Food Chemistry 169 (2015) 320–326

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Simulated gastrointestinal digestion, intestinal permeation and plasma protein interaction of white, green, and black tea polyphenols

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ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form 13 May 2014
Accepted 2 August 2014
Available online 10 August 2014

Keywords:
White tea
Polyphenols
Digestion
Caco2 cells
Plasma proteins

ABSTRACT

The gastrointestinal digestion, intestinal permeation, and plasma protein interaction of polyphenols from a single tea cultivar at different stages of processing (white, green, and black teas) were simulated. The salivary phase contained 74.8–99.5% of native polyphenols, suggesting potential bioavailability of significant amounts of antioxidants through the oral mucosal epithelium that might be gastric sensitive and/or poorly absorbed in the intestine. White tea had the highest content and provided the best intestinal bioaccessibility and bioavailability for catechins. Since most of native catechins were not absorbed, they were expected to accumulate in the intestinal lumen where a potential inhibition capacity of cellular glucose and cholesterol uptake was assumed. The permeated catechins (approximately, 2–15% of intestinal levels) significantly bound (about 37%) to plasma HDLs, suggesting a major role in cholesterol metabolism. White tea and its potential nutraceuticals could be effective in the regulation of plasma glucose and cholesterol levels.

1. Introduction

In spite of numerous data about the effects of green and black teas on human health, very little is known about white tea, which is the rarest and the least processed. White tea is, generally, composed only of the first white hairy leaves while green and black teas are the products of postharvest treatments that, along with genotype and growing conditions, influence the chemical content of the tea and its flavour, taste, and health characteristics (van der Hooft et al., 2012).

Flavan-3-ols, also known as catechins, constitute up to 30% of tea leaves dry weight. (−)-Epigallocatechin-3-gallate (EGCG) has been identified as the major polyphenol in both white and green teas, but (−)-epigallocatechin (EGC) and (−)-epicatechin-3-gallate (ECG), along with gallic acid, caffeine, and theobromine, are present at higher concentrations in white tea (Santana-Rios et al., 2001). EGCG is thought to be responsible primarily for many of the health benefits associated with tea consumption, including reduced oxidation (Mildner-Szkudlarz, Zawirska-Wojtasiak, Obuchowski, & Golinski, 2009) and inflammatory (Cao et al., 2007) processes, glucose/insulin regulation (Nishiumi et al., 2010) and lipid metabolism (Ikeda, Yamahira, Kato, & Ishikawa, 2010). The higher concentrations of several white tea constituents compared with black and green teas may be responsible for the apparent increase in biological activity (Santana-Rios et al., 2001). White tea has been reported to alleviate inflammation and rheumatoid arthritis more effectively than some green teas (Thring, Hili, & Naughton, 2009). Sõhle et al. (2009) showed white tea lipolytic activity in human subcutaneous (pre)-adipocytes and inhibition of adipogenesis.

To achieve a specific effect, polyphenols must be bioavailable, i.e. extracted from the food matrix and absorbed from the gut. In this sense, the term bioaccessibility has been defined as the fraction of a compound that is released from the food matrix in the gastrointestinal tract and, thus, becomes available for intestinal absorption (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Bioavailability is used to describe the proportion of the ingested compound that reaches the systemic circulation (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Digestion is a physiological process that allows the release of both nutrients and non-nutrients (e.g., polyphenols) from the food matrix (Hinsberger & Sandhu, 2004; Pedersen, Bardow, Jensen, & Nauntofte, 2002). In humans, the digestive process starts in the mouth where the initial degradation of polysaccharides and triglycerides takes place due to mastication and the action of salivary enzymes (α-amylase and lipase) (Hinsberger & Sandhu, 2004; Pedersen et al., 2002). Then, the food bolus is subjected to gastrointestinal (GI) digestion, where both

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http://dx.doi.org/10.1016/j.foodchem.2014.08.006
0308-8146/

digestive enzymes of the stomach and the small intestine, and colonic fermentative bacteria in the large intestine, are decisive for making nutrients and bioactive compounds available for the absorption through the intestinal wall (Hinsberger & Sandhu, 2004; Pedersen et al., 2002). These physiological conditions may result in structural changes that affect the stability, bioavailability and bioactivity of food constituents (Cilla, González-Sarrías, Tomás-Barberán, Espín, & Barberá, 2009). It has been demonstrated that digestion decreases the phenolic content by at least 47% in fruit beverages compared with pre-digestion (Cilla et al., 2009). Tagliazucchi et al. (2010), found only 62% of polyphenols originally present in grapes were bioaccessible after GI digestion, and radical-scavenging activities of polyphenols may be pH-dependent, suggesting a greater scavenging capacity in the intestine than in the stomach.

Polyphenols have a high affinity for proteins and bind to them by hydrophobic interactions, and hydrogen and covalent bonds (Brunet, Bladé, Salvadó, & Arola, 2002). However, the formation of protein–phenol complexes depends considerably on individual structures. EGCG binds to several human plasma proteins when serum is incubated *in vitro* with tea catechins (Brunet et al., 2002). Evidently, the processes of absorption and transport affect distribution of polyphenols to tissues, metabolism, and excretion and, despite considerable interest in polyphenols, little is known about these *in vivo*.

Thus, the aim of this work was to evaluate the bioaccessibility, bioavailability and plasma protein interaction of white tea polyphenols *in vitro* using a GI digestion model. GI digestion was categorised into salivary, gastric and duodenal digestion; Caco2 cell lines were used to explore intestinal transit; the interaction of white tea polyphenolic compounds with plasma albumins and lipoproteins after absorption was evaluated *in vitro*.

2. Materials and methods

2.1. Reagents and standards

All chemicals and reagents used were either analytical-reagent or HPLC grade. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA) before use. Standards used for the identification and quantification of phenolics were: C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechingallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechingallate; GC, (−)-gallocatechin; CG, (−)-catechingallate; TTF, total theaflavins from black tea (Sigma–Aldrich Co., St. Louis, USA). Chemicals and reagents used to simulate the GI digestion, and interaction with plasma proteins, were: potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulphate (Na_2SO_4), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amylase, hydrochloric acid (HCl), pepsin, pancreatin, bile salts, human serum albumin (HSA, ⩾97.0%), high density lipoprotein (HDL, ⩾95.0%), low density lipoprotein (LDL, ⩾95.0%) and very low density lipoprotein (VLDL, ⩾95.0%) (Sigma–Aldrich Co.). Acetonitrile (HPLC grade), methyl alcohol (HPLC grade) and formic acid were purchased from Carlo Erba Reagents (Milan, Italy).

2.2. Tea samples and preparation

White, green and black tea samples were obtained from the same tea cultivar Chun Mee 41022 (Vicony Teas Company, Huangshan, China). Green leaves had been harvested from the same field in spring, summer and autumn 2011. Fresh leaves were then processed as follows: withering in natural sunlight (12% moisture content, 3 days) and drying (15 min, 90 °C), for white tea; steaming (1 min), withering (75% moisture content, 24 h), and drying (20 min, 90 °C), for green tea; withering (72% moisture content, 12–18 h), crush-tear-curl in Lawrie tea processor (30 s), oxidation (55 min, from 27–30 °C to 22–23 °C) and drying (20 min, 90 °C), for black tea. The teas were ground to obtain a homogeneous fine powder. The infusions were prepared by pouring 20 mL of water at 90 °C on 0.5 g of tea and brewed for 7 min. They were then filtered through Whatman paper filters 43–48 μm and diluted appropriately with water according to each specific assay.

2.3. In vitro gastrointestinal digestion

The assay was performed according to the procedure described by Raiola, Meca, Mañes, and Ritieni (2012) with slight modifications, as follows. GI digestion was split into three categories: salivary, gastric and duodenal digestion. For the salivary digestion, the tea infusions (20 mL) were mixed with 6 mL of artificial saliva composed of: KCl (89.6 g/L), KSCN (20 g/L), NaH_2PO_4 (88.8 g/L), Na_2SO_4 (57.0 g/L), NaCl (175.3 g/L), $NaHCO_3$ (84.7 g/L), urea (25.0 g/L) and 290 mg of α-amylase. The pH of the solution was adjusted to 6.8 with HCl 0.1 mol/L. The mixture was put in a plastic bag containing 40 mL water, and homogenised in a Stomacher 80 Microbiomaster (Seward, Worthing, UK) for 3 min at 37 °C. Pepsin (0.5 g, 14,800 U) dissolved in HCl 0.1 mol/L was added, pH adjusted to 2 with HCl (6 mol/L), and the mixture incubated at 37 °C in a Polymax 1040 orbital shaker (250 rpm) (Heidolph, Schwabach, Germany) for 2 h. After the gastric digestion, the pancreatic digestion was simulated as follows: pH was adjusted to 6.5 with $NaHCO_3$ (0.5 mol/L) and 5 mL of a mixture containing pancreatin (8 mg/mL) and bile salts (50 mg/mL) (1:1; v/v), dissolved in water (20 mL), was added before the mixture was incubated at 37 °C in an orbital shaker (250 rpm) for 2 h. After each step of digestion, 10 mL was removed, centrifuged at 4000 rpm and 4 °C for 1 h, and the supernatant was freeze-dried. To determine the polyphenolic profile, the residues were extracted with an acetonitrile-water (84:16; v/v) mixture, centrifuged at 4000 rpm and 4 °C for 1 h, and then the supernatants were analysed by HPLC.

2.4. Cell culture and in vitro study of polyphenolic transepithelial transport

The human colon carcinoma cell line Caco-2 (HTB-37) was obtained from the American Type Culture Collection (LGC Promochem, Molsheim, France). Cells were cultured routinely in HEPES buffered Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose and supplemented with 12.5% heat-decomplemented fetal calf serum (FCS), 1% nonessential amino acids, 5 mmol/L L-glutamine, 40 U/mL penicillin, 100 μg/mL gentamycin, and 40 μg/mL streptomycin (DMEMc). Cells were maintained at 37 °C in a humidified atmosphere of CO_2/air (5:95) and passaged every 7 days by trypsinisation. They were seeded in transwells at 6×10^4 cells/cm^2. The medium (15 mL DMEM containing 10% FCS) was changed every 2 days until cells reached confluence (7–8 days). The integrity of the monolayers was evaluated by measurement of the transepithelial electrical resistance (TEER) using a Millicell-ERS device (Millipore, Zug, Switzerland) before and after the treatments. To evaluate transepithelial permeability, medium was removed from the apical and basal sides of the cultures and replaced by 2 mL of the transport solution consisting of Hanks' balanced salt solution (HBSS) and intestinal tea polyphenols, and pH was adjusted to 6 or 7.4. After 4 h of incubation at 37 °C, apical and basal solutions were collected and to determine the polyphenolic profile, aliquots (5 mL) were immediately mixed with 1 mL

of methanol and filtered on 0.45 μm Millex-HV filter units. Samples were stored at −20 °C until HPLC analysis.

2.5. In vitro study of plasma protein interaction with polyphenols

The *in vitro* trans-epithelial "permeated" polyphenols were added to plasma protein solutions simulating average fasting plasma protein concentrations. The basic composition of plasma protein solutions consisted in 20 mmol/L HEPES (pH 7.5), 0.9% NaCl, 0.5 mol/L $NaHCO_3$. Each plasma protein solution was: HAS, 42 mg/mL; HDL, 1450 μg/mL; LDL, 780 μg/mL, VLDL, 120 μg/mL. Polyphenols were incubated with plasma proteins for 30 min at 37 °C to allow equilibration. Then, the samples were centrifuged at 25 °C for 30 min at 14,000*g*. The supernatant, containing the free polyphenolic fraction, was collected and subjected to HPLC analysis for the identification and quantification of the unbound polyphenols.

2.6. HPLC-DAD/ESI-MS^n analysis

HPLC separation and quantification of phenolic compounds in tea extracts and samples obtained from *in vitro* digestion, trans-epithelial permeation, and plasma protein binding experiments, were performed according to earlier studies (Ikeda et al., 2010; Wu, Xu, Héritier, & Andlauer, 2012) with some modifications, as follows. Identification of individual polyphenols was achieved by recording chromatograms at 280 nm and comparison with spectra and retention times for the standards and those reported in the literature. The mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile (solvent B) and a gradient flow rate of 0.7 mL/min. The column selected was a Kinetex C18 100A (250 mm × 4.6 mm, 5 μm) (Phenomenex, Torrance, CA) protected with a Kinetex XB-C18/C18 Enhancement Kit (Phenomenex). Analyses were run on a Finnigan HPLC system (Thermo Electron Corporation, San Jose, CA) fitted with a photodiode array detector (DAD). Gradient elution started at 95% solvent A and 5% solvent B, solvent B was increased linearly to 15% over 14 minutes and maintained for 11 min. Then, solvent B content was increased to 35% over 28 min, to 85% over 12 min, and to 95% over 5 min, and maintained for 3 min. Finally, solvent B was returned to 5% over 10 min. HPLC separation was performed at room temperature. The proposed identity of some polyphenolics was confirmed by LC-ESI/MS/MS and data were compared with standards and those reported in the literature. The same chromatographic conditions were applied to a HP1100 HPLC system (Agilent, Palo Alto, CA) coupled to a PE-Sciex API-2000 triple-quadrupole mass spectrometer (AB Sciex, Warrington, U. K.) equipped with a Turbospray (TSI) source. Individual phenols were separated on a Hypersil BDS C18 column (250 mm × 2.1 mm, 5 μm) (Thermo Electron, Bellefonte, PA) at a flow rate of 200 μL/min. Mass spectra were recorded from m/z = 50 to 1500, in negative ionisation mode. The capillary voltage was set at −13 V, the spray voltage was at 4.5 kV and the tube lens offset was at −15 V. The capillary temperature was 275 °C. Data were acquired in MS, MS/MS and MS^n scanning modes.

2.7. Statistics

Unless otherwise stated, all of the experimental results are expressed as mean ± standard deviation (SD) of at least five replications. Statistical analysis of data was performed using the Student's *t* test or two-way ANOVA, followed by the Tukey–Kramer multiple comparison test to evaluate significant differences between a pair of means. The level of significance (α-value) was 95% in all cases ($P < 0.05$). The degree of linear relationship between two variables was measured using the Pearson product moment correlation coefficient (R). Correlation coefficients (R) were calculated using Microsoft Office Excel.

3. Results and discussion

The polyphenolic pattern of white, green and black tea extracts have been reported previously (Almajano, Carbó, López Jiménez, & Gordon, 2008; Ikeda et al., 2010; Wu et al., 2012). White and green tea leaves are characterised by higher levels of catechins than black tea, since the postharvest treatments, specifically fermentation, convert these compounds to other higher molecular mass compounds, mainly, theaflavins (Fig. 1). High catechin levels have been positively correlated with tea radical scavenging properties, which may be responsible for the protective effects of tea in conditions associated with oxidative stress, neoplastic transformations, and cardiovascular disease (Dufresne & Farnworth, 2001; Rietveld & Wiseman, 2003).

Tea catechins were detected in the salivary phase at levels ranging from 74.8% to 99.5% of undigested samples, indicating good

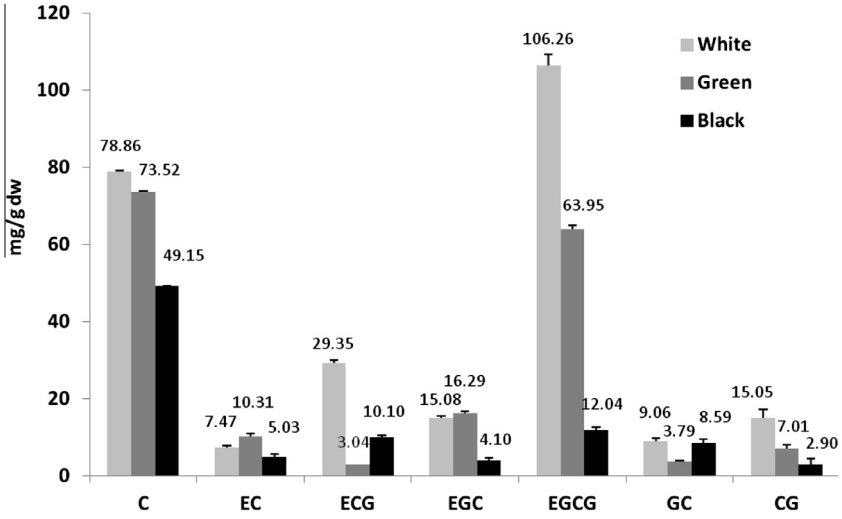

Fig. 1. Polyphenolic profile of tea samples. Polyphenolic contents are expressed as mean value (mg/g dry weight of tea leaves) ± SD (n = 5). Means are significantly different by the Tukey–Kramer multiple comparison test ($P < 0.05$). C: (+)-catechin; EC: (−)-epicatechin; ECG: (−)-epicatechingallate; EGC: (−)-epigallocatechin; EGCG: (−)-epigallocatechingallate; GC: (−)-gallocatechin; CG: (−)-catechingallate.

stability under oral biochemical conditions *in vitro* (Fig. 2). It is well known that oral mucosa can promote bioavailability of a wide range of both polar and hydrophobic compounds that rapidly reach the blood circulation, by-passing the GI tract, and, recently, some authors have tried to clarify the molecular mechanisms at the base of this process (Oulianova, Cheng, Huebert, & Chen, 2007). Moreover, since a large part of nutrients and non-nutrients are gastro-sensitive and/or poorly absorbed by the intestinal tract, the salivary extraction and absorption via oral mucosal epithelium would allow bioactive compounds to target specific tissues and organs directly, without undergoing the potential degradation in the GI tract and/or excretion in the feces (Oulianova et al., 2007). Interestingly, it has been reported that procyanidins and condensed tannins are able to inhibit digestive enzymes, decreasing the bioaccessibility of food nutrients, thus, acting as antinutritional metabolites (Fraziera et al., 2010). Consequently, mastication rather than salivary enzymatic action may enable bioaccessibility of food constituents from food rich in these polyphenols. Therefore, salivary digestion must be considered as a key step in a simulated GI digestion model for the evaluation of bioaccessibility and bioavailability of food components. Our experimental results for salivary digestion indicated bioaccessibility of EGCG, C, ECG, CG and GC was greatest from white tea, while green tea provided the most EGC and EC (Fig. 2). Means were significantly different by the Tukey–Kramer multiple comparison test ($P < 0.05$).

Our experimental results (Figs. 3 and 4) also confirmed tea catechin degradation might occur primarily in the intestine (Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007). On average 44.4% and 91.8% of native catechin were lost following gastric and intestinal digestion, respectively (Figs. 3 and 4). It has been demonstrated that pH conditions rather than digestive enzymes are responsible for this sensitivity (Green et al., 2007). In fact, preliminary experiments have shown the degree of tea catechin degradation is similar to that obtained from simplified gastric and small intestinal model systems without the presence of digestive enzymes, and the addition of digestive enzymes does not

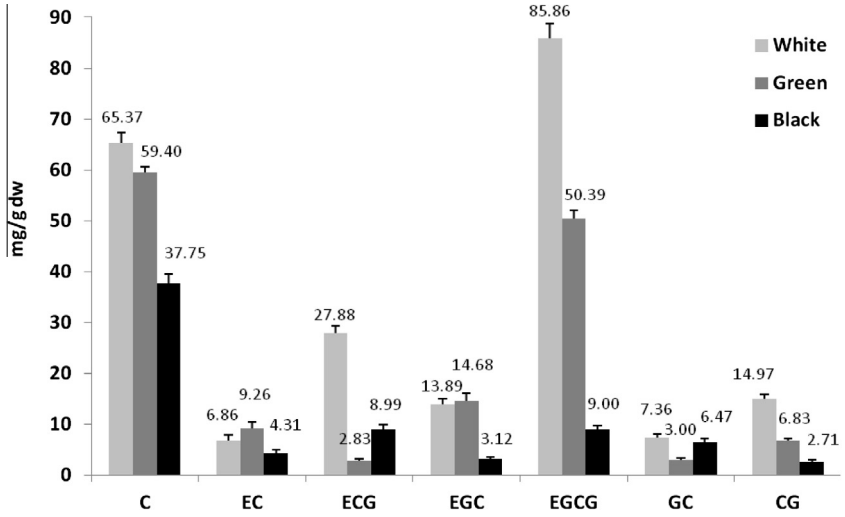

Fig. 2. Salivary bioaccessibility of polyphenols from tea samples. Polyphenolic contents are expressed as mean value (mg/g dry weight of tea leaves) ± SD ($n = 5$). Means are significantly different by the Tukey–Kramer multiple comparison test ($P < 0.05$). C: (+)-catechin; EC: (−)-epicatechin; ECG: (−)-epicatechingallate; EGC: (−)-epigallocatechin; EGCG: (−)-epigallocatechingallate; GC: (−)-gallocatechin; CG: (−)-catechingallate.

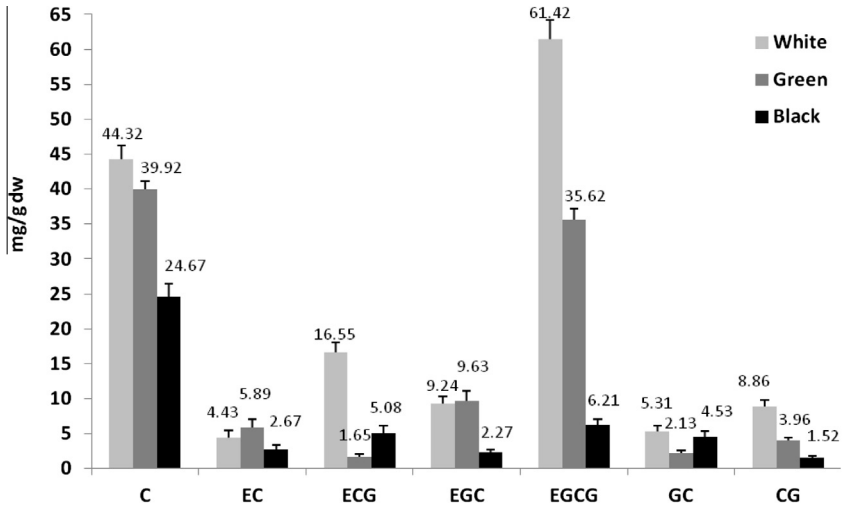

Fig. 3. Gastric bioaccessibility of polyphenols from apple peels and flesh from tea samples. Polyphenolic contents are expressed as mean value (mg/g dry weight of tea leaves) ± SD ($n = 5$). Means are significantly different by the Tukey–Kramer multiple comparison test ($P < 0.05$). C: (+)-catechin; EC: (−)-epicatechin; ECG: (−)-epicatechingallate; EGC: (−)-epigallocatechin; EGCG: (−)-epigallocatechingallate; GC: (−)-gallocatechin; CG: (−)-catechingallate.

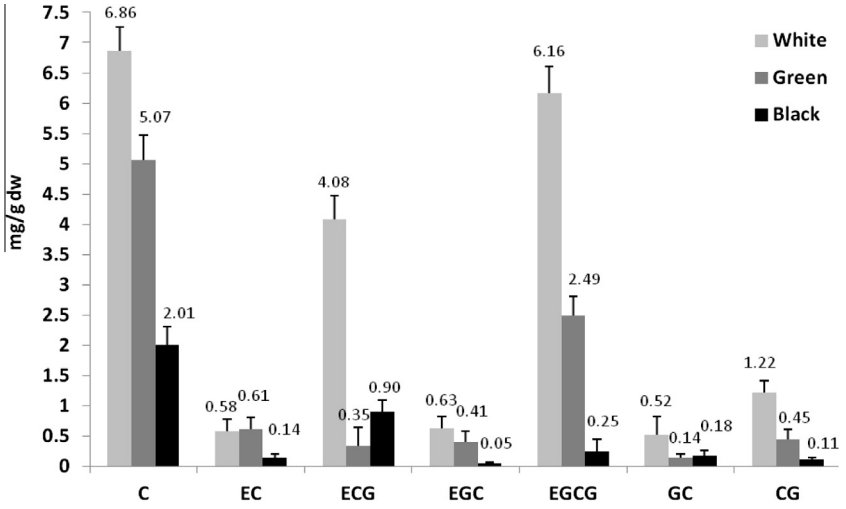

Fig. 4. Intestinal bioaccessibility of polyphenols from tea samples. Polyphenolic contents are expressed as mean value (mg/g dry weight of tea leaves) ± SD (n = 5). Means are significantly different by the Tukey–Kramer multiple comparison test (P < 0.05). C: (+)-catechin; EC: (−)-epicatechin; ECG: (−)-epicatechingallate; EGC: (−)-epigallocatechin; EGCG: (−)-epigallocatechingallate; GC: (−)-gallocatechin; CG: (−)-catechingallate.

significantly alter catechin recovery (Green et al., 2007). Intestinal pH, along with residual dissolved oxygen, allows epimerisation of catechins as well as auto-oxidation (Shim et al., 2012). Vitamin C may enhance tea catechins stability simply because it is a strong antioxidant, protecting catechins from oxidation under alkaline condition typically found in the intestinal phase of digestion (Shim et al., 2012). Our data (Fig. 4) indicated that EGCG and EGC (average, 3.9% and 2.6% of undigested content, respectively, intestinal phase) are more vulnerable to loss during digestion than ECG and EC (average, 11.5% and 5.5% of undigested content, respectively, intestinal phase) probably because of their tendency to form semiquinone free radicals in the pyrogallol moiety of the B ring at near neutral pH (Green et al., 2007). Nevertheless, EGCG has been reported to be the most effective antioxidant compound among tea catechins, both in radical-scavenging and ferric-reducing capacities, followed by EGC and ECG (Almajano et al., 2008). Our experimental results (Fig. 4) are of interest since they indicate white tea would facilitate bioaccessibility of EGCG in the intestinal lumen, thus suggesting greater antioxidant protection from white tea than green or black teas. Means were significantly different by the Tukey–Kramer multiple comparison test (P < 0.05).

The bioavailability of polyphenols from tea was evaluated using single layers of Caco-2 cells as a model of absorption in the small intestine. Data reported in Fig. 5, regarding permeated tea polyphenols (ca. 2–15% of intestinal concentrations), demonstrate previous reports may have underestimated the bioavailability of these polyphenols (Xie, Kosińska, Xu, & Wilfried, 2012). Most tea catechins seemed to concentrate in the intestinal medium, confirming that the apical-to-basolateral transport of catechins in Caco-2 cells is very low, perhaps due to their instability at near neutral pH value and active excretion to the apical side by efflux transporters (Xie et al., 2012). Interestingly, such catechin accumulation would exert some potential health effects including localised protection of the intestinal tract, which is exposed to oxidising agents and affected by inflammation, and increased risk of cancer (Halliwell, Zhao, & Whiteman, 2000). Non-glycosylated polyphenols, specifically those containing a 3,4,5-trihydroxy benzene residue (in either the flavanol B-ring or the galloyl residue) have been shown to exert

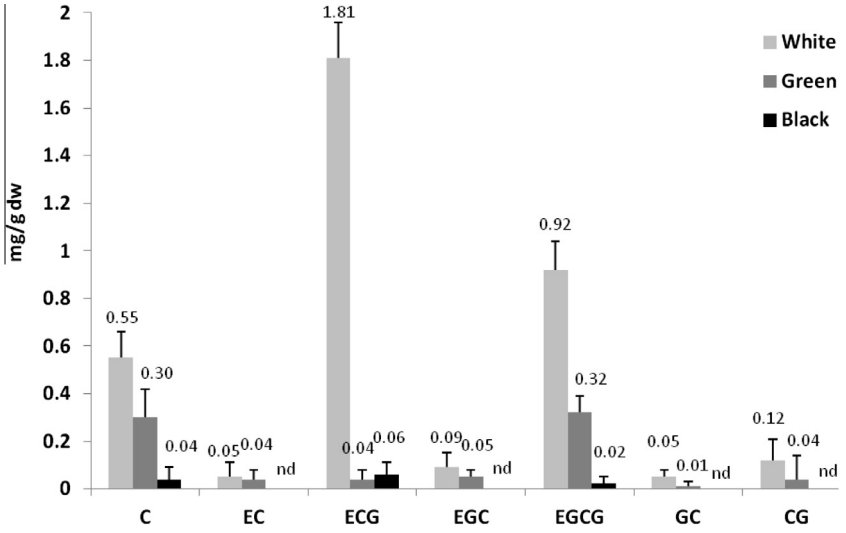

Fig. 5. Bioavailability of polyphenols from tea samples evaluated by using single layers of Caco-2 cells as a model of absorption in the small intestine. Polyphenolic contents are expressed as mean value (mg/g dry weight of tea leaves) ± SD (n = 5). Means are significantly different by the Tukey–Kramer multiple comparison test (P < 0.05). C: (+)-catechin; EC: (−)-epicatechin; ECG: (−)-epicatechingallate; EGC: (−)-epigallocatechin; EGCG: (−)-epigallocatechingallate; GC: (−)-gallocatechin; CG: (−)-catechingallate; nd: not detected.

in vitro inhibitory effect on glucose uptake under sodium-free conditions (Johnston, Sharp, Clifford, & Morgan, 2005). Experiments conducted on HepG2 cell lines (human hepatocellular carcinoma) indicated an appreciable inhibition capacity of glucose uptake associated with white tea (+17.7%) compared with green and black teas (+11.9% and +2.6%, respectively) (Tenore, Stiuso, Campiglia, & Novellino, 2013). In particular, EGCG, EGC and ECG have been reported to cause a significant reduction in glucose uptake (37%, 60% and 65%, respectively) while the rest of the catechins have shown no effects (Johnston et al., 2005). The effects of gallate-type catechins on glucose are likely to be the result of steric hindrance, caused by incorporation into the lipid-bilayer membrane, decreasing the functionality of the glucose transport system compared with non-gallate-type catechins (Johnston et al., 2005). Kirana, Rogers, Bennett, Abeywardena, and Patten (2005) also evaluated the effects of green tea catechins on cholesterol uptake, using cultured Caco-2 cells. EGCG significantly reduced the solubility of cholesterol, and was shown to be more active than ECG and CG at the same molar concentration; catechin were shown to have no effect on cholesterol. Tenore et al. (2013) proved that white tea polyphenols were more active in reducing HepG2 cell cholesterol uptake (+32.4%) than green and black teas (+5.8% and +2.04%, respectively). Catechin gallate esters have a hydrophobic domain and greater affinity for lipid bilayers and, therefore, for cholesterol than free catechins (Kirana et al., 2005). Gallic acid is also effective at reducing cholesterol micellar solubility, suggesting the gallic acid moiety is key in determining cholesterol uptake activity (Kirana et al., 2005). It could be assumed that catechin gallate esters are capable of acting as β-cyclodextrins by promoting the formation of micelle-like complexes in which cholesterol is selectively, and intensively, incorporated, preventing its uptake and giving the false impression of inhibited cholesterol membrane transport in these cells (Ikeda et al., 2010). Our data (Figs. 4 and 5) indicated a higher accumulation of EGCG, EGC and ECG in the intestinal lumen from white tea extract *in vitro*, than green and black teas, highlighting white tea as potentially the best for antioxidant protection, and reduced glucose and cholesterol uptake among the samples tested. Means were significantly different by the Tukey–Kramer multiple comparison test ($P < 0.05$).

The interaction of permeated tea polyphenols with plasma proteins was also evaluated. Our experimental results (Table 1) confirmed galloylation can strengthen catechin binding to proteins, due to an increased affinity directly associated with the number of OH groups (Xiao & Kai, 2012). Generally, a high percentage of gallated catechin (EGCG-, ECG- and CG-) binding to whole plasma proteins (up to 92%), with the exception of VLDL, was revealed (Table 1). Obviously, the reversible, and irreversible, protein–polyphenol interactions depend on several factors, mainly pH, temperature, and protein and ligand concentrations (Xiao & Kai, 2012). The effect of plasma protein binding on the biological activity of polyphenols is still unclear. According to previous studies, the interactions of polyphenols with proteins weaken polyphenol antioxidant capacity, both *in vitro* and *in vivo*, provided the catechol moiety of protein-bound polyphenols remains accessible to oxidising agents (Manach et al., 2004). In fact, the low stability of gallated catechins at neutral pH, typical of human serum, would be significantly improved by interaction with HSAs and antioxidant capacity would be enhanced (Xiao & Kai, 2012). Overall, all of the polyphenolic compounds bound to LDLs (av. 17%) (Table 1) suggesting a possible role in cardiovascular protection from oxidation and atherosclerosis (Manach et al., 2004). Nevertheless, antioxidant capacity is only one of the many potential bioactivities of polyphenols, so binding to plasma proteins could lead to different biological effects some of which have been reported but remain controversial (Manach et al., 2004; Xiao & Kai, 2012). Interestingly, our data indicated gallated catechins had good interaction (av. 37%) with HDLs (Table 1). Brunet et al. (2002) demonstrated that apo A-I is the major protein in human plasma HDL with which catechin derivatives would form a stable complex. Apo A-I activates lecithin:cholesterol acyltransferase (LCAT) and is, therefore, essential for the reverse cholesterol pathway (Jonas, 1998). LCAT would be more active after interaction of catechins and procyanidins with apo A-I and, subsequent, changes of its conformation (Jonas, 1998). Therefore, it could be suggested that foods rich in catechins may have a role in the reverse transport of cholesterol from tissues to the liver for excretion.

Table 1
Percentages of bioavailable tea polyphenolic compounds bound to plasma proteins. Data are mean values ± SD ($n = 5$). Means are significantly different by the Tukey–Kramer multiple comparison test ($P < 0.05$).

	HSA	HDL	LDL	VLDL
C	0.5 ± 0.1	0.1 ± 0.2	0.1 ± 0.2	nd
EC	0.6 ± 0.4	0.2 ± 0.1	0.2 ± 0.3	nd
ECG	38.1 ± 0.9	38.7 ± 1.6	18.7 ± 0.8	0.4 ± 0.5
EGC	0.6 ± 0.2	0.2 ± 0.3	0.2 ± 0.1	nd
EGCG	39.2 ± 0.9	37.6 ± 1.6	16.9 ± 0.8	0.5 ± 0.5
GC	0.5 ± 0.3	0.2 ± 0.1	0.1 ± 0.2	nd
CG	39.7 ± 1.5	36.8 ± 1.4	17.9 ± 0.9	0.4 ± 0.3

C: (+)-catechin; EC: (−)-epicatechin; ECG: (−)-epicatechingallate; EGC: (−)-epigallocatechin; EGCG: (−)-epigallocatechingallate; GC: (−)-gallocatechin; CG: (−)-catechingallate; HSA: human serum albumin; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; nd: not detected.

4. Conclusion

Salivary digestion is an important preliminary step in a simulated digestive extraction process. On average, tea catechins were detected in the salivary phase at levels ranging from 74.8% to 99.5% of undigested samples, which indicates a good stability under oral biochemical conditions. These data are indicative of potential absorption through the oral mucosal epithelium of significant amounts of tea catechins and perhaps other bioactive compounds that are degraded during GI digestion and/or poorly absorbed in the intestine. White tea was richest in catechin derivatives, and was associated with the best intestinal bioaccessibility and bioavailability, among all of the tea samples tested, and could have a major role in the regulation of glucose and plasma cholesterol. White tea catechins may accumulate in the intestinal lumen, where it could be assumed to inhibit cellular glucose and cholesterol uptake (Tenore et al., 2013). Permeated polyphenols may also bind to plasma HDLs, stimulating reverse transport and metabolism of cholesterol. Undoubtedly, further studies *in vivo* such as dietary intervention, are needed to support these results. Overall, our findings contribute to the little knowledge existing about this rare tea and suggest its use and that of related products may have potential health benefits.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.08.006.

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