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Study of the chlorogenic acid content in yerba mate (*Ilex paraguariensis* St. Hil.): Effect of plant fraction, processing step and harvesting season

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ABSTRACT

Chlorogenic acid (CGA) is a fine chemical used in different food and pharmaceutical industries. CGA is currently extracted from various plant materials, particularly green coffee beans. Yerba mate (*Ilex paraguariensis* St. Hil.) contains significant amounts of CGA with very low levels of extraction interfering substances (fatty materials). Both of these reasons prompted us to evaluate yerba mate as a novel source for extraction of this compound.

CGA content was quantified in various yerba mate fractions during different processing steps, and in samples taken from two companies at early and late harvesting seasons. Samples were exhaustively extracted with hot water and total CGA content (including its three isomeric compounds) was determined by HPLC. Total CGA content (on a dry weight basis) ranged from 45.8 ± 0.4 to 80.8 ± 1.0 g CGA kg^{-1} of leaves and from 31.6 ± 0.6 to 78.9 ± 5.3 g CGA kg^{-1} of stems. A substantial reduction in CGA content was found along the processing steps. The highest CGA content was found in samples from freshly harvested (green) yerba mate, for both leaves and stems; with no significant differences in their CGA content ($P < 0.05$). CGA content at the early harvesting season was substantially higher to that obtained at the end of the harvesting season, for both green leaves and stems.

Green stems, a residue from yerba mate processing obtained at the early harvesting season, could be considered as a promising and valuable raw material for the production of CGA-enriched extracts.

1. Introduction

Structurally, chlorogenic acid (CGA, CAS number: 327-97-9) is an ester formed between caffeic and L-quinic acids (5-*O*-caffeoyl-quinic acid) (Fig. 1). Isomers of CGA include 3-*O*-caffeoyl-quinic acid (neochlorogenic acid, neo-CGA) and 4-*O*-caffeoyl-quinic (cryptochlorogenic acid, crypto-CGA). CGA is a kind of polyphenol derivative widely present in higher plants (Delage et al., 1991) that is extensively used in medicine and industries such as in consumer chemicals and food industries (Kweon et al., 2001). It is employed as additive in beverages, cosmetics, tea products and foods as well as medical substances (Jiang et al., 2000; Jin et al., 2005). Potentially beneficial properties to humans such as antioxidant, hypoglycaemic, antiviral and hepatoprotective activities have been also attributed to CGA in *in vitro*, *in vivo* and epidemiological studies (Farah and Donangelo, 2006).

CGA is currently available in the international market both as analytical grade reagent, as well as food grade and bulk product, being considered within the category of Fine Chemicals. Current FOB price of CGA (food grade, bulk product, 95% w/w purity) is estimated in around US$ 3500 per kilogram (personal communication from a trader, 2014). Its present commercial sources are from plant extracts of *Lonicera japonica* Thunb and *Eucommia ulmoides* Oliver (Chun and Kim, 2004; Clifford et al., 2006; Li et al., 2005; Rønsted et al., 2002). These sources are generally limited and therefore expensive. CGA is also present in relative high concentrations in other plant resources, such as apples, pears and potato tubers, and mainly in coffee berries, particularly in green (or raw) coffee beans, material which is considered to date as the most important CGA natural source (Clifford, 1999, 2000). Green coffee beans are

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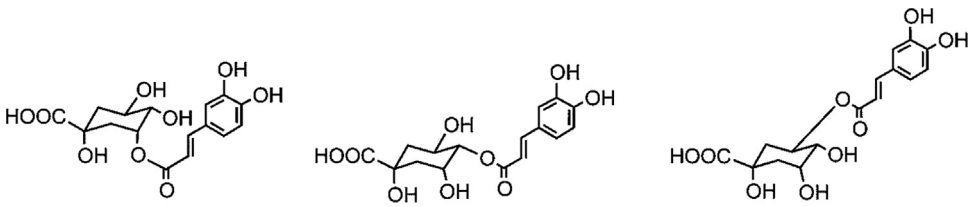

Fig. 1. Chemical structure of chlorogenic acid and its isomeric compounds.

basically just unroasted coffee beans. Total CGA content of green coffee beans may vary according to genetics—species and cultivar, degree of maturation and, less importantly, agricultural practices, climate and soil (Farah and Donangelo, 2006). The diversity of methodology employed in the analysis of CGA is another important factor when comparing levels. Depending on the species, green coffee beans contain some 6–10% CGA on a dry weight basis. Due to thermal instability, CGA is degraded into low molecular weight phenol derivatives during coffee roasting, resulting in a progressive destruction and transformation of CGA with some 8–10% being lost for every 1% loss of dry weight (Clifford, 1999, 2000).

Ilex paraguariensis St. Hil. (Aquifoliaceae) (yerba mate) is an arboreal species that naturally grows and is cultivated in the temperate and subtropical climatic regions of Argentina, Brazil, and Paraguay. Argentina is the main producer of yerba mate (≈60% of the worldwide production accounting for 750,000 ton/year in average). Yerba mate is traditionally employed as a decoction or infusion due to its nutritional and medicinal properties (tonic, choleretic, diuretic, anti-rheumatic, etc.). Values as high as 8–10% (w/w) CGA (including its various isomeric compounds) on a dry weight basis have been reported for different yerba mate derived materials (Isolabella et al., 2010; Marques and Farah, 2009; Pagliosa et al., 2010). Therefore, yerba mate could be considered as a completely new, still untapped and highly competitive source for CGA extraction.

The production process of yerba mate involves harvesting of the leaves and stems, which are roasted, dried over fire, minced, aged, milled, and packed for their commercialization. Processing may vary among industries although the procedure is basically the same. Fig. 2 shows a typical process flow chart for yerba mate (Schmalko and Alzamora, 2001).

During the industrial processing steps of yerba mate (harvesting, roasting, drying, and aging) some changes in the profile and concentration of bioactive compounds may be produced (López et al., 2006). Only a few recent studies have been carried out to assess the changes that occur in yerba mate during its processing. Bastos et al. (2006) and Isolabella et al. (2010) have studied CGA variation, among other compounds, of yerba mate leaves along the different processing steps. Nevertheless, to date there is no detailed information of the CGA content in the different fractions of yerba mate (particularly leaves and stems) during its industrial processing and at different harvesting seasons.

Scientific information available on yerba mate includes studies on its composition, physiological effects, and potential health implications as well as technological considerations for its processing (Heck and Gonzalez de Mejia, 2007). Nevertheless, no examples about yerba mate utilization as raw material for bio-transformations have been described. A novel strategy for industrial production of various useful materials of pharmaceutical significance from our traditional yerba mate is under development. This strategy is based on its high CGA content, comparable to that in coffee products, but with a lower level of extraction interfering substances, particularly fatty materials. Therefore, yerba mate seems to be a quite special and unique source of CGA, which can be bio technologically converted into value added compounds of pharmaceutical significance. For instance, CGA can be readily converted to shikimate via quinate, 3-dehydroquinate and 3-dehydroshikimate (Adachi et al., 2006a,b,c, 2008). Shikimate is important as the direct precursor for Oseltamivir synthesis (Roche's brand name Tamiflu®), the potent and selective competitive inhibitor of influenza A and B neuraminidase (Enserink, 2006) preventing people from pandemic flu infection, as well as for the synthesis of antibiotics, amino acids and agrochemicals.

According to the previously described, we investigated the CGA content, in both leaves and stems, along the different processing steps in two yerba mate companies at the beginning and at the end of the harvesting season. This information is mandatory for selecting the most adequate yerba mate fraction as raw material for the preparation of CGA-enriched aqueous extracts for subsequent CGA-biotransformation into valuable products.

2. Materials and methods

2.1. Yerba mate materials

Samples of fresh (just harvested) yerba mate branches and processed yerba mate were supplied by two yerba mate processing plants (Plant A and Plant B) located in Apóstoles, Misiones, Argentina. Collection and storage of the plant material were carried out under strict controlled conditions. Plant material was mechanically harvested during the beginning (April and May, 2014) and at the end of the harvesting season (September 2014). Analysis was carried out on samples taken from the same lot (≅10 ton) during the following processing steps:

i) Harvesting (samples labeled H): samples of fresh yerba mate branches (≅20 kg, randomly chosen) just harvested were conveniently cut into pieces and treated in our laboratory in a microwave oven at maximum power (700 W) for 5 min to inhibit enzymatic activity.
ii) Roasting (R, locally called zapecado): in this stage, green yerba mate branches are exposed to direct fire at temperatures between 250 and 550 °C during 2–4 min depending on processing plant to inactivate oxidizing enzymes. Samples of yerba mate branches (≅20 kg, randomly chosen) were analyzed.
iii) Drying (D): in both processing plants it was performed employing a continuous belt system. In the case of Plant A drying consisted of the following sub-steps: pre-drying (PD) (250 °C for 7 min), first drying belt (1st D) and second drying belt (2nd D) (105–120 °C for 1.5 h). In Plant B drying was performed at 90 °C during 5 h. Samples (≅20 kg, randomly chosen from each drying process) of yerba mate branches were analyzed. In addition, in the case of Plant B, samples taken after coarse milling were also analyzed. In these cases (processing steps i, ii and iii),