* ($n = 1$) (boldo tea), *Matricaria recutita* ($n = 1$) (chamomile tea), *Baccharis ginistelloides* ($n = 1$) ('carqueja' tea), *Cymbopogon citratus* ($n = 1$) (lemon grass tea) and *Ilex paraguariensis* ($n = 1$) (yerba mate or mate).

Extraction procedures

Hot extract followed methodology by Zielinski *et al*. (2014a). A total of 2.0 g of the sample was extracted with 100 mL of water distilled at 80 °C. Extraction was conducted by magnetic stirring for 450 s. Cold extraction consisted of 0.5 g of tea with 20 mL of distilled water. The infusion was kept still, at room temperature (20–25 °C) for 2 h, and stirred manually at every 30 min (Damiani *et al*., 2014). All extracts were filtered, transferred to Falcon tubes and immediately frozen at −20 °C (dark) for later analysis in order to prevent oxidation by light and oxygen.

Total phenolic compounds

Phenolic compounds were determined with the Folin–Ciocalteu method, following Singleton & Rossi (1965), with modifications. The values of total phenolic compounds were determined by interpolating samples' absorbance against the calibration curve constructed by gallic acid standard [$y = 0.001x + 0.009$; $R^2 = 0.999$; $P < 0.001$] and expressed in milligrams of gallic acid equivalent per litre (mg GAE L^{-1}). Analyses were carried out in triplicate, and results were given in means and standard deviation.

Total flavonoids

Contents of total flavonoids were quantified in triplicate by the colorimetric method with aluminium chloride, following methodology by Chang *et al*. (2002).

Results were interpolated with catechin calibration curve [$y = 0.002x + 1.043$; $R^2 = 0.996$; $P < 0.001$] and given in milligrams of catechins equivalents per litre of tea (mg CE L^{-1}). Results were given in means and standard deviation.

Determination of *in vitro* antioxidant capacity

The antioxidant capacity of the samples was determined by DPPH and $ABTS^{\cdot+}$ methods. DPPH was performed following methodology by Mensor *et al.* (2001), with modifications, or rather, 2500 μL of the diluted extract was added to 1000 μL of DPPH 0.3 mmol L^{-1} methanol solution. At the same time, a negative control with 2500 μL of the extracting solution (water) and 1000 μL of DPPH was prepared, likewise a control (blank) to discard the colour of the extracts (2500 μL extract and 1000 μL methanol). The mixtures were stored away from light during 30 min, and absorbance was set at 517 nm in spectrophotometer (UV/VIS T-80; PG Instruments Limited, Beijing, China). Methanol was used as control for the calibration of the equipment. Results were given in percentage of inhibition.

Antioxidant activity by $ABTS^{\cdot+}$ was assessed following Thaipong *et al.* (2006). A working solution was prepared by stirring two stock solutions (solution 7.4 mmol L^{-1} $ABTS^{\cdot+}$ and solution 2.6 mmol L^{-1} of potassium persulfate), in equal amounts, and reacting for 12 h at room temperature in the dark. After reaction time, the solution was diluted by a mixture of 1 mL $ABTS^{\cdot+}$ solution with 60 mL methanol for an absorbance between 1.1 and 734 nm. Analysis comprised 150 μL of the extract mixed with 2850 μL of $ABTS^{\cdot+}$ solution. After 2 h in the dark, the samples' absorbance was read at 734 nm; water was used as negative control. Results were compared with the trolox calibration curve [$y = -0.001x + 1.009$; $R^2 = 0.999$; $P < 0.001$] and given in μmol of trolox equivalent per litre (μmol TE L^{-1}). Analyses were carried out in triplicate, and results were given in means and standard deviation.

Identification of phenolic acids by HPLC

The analysis of phenolic compounds was based on methodology by (Haminiuk *et al.* 2012b; Iora *et al.* 2014), with modifications. Extracts were filtered by 0.22-μm filter nylon syringe (Millipore). Mobile phases comprised acetic acid 1% (A) and methanol (B) for the total execution time of 65 min. High-performance liquid chromatography (HPLC) analysis was carried out using a chromatograph Dionex Ultimate 3000 (Dionex, Idstein, Germany) equipped with a Ultimate 3000 pump, sample compartment column Ultimate 3000 and photodiode detector Ultimate 3000. Injection volume was 3 μL, with the following gradient: 5–10% B (0–2 min), 10–12% B (2–3 min), 12–16% B (3–5 min), 16–23% B (5–10 min), 23–33% B (10–20 min), 33–45% B (20–30 min), 45–65% B (30–40 min), 65–80% B (40–45 min), 80–100% B (45–50 min), 100% B (50–55 min), 100–5% B (55–60 min) and 5% B (60–65 min). Data were collected by Chromeleon software (Dreieich, Germany). Separation was performed on an Acclaim® 120 C18 measuring 4.6 mm × 250 mm, 5 μm (Dionex, Salt Lake City, UT, USA) at 40 °C with a flow rate of 1.0 mL min^{-1}. Standards of gallic acid ($y = 0.0457x$, $R^2 = 0.9993$), catechin ($y = 0.0107x$, $R^2 = 0.9996$), syringic acid ($y = 0.0550x$, $R^2 = 0.9996$), chlorogenic acid ($y = 0.0531x$, $R^2 = 0.9996$), caffeic acid ($y = 0.0965x$, $R^2 = 0.9996$), p-coumaric acid ($y = 0.1005x$, $R^2 = 0.9996$), ferulic acid ($y = 0.0929x$, $R^2 = 0.9996$), rutin ($y = 0.0232x$, $R^2 = 0.99959$), quercetin ($y = 0.0682x$, $R^2 = 0.9994$), theobromine ($y = 0.0512x$, $R^2 = 0.9996$) and caffeine ($y = 0.0424x$, $R^2 = 0.9996$) were used to identify the phenolic compounds of the samples. Analysis was performed in triplicate.

In vitro determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) of tea extracts were evaluated by the microdilution broth method following the protocol described in M100-S22/2012 of the Clinical and Laboratory Standards Institute (CLSI, 2012). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used in the experiments to determine MIC and MBC of each tea extract. Two controls were established: the first control with Mueller–Hinton broth (MHB) and bacterial isolates to test the viability of micro-organisms; and the second control with MHB and the extract in the initial concentration to discard any possible turbidity in the medium produced by the colour of the teas.

Bacterial isolates and antimicrobial agents

The study was conducted with isolates of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, stocked in a brain–heart infusion broth (BHI) with 20% glycerol at −20 °C.

Analysis of the Principle Components (PCA) and Hierarchical Cluster Analysis (HCA)

Results were analysed by the multivariate statistical methods PCA and HCA. A matrix composed of the samples ($n = 16$) and responses ($n = 16$) was built, totalling 256 points. Results for each parameter were

the variables (columns), whilst the teas were the individual samples (rows). Before the chemometric analysis, a autoscaled pretreatment of data was performed to standardise the significant importance of all the variables (Zielinski *et al.*, 2014c). Therefore, PCA separated the samples of the teas, whilst results of the samples and variables were plotted in two-dimension graphs. HCA was performed to verify the similarity of the samples based on the Euclidian distance, whereas Ward's method was employed to form and suggest the clusters of similar samples. Results were plotted on a dendrogram. Then, the results between clusters could be compared, and Levene test checked the homoscedasticity of results, whilst the analysis of variance (one-way ANOVA or Kruskal–Wallis's test) was applied to identify the difference between clusters at $P \leq 0.05$.

Results and discussion

Table 1 shows the concentrations of the phenolic compounds of the teas samples. The values of the phenolic compounds ranged between 267.27 and 2896.00 mg GAE L^{-1} and between 215.10 and 7351.33 mg GAE L^{-1}, respectively, in hot and cold extractions. Only the chamomile tea sample did not present any significant difference between the two types of extraction. Green, boldo, white, black and mate teas showed the highest concentrations in phenolic compounds in hot and cold extraction, respectively. Among the samples evaluated, black tea had the highest increase in the concentration of total phenolic compounds with the change in the extraction process (hot to cold), that is an increase of fivefold.

According to Venditti *et al.* (2010), as a rule, the contents of phenolic compounds are always higher in hot infusions. Few studies have investigated whether the contents of phenolic compounds and antioxidant activity of teas are affected by different temperatures (Yang *et al.*, 2007; Lin *et al.*, 2008). In a recent study, Damiani *et al.* (2014) assessed hot and cold infusions of different types of teas, where the highest concentration were found in the cold infusions. The above results are similar to the results found in this research, probably associated with the thermosensitiveness of phenolic compounds, as reported in some studies (Larrauri *et al.*, 1997; Henríquez *et al.*, 2014). According to Venditti *et al.* (2010), in the infusions at room temperature, the leaves are left in the water for two or more hours. As they become fully swelled (Yang *et al.*, 2007), their chemical components migrate to the solution and a beverage rich in phenolic compounds is produced.

Total flavonoids ranged between 53.33 and 969.17 mg CE L^{-1} in the hot extraction and between 95.33 and 510.00 mg CE L^{-1} in the cold extraction (Table 1). Only black and chamomile teas showed an increase in flavonoids concentration with the extraction process, that is hot to cold. The mate sample presented higher concentration in total flavonoids in relation to the total phenolic compounds. Similar results were found by Horžić *et al.* (2009). According to Baptista *et al.* (1998), this phenomenon is possible due to hydrophobic interactions of some phenolic compounds in the tea infusions extracted at different temperatures and to the synergic or antagonistic effect of several compounds, including methylxanthine, on flavan-3-ol content.

Other studies assessed the concentrations of phenolic compounds and flavonoids in different types of samples. Almajano *et al.* (2008) showed values between 234.00 and 2247.00 mg GAE L^{-1} for phenolic compounds in different types of infusion, very similar to those in current assay. In their evaluation of certain

Table 1 Contents of total phenolic compounds and total flavonoids from hot and cold infusions

	Hot extraction		Cold extraction	
Samples	**Total phenolic compounds (mg GAE L^{-1})**	**Total flavonoids (mg CE L^{-1})**	**Total phenolic compounds (mg GAE L^{-1})**	**Total flavonoids (mg CE L^{-1})**
Green (1)	$2896.00^{a} \pm 104.40$	$969.17^{a} \pm 23.23$	$3404.67^{b} \pm 174.74$	$433.33^{b} \pm 22.68$
Lemon grass (2)	$267.27^{a} \pm 8.50$	$195.67^{a} \pm 5.25$	$215.10^{b} \pm 11.82$	$133.00^{b} \pm 1.50$
Carqueja (3)	$373.93^{a} \pm 43.98$	$300.50^{a} \pm 7.70$	$264.93^{b} \pm 19.50$	$135.50^{b} \pm 3.12$
Boldo (4)	$1809.67^{a} \pm 61.10$	$904.17^{a} \pm 17.68$	$6956.33^{b} \pm 303.99$	$433.33^{b} \pm 41.26$
White (5)	$2546.00^{a} \pm 245.15$	$916.67^{a} \pm 18.76$	$5922.67^{b} \pm 225.02$	$510.00^{b} \pm 34.73$
Chamomile (6)	$399.60^{a} \pm 38.11$	$53.33^{a} \pm 6.25$	$336.27^{a} \pm 16.44$	$95.33^{b} \pm 4.16$
Black (7)	$1446.33^{a} \pm 45.37$	$175.67^{a} \pm 4.54$	$7351.33^{b} \pm 547.18$	$210.67^{b} \pm 14.25$
Mate (8)	$883.29^{a} \pm 48.50$	$934.17^{a} \pm 23.63$	$1639.87^{b} \pm 106.31$	$397.50^{b} \pm 13.92$

Results in mean ± standard deviation. Means on the same line followed by different letters are significantly different ($P \leq 0.05$). 1: (*Camellia sinensis* (L.) Kuntze); 2: (*Cymbopogon citratus* Stapf); 3: [*Baccharis ginistelloides* (Lamark_Persoon)]; 4: (*Peumus boldus* Molina); 5: (*Camellia sinensis* (L.) Kuntze); 6: (*Matricaria recutita* L.); 7: (*Camellia sinensis* L.) Kuntze; 8: (*Ilex paraguariensis* St. Hil.).

Table 2 Antioxidant activity of hot and cold infusions by assays DPPH and $ABTS^{\cdot+}$

	Hot extraction		Cold extraction	
Sample (Tea)	**DPPH %**	**$ABTS^{\cdot+}$ (μmol TE L^{-1})**	**DPPH %**	**$ABTS^{\cdot+}$ (μmol TE L^{-1})**
Green (1)	$58.57^{a} \pm 1.80$	$33\,300.17^{a} \pm 804.67$	$56.97^{a} \pm 0.64$	$38\,300.67^{b} \pm 444.41$
Lemon grass (2)	$43.10^{a} \pm 2.03$	$2535.43^{a} \pm 128.16$	$46.80^{b} \pm 0.44$	$1710.21^{b} \pm 91.24$
Carqueja (3)	$57.47^{a} \pm 0.70$	$3255.54^{a} \pm 112.58$	$50.53^{b} \pm 1.42$	$1110.34^{b} \pm 7.15$
Boldo (4)	$50.47^{a} \pm 1.27$	$19\,950.23^{a} \pm 278.76$	$50.67^{a} \pm 2.05$	$8350.70^{b} \pm 486.70$
White (5)	$62.07^{a} \pm 1.73$	$27\,100.91^{a} \pm 707.70$	$59.07^{a} \pm 1.05$	$32\,050.41^{b} \pm 557.52$
Chamomile (6)	$47.53^{a} \pm 1.15$	$2535.57^{a} \pm 98.36$	$47.27^{a} \pm 0.46$	$1680.84^{b} \pm 108.17$
Black (7)	$71.20^{a} \pm 1.22$	$17\,875.73^{a} \pm 737.82$	$68.93^{a} \pm 1.02$	$11\,850.81^{b} \pm 354.44$
Mate (8)	$73.67^{a} \pm 1.10$	$7275.95^{a} \pm 8.66$	$77.13^{b} \pm 0.38$	$3465.66^{b} \pm 65.38$

Results given in means ± standard deviation. Means on the same line followed by different letters are significantly different ($P \leq 0.05$). 1: (*Camellia sinensis* (L.) Kuntze); 2: (*Cymbopogon citratus* Stapf); 3: [*Baccharis ginistelloides* (Lamark_Persoon)]; 4: (*Peumus boldus* Molina); 5: (*Camellia sinensis* (L.) Kuntze); 6: (*Matricaria recutita* L.); 7: (*Camellia sinensis* (L.) Kuntze; 8: (*Ilex paraguariensis* St. Hil.).

tea species, Zielinski *et al*. (2014a) presented results between 100.45 and 1034.48 mg GAE L^{-1} for phenolic compounds and between 34.09 and 179.88 mg CE L^{-1} for flavonoids.

Table 2 demonstrates results of the antioxidant activity of the samples. DPPH is a stable free radical featuring an intense purple colour and a strong absorption band at wave length 515–520 nm. In antioxidant compounds, the radical may receive an electron or an atom of hydrogen from the sequestering molecule of the antioxidant to be converted into a more stable one. As the reduced form of DPPH is pale yellow, the antioxidant activity of certain compounds may be spectrophotometrically determined. The highest the elimination capacity of free radicals of an antioxidant compound, the greater is the DPPH reduction and the lower is the intensity of the purple colour in the sample (Carmona-Jiménez *et al*., 2014). Mate tea showed the highest antioxidant activity in the DPPH assay, with a slight increase ($P \leq 0.05$) with the change in the extraction process (hot to cold). Results did not show any significant difference in the antioxidant activity of the other types of tea, excepting the lemon grass and carqueja. Results varied between 43.10 and

Table 3 Bioactive compounds which were identified and quantified by HPLC analysis (mg L^{-1})

FC standards	Gallic acid	Theobromine	Catechin	Chlorogenic acid	Caffeine	Caffeic acid	Syringic acid	p-coumaric acid	Ferulic acid	Rutin	Trans-cinnamic acid	Quercetin
λ (nm)	279	279	279	324	279	324	279	324	324	370	279	370
Cold (1)	43.69	17.25	112.8108	4.97	399.59	–	–	–	–	45.99	–	1.05
Hot (1)	81.41	24.69	40.89707	4.54	446.94	–	–	–	–	53.67	–	–
Cold (2)	–	–	–	15.43	–	2.33	–	3.11	0.73	–	0.37	–
Hot (2)	–	–	–	34.71	–	0.47	–	3.13	0.54	–	–	–
Cold (3)	–	1.92	–	39.74	9.66	9.18	0.50	–	–	–	0.30	–
Hot (3)	–	1.70	–	47.35	8.42	0.98	0.23	–	–	–	–	–
Cold (4)	–	–	21.26	–	–	–	–	–	–	21.33	–	–
Hot (4)	–	–	26.12	–	–	–	–	–	–	19.39	–	–
Cold (5)	141.19	24.80	16.18	4.58	485.95	–	–	–	–	49.78	–	0.33
Hot (5)	102.03	26.75	19.53	4.57	573.95	–	–	–	–	–	–	–
Cold (6)	–	–	–	30.15	–	–	–	–	–	–	–	–
Hot (6)	–	–	–	29.77	–	–	–	–	–	–	–	–
Cold (7)	37.56	2.52	–	2.60	256.05	–	–	–	–	53.97	–	–
Hot (7)	60.84	3.29	–	–	333.82	–	–	–	–	59.56	–	–
Cold (8)	–	18.34	–	86.07	98.76	–	–	–	–	–	–	–
Hot (8)	–	19.64	–	81.90	111.16	–	–	–	–	–	–	–

FC: Phenolic compounds; λ: wave length, 1: Green (*Camellia sinensis* (L.) Kuntze); 2: Lemon grass (*Cymbopogon citratus* Stapf); 3: 'Carqueja' [*Baccharis ginistelloides* (Lamark_Persoon)]; 4: Boldo (*Peumus boldus* Molina); 5: White (*Camellia sinensis* (L.) Kuntze); 6: Chamomile (*Matricaria recutita* L.); 7: Black (*Camellia sinensis* (L.) Kuntze; 8: Mate (*Ilex paraguariensis* St. Hil.).

73.67% for hot extraction and between 46.80 and 77.13% for cold extraction.

$ABTS^{\cdot+}$ is frequently employed to measure the antioxidant capacity of food. Cation $ABTS^{\cdot+}$ is reactive for most antioxidants which include phenolic compounds (Walker & Everette, 2009). The radical cation $ABTS^{\cdot+}$ during the reaction is transformed again into its neutral colourless form. The antioxidant activity of plants' extracts is related to the contents of phenolic compounds due to the compounds' capacity to donate electrons or hydrogen atoms and to capture free radicals (Awe *et al*., 2013). In the $ABTS^{\cdot+}$ method, the values ranged between 2535.43 and 33 300.17 μmol TE L^{-1} and between 1110.34 and 38 300.67 μmol TE L^{-1}, respectively, for hot and cold extractions.

Samples were analysed by HPLC, and compounds identified are shown in Table 3. Dufresne & Farnworth Edward (2001) listed the main constituent clusters of teas, namely catechins, theaflavins, thearubigins, theogallins, proanthocyanidins, flavonols, methylxanthines, phenolic acids and others. All identified compounds fit in the above-mentioned clusters.

The phenolic compounds identified in most of the samples evaluated were theobromine, chlorogenic acid and caffeine. Similar results were also obtained in studies by Bae *et al*., 2015 and He *et al*., 2015. Gallic acid, catechin, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, rutin, trans-cinnamic acid and quercetin were also identified in the samples.

The principal components analysis (PCA) was performed to evaluate the data set of the individual phenolic compounds determined by HPLC, TFC (total phenolic compounds), TF (total flavonoids) and antioxidant activity (DPPH and $ABTS^{\cdot+}$ assays) of the different herbal (hot and cold extraction processes). Principal component (PC) 1 explained 39.95% of total variance and PC 2 explained 15.74%, totalising 55.69% of total variance explained by the first two principal components (Fig. S1). The variables that indicate the separation of herbal teas along the PC 1 were TFC, $ABTS^{\cdot+}$, gallic acid, theobromine, catechin, caffeine, caffeic acid, p-coumaric acid, ferulic acid, rutin, trans-cinnamic acid and quercetin, whilst in PC 2, the samples were separated in relation to antioxidant activity (DPPH) and phenolic acids (chlorogenic, p-coumaric and ferulic). According to scatter plots (PC1 vs. PC2) (Fig. S1), the samples grouped at the right side were separated owing to their higher concentrations in TFC, $ABTS^{\cdot+}$, gallic acid, theobromine, catechin, caffeine, rutin and quercetin, whereas samples on the left side were separated owing to their high values in caffeic acid, p-coumaric acid, ferulic acid, trans-cinnamic acid, chlorogenic acid and DPPH.

Hierarchical cluster analysis (HCA) was performed to verify the similarity among the samples, and three clusters were proposed (Fig. S2) corroborated with the results obtained by PCA. Cluster 1 was formed by all *Camellia sinensis* teas (red, white and black), regardless of the extraction process. Multiple comparison between the groups revealed that the cluster had a significant difference ($P < 0.01$) with the highest values of TFC, the antioxidant activity measured by $ABTS^{\cdot+}$, caffeine and rutin (Table S1). On the other hand, Cluster 2 had the lowest TFC values, the antioxidant activity measured by $ABTS^{\cdot+}$, caffeine and rutin, formed by lemon grass and 'carqueja' teas (cold extraction). However, these teas had the highest concentrations of caffeic and syringic acids. Cluster 3, formed by boldo, chamomile, 'carqueja' (hot extraction) and mate, provided intermediate results for variables assayed. The association of the groups by HCA revealed that the samples in Cluster 1 had a 'high functionality', Cluster 2 showed 'low functionality' and Cluster 3 'intermediate functionality'.

Most phenolic compounds show antimicrobial activities. Several studies investigated the effects of phenolic compounds in intestine pathogens, even though disagreement exists on the precise bacteria inhibited by the antioxidants. Tolerance of bacteria to phenolic compounds depends on the bacterial species and on the phenolic compound structure (Campos *et al*., 2003; Taguri *et al*., 2004; Almajano *et al*., 2008).

Staszewski *et al*. (2011) suggest that flavonoids may inhibit the synthesis of nucleic acid and influence enzyme activity, especially those associated with the production of energy. Protein and lipid synthesis is also slightly affected. Further, the phenolic compounds may interfere in the membrane's function (the transport of electrons, absorption of nutrients) and interact with the membrane's proteins, with a deformation in the structure and its functionality. The same authors related that the disorganisation of the cell wall structure, reported in electron microscopy studies, suggests that tea components interfere in the synthesis of peptidoglycan and compromise the cell separation process.

Only the green and black tea samples among the hot infusions had any bacteriostatic activity on *S. aureus* and *E. coli* in the initial concentration of the extracts (2.5%), whereas in the case of cold extraction, only white tea had a similar effect on *S. aureus*, in the initial concentration. No extract had any bactericide activity as from the initial concentration evaluated. Inhibition depends on the tested strain, type and concentration of the extract. Probably a higher concentration or a greater inoculated infusion volume provides a greater effect (Almajano *et al*., 2008).

Conclusions

The method of extraction clearly influenced the amount and profile of bioactive compounds in tea

samples. Despite the fact that the use of hot water in the preparation of tea infusion is more popular, the cold extraction is presented as an alternative to the consumers, resulting in a beverage with higher concentrations of bioactive compounds. Although, the tea infusions present high antioxidant capacity *in vitro* and bacteriostatic activity, as demonstrated in this study, the *in vivo* effects are not clear and further studies are still necessary to better understand the action and the mechanisms.

Acknowledgments

The authors thank the National Council for Scientific and Technological Development (CNPq; Process Number 303238/2013-5), Araucaria Foundation (Process 41013) and UTFPR for financial support.

References

Almajano, M.P., Carbo, R., Jiménez, J.A.L. & Gordon, M.H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, **108**, 55–63.

Awe, F.B., Fagbemi, T.N., Ifesan, B.O.T. & Badejo, A.A. (2013). Antioxidant properties of cold and hot water extracts of cocoa, Hibiscus flower extract, and ginger beverage blends. *Food Research International*, **52**, 490–495.

Bae, I.K., Ham, H.M., Jeong, M.H., Kim, D.H. & Kim, H.J. (2015). Simultaneous determination of 15 phenolic compounds and caffeine in teas and mate using RP-HPLC/UV detection: method development and optimization of extraction process. *Food Chemistry*, **172**, 469–475.

Balasundram, N., Sundram, K. & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chemistry*, **99**, 191–203.

Baptista, J.A.B., Tavares, J.F.P. & Carvalho, R.C.B. (1998). Comparison of catechins and aromas among different green teas using HPLC/SPME-GC. *Food Research International*, **31**, 729–736.

Campos, F.M., Couto, J.A. & Hogg, T.A. (2003). Influence of phenolic acids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. *Journal of Applied Microbiology*, **94**, 167–174.

Carmona-Jiménez, Y., García-Moreno, M.V., Igartuburu, J.M. & Barroso, C.G. (2014). Simplification of the DPPH assay for estimating the antioxidant activity of wine and wine by-products. *Food Chemistry*, **165**, 198–204.

Chang, C.C., Yang, M.H., Wen, H.M. & Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, **10**, 178–182.

Clinical Laboratory Standards Institute (2012). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement*. CLSI document M100-S22, Wayne, PA: Clinical and Laboratory Standards Institute, 2012.

Damiani, E., Bacchetti, T., Padella, L., Tiano, L. & Carloni, P. (2014). Antioxidant activity of different white teas: comparison of hot and cold tea infusions. *Journal of Food Composition and Analysis*, **33**, 59–66.

Dufresne, C.J. & Farnworth Edward, R. (2001). A review of latest research findings on the health promotion properties of tea. *Journal of Nutritional Biochemistry*, **12**, 404–421.

Francisco, V., Figueirinha, A., Neves, B.M. *et al.* (2011). *Cymbopogon citratus* as source of new and safe anti-inflammatory drugs: bio-guided assay using lipopolysaccharide-stimulated macrophages. *Journal of Ethnopharmacology*, **133**, 818–827.

Grance, S.R.M., Teixeira, M.A., Leite, R.S. *et al.* (2008). *Baccharis trimera*: effect on hematological and biochemical parameters and hepatorenal evaluation in pregnant rats. *Journal of Ethnopharmacology*, **117**, 28–33.

Haminiuk, C.W.I., Maciel, G.M., Plata-Oviedo, M.S.V. & Peralta, R.M. (2012a). Phenolic compounds in fruits – an overview. *International Journal of Food Science and Technology*, **46**, 1529–1537.

Haminiuk, C.W.I., Plata-Oviedo, M.S.V., Guedes, A.R., Stafussa, A.P., Bona, E. & Carpes, S.T. (2011). Chemical, antioxidant and antibacterial study of Brazilian fruits. *International Journal of Food Science and Technology*, **46**, 1529–1537.

Haminiuk, C.W.I., Mattos, G., Carpes, S.T., Plata-Oviedo Manuel, V. & Branco, I.G. (2012b). Extraction and quantification of phenolic acids and flavonols from Eugenia pyriformis using different solvents. *Journal of Food Science and Technology*, **49**, 1–5.

He, X., Li, J., Zhao, W., Liu, R., Zhang, L. & Kong, X. (2015). Chemical fingerprint analysis for quality control and identification of Ziyang green tea by HPLC. *Food Chemistry*, **171**, 405–411.

Henríquez, C., Córdova, A., Almonacid, S. & Saavedra, J. (2014). Kinetic modeling of phenolic compound degradation during drum-drying of apple peel by-products. *Journal of Food Engineering*, **143**, 146–153.

Horžić, D., Draženka, K., Belščak, A., Ganič, K.K., Iveković, D. & Karlović, D. (2009). The composition of polyphenols and methylxanthines in teas and herbal infusions. *Food Chemistry*, **115**, 441–448.

Hu, B., Wang, L., Zhou, B. *et al.* (2009). Efficient procedure for isolating methylated catechins from green tea and effective simultaneous analysis of ten catechins, three purine alkaloids, and gallic acid in tea by high performance liquid chromatography with diode array detection. *Journal of Chromatography A*, **1216**, 3223–3231.

Iora, S.R.F., Maciel, G.M., Zielinski, A.A.F., Silva, M.V., Pontes, P.V.A. & Haminiuk, C.W.I. (2014). Evaluation of the bioactive compounds and the antioxidant capacity of grape pomace. *International Journal of Food Science & Technology*, **49**, 2717–2725.

Larrauri, J., Ruperez, P. & Saura-Calixto, F. (1997). Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *Journal of Agriculture and Food Chemistry*, **45**, 1390–1393.

Lin, S.D., Liu, E.H. & Mau, J.L. (2008). Effect of different brewing methods on antioxidant properties of steaming green tea. *LWT – Food Science and Technology*, **41**, 1616–1623.

Mckay, D.L. & Blumberg, J.B. (2006). A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). *Phytotherapy Research*, **20**, 519–530.

Mensor, L.L., Menezes, F.S., Leitao, G.G., Reis, A.S. & Santos, T.C. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, **15**, 127–130.

Murakami, A.N.N., Amboni, R.D.M.C., Prudêncio, E.S. *et al.* (2011). Concentration of biologically active compounds extracted from *Ilex paraguariensis* St. Hil. by nanofiltration. *Food Chemistry*, **141**, 60–65.

Petronilho, S., Maraschin, M., Coimbra, M.A. & Rocha, S.M. (2012). In vitro and in vivo studies of natural products: a challenge for their valuation. The case study of chamomile (*Matricaria recutita* L.). *Industrial Crops and Products*, **40**, 1–12.

Rice-Evans, C., Miller, N. & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, **2**, 152–159.

Sharangi, A.B. (2009). Medicinal and therapeutic potentialities of tea (*Camellia sinensis L.*) – a review. *Food Research International*, **42**, 529–535.

Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144–158.

Soto, C., Caballero, E., Péreza, E. & Zúñiga, M.E. (2014). Effect of extraction conditions on total phenolic content and antioxidant capacity of pretreated wild *Peumus boldus* leaves from Chile. *Food and Bioproducts Processing*, **92**, 328–333.

Staszewski, M.V., Pilosof, A.M.R. & Jagus, R.J. (2011). Antioxidant and antimicrobial performance of different Argentinean green tea varieties as affected by whey proteins. *Food Chemistry*, **125**, 186–192.

Taguri, T., Tanaka, T. & Kouno, I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biological & Pharmaceutical Bulletin*, **27**, 1965–1969.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. & Byrne, D.H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, **19**, 669–675.

Unachukwu, U.J., Ahmed, S., Kavalier, A., Lyles, J.T. & Kennelly, E.J. (2010). White and green teas (*Camellia sinensis var. sinensis*): variation in phenolic, methylxanthine, and antioxidant profiles. *Journal of Food Science*, **75**, 541–548.

Venditti, E., Bacchetti, T., Tiano, L., Carloni, P., Greci, L. & Damiani, E. (2010). Hot vs. cold water steeping of different teas: do they affect antioxidant activity? *Food Chemistry*, **119**, 1597–1604.

Walker, R.B. & Everette, J.D. (2009). Comparative reaction rates of various antioxidants with ABTS radical cation. *Journal of Agricultural and Food Chemistry*, **57**, 1156–1161.

Yang, D.-J., Hwang, L.S. & Lin, J.-T. (2007). Effects of different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. *Journal of Chromatography A*, **1156**, 312–320.

Zielinski, A.A.F., Haminiuk, C.W.I., Alberti, A., Nogueira, A., Demiate, I.M. & Granato, D. (2014a). A comparative study of the phenolic compounds and the in vitro antioxidant activity of different Brazilian teas using multivariate statistical techniques. *Food Research International*, **60**, 246–254.

Zielinski, A.A.F., Nogueira, A., Wosiacki, G. & Haminiuk, C.W.I. (2014b). The association between chromaticity, phenolics, carotenoids, and antioxidant activity of frozen fruit pulp in Brazil: an application of chemometrics. *Journal of Food Science*, **79**, C510–C516.

Zielinski, A.A.F., Haminiuk, C.W.I., Nunes, C.A., Schnitzler, E., Van-Ruth, S.M. & Granato, D. (2014c). Chemical composition, sensory properties, provenance, and bioactivity of fruit juices as assessed by chemometrics: a critical review and guideline. *Comprehensive Reviews in Food Science and Food Safety*, **13**, 300–316.

Zhu, Q.Y., Hackman, R.M. & Ensunsa, J.L. (2002). Antioxidative activities of oolong tea. *Journal of Agricultural and Food Chemistry*, **50**, 6929–6934.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Scatter plots (PC1 vs. PC2) for the different types of herbal teas by hot and cold extractions (a) and their responses (b).

Figure S2. Dendrogram for the different types of herbal teas by hot and cold extraction.

Table S1. Data of teas grouped by TFC, TF, antioxidant activity and individual phenolic compounds.