Polyphenol content, *in vitro* bioaccessibility and antioxidant capacity of widely consumed beverages

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ABSTRACT

BACKGROUND: Beverages prepared from antioxidant rich plants are sources of polyphenols, which bioactivity depends on bioaccessibility in the gastrointestinal tract. This work evaluated the polyphenol content and antioxidant capacity of widely consumed beverages such as chamomile tea, yerba mate, a coffee blend (65% roasted:35% green), and coffee-like substitutes such as chicory, malt and a soluble cereal mixture. Additionally, the bioaccessibility of the two beverages with the highest antioxidant capacity was evaluated using an *in vitro* digestion model.

RESULTS: Total phenolic contents ranged from 11.15 mg/200 mL in chamomile tea up to 154.53 mg/200 mL in mate or 215.05 mg/200 mL in the blend. These results correlated with the antioxidant capacity analysed by FRAP, ORAC and ABTS methods. Yerba mate and the coffee blend showed an average polyphenol recovery after *in vitro* gastrointestinal digestion of 57% and 78%, respectively. Although both beverages showed similar phenolic composition, polyphenols in coffee were more stable than in yerba mate. Alkaline pH in the intestinal digestion stage was responsible for the observed reduction in polyphenol stability.

CONCLUSION: Regular consumption of the studied beverages provides considerable amounts of antioxidants which are relatively stable after simulated digestion, and thus have the potential to prevent oxidative stress-related disorders.

KEYWORDS: yerba mate, green/roasted coffee blend, chamomile, chicory, malt coffee, phenolic content, antioxidant capacity, gastrointestinal bioaccessibility.

ABBREVIATIONS: AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; ABTS, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical scavenging capacity; TE, Trolox equivalents; TPTZ, 2,4,6-tripyridyl-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

INTRODUCTION

Increase of oxygen free radicals can cause molecular injury that may contribute to aetiopathogenic mechanisms involved in the development of important non-communicable diseases such as cancer, cardiovascular diseases or diabetes.¹ Intake of natural antioxidants may be a strategy to inhibit or delay oxidative damage. Accordingly, regular consumption of fruits, vegetables or beverages rich in antioxidant phytochemicals has been highly recommended. However, the bioavailability and metabolism of these antioxidants in the digestive system determine their beneficial health effects since changes in the chemical structure after digestion and absorption might affect their bioactivity.²⁻⁵ The bioavailability of a compound strongly depends on its bioaccessibility, i.e. the amount of a compound available for absorption in the gut. Bioaccessibility may be determined by using an *in vitro* digestion model that simulates the conditions in the digestive system, and thus assess the stability of food constituents throughout the gastrointestinal tract.⁶

Consumption of beverages prepared from plants, such as herbal teas, tisanes or coffee, is a common practice in many cultures due to the well-accepted taste and stimulant effects, along with their health-promoting benefits. Among these beverages is yerba mate, commonly consumed in South America. Prepared from dried leaves of *Ilex paraguariensis*, mate has become increasingly popular in other parts of the world due to its nutritional and medicinal properties, having been considered as a functional food.⁷ Chamomile is among the most ancient infusions, with daily consumption over one million cups around the world.⁸ Prepared from the dried flowers of *Matricaria chamomile*, chamomile's popularity is due to its therapeutic properties against fever, inflammation, gastrointestinal disorders or insomnia, even being considered as a medicine in the pharmacopoeia in 26 countries.⁹ Other popular herbal teas prepared from dried leaves or flowers are rosemary (*Rosmarinus officinalis*), Jamaica calices (*Hibiscus sabdariffa*), lime (*Tilia x europaea*), pennyroyal (*Mentha pulegium*), etc., although the most widely consumed herbal infusion is tea (*Camelia sinensis*), consumed either as non-fermented green tea, semifermented oolong tea or fermented black tea. A large body of scientific evidence supports the antioxidant, anti-carcinogen, anti-obesity, anti-depressant, and cardioprotective effects of tea¹⁰⁻¹¹. However, nowadays the most widely consumed beverage is coffee. Due to its flavour, aroma and stimulatory effects, over two billion cups of coffee are consumed daily. In spite of the adverse effects on blood pressure, related to content in caffeine, recent studies have evidenced that moderate coffee intake may have cardioprotective effects.¹²⁻¹³ In order to understand coffee's health benefits, attention has been focused on its phenolic composition, which drastically decreases with green coffee bean roasting.¹⁴⁻¹⁶ Therefore, the intake of green coffee products has increased in recent years as a healthier option to roasted coffee. Likewise, the intake of other coffee-like, caffeine-free brews, such as chicory or malt coffee with sensory attributes similar to those of natural coffee has increased. The former is prepared from dried and roasted chicory roots (*Chicorium intybus*) and has been widely used to treat various ailments, having become popular as a "wellness" product recently.¹⁷ On the other

hand, malt coffee is prepared from malted barley (*Hordeum vulgare*) and has also shown protective health effects against gastric ulcers, lowering the incidence of circulatory diseases.¹⁸

The biological activity associated with the intake of these beverages has been mostly attributed to their high content in phytochemicals, in particular polyphenols with important antioxidant capacity. The redox properties of polyphenols are the basis of their antioxidant capacity, being hydrogen donors, single oxygen quenchers or metal chelators.¹⁹⁻²⁰ In addition, these secondary plant metabolites exhibit a wide spectrum of health benefits such as anti-inflammatory, anti-carcinogenic, anti-diabetic or neuroprotective properties.²¹⁻²²

The main objective of this work was to determine the polyphenol content and antioxidant capacity of certain traditionally consumed infusions: chamomile tea (*Matricaria chamomile* L.), yerba mate (*Ilex paraguariensis* St-Hil.), a green/roasted coffee blend, and other coffee substitutes such as chicory (*Cichorium intybus* L.) and malt coffee (*Hordeum vulgare* L.) as well as a soluble cereal mix (Eko®). Additionally, the potential bioaccessibility of the two beverages with highest antioxidant capacity, coffee and yerba mate, was evaluated using an *in vitro* digestion model to evaluate the gastrointestinal stability of polyphenols in the selected beverages.

MATERIALS AND METHODS

Materials and reagents

Four different commercial brands (named mate A-D) of yerba mate (*Ilex paraguariensis* Saint Hilaire), a chamomile tea (*Matricaria chamomile* L.), a green/roasted coffee blend (35:65) (*Coffea arabica* L.), a chicory coffee (*Chicorium intybus* L.), a malt coffee (*Hordeum vulgare* L.), and a soluble cereals coffee mix Eko® (78.8% barley, malted barley and rye, and 19.7% chicory) were purchased in a local supermarket in Madrid (Spain). 3,5-dicaffeoylquinic acid was acquired from PhytoLab (Vestenbergsgreuth, Germany). 2,4,6-tripyridyl-s-triazine (TPTZ) and potassium persulfate were from Fluka (Madrid, Spain). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), fluorescein sodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 5-caffeoylquinic acid, caffeic acid, ferulic acid, gallic acid, rutin, pepsin from porcine gastric mucose (3,260 Units/mg protein) and pancreatic from porcine pancreas (activity equivalent to 4 x U.S.P.) were obtained from Sigma-Aldrich (Madrid, Spain). Folin-Cicalteau reagent, phosphoric, acetic and boric acid were from Panreac (Madrid, Spain). All other reagents were of analytical or chromatographic grade.

Sample preparation

Beverages were prepared according to manufacturer's instructions. 2.5 g of green/roasted coffee blend (G/R:35/65), 2.12 g of chicory coffee and 3.75 g of cereal mixture were dissolved in

240 mL of boiling water and shaken for 5 min. Chamomile and yerba mate tea infusions were prepared by brewing tea bags (1.1 g chamomile; 2.5 g yerba mate) for 5 min in 240 mL boiling water. Malt coffee was prepared by decoction of 7 g in 240 mL of water for 5 min. After cooling at room temperature, beverages were made up to 250 mL with water and filtered through filter paper. Beverages were prepared in duplicate and analyses were performed in duplicate in each replica.

An aliquot from mate (C) commercial brand and the green/roasted coffee blend was evaporated under a stream of nitrogen, dissolved in 1% formic acid in deionized water, filtered through 0.45 µm membrane filters and stored at -20 °C until chromatographic analysis.

Polyphenolic content

Total phenolic content of beverages was quantified spectrophotometrically at 750 nm using the Folin-Cicalteau reagent.²³ The test samples were mixed with the reagent, 75 g/L sodium carbonate solution and distilled water (1:1:20:28, v/v/v/v). After 1 h of incubation at room temperature (25 °C), the absorbance was measured in a Beckman DU-640 UV-Visible Spectrophotometer (Fullerton, CA, USA). Gallic acid was used as standard and results were expressed as mg gallic acid equivalent (GAE) per 200 mL of beverage, considered as a standard serving size.

Determination of antioxidant capacity

Ferric reducing antioxidant power (FRAP) assay: the method modified by Pulido *et al.*²⁴ was used to determine the reducing activity of the beverages. Briefly, FRAP reagent [10 mM TPTZ solution in 40 mM HCl, 20 mM FeCl₃ and 0.3 M acetate buffer pH 3.6 (1:1:3, v/v/v)] was mixed with distilled water, 0.3 M acetate buffer and test sample (6:3:20:1, v/v/v/v). Absorbance readings were monitored at 595 nm for 30 min at 37 °C using a Beckman DU-640 UV-Visible spectrophotometer. Solutions of known Trolox concentrations in the range of 50 to 1000 µmol/L were used for calibration and results were expressed as µmol Trolox equivalent (TE) per 200 mL of beverage.

ABTS assay: the ABTS method of Re *et al.*²⁵ was used. The ABTS radical cation was performed by reaction of ABTS with 2.45 mM potassium persulfate during 12-16 h at room temperature in the dark and diluted with methanol to get an absorbance value of 0.70 ± 0.02 at 730 nm. The change of absorbance at 730 nm in presence of the samples (1:39, v/v of sample and free radical, respectively) was recorded for 6 min in a Beckman DU-640 UV-Visible spectrophotometer. Trolox was used as a standard in the range of 50 to 1000 µmol/L and results were expressed as µmol TE per 200 mL of beverage.

Oxygen radical scavenging capacity (ORAC) assay: this method is based on the fluorescence decay of a reference substance, fluorescein, after the addition of a peroxy radical (AAPH), which acts as an initiator of the oxidative reaction.²⁶ Samples were mixed with 8.5x10⁻⁵

mM fluorescein in 75 mM phosphate buffer pH 7.4 and 153 mM AAPH (1:6:1.2, v/v/v) in 96-multiwell and fluorescence ($\lambda_{\text{excitation}}$ 485 nm, $\lambda_{\text{emission}}$ 528 nm) was measured for 90 min at 37 °C in an automatized plate reader (Bio-Tek, Winooski, VT, USA). **Solutions of known Trolox concentrations in the range of 1.25 to 50 $\mu\text{mol/L}$ were used for calibration and results were expressed as $\mu\text{mol TE}$ per 200 mL of beverage.**

Evaluation of the digestive stability of yerba mate and green/roasted coffee blend

The digestive stability of polyphenols contained in yerba mate infusion and the green/roasted coffee blend was evaluated following the method described by Pereira-Caro *et al.*⁶ with slight modifications. Briefly, samples were mixed with 0.4 mg/mL pepsin from pig gastric mucosa and acidified to pH 2 with HCl. Then, samples were incubated in a shaking water bath for 2 h at 37 °C in the absence of light and under nitrogen atmosphere to simulate gastric digestion. After incubation, pH was adjusted to 7.5 with NaOH and 4 mg/mL of pancreatin from porcine pancreas in Britton-Robinson buffer (1 M phosphoric acid, 1 M acetic acid, 0.1 M boric acid in distilled water, 1:1:10:68, v/v/v/v) was added to simulate intestinal digestion. After 2 h at 37 °C under nitrogen atmosphere, samples were acidified to pH 2 with HCl to stop enzyme activity. Two different blank samples (i) without phenolic compounds and (ii) without enzymes were also incubated under the same conditions. Different aliquots were taken before and after gastric and intestinal digestions, centrifuged at 3400 g for 10 min, filtered (0.45 μm pore size) and kept at -20 °C until chromatographic analyses.

Chromatographic characterization of phenolic compounds in yerba mate and green/roasted coffee blend

Phenolic compounds in yerba mate and green/roasted coffee blend infusions were analyzed using an Agilent 1200 series liquid chromatographic system equipped with an autosampler, quaternary pump, diode-array detector (DAD) and simple quadrupole (sQ) mass spectrometer (Agilent Technologies, Waldrom, Germany). Samples (5 μL) were injected into a Superspher RP18 column (4.6 x 250 mm, 4 μm ; Agilent Technologies) protected with an ODS RP18 guard column. Elution was performed at a flow rate of 1 mL/min at 30 °C using a binary system consisting in 1% formic acid in deionized water (solvent A) and acetonitrile (solvent B). The solvent gradient changed from 10 to 20% solvent B in 25 min, to 25% solvent B in 10 min, to 35% solvent B in 10 min followed by 5 min maintenance and returning to initial conditions in 10 min. Chromatograms were acquired at 280, 320 and 360 nm. The MS was fitted to an atmospheric pressure electrospray ionization (ESI) source which operated in negative ion mode. Capillary voltage was set to 3500 V, with nebulizing gas flow rate of 12 L/h, drying gas temperature of 350 °C, and nebulizer pressure of 45 psig. Mass spectrometry data were acquired in scan mode (mass range m/z 100-1000). Data acquisition and analysis were carried out in an Agilent ChemStation.

Quantitative analysis of polyphenols in yerba mate and green/roasted coffee blend

An Agilent 1200 series liquid chromatographic system (Agilent Technologies) equipped with a quaternary pump, column oven, autosampler and diode-array detector (DAD) was used to quantify the identified polyphenols in yerba mate and green/roasted coffee blend by HPLC-MS before and after each digestion step. The chromatographic conditions (column, guard column, binary gradient, injection volume, etc.) were as described above. Standards 5-caffeoylquinic and 3,5-dicaffeoylquinic acids were used to quantify mono- and diacyl derivatives of hydroxycinnamic acids, respectively; caffeic acid was used to quantify other caffeic acid derivatives, and rutin to determine flavonol content.

Statistical analysis

Statistical analyses were carried out using the program SPSS (version 21.0, SPSS, Inc., IMB Company). Prior to analysis, data were tested for homogeneity of variances using Levene test; for multiple comparisons, one-way ANOVA was carried out followed by the Bonferroni test when variances were homogeneous or by the Games-Howell test when not. The significance level was $p < 0.05$. Results were expressed as mean $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

Total phenolic content and antioxidant capacity

The extended awareness on the importance of diet in disease prevention has raised the interest on traditional herbal teas and beverages as a source of antioxidants with potential health promoting properties. In the present work, the antioxidant capacity of chamomile, yerba mate, a green/roasted coffee blend, chicory, malt and a soluble cereal mixture was assessed in order to evaluate the relationship with their polyphenol concentration.

The total phenolic content determined by Folin-Ciocalteu assay (Table 1) varied considerably, ranging from 11.15 mg/200 mL in chamomile tea up to 154.53 mg/200 mL in yerba mate. The coffee blend was the beverage with the highest phenolic content, 215.05 mg/200 mL. These results are in agreement with data reported by Zielinski *et al.*²⁷ who showed that yerba mate and chamomile presented, respectively, the highest and the lowest phenolic content among 51 Brazilian teas made from various plant species. Moreover, the polyphenol concentration in chamomile tea was in line with values determined by Raal *et al.*²⁸ (8.3-69.1 mg/200 mL) who interestingly observed a higher phenolic content in infusions bought in pharmacies than in food supermarkets. Regarding the other beverages tested, the present results agree with those previously described in the literature^{27,29-30} and confirm that these beverages, particularly the coffee blend and yerba mate, are important sources of polyphenols.

The reducing power, measured by the FRAP assay, and the free radical scavenging capacity of the beverages determined by the ABTS and ORAC methods showed that the antioxidant

capacity of these beverages was related to their phenolic content, so that chamomile tea, chicory and malt coffees had the lowest antioxidant activity and the green/roasted coffee blend the highest (Table 1). Accordingly, the correlation coefficient between total phenolic content and antioxidant capacity determined by different assays was high ($R^2 = 0.92, 0.97$ and 0.94 for FRAP, ABTS and ORAC, respectively), which confirms the important contribution of the phenolic fraction to the antioxidant capacity of the beverages. The direct relationship between these parameters had already been reported for chamomile, yerba mate, green coffee, chicory and barley malt coffee.^{27,31-34} The slight differences in the phenolic content and antioxidant capacity among the four commercial brands of yerba mate here studied might be due to genetic or environmental factors that affect the biosynthetic pathways of polyphenol formation, as well as differences in procedures during mate production (e.g. drying temperature of mate leaves, presence of stems, etc.).³⁵ Interestingly, the soluble cereal mixture presented higher antioxidant capacity than the malt coffee, which might account for the contribution of hydroxycinnamic acids and alkylresorcinols in barley and rye, the former also containing flavanols and dimeric procyanidins³⁶.

Characterization of the phenolic composition of yerba mate and the green/roasted coffee blend

Based on the higher total phenolic content determined by Folin-Ciocalteu assay, yerba mate C and the green/roasted coffee blend were selected to evaluate their stability during *in vitro* gastrointestinal digestion. Firstly, the individual phenolic composition of the selected yerba mate and the green/roasted coffee blend was characterized by high performance liquid chromatography (HPLC-DAD). Most of the constituents in yerba mate infusion presented UV spectra with a maximum at 324-328 nm and a shoulder at 292-298 nm, characteristic of hydroxycinnamic acid derivatives which is a group of polyphenols formed by one or more hydroxycinnamic acids esterified with quinic or shikimic acids. The second group of phenolic compounds observed in yerba mate were flavonols, with a specific UV-spectra, showing two absorption maxima at 256-266 and 346-356 nm.³⁷ In the green/roasted coffee blend infusion, besides compounds with typical UV spectrum of hydroxycinnamic acid derivatives (λ_{max} at 324-328 and shoulder at 296 nm) a compound was detected with UV-vis spectrum showing two absorption maxima at 290 and 320 nm, typical of hydroxycinnamic acids conjugated with an amino acid.³⁸ In a second set of experiments, both infusions were analysed by high-resolution mass spectrometry using an LC-ESI-sQ instrument with ionization in the negative ion mode. Selected ion monitoring located a total of 16 phenolic compounds in both infusions. Tables 2 and 3 include the chemical characterization of the identified polyphenols classified according to peak elution order, retention time, UV absorption maxima, quasimolecular ion, fragment ions and tentative nomenclature.

Polyphenols characterized in yerba mate tea: MS analysis showed three peaks (1, 3 and 4) with parent ion at m/z 341 in negative mode. Additionally, compounds 3 and 4 presented a

fragment ion at m/z 281 which is characteristic of caffeoyl-glucosides.³⁹ These compounds were assigned as caffeoylglucose which have been previously described in yerba mate (Table 2).^{39,40}

Three major compounds (peaks **2**, **5** and **6**) showed a MS spectrum with quasimolecular ion at m/z 353, corresponding to caffeoylquinic acid. Compound **5** was positively identified as 5-caffeoylquinic acid based on the commercial standard. The fragment ion at m/z 173 derived of the dehydration of quinic acid moiety clearly indicates substitution at position 4;⁴¹ therefore, MS spectrum of peak **6** with this characteristic ion allowed its identification as 4-caffeoylquinic acid. Peak **2** gave two fragment ions at m/z 179 and 191, corresponding to deprotonated caffeic and quinic acids, respectively, being assigned as 3-caffeoylquinic acid (Table 2).⁴¹

Peaks **7-9** presented a common quasimolecular ion at m/z 367. Compound **7** showed a fragment ion at m/z 193 corresponding to deprotonated ferulic acid, which allowed its identification as 3-feruloylquinic acid. Compound **8** presented a fragment ion at m/z 173, suggesting the presence of a substituent at position 4 of quinic acid being, therefore, assigned as 4-feruloylquinic acid, whereas compound **9**, with a fragment ion at m/z 191 corresponding to deprotonated quinic acid, was identified as 5-feruloylquinic acid (Table 2).⁴¹

Chromatographic peak **10** showed the quasimolecular ion at m/z 335 and no fragment ions, being identified as caffeoylquinic lactone based on the literature.³⁹⁻⁴⁰ Lactones result from the dehydration of caffeoylquinic acids during thermal treatments and are likely formed in mate during drying of the fresh leaves (Table 2).

Three dicaffeoylquinic acid isomers (peaks **13**, **15** and **16**) were identified by their quasimolecular ion at m/z 515 in negative mode along with their fragment ion at m/z 353, originated from the loss of one caffeoyl residue. Peak **15** was positively identified as 3,5-dicaffeoylquinic acid by comparison with a commercial standard. Based on the elution order for these compounds previously described in the literature,⁴² compounds **13** and **16** were assigned as 3,4- and 4,5-dicaffeoylquinic acids, respectively (Table 2).

Finally, three compounds (**11**, **12** and **14**) had a UV spectrum compatible with flavonols, characterized by two absorption maxima at 256-266 and 346-356 nm. The first (**11**) was positively identified as rutin by comparison with the commercial standard and its deprotonated parent ion at m/z 609. The parent ion at m/z 463 in negative mode along with the fragment ion at m/z 301, derived from the loss of a sugar moiety, allowed confirming that peak **12** was a quercetin-glycoside.²³ At last, compound **14** was assigned as kaempferol-rhamnoglucoside based on its deprotonated parent ion at m/z 593 (Table 2).^{23,37}

Polyphenols characterized in the green/roasted coffee blend brew: Eight compounds (peaks **1'-4'**, **7'** and **11'-13'**) (Table 3) showed UV and MS spectra coincident with those described above for yerba mate infusion, permitting their identification as 3-caffeoylquinic acid (**1'**), 5-caffeoylquinic acid (**2'**), 4-caffeoylquinic acid (**3'**), 3-feruloylquinic acid (**4'**), 4-feruloylquinic acid (**7'**), 3,4-dicaffeoylquinic acid (**11'**), 3,5-dicaffeoylquinic acid (**12'**) and 4,5-dicaffeoylquinic acid (**13'**) (Table 3).

Additionally, two compounds (peaks **5'** and **8'**) with parent ion at m/z 367 and fragment ion at m/z 191, were characterized in the green/roasted coffee blend beverage. Their MS spectra were compatible with feruloylquinic acids, although the only fragment ion at m/z 191 hindered identifying the substituted position in quinic acid.

Peak **6'** at 13.76 min had a parent ion at m/z 335 similar to peak 10 in yerba mate, also lacking fragment ion and with similar UV-Vis spectra. However, differences in the retention time show both peaks being different compounds. Indeed, caffeoylshikimic acid has been recently reported in green coffee beans by LC-QTOF,⁴³ supporting the identification of peak **6'** as caffeoylshikimic acid. Likewise, peaks **9'** and **10'** also had a quasimolecular ion at m/z 335. Despite similarities with caffeoylshikimic acid in the m/z value, the presence of a fragment ion at m/z 161 in both MS spectra, originated by the loss of a lactone moiety, allowed their identification as caffeoylquinic lactones⁴⁴ formed during coffee roasting (Table 3).⁴⁵

Two peaks (**14'** and **15'**) presented a common quasimolecular ion at m/z 529, corresponding to caffeoyl-feruloylquinic acid isomers.⁴² The only fragment ion at m/z 353 in MS spectrum of compound **15** was insufficient to identify the specific isomer (Table 3).

Finally, compound **16'** had a UV spectrum typical of hydroxycinnamic acid conjugated with tryptophan, showing two maxima at 290 and 320 nm. In agreement with its parent ion at m/z 365 in negative ion mode and data previously reported by other authors,^{38,46} this compound was identified as caffeoyl-*N*-tryptophan (Table 3).

Quantification of phenolic compounds in yerba mate and green/roasted coffee

The green/roasted coffee blend and yerba mate C were further analysed by HPLC-DAD to quantify their individual phenolic composition. Both beverages showed a similar total content of polyphenols, about 74 mg/g (Table 4), in contradiction with the phenolic content quantified by the Folin method (Table 1) where coffee showed a higher total concentration of polyphenols. This may be due the colorimetric method being a rather unspecific procedure that overestimates the real content of phenolic compounds in foods, although it is commonly used in the literature.

Despite the similar total phenolic content, important differences in the major groups of phenolic compounds in the mate and coffee drinks were observed. Thus, although in both beverages caffeoylquinic acids were the predominant phenols (50.1 mg/g in mate vs. 51.2 mg/g in coffee), 5-caffeoylquinic acid was the major hydroxycinnamic acid in coffee while the 3-isomer was more abundant in yerba mate. Another important difference was the content in feruloylquinic acids, amounting to only 1.457 mg/g in mate whilst coffee had a much higher concentration (up to 10.365 mg/g). In contrast, yerba mate was richer in dihydroxycinnamoylquinic acids (12.917 mg/g vs. 9.365 mg/g in the green/roasted coffee blend) (Table 4). Lactones were more abundant in coffee, which also contained caffeoylshikimic acid and caffeoyl-*N*-tryptophan, absent in mate. In turn, yerba mate contained minor amounts of

caffeoylglucose isomers (1.447 mg/g) as well as flavonols (up to 7.368 mg/g) that were not present in coffee.

Assessment of digestive stability of phenolic compounds in yerba mate and green/roasted coffee

The biological activity of a compound is partly determined by its chemical stability in the gastrointestinal tract. Therefore, evaluating its digestive stability is the first step to estimate the amount of a phytochemical that might be available for absorption in the intestine. In order to evaluate the stability of the main polyphenols of yerba mate and green/roasted coffee blend infusions during simulated digestion a double incubation with pepsin and pancreatin, the main enzymes involved in human gastric and intestinal digestions, respectively, was performed. Table 5 shows the amount of individual compounds recovered after each digestion step as a percentage of the total initial phenolic content.

As a whole, no new compound was observed after comparison of chromatographic profiles before and after gastrointestinal digestion. Moreover, neither caffeic or ferulic acids were detected after gastrointestinal digestion of yerba mate and green/roasted coffee blend, indicating that hydroxycinnamate esters are not hydrolysed by digestive enzymes. In agreement, previous studies with soluble coffee did not show the presence of free caffeic and/or ferulic acid or the formation of new compounds after *in vitro* digestion.^{29,47}

At least 90% of the initial amount of polyphenols in yerba mate and green/roasted coffee blend beverages were recovered after gastric digestion (Table 5, Figure 1), suggesting great stability of the phenolic compounds under conditions mimicking gastric digestion, as no differences were observed in the phenolic fraction of samples with and without enzymes (data not shown). Hydroxycinnamoylquinic acids were more stable than hydroxycinnamoylshikimic acids, tryptophan derivatives and lactone derivatives contained in green/roasted coffee blend, which showed recoveries of approximately 94-100%, 80%, 75% and 40%, respectively. Interestingly, caffeoylquinic lactone in mate showed a higher recovery (99%) compared to lactones in coffee (38-40%) (Table 5). Flavonols, present only in yerba mate, were highly stable after gastric digestion and were almost completely recovered (96% of the initial content). These data are in agreement with those described by Tagliazucchi *et al.*⁴⁷ who observed high stability of hydroxycinnamoylquinic acids at pH 2, with caffeoylshikimic acid, caffeoyl-*N*-tryptophan and, specially, caffeoylquinic lactones as the most unstable compounds in the phenolic fraction, reporting recoveries of 80, 70 and 35% regarding the initial levels, respectively.

After gastric digestion, samples were subjected to intestinal digestion with pancreatin at pH 7.5. Higher losses of phenols in both beverages were observed compared to gastric digestion, confirming the influence of alkaline pH (7.5) in the stability of the phenolic compounds as previously reported.^{6,47} Alike pepsin, pancreatin did not affect polyphenol stability since no differences were observed in samples incubated without enzyme compared with samples with

enzyme (data not shown). Oxidation of polyphenols to quinone derivatives is the possible biotransformation pathway followed by phenols in alkaline media, the absence of free caffeic and ferulic acids after intestinal digestion ruling out hydroxycinnamoylquinic acid hydrolysis. These data are in agreement with results previously reported in coffee⁴⁷ and 5-caffeoylquinic acid, the main polyphenol contained in both coffee and mate.⁴⁸

Dawidowicz & Typek⁴⁹ studied the influence of pH on the thermal stability of 5-*O*-caffeoylquinic acids in aqueous solutions, reporting up to fourteen different isomeric compounds (chlorogenic acid derivatives and their reaction products with water) at different pH values. Although the relative amount of the different compounds depended on time and pH, the predominant formation of 4-caffeoylquinic acid over 3-caffeoylquinic acid from 5-caffeoylquinic acid was key in its biotransformation pathway. In agreement with the results described by Dawidowicz and Typek⁴⁹, 4-caffeoylquinic acid was more abundant than 5-caffeoylquinic acid in both beverages after intestinal digestion, while 3-caffeoylquinic acid was similar and even higher than 5-caffeoylquinic acid in mate and coffee, respectively. This outcome might explain the relative amounts of the different dicaffeoylquinic acids isomers recovered after digestion of yerba mate (74% of 3,4-dicaffeoylquinic acid *versus* 43% and 46% of 3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic acids, respectively). However, the amount of the three dicaffeoylquinic acid isomers recovered in coffee was surprisingly high (> 100%). Regarding feruloylquinic acids, yerba mate showed a similar amount of the three identified isomers and recoveries in agreement with the rest of polyphenols, while coffee showed a relatively high amount of 3-feruloylquinic acid (108%) in comparison with the rest of isomers (58-77%) after the gastrointestinal digestion. The high recovery determined for 3-feruloylquinic acid in coffee could derive from the transformation of other feruloylquinic acid isomers at pH 7.5, as occurred with caffeoylquinic acids,⁴⁹ although this would be in disagreement with the results reported by Tagliazucchi et al.⁴⁷ who observed that this compound in soluble coffee was the least stable after *in vitro* digestion (up to 60% loss). This beverage also contains caffeoyl-feruloylquinic acids that were barely affected by the gastrointestinal digestion (recoveries of approximately 108%).

Recovery of the minor polyphenols (hydroxycinnamoylshikimic acids (55%) and caffeoyl-N-tryptophan (63%) in coffee, and flavonols (54%) in yerba mate) showed similar behaviour than other hydroxycinnamoylquinic acids. However, caffeoylquinic lactones in coffee were drastically affected (29-38% recovery), whereas the only lactone in yerba mate was slightly more stable (45% recovery) (Table 5).

The recovered amount of polyphenols in yerba mate and green/roasted coffee blend infusions after simulated gastrointestinal digestion was in line with other studies focussed on determining *in vitro* bioaccessibility of polyphenols in different food matrices, showing recoveries of approximately 40% of flavanones in orange juice,⁵⁰ 55% of quinic acid and up to 40% of hydroxycinnamoyl acid derivatives in different soluble coffees,²⁹ or up to 70% of flavonols in chokeberry juice.⁵¹ The variability observed between studies can be due to differences in the

gastrointestinal procedure followed (incubation time, pH conditions, etc.), in addition to differences in the type and chemical structure of the phenolic compounds. In general terms, polyphenols show acceptable stability in acidic pH, being more sensitive to alkaline conditions. Other nutrients in yerba mate and coffee such as fat, carbohydrates, proteins, vitamins and minerals, as well as other bioactive compounds such as methylxanthines, saponins, terpenes, etc. may have influenced polyphenol stability during the simulated gastrointestinal digestion. In fact, polyphenols contained in green/roasted coffee blend were slightly more stable than in yerba mate, especially 3-feruloylquinic acid and the dicaffeoylquinic acids (Table 5, Figure 1), showing higher bioaccessibility at the end of the digestion. These results are of great interest considering the health benefits associated to polyphenols, which can ameliorate other negative effects associated to the intake the coffee.¹³

In summary, this study shows that yerba mate and green/roasted coffee blend beverages are richer in phenolic compounds than chamomile tea, chicory coffee, malt coffee, and a soluble cereal mixture and, accordingly, show the highest antioxidant capacity. High stability of the predominant polyphenols (hydroxycinnamate esters) contained in yerba mate and green/roasted coffee blend drinks was recorded after an *in vitro* bioaccessibility study, showing the important influence of intestinal conditions (i.e. alkaline pH) on their stability. The fraction of polyphenols recovered after gastrointestinal digestion (57% and 78% in yerba mate and green/roasted coffee blend, respectively) would be available for intestinal absorption. Interestingly, in spite of the similar composition in hydroxycinnamoylquinic esters, a higher stability of polyphenols in coffee than in yerba mate was observed, pointing to the influence of other food ingredients on the stability of polyphenols. It may be concluded that regular consumption of coffee and yerba mate, as part of a healthy diet, provides important amounts of phenolic antioxidants relatively stable during digestion and thus these beverages may contribute to prevent oxidative stress-related disorders.

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Figure 1. Recovery of hydroxycinnamoyl esters in yerba mate (A) and green/roasted coffee (B) after *in vitro* gastric (open bars) and intestinal (dashed bars) digestion as percentage of the total initial compounds. CSAs: caffeoylshikimic acids; MonoCQAs: monohydroxycinnamoylquinic acids (including caffeoylquinic and feruloylquinic acids); DiCQAs: dihydroxycinnamoylquinic acids (including dicafeoylquinic acids and caffeoyl-feruloylquinic acids).

Table 1. Total phenolic content and antioxidant capacity of widely consumed beverages. Values are means $\pm$ SD (n = 2). Different letters within a column indicate statistically significant differences (p < 0.05).

	Total phenolic content	Antioxidant capacity		
	Folin method (mg GAE /200 mL)	FRAP (μ mol TE/200 mL)	ABTS (μ mol TE/200 mL)	ORAC (μ mol TE/200 mL)
Chamomile	11.15 $\pm$ 0.17 ^f	81.85 $\pm$ 3.81 ^h	66.54 $\pm$ 2.98 ^h	465.30 $\pm$ 46.45 ^g
Mate A	148.80 $\pm$ 4.60 ^b	1256.26 $\pm$ 81.03 ^c	786.12 $\pm$ 6.36 ^c	5164.32 $\pm$ 403.95 ^b
Mate B	135.02 $\pm$ 12.53 ^c	805.07 $\pm$ 77.46 ^e	556.91 $\pm$ 36.27 ^e	3984.07 $\pm$ 301.68 ^d
Mate C	137.58 $\pm$ 8.20 ^c	1165.00 $\pm$ 72.64 ^d	680.29 $\pm$ 47.30 ^d	4542.04 $\pm$ 445.13 ^c
Mate D	154.53 $\pm$ 5.78 ^b	1449.22 $\pm$ 66.44 ^b	859.46 $\pm$ 38.71 ^b	5368.46 $\pm$ 348.16 ^b
Green/roasted coffee blend	215.05 $\pm$ 12.05 ^a	1539.80 $\pm$ 75.47 ^a	1071.37 $\pm$ 26.78 ^a	6187.42 $\pm$ 473.26 ^a
Chicory coffee	56.23 $\pm$ 1.14 ^e	311.04 $\pm$ 12.90 ^g	290.55 $\pm$ 21.03 ^f	786.55 $\pm$ 74.82 ^{fg}
Malt coffee	54.49 $\pm$ 1.99 ^e	295.08 $\pm$ 10.45 ^g	204.42 $\pm$ 8.42 ^g	1098.52 $\pm$ 106.26 ^f
Soluble cereal mixture	72.62 $\pm$ 2.21 ^d	404.40 $\pm$ 13.04 ^f	296.81 $\pm$ 3.59 ^f	1544.12 $\pm$ 139.26 ^e

Table 2. LC-DAD-MS characterization of the main phenolic compounds in yerba mate infusion.

No.	RT (min)	λ_{max}	[M-H] ⁺	Fragment	Tentative compound
1	3.92	326, 292sh	341		Caffeoylglucose
2	4.99	324, 296sh	353	179, 191	3-Caffeoylquinic acid
3	6.27	324, 296sh	341	281	Caffeoylglucose
4	7.27	324, 296sh	341	281	Caffeoylglucose
5	8.05	326, 296sh	353	191	5-Caffeoylquinic acid
6	8.33	326, 296sh	353	173	4-Caffeoylquinic acid
7	8.66	324, 296sh	367	193	3-Feruloylquinic acid
8	14.28	326, 296sh	367	173, 193	4-Feruloylquinic acid
9	14.93	326, 296sh	367	191, 193	5-Feruloylquinic acid
10	15.45	328, 296sh	335		Caffeoylquinic lactone
11	21.31	256, 356	609		Rutin
12	22.75	256, 354	463	301	Quercetin-glycoside
13	25.21	324, 296sh	515	353	3,4-Dicaffeoylquinic acid
14	26.10	266, 346	593		Kaempferol-rhamnoglucoside
15	26.73	326, 296sh	515	353	3,5-Dicaffeoylquinic acid
16	30.38	326, 296sh	515	353	4,5-Dicaffeoylquinic acid

Table 3. LC-DAD-MS characterization of the main phenolic compounds in the green/roasted coffee blend beverage.

No.	RT (min)	λ_{max}	$[\text{M}-\text{H}]^-$	Fragment ions	Tentative compound
1'	4.97	324, 296sh	353	179, 191	3-Caffeoylquinic acid
2'	7.98	326, 296sh	353	191	5-Caffeoylquinic acid
3'	8.30	326, 296sh	353	173	4-Caffeoylquinic acid
4'	8.67	324, 296sh	367	193	3-Feruloylquinic acid
5'	13.11	326, 296sh	367	191	Feruloylquinic acid
6'	13.76	328, 296sh	335		Caffeoylshikimic acid
7'	14.20	326, 296sh	367	173, 193	4-Feruloylquinic acid
8'	14.81	324, 296sh	367	191	Feruloylquinic acid
9'	15.40	326, 296sh	335	161	Caffeoylquinic lactone
10'	16.40	328, 296sh	335	161	Caffeoylquinic lactone
11'	25.15	324, 296sh	515	353	3,4-Dicaffeoylquinic acid
12'	26.68	326, 296sh	515	353	3,5-Dicaffeoylquinic acid
13'	31.37	326, 296sh	515	353	4,5-Dicaffeoylquinic acid
14'	32.87	324, 296sh	529		Caffeoyl-feruloylquinic acid
15'	37.80	326, 296sh	529	353	Caffeoyl-feruloylquinic acid
16'	38.77	290, 320	365		Caffeoyl- <i>N</i> -tryptophan

Table 4. Quantification of phenolic compounds identified in the yerba mate and green/roasted coffee.

Phenolic Group	Compound	Mate Peak No.	Coffee Peak No.	Mate (mg/g)	Coffee (mg/g)
Hydroxycinnamic-glycosides	Caffeoylglucose	1		0.256 ± 0.016	
	Caffeoylglucose	3		0.476 ± 0.018	
	Caffeoylglucose	4		0.715 ± 0.031	
Monohydroxycinnamoyl-quinic acids	3-Caffeoylquinic acid	2	1'	22.055 ± 0.938	10.902 ± 0.759
	4-Caffeoylquinic acid	6	3'	10.871 ± 0.466	13.249 ± 0.544
	5-Caffeoylquinic acid	5	2'	17.167 ± 0.777	27.069 ± 2.437
	3-Feruloylquinic acid	7	4'	0.503 ± 0.032	2.187 ± 0.221
	4-Feruloylquinic acid	8	7'	0.232 ± 0.011	1.751 ± 0.117
	5-Feruloylquinic acid	9		0.722 ± 0.027	
	Feruloylquinic acid		5'		0.167 ± 0.009
	Feruloylquinic acid		8'		6.260 ± 0.465
Dihydroxycinnamoyl-quinic acids	3,4-Dicaffeoylquinic acid	13	11'	1.739 ± 0.154	2.892 ± 0.287
	3,5-Dicaffeoylquinic acid	15	12'	7.265 ± 0.567	2.294 ± 0.219
	4,5-Dicaffeoylquinic acid	16	13'	3.913 ± 0.349	3.134 ± 0.328
	Caffeoyl-feruloylquinic acid		14'		0.571 ± 0.053
	Caffeoyl-feruloylquinic acid		15'		0.474 ± 0.052
Hydroxycinnamoylquinic lactones	Caffeoylquinic lactone	10	9'	0.459 ± 0.016	1.149 ± 0.050
	Caffeoylquinic lactone		10'		0.546 ± 0.034
Hydroxycinnamoylshikimic acids	Caffeoylshikimic acid		6'		0.264 ± 0.021
Hydroxycinnamoyl-amino acids	Caffeoyl-N-tryptophan		16'		1.304 ± 0.108
Flavonols	Rutin	11		6.030 ± 0.330	
	Quercetin-glycoside	12		0.458 ± 0.023	
	Kaempferol-rhamnogluconide	14		0.880 ± 0.080	
TOTAL				73.741	74.213

Table 5. Bioaccessibility of phenolic compounds in yerba mate and green/roasted coffee after *in vitro* gastric (GD) and gastrointestinal (GID) digestion. Values are percentage with respect to the total initial phenolic content recovered after each step $\pm$ SD (n = 2).

Phenolic Group	Family	Compound	Peak No. Mate	Peak No. Coffee	GD (%)		GID (%)	
					Mate	Coffee	Mate	Coffee
Hydroxycinnamic-glycosides	Caffeoyl-glycosides	Caffeoylglucose	1		85.49 $\pm$ 5.43		53.58 $\pm$ 5.05	
		Caffeoylglucose	3		94.61 $\pm$ 0.96		60.47 $\pm$ 4.97	
		Caffeoylglucose	4		90.95 $\pm$ 2.65		53.86 $\pm$ 5.34	
Monohydroxycinnamoylquinic acids	Caffeoylquinic acids	3-Caffeoylquinic acid	2	1'	93.02 $\pm$ 0.85	100.98 $\pm$ 15.05	52.97 $\pm$ 3.22	75.23 $\pm$ 8.93
		4-Caffeoylquinic acid	6	3'	92.77 $\pm$ 0.87	98.32 $\pm$ 13.79	68.26 $\pm$ 5.03	76.59 $\pm$ 10.53
		5-Caffeoylquinic acid	5	2'	94.08 $\pm$ 4.10	94.87 $\pm$ 14.37	55.69 $\pm$ 3.04	57.19 $\pm$ 6.10
	Feruloylquinic acids	3-Feruloylquinic acid	7	4'	76.16 $\pm$ 5.72	114.03 $\pm$ 17.58	63.45 $\pm$ 6.87	107.66 $\pm$ 13.80
		4-Feruloylquinic acid	8	7'	102.63 $\pm$ 7.92	96.23 $\pm$ 11.46	67.44 $\pm$ 4.11	77.48 $\pm$ 7.53
		5-Feruloylquinic acid	9		90.14 $\pm$ 0.96		65.91 $\pm$ 3.42	
		Feruloylquinic acid		5'		101.57 $\pm$ 13.56		65.10 $\pm$ 7.42
		Feruloylquinic acid		8'		95.17 $\pm$ 10.82		57.96 $\pm$ 6.34
Dihydroxycinnamoylquinic acids	Dicafeoylquinic acids	3,4-Dicafeoylquinic acid	13	11'	91.04 $\pm$ 3.72	101.36 $\pm$ 2.08	73.76 $\pm$ 8.94	104.22 $\pm$ 15.22
		3,5-Dicafeoylquinic acid	15	12'	92.16 $\pm$ 3.08	99.97 $\pm$ 5.77	43.19 $\pm$ 2.27	114.16 $\pm$ 18.44
		4,5-Dicafeoylquinic acid	16	13'	91.48 $\pm$ 3.23	100.93 $\pm$ 5.54	45.69 $\pm$ 5.23	105.62 $\pm$ 20.23
	Caffeoyl-feruloylquinic acids	Caffeoyl-feruloylquinic acid		14'		107.20 $\pm$ 6.04		107.92 $\pm$ 13.26
		Caffeoyl-feruloylquinic acid		15'		103.93 $\pm$ 15.49		109.78 $\pm$ 22.26
Hydroxycinnamoylquinic lactones		Caffeoylquinic lactone	10	9'	98.83 $\pm$ 4.69	40.24 $\pm$ 9.70	44.90 $\pm$ 3.89	38.52 $\pm$ 8.28
		Caffeoylquinic lactone		10'		37.78 $\pm$ 8.15		29.29 $\pm$ 8.58
Hydroxycinnamoylshikimic acids		Caffeoylshikimic acid		6'		80.31 $\pm$ 10.73		55.21 $\pm$ 8.62
Hydroxycinnamoyl-amino acids		Caffeoyl-N-tryptophan		16'		74.37 $\pm$ 3.80		62.60 $\pm$ 12.77
Flavonols		Rutin	11		96.16 $\pm$ 2.27		53.86 $\pm$ 4.60	
		Quercetin-glycoside	12		103.07 $\pm$ 8.74		52.41 $\pm$ 3.72	
		Kaempferol-rhamnoglucoside	14		100.88 $\pm$ 1.14		56.82 $\pm$ 4.02	