Food Research International 61 (2014) 228–235

Contents lists available at ScienceDirect

Food Research International

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

Scavenging capacity of coffee brews against oxygen and nitrogen reactive species and the correlation with bioactive compounds by multivariate analysis

Naira Poerner Rodrigues [a], Marta Toledo Benassi [b], Neura Bragagnolo [a,*]

[a] Department of Food Science, School of Food Engineering, University of Campinas (UNICAMP), 13083-862 Campinas, São Paulo, Brazil
[b] Department of Food Science and Technology, State University of Londrina (UEL), PO Box 6001, 86051-970 Londrina, Paraná, Brazil

ARTICLE INFO

Article history:
Received 12 June 2013
Received in revised form 17 September 2013
Accepted 18 September 2013
Available online 27 September 2013

Keywords:
Soluble coffee
Roasted ground coffee
Antioxidant capacity
Reducing capacity
Cinnamic acids
Methylxanthines

ABSTRACT

In the present work the scavenging capacity of coffee brews against ROS ($ROO^{\bullet}$, H_2O_2, $HO^{\bullet}$ and HOCl) and RNS ($NO^{\bullet}$ and $ONOO^-$) was evaluated and correlated with their bioactive compounds by multivariate analysis. It is the first time that the complete antioxidant capacity profile of coffee brews against different ROS and RNS is evaluated and that the capacity of coffee brews to scavenge $NO^{\bullet}$ is reported. The coffee brews were able to scavenge all the reactive species tested and possibly $NO_2^{\bullet}$ and $CO_3^{\bullet-}$. The capacity of the coffee brews to scavenge $ROO^{\bullet}$ (2523 to 3673 μmol TE/g), $HO^{\bullet}$ (IC_{50} = 2.24 to 4.38 μg/mL), $NO^{\bullet}$ (IC_{50} = 3.07 to 5.67 μg/mL) and $ONOO^-$ (IC_{50} = 1.29 to 2.88 μg/mL) was positively correlated to the contents of chlorogenic acids, chlorogenic acid lactones and *p*-coumaric acid, while the scavenging capacity against H_2O_2 (IC_{50} = 336 to 531 μg/mL) was positively correlated to the content of browned compounds, mainly melanoidins. The coffee brews showed to be potent HOCl scavengers (IC_{50} = 5.12 to 11.20 μg/mL), which was correlated to the contents of caffeic acid, 5-hydroxymethylfurfural and browned compounds. These results reinforce the hypothesis that the scavenging capacity against ROS/RNS is one of the mechanisms that can explain the association between the consumption of coffee brews and the decreased risk of chronic-degenerative diseases.

1. Introduction

Coffee is one of the most widely consumed beverages in the world due to its sensory characteristics and stimulating effects (Pérez-Martínez, Caemmerer, De Peña, Cid, & Kroh, 2010). Additionally, coffee has gained attention because of its benefic health effects, hypothetically attributed to the antioxidant properties of bioactive compounds, which would reduce the *in vivo* oxidative damages induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Hoelzl et al., 2010). Moreover, the moderate consumption of coffee is associated to the decreased risk of several chronic-degenerative diseases such as neurodegenerative disorders (Parkinson and Alzheimer), cirrhosis, asthma and type 2 diabetes (Alves, Casal, & Oliveira, 2009).

The main bioactive compounds of coffee brews are derived from the raw coffee beans, such as chlorogenic acids, trigonelline and caffeine, and from the roasting process, when Maillard reaction products, chlorogenic acid lactones and nicotinic acid are formed (Esquivel & Jiménez, 2012). In addition, the content of bioactive compounds of coffee can vary according to the type of processing, such as decaffeination (Farah, De Paulis, Moreira, Trugo, & Martin, 2006) and production of soluble coffee (Vignoli, Bassoli, & Benassi, 2011).

Coffee is the main source of chlorogenic acids in the human diet (Clifford, 1999). Among the bioactive compounds found in coffee, the chlorogenic acids have gained special attention since they are found in high content in coffee and possess *in vitro* and *in vivo* antioxidant capacity (Shahidi & Chandrasekara, 2010). However, other bioactive compounds found in coffee, such as the Maillard reaction products, caffeine and trigonelline can also contribute to its antioxidant capacity (López-Galilea, De Peña, & Cid, 2008).

The capacity of coffee brews to scavenge non-biological radicals, such as $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, can be found in several published studies (López-Galilea et al., 2008; Ludwig et al., 2012; Niseteo, Komes, Belščak-Cvitanovic, Horzic, & Budec, 2012; Pérez-Martínez et al., 2010). However, studies on the scavenging capacity of coffee brews

Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; $O_2^{\bullet-}$, superoxide anion; $ROO^{\bullet}$, peroxyl radical; H_2O_2, hydrogen peroxide; $HO^{\bullet}$, hydroxyl radical; HOCl, hypochlorous acid; $NO^{\bullet}$, nitric oxide radical; $ONOO^-$, peroxynitrite anion; $ABTS^{\bullet+}$, 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid); $DPPH^{\bullet}$, 2, 2-diphenyl-1-picrylhydrazyl; AAPH, α,α′-azodiisobutyramidine dihydrochloride; DHR, dihydrorhodamine 123; DAF-2, 4,5-diaminofluorescein; DAF-2T, triazolofluorescein; DMSO, dimethyl sulfoxide; NOC-5, 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene; EDTA, ethylenediaminetetraacetic acid; DE, dry extract; GAE, gallic acid equivalents; TE, trolox equivalents; IC_{50}, *in vitro* inhibitory concentration necessary to decrease by 50% the oxidative effect of the reactive species in the tested media; CA, caffeic acid; 5-HMF, 5-hidroxymethylfurfural; BC, browned compounds; RR, regular roasted ground coffee; DR, decaffeinated roasted ground coffee; RS, regular soluble coffee; DS, decaffeinated soluble coffee.

* Corresponding author. Tel.: +55 19 35212162; fax: +55 19 35212153.
E-mail addresses: nairapoernersa@gmail.com (N.P. Rodrigues), martatb@uel.br (M. Toledo Benassi), neurabragagnolo@gmail.com (N. Bragagnolo).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.09.028

against ROS and RNS of biological relevance are scarce or inexistent. The ROS and RNS have different chemical properties, especially different reactivities, ranging from the hydrogen peroxide (H_2O_2), which is little reactive, to the hydroxyl radical ($HO^{\bullet}$), which is highly reactive (Winterbourn, 2008). These differences are more pronounced when we compare non-biological radicals with ROS and RNS. Considering that each reactive species has specific chemical properties, the capacity of a particular matrix to scavenge a specific reactive species does not necessarily indicate that it is capable to scavenge any other reactive species. This suggests that the antioxidant capacity of a particular matrix must be evaluated against different ROS and RNS in order to establish the complete antioxidant capacity profile. The knowledge of the complete antioxidant capacity profile could help the interpretation of studies that aim to correlate the *in vitro* antioxidant properties of coffee brews and the decreased risk of chronic-degenerative diseases associated to the oxidative stress.

Thus, in the present work we determined the scavenging capacity of coffee brews against the main ROS and RNS of biological relevance, namely peroxyl radical ($ROO^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), hypochlorous acid (HOCl), nitric oxide radical ($NO^{\bullet}$) and peroxynitrite anion ($ONOO^{-}$), and correlated it to the content of bioactive compounds by multivariate analysis. It should be pointed out that it is the first time that the capacity of coffee brews to scavenge $NO^{\bullet}$ is reported.

2. Materials and methods

2.1. Chemicals

Standards of caffeine (99% purity), 5-caffeoylquinic acid (95% purity), caffeic acid (98% purity), *p*-coumaric acid (98% purity), trigonelline hydrochloride (98% purity), 5-hydroxymethyl-2-furaldehyde (99% purity), theophylline (99% purity), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox, 97% purity), ascorbic acid (99% purity), quercetin (98% purity), cysteine (97% purity) and rutin (94% purity) were supplied by Sigma-Aldrich (St. Louis, MO, USA) and gallic acid, by Extrasynthèse (Genay, France). Fluorescein sodium salt, α,α′-azodiisobutyramidine dihydrochloride (AAPH), lucigenin, 30% hydrogen peroxide, luminol, dihydrorhodamine 123 (DHR), N,N-dimethylformamide (DMF), sodium hypochlorite solution with 10–15% available chlorine, 4,5-diaminofluorescein (DAF-2), dimethyl sulfoxide (DMSO), 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5), sodium phosphate tribasic dodecahydrate and 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS) were obtained from Sigma-Aldrich. Folin–Ciocalteau reagent was obtained from Dinâmica (São Paulo, SP, Brazil). Sodium carbonate, sodium phosphate monobasic monohydrate, sodium phosphate dibasic, sodium hydroxide, sodium nitrite, sodium chloride, potassium chloride, and sodium bicarbonate were obtained from Synth (São Paulo, SP, Brazil). Ferrous chloride tetrahydrate was supplied by J. T. Baker (Phillipsburg, NJ, USA) and ethylenediaminetetraacetic acid (EDTA), by Quemis (Joinvile, SC, Brazil). Ultrapure water was obtained from a Millipore system (Billerica, MA, USA).

2.2. Coffee samples and preparation of coffee brews

Ten samples of roasted ground coffee (6 regular and 4 decaffeinated) and four samples of soluble coffee (2 regular and 2 decaffeinated) of different brands were acquired in the local market in Campinas, São Paulo, Brazil. The roasted ground coffees had different classifications of identity and quality according to the characteristics described in their packages. The samples RR9 and DR10 were classified as Gourmet (only *Coffea arabica* beans); DR4 and DR5, as Superior (up to 15% of *C. canephora* beans); and RR1, RR2, RR3, RR6, RR7 and RR8, as Traditional (blend with *Coffea canephora* beans). The roasted ground coffees and soluble coffees had medium (DR4, DR5, RR6, RR7, RR8, RR9, DR10, DS52 and DS53) to dark (RR1, RR2, RR3, RS51 and RS54) degrees of roasting according to the information provided on the labels and to the color parameters (L^* and $h°$) assayed by Rodrigues and Bragagnolo (2013).

The brews of roasted ground coffee were prepared by percolation using a filter paper (Whatman no. 4) and a proportion of 5 g of roasted ground coffee to 50 mL of ultrapure water ($T = 92.3 \pm 0.1$ °C). The brews of soluble coffee were prepared by dissolving 0.2 g of coffee in ultrapure water in a 10 mL volumetric flask. The coffee brews were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

The dry extract (DE) was determined by drying 5 mL of brew in an oven until constant weight (16 h at 70 °C) (AOAC, 2000). The determination of the dry extract was carried out in triplicate. DE varied between 13.19 and 20.43 g/L of roasted ground coffee brews and between 20.01 and 20.09 g/L of soluble coffee brews.

2.3. Reducing capacity

The reducing capacity of the coffee brews was determined by using the Folin–Ciocalteau colorimetric method (Singleton & Rossi, 1965), which was adapted for analysis in a microplate reader (Bio-Tek Instruments, model Synergy Mx, USA). Reaction mixtures in the wells contained the following reagents in a final volume of 300 μL: 3 concentrations of coffee brews (22 to 84 μg/mL of ultrapure water) or standards (caffeic acid and *p*-coumaric acid were dissolved in methanol and diluted in ultrapure water, the other standards (5-caffeoylquinic acid, trigonelline, 5-hydroxymethylfurfural, caffeine and theophylline) were dissolved and diluted in ultrapure water, Folin–Ciocalteau reagent (8.3%, *v*/*v*) and sodium carbonate solution (2.3%, *w*/*v*). The absorbance signal was monitored at 765 nm for 120 min (25 °C). Quantitation was carried out using curves constructed with gallic acid in the range of 2 to 12.5 μg/mL and the reducing capacity was expressed as mg of gallic acid equivalents per g of coffee brew DE (mg GAE/g DE). Ascorbic acid was used as positive control (721 ± 45 mg GAE/g).

2.4. ROS and RNS scavenging capacity

The assays were carried out in a microplate reader (Bio-Tek Instruments, model Synergy Mx, USA) for fluorescence, UV/vis and luminescence measurements, equipped with a thermostat set at 37 °C and dual reagent dispenser. Two control assays were conducted in all microplates, (1) to verify the interaction between the probe and the coffee brew, without radical generator or reactive species addition and (2) to analytical quality control, adding a compound with known capacity to scavenge the specific reactive species. No interaction between the probes and the coffee brews was observed and the maximum variation in the response of the positive controls during the assays was 15%. Each ROS and RNS scavenging assay corresponds to three independent experiments, performed in triplicate. The peroxyl radical ($ROO^{\bullet}$) scavenging capacity was reported as μmol of trolox equivalents per g of coffee brew DE (μmol TE/g DE). For all the other ROS and RNS evaluated, the results were presented as IC_{50} values, expressed as μg of coffee brew DE per mL (μg/mL) and calculated by non-linear regression analysis using the GraphPad Prism 5 software.

2.4.1. Peroxyl radical scavenging capacity

The $ROO^{\bullet}$ scavenging capacity was measured by monitoring the effect of the coffee brews on the fluorescence decay resulting from $ROO^{\bullet}$-induced oxidation of fluorescein (Ou, Hampsch-Woodill, & Prior, 2001). The $ROO^{\bullet}$ was generated by thermodecomposition of AAPH at 37 °C. Reaction mixtures in the wells contained the following reagents, dissolved in 75 mM phosphate buffer (pH 7.4), at the indicated final concentrations (final volume of 200 μL): fluorescein (61 nM), 3 concentrations of coffee brews (0.7 to 5 μg/mL) or standards (caffeic acid and