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Chlorogenic acid isomer contents in 100 plants commercialized in Brazil

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ABSTRACT

This study analysed 100 plants employed in Brazil as ingredients to infusions for their caffeic acid, 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), 3,4-dicaffeoylquinic acid (3,4-DQA), 3,5-dicaffeoylquinic acid (3,5-DQA), and 4,5-dicaffeoylquinic acid (4,5-DQA) contents. The samples were collected from public markets and analysed using ultra-high performance liquid chromatography (UPLC). The highest concentrations of chlorogenic acids were found in yerba mate (*Ilex paraguariensis*), 9,2 g·100 g^{-1}, white tea (*Camellia sinensis*), winter's bark (*Drimys winteri)*, green tea (*Camellia sinensis*), elderflower (*Sambucus nigra*), and *Boehmeria caudata* (known as *assa-peixe* in Brazil), 1,1 g·100 g^{-1}. The present work showcased the investigation of chlorogenic acids in a wide range of plants not yet studied in this regard and also resulted in a comparative table which explores the content of six isomers in the samples.

1. Introduction

Chlorogenic acids are present in several foods and drinks such as coffee, teas, fruits, and vegetables. The term was first used to describe one compound from coffee, now known as 5-CQA, which then led to the characterization of a whole class of hydroxycinnamic acid and quinic acid esters (Kremr, Cocovi-Solberg, Bajerová, Ventura, & Miró, 2015; Morishita & Ohnishi, 2001; Shin et al., 2015). To date, 5-CQA is thought to be the most abundant among the various isomers (Wang, Clifford, & Sharp, 2008).

Chlorogenic acids are known for being bioactive compounds that have antioxidant, antimicrobial, antibacterial, antiviral, anti-inflammatory properties and may be responsible for preventing the risk of chronic and cardiovascular diseases, as reported by several *in vivo* and *in vitro* studies (Bajko, Kalinowska, Borowski, Siergiejczyk, & Lewandowski, 2010; Butiuk, Martos, Adachi, & Hours, 2016; García-Tirado, Rieger-Reyes, & Saz-Peiró, 2012; Jiang, Wei, & He, 2015; Liu et al., 2014; Peng, Zhu, Zhong, Xu, & Wang, 2015). Peng et al. (2015) reported that chlorogenic acids can alter the metabolism of glucose and maintain homeostasis. It is also possible that using chlorogenic acids has a positive effect on the prevention of diabetes and in the treatment of its symptoms (Bagdas et al., 2015). These acids may also play roles in the absorption of glucose (Wang et al., 2008) and in protecting endothelial cells from oxidative stress (Jiang, Hodgson, Mas, Croft, & Ward, 2016). They also have anti-glycation activity (Bhattacherjee & Datta, 2015) and may protect neurons from the toxic effect of glutamate (Mikami & Yamazawa, 2015).

Previous studies have reported that chlorogenic acids can influence the treatment of liver diseases. Shi et al. (2016) suggested that these compounds protect the liver against the formation of CCl_4-induced fibroses by decreasing oxidative stress. Wu et al. (2015) orally administered chlorogenic acid doses to rats and observed decreased cholestasis-induced swelling and liver fibrosis, whereas Zheng, Sheng, Lu, and Ji (2015) demonstrated the detoxifying capacity of chlorogenic acid in liver damage caused by paracetamol. In addition to other effects associated to the liver, Wang et al. (2009) also reported the ability of chlorogenic acid to inhibit the duplication of Hepatitis B virus.

Chlorogenic acids have been correlated to lipid metabolism and to a positive interference in the absorption of cholesterol (Arantes et al., 2016). Cho et al. (2010) observed that some of these compounds inhibit the biosynthesis of cholesterol, which also suggests chlorogenic acid has anti-obesity potential in rats with high-fat diets. Gugliucci and Bastos (2009) concluded that chlorogenic acids improve protection regarding the activity of the enzyme paraoxonase, which is part of the oxidation mechanism of LDL and HDL. De Morais et al. (2009) showed that consuming yerba mate (*Ilex paraguariensis*), which has a high content of chlorogenic acids, improved the lipid parameters of the studied subjects and resulted in decreased LDL levels.

Brazil's large available biodiversity is consistent with its popular tradition of using plants in the preparation of aqueous infusions,

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Table 1
Figures of merit for the validation of the method for analysis of chlorogenic acids in plants through ultra-high performance liquid chromatography.

Parameters		Compounds					
		Caffeic acid	5-CQA	4-CQA	3,4-DQA	3,5-DQA	4,5-DQA
Linear range ($mg{\cdot}L^{-1}$)		0.05 a 35.0	0.04 a 35.0	0.05 a 35.0	0.05 a 35.0	0.04 a 35.0	0.04 a 35.0
Lack of fit test (F)(1)		1.01	2.31	1.70	2.42	2.58	2.82
Precision on the day in relative standard deviation (n = 10)	Level 1	5.31	5.36	5.33	5.81	5.72	5.99
	Level 2	2.26	2.31	2.45	2.19	2.23	2.21
	Level 3	2.72	2.70	2.76	2.82	2.93	3.02
Precision between days in relative standard deviation (n = 3)	Level 1	0.22	0.78	0.42	0.23	0.46	0.64
	Level 2	0.28	0.08	0.57	1.12	1.62	2.08
	Level 3	0.52	0.68	0.87	0.33	0.05	0.41
Limit of quantification (mg·L-1)		0.05	0.04	0.05	0.05	0.04	0.04

(1): The model has proper fit as long as calculated F is less than critical F (3.49). Compounds: 3-CQA - 3-caffeoylquinic acid, 4-CQA - 4-caffeoylquinic acid, 5-CQA - 5-caffeoylquinic acid, 3,4-DQA - 3,4-dicaffeoylquinic acid, 3,5-DQA - 3,5-dicaffeoylquinic acid, 4,5-DQA - 4,5-dicaffeoylquinic acid.

popularly known as tea. Taking in consideration the chlorogenic acids potential regarding health effects, the great plant diversity used in Brazil and the lack of information concerning the content of these compounds, this study aims to identify and quantify the chlorogenic acids (3-CQA, 4-CQA, 5-5-CQA, 3,4-DQA, 3,5-DQA, 4,5-DQA and caffeic acid) contents of 100 plants that are traditionally used in Brazil, in order to make them more present in the diet.

2. Materials and methods

2.1. Samples and reagents

The plants were purchased dry from three different suppliers in public markets and fairs in the municipalities of Campinas and Americana, São Paulo state, Brazil. Forty to 200 g were purchased from each supplier. The samples are commercialized in dry form, which is how they are usually consumed, therefore all the plants were analysed without further process of drying to maintain the original content of chlorogenic acids.

Standards of cafeic acid, 4-CQA, 5-CQA, 3,4-DQA, and 4,5-DQA were purchased from Biopurify (Chengdu, China). No commercial standard was found for 3-CQA. Its identity was confirmed by electrospray ionization mass spectrometry in negative ion mode (Thermo, USA) from a yerba mate sample (*Ilex paraguariensis*); its chromatographic behaviour and fragmentation was previously characterized by Bravo, Goya, and Lecumberri (2007). Stock solutions of the standards were prepared in methanol (J.T. Baker, Brazil) at a concentration of 1 $mg{\cdot}mL^{-1}$ and were stored at − 80 °C. Formic acid was obtained from Merck (Brazil); acetonitrile was obtained from JT Baker (Brazil); and ethanol F.A. was obtained from Synt (Brazil). The water that was used in the experiments was ultra-purified in a Milli-Q® system (Millipore, USA). All solutions were filtered through PVDF membranes with a 0.22-μm pore size (Millipore, USA).

2.2. Sample preparation

The sampling was conducted by weighing approximately 40 g of sample from each supplier, which was then were homogenized and crushed in a hammermill (Marconi TE 600, Brazil) with a 200 mesh sieve. The hardest samples, such as bark, thalli, stems, and tubercles, were reduced in size and cut with a knife before being crushed.

To extract the chlorogenic acids, 0.5 g of the crushed sample was weighed in lid tubes, and 15 mL of extracting solution was added (74% water: 26% ethanol), the tubes were then sealed and heated in a *water bath* at 60 °C under agitation at 240 rpm (rotations per minute) for 22 min. The volume was then measured to − 100 mL, filtered through a PVDV membrane with a 0.22-pore size, and injected into the chromatographer.

2.3. Method for the analysis of the chlorogenic acids

The employed analytical method was based on a study by Da Silveira, Meinhart, De Souza, Teixeira Filho, and Godoy (2016), with adaptations. The ultra-high performance liquid chromatography was performed using an Acquity® UPLC (Waters, USA) with a diode array detector, a ternary pump, an automated injector, and a controlled column oven at 30 °C. We used a C18 column (Kinetex, code number 00D-4475-AN) with a length of 100 mm, an internal diameter of 2.1 mm, and a particle size of 1.7 μm (Phenomenex, USA). The mobile phase was water acidified with formic acid (0.1%, pH 2.4) (solvent A) and acetonitrile (solvent B). The elution was conducted with a linear gradient that started at 100% A and decreased until it reached 92% A at 10 min; subsequently, it decreased until it reached 70% A at 14 min and then held constant for another minute. To clean the column, a linear gradient from 70% to 0% A was applied between minute 15 and 16, which was held for an additional minute. From minute 17 to 23, the column was reconditioned to 100% A. The flow rate was 0.3 $mL{\cdot}min^{-1}$, and the injection volume was 10 μL. The identification of the

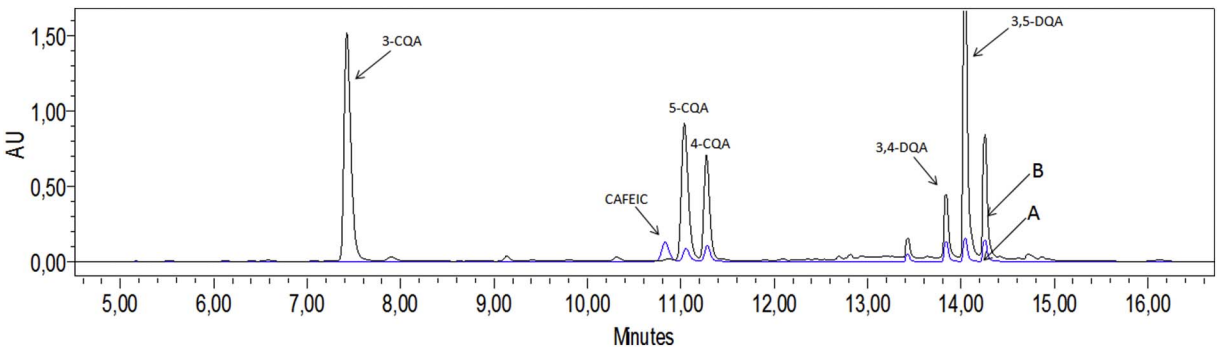

Fig. 1. Chromatograms of analytical standards (A) and yerba mate (*Ilex paraguariensis*) (B) sample. Chromatographic conditions described in the text.