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Increasing the antioxidant power of tea extracts by biotransformation of polyphenols

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ABSTRACT

Green tea (*Camellia sinensis*) and yerba mate (*Ilex paraguariensis*) are rich in polyphenolic compounds, which are thought to contribute to the health benefits of tea. The aim of this study was to evaluate the potential antioxidant properties of green tea and yerba mate extracts before and after the enzymatic biotransformation reaction catalysed by the *Paecilomyces variotii* tannase. The antiradical properties of the tea extracts, as well as the standards of chlorogenic acid and EGCG, were assessed using the ORAC and DPPH assays before and after the tannase biotransformation. The antioxidant power of enzyme-treated green tea and yerba mate increased by 55% and 43%, respectively, compared with that of untreated teas. The antioxidant power of the standards was also highly increased by enzyme treatment. These results provide relevant data about the potential of the tannase application on various polyphenol sources and to increase the antioxidant power of two widely consumed beverages.

1. Introduction

Tea is the second most widely-consumed beverage worldwide (after water) and is rich in polyphenolic compounds, known as tea flavonoids. Green tea contains several tea polyphenols, including epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) (Suganuma et al., 1999). These flavonoids (also known as catechins) possess strong antioxidant properties (Majchrzak, Mitter, & Elmadfa, 2004). Catechins have been proven to have antioxidant, antimutagenic, and anticarcinogenic properties, and they can also prevent cardiovascular diseases (Cao & Ito, 2004).

Yerba mate (*Ilex paraguariensis*) is a plant originally from the subtropical region of South America and is present in the south of Brazil, the north of Argentina, Paraguay and Uruguay. Mate beverages have been widely consumed for hundreds of years as infusions popularly known as chimarrão, tererê (both from green dried mate leaves) and mate tea (roasted mate leaves). Mate beverages are rich in polyphenolic compounds, which are mainly caffeoyl derivatives, such as dicaffeoylquinic and chlorogenic acids, saponins and purine alkaloids (Martins et al., 2009).

The considerable antioxidant potential of green tea and yerba mate has long been recognised and is dependent on many factors involved in tea preparation. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, singlet-oxygen quenchers and metallic-ion chelators (Atoui, Mansouri, Boskou, & Kefalas, 2005).

Despite the proven antioxidant capacity of tea polyphenols, many clinical studies and animal models have shown that these compounds, especially the polymers, esters, and glycosides, are abundant, but are not always absorbed by oral administration. The functional effect of the compound depends not only on the amount ingested, but on its bioavailability (Holst & Williamson, 2008). Therefore, the enzymatic hydrolysis of polyphenols from food is a subject worth investigating.

Tannin acylhydrolases, commonly referred to as tannases (E.C. 3.1.1.20), are inducible enzymes produced by fungi, yeast and bacteria. Tannases have mostly been characterised by their activity on complex polyphenolics, and are able to hydrolyse the "ester" bond (galloyl ester of an alcohol moiety), as well as the "depside" bond (galloyl ester of gallic acid) of substrates such as tannic acid, epicatechin gallate, epigallocatechin gallate, and chlorogenic acid (Fig. 1) (Garcia-Conesa, Ostergaard, Kauppinen, & Williamson, 2001).

In this paper, the activity of tannase on the extracts of green tea and yerba mate was investigated. The aim of this work was to study the potential antioxidant properties of extracts of green tea and yerba mate before and after an enzymatic reaction, catalysed by the tannase, produced by *Paecilomyces variotii* (Battestin, Pastore, & Macedo, 2005). The antiradical properties of these samples were assessed using the oxygen radical-absorbance capacity (ORAC) (Cao, Sofic, & Prior, 1996) and 2,2-diphenyl-1-picrylhydrazyl

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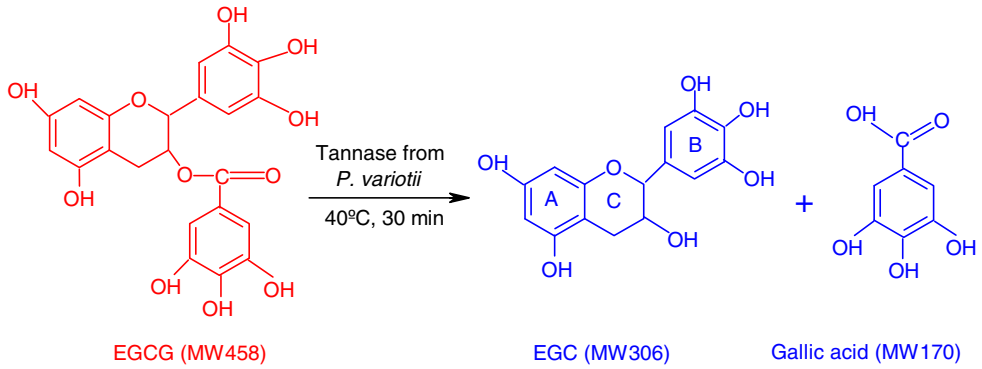

Fig. 1. Degalloylation of EGCG by the tannase from *Paeciloyces variotii*.

(DPPH) assays (Benzie & Strain, 1996; Bondet, Brand-Williams, & Berset, 1997). To date, the ORAC assay has been largely applied to the assessment of the free-radical scavenging capacity of human plasma, proteins, DNA, pure antioxidant compounds and antioxidant plant/food extracts (Dávalos, Goméz-Cordovés, & Bartolomé, 2004).

2. Material and methods

2.1. Reagents

Epigallocatechin gallate (EGCG, 95%), epigallocatechin (EGC, 98%), 2,2′- azobis (2-methylpropionamidine) (97%), and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals were purchased in the grade commercially available. The fluorescein was from ECIBRA, and the Trolox® (97%) was from ACROS Organics.

2.2. Enzyme

The tannase from *Paecilomyces variotii* was obtained according to a previously published procedure (Battestin & Macedo, 2007). A 250 ml conical flask containing 5 g of wheat bran, 5 g of coffee husk, 10 ml of distilled water and 10% tannic acid (w/w) (Ajinomoto OmniChem Division, Wetteren, Belgium) was used for the fermentation process. The culture medium (pH 5.7) was sterilised at 120 °C for 20 min. After sterilization, the flasks were inoculated with 2.5 ml (5.0×10^7 spores/ml) of the pre-inoculum suspension and incubated at 30 °C for 120 h. After fermentation, 80 ml of 20 mM acetate buffer at pH 5.0 was added and shaken at 200 rpm for 1 h. The solution was filtered and centrifuged at 9650*g* for 30 min at 4 °C (Beckman J2–21 centrifuge, Beckman-Coulter, Inc. Fullerton, CA, USA). The supernatant was then treated with solid ammonium sulphate (80% saturation) and stood overnight at 4 °C. The precipitate was collected by centrifugation (9650*g* for 30 min), resuspended in distilled water and dialysed against distilled water. The dialysed preparation was freeze-dried and used as crude tannase.

2.3. Sample preparation

2.3.1. Preparation of tea extracts for LC–MS analysis

The extraction of green tea (*Camellia sinensis*) and yerba mate (*Ilex paraguariensis*) (1 g) were performed with 20 ml of ethanol/water (50% v/v) and 20 ml of chloroform using a blender (Ultra-Turrax) for 5 min, according to the procedure described by De Freitas, Carvalho, and Mateus (2003). The 50%-ethanol upper aqueous layer was separated from the chloroform layer containing the chlorophylls, lipids and other undesirable compounds. The ethanol was removed using a rotary evaporator, before the resulting aqueous solution, containing catechins, was dissolved in acetate buffer (pH 6.0, 0.2 M) for identification of the compounds present.

2.3.2. Preparation of tea extracts for antioxidant assays

Fifty milliliter of distilled water and 250 mg of each sample of tea were combined in 125 ml Erlenmeyer flasks. The extraction of compounds from green tea and yerba mate was performed in a water bath at 100 °C for 30 min. After being filtered on filter paper, the extracts were freeze-dried. The resulting powder was called dried tea extract and used for antioxidant assays (Cao et al., 1996).

2.3.3. Polyphenol commercial standards

As an identified representative polyphenol from green tea, the commercial standard epigallocatechin gallate (EGCG, 95%) was used as a control sample, as was the chlorogenic acid (95%) from yerba mate tea. These samples were tested for antioxidant power (by DPPH and ORAC assays) and treated with tannase, using the same procedures that were employed on the tea extracts.

2.4. Enzymatic biotransformation

The extracts obtained from the green tea, yerba mate and the commercial control samples were used as substrates for enzymatic hydrolysis by tannase isolated from *Paecilomyces variotii* (Battestin, Macedo, & Freitas, 2008). The dried tea extract (5 mg) was dissolved in 1 ml of phosphate buffer (pH 7.4, 75 mM) and incubated with 5 mg of tannase at 40 °C for 30 min. The hydrolysis process was stopped by placing the reaction in an ice bath for 15 min. The biotransformed tea was used for the antioxidant assay after suitable dilution with the same phosphate buffer (pH 7.4, 75 mM) for ORAC and with a 70% methanol solution for DPPH.

2.5. LC–MS analysis

A Finnigan Surveyor-series liquid chromatograph, equipped with a 150 × 4.6 mm i.d., 5 μm LicroCART® (Merck, Darmstadt, Germany), reversed-phase C18 column maintained at 25 °C by a thermostat, was used. Mass detection was carried out using a Finnigan LCQ DECA XP MAX (Finnigan Corp., San José, CA, USA) mass detector with an API (atmospheric pressure ionisation) source of ionisation and an ESI (ElectroSpray ionisation) interface. The solvents used were formic acid in H_2O (1%, v/v) and acetonitrile. The capillary voltage was 4 V and the capillary temperature was 275 °C. The spectra were recorded in the positive-ion mode between 120 and 1500 *m/z*. The mass spectrometer was programmed to carry out a series of three scans: a full mass, a zoom scan of the most intense ion in the first scan, and a MS–MS of the most intense ion using relative collision energy of 30 and 60.