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Angiotensin I-converting enzyme (ACE) inhibition and biological activities of green and black tea samples from Azorean *Camellia sinensis*

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ABSTRACT

The inhibition of ACE can be targeted in order to control hypertension and much attention has recently been paid towards the search for natural products as alternatives to synthetic drugs due to their adverse side effects associated with them. *Camellia sinensis* tea has received considerable attention due to the beneficial effects on health, particularly as a result of its antioxidant properties. The objective of this study was to investigate the ACE-inhibition of different types of Azorean *C. sinensis* tea samples and consequently its ability to reduce hypertension, relating it to antioxidant activity, catechin profiles, total phenolic content (TPC) and total flavonoid content (TFC) in different seasons. The results clearly highlighted differences in ACE-inhibition, FRSA, FRAP, FIC activity, TPC and epicatechin content among samples and the best results were observed in green tea collected in the summer. For TFC, higher values were observed in black tea harvested during the summer. In conclusion, the differences are related to the effect of collecting seasons, with the higher values being found in the summer with respect to the spring season.

1. Introduction

Tea, produced from *Camelia sinensis* (L.) Kuntze plant (Theaceae), is one of the most frequently consumed beverages worldwide, following water, due to its pleasant fragrance and taste, antioxidant properties, well documented therapeutic applications, and relatively low retail cost (Macfarlane & Macfarlane, 2004).

Originated from China, tea plant gradually expanded into many tropical and sub-tropical countries with adequate rainfall and slightly acidic soil, and since the last decade of the 19th century is also produced in one unique place in Europe: S. Miguel Island, Azores (Baptista et al., 2012). The composition of tea beverages differs in terms of the species, type of leaves, climate, seasons, location, horticultural practices, as well as harvesting and processing conditions. Tea is typically classified into three major types based on the degree of fermentation: unfermented green tea, semi-fermented Oolong tea, and totally fermented black tea (Macfarlane & Macfarlane, 2004). Recently, a strong focus has been placed on bioactive compounds of natural origin, such as tea extracts, in particular for their catechin content (catechin, epicatechin, epigallocatechin 3-gallate, epigallocatechin and epicatechin 3-gallate) (Rohadi et al., 2019) as well as other polyphenols compounds as theaflavins, in black tea that ranged from 6.54 to 27.98 mg/g of dry weight (DW) from spring and summer, respectively (Paiva, Lima, Motta, Marcone, & Baptista, 2022), theanine, the major amino acid (averaged 3.10 g/100 g DW) in green tea samples (Baptista et al., 2012), and tea polysaccharides that also contribute to the health benefits of tea (Gao et al., 2021). In general, green tea contains more polyphenolic compounds than black tea and many studies have shown that green tea is rich in catechins, which accounts for approximately 30 % of their dry mass (Crozier et al., 2009), whereas black tea contains approximately 3–10 % (Hung et al., 2018). However, several studies have shown that the regular consumption of green and/or black tea can have health benefits and it is seen as an option to improve antioxidant status *in vivo* and has been shown to reduce the risk of cardiovascular diseases as well as some forms of cancer. Moreover, it has also been observed to improve oral health, facilitate weight gain control, and increase antiviral as well

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as antibacterial activities (Hodgson & Croft, 2010).

It is well known that diet plays an important role in the treatment and control of high blood pressure (Yang & Hong, 2013) and research has shown that flavonoids play an important role in the treatment and control of high blood pressure (Zhang et al., 2013). Consequently, the anti-hypertension effects of drinking tea have become an important topic for molecular nutrition and food research. Hypertension is the major risk factor for cardiovascular diseases and is one of the most common disorders in the world, leading to major health complications such as cardiovascular disease, strokes, as well as renal and kidney dysfunctions (Mendis et al., 2011).

On the other hand, angiotensin I-converting enzyme (ACE) plays an important role in controlling hypertension and is a key component in the renin angiotensin aldosterone system (RAAS) (Balasuriya & Rupasinghe, 2011). ACE converts inactive angiotensin I to angiotensin II, a powerful vasoconstrictor, and inactivates bradykinin, the vasodilator which is favorable to lower blood pressure. ACE-inhibitors were technologically advanced as therapeutic agents for hypertension treatment and can block the production of angiotensin II and prevent the constriction of blood vessels, thus lowering blood pressure (Sweitzer, 2003). Several ACE-inhibitors (captopril, enalapril, lisinopril, etc.) are approved for the clinical treatment of hypertension but these drugs also generate side effects, and, thus, the search for natural ACE-inhibitors has been accelerating due to their potentially beneficial effects, safety profile, and cost-effectiveness (Coates, 2003). Several different plant-based foods (Acharya et al., 2016), along with several phytochemicals such as isoquercetin, glycosides of quercetin, apigenin, cyanidin, kaempferol and luteolin (Balasuriya & Rupasinghe, 2011), were reported to inhibit ACE. According to several studies (Naveed et al., 2018; Wang et al., 2011), the regular consumption of green and black teas has been reported to lower blood pressure (Dong et al., 2011). Moreover, the same authors reported that *in vitro* ACE-inhibitory activity was affected by the processing conditions of the tea and presented the followed IC_{50} values for ACE-inhibition: green < oolong < white < black < dark teas.

The objective of this study was to investigate the ACE-inhibition of different types of Azorean *C. sinensis* tea samples and its potential effects on the hypertension reduction as well as its relationship with antioxidant activities, catechin profiles, total phenolic content (TPC) and total flavonoid content (TFC) in different harvesting seasons characterized by an oceanic climate with mild temperature all year around.

2. Material and methods

2.1. Chemicals and reagents

Angiotensin I-converting enzyme (ACE) from porcine kidney, hippuric acid (HA), hippuryl-L-histidyl-L-leucine (HHL), catechins, namely (+)-catechin (C, 98 %–C1251), (–)-epicatechin (EC, 98 %–E4018), (–)-epigallocatechin (EGC, 98 %–E3768), (–)-epigallocatechin-3-gallate (EGCG, 95 %–E4143), (–)-epicatechin-3-gallate (ECG, 98 %–E3893), (+)-gallocatechin (GC, 98 %–G6657) and (–)-gallocatechin-3-gallate (GCG, 98 %–G6782), caffeine (CAF, 99 %–C0750), gallic acid (98 %–G7384), rutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ethylenediaminetetraacetic disodium salt (EDTA), Folin–Ciocalteu reagent (FCR), potassium ferricyanide, iron (II) chloride ($FeCl_2$), iron (III) chloride ($FeCl_3$), aluminum chloride ($AlCl_3$), ferrozine, trichloroacetic acid (TCA), hydrochloric acid (HCl), trizma base, and zinc chloride were all obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium carbonate (Na_2CO_3), potassium acetate (KCH_3CO_2), sodium phosphate, sodium chloride (NaCl), and orthophosphoric acid were obtained from E. Merck (Darmstadt, Hessen, Germany). Methanol (MeOH) and acetonitrile (ACN) HPLC grade were purchased from Fluka Chemika (Steinheim, Switzerland). Chloroform and ethyl acetate, HPLC-grade, were obtained from Riedel-de Häen (Aktiengesellschaft, Seelze, Germany). Ultrapure glass distilled water that was deionized with the Millipore Milli-Q purification system (Millipore, Bedford, MA, USA) was used throughout all the experiments.

2.2. Tea samples preparation

Tea samples from Azorean *Camellia sinensis* (L.) Kuntze var. *sinensis* were provided by Gorreana Tea Plantation (São Miguel Island, Azores, Portugal – 37°49005.900 N 25°24008.200 W) and were composed of a bud and the two youngest leaves. The leaves for the tea (green and black) samples were plucked in spring and summer of 2019 and were prepared under the following conditions: freshly plucked tea leaves were indoor-withered at 25–30 °C for several hours to achieve 70 % of relative humidity. For green tea, the leaves were heated with boiling water vapor to inactivate the polyphenol oxidase enzyme that promotes the oxidation of catechins, while for black tea, the leaves were oxidized for 3 h. Tea leaves were then dried in a heating chamber at 70 °C for 30 min and ground in a mortar to a particle size of 20–30 μm. The aqueous extracts of *C. sinensis* samples were prepared using 1 g of dried powder material extracted in 20 mL of distilled water under an atmosphere of N_2 to prevent oxidation. These solutions were then heated at 70 °C in a water bath for 15 min to avoid degradation of catechins that can occur at temperatures higher than 70 °C. The extraction process was repeated three times, under the same conditions, and the combined extract was filtered under vacuum through a cellulose acetate membrane (porosity of 0.45 μm) to remove particulate matter. Then, it was dried on a rotary evaporator and lyophilized for further analysis.

2.3. Determination of ACE-inhibitory activity of tea extracts

The *in vitro* determination of ACE-inhibitory activity was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) adapted from the spectrophotometric method described by Cushman and Cheung (1971) with slight modifications (Paiva et al., 2016). This method is based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (Hip-His-Leu) catalyzed by ACE. For the assay, the tea sample extract (2 mg/mL), obtained by the methodology described in section 2.2, was pre-incubated for 5 min at 37 °C with 10 μL of ACE (0.6 mU/mL) enzyme. The mixture was further incubated for 60 min at 37 °C with 20 μL of the substrate HHL (5 mM) in zinc chloride (10 μM) containing NaCl (300 mM) at pH 8.3 and sodium trizma base (100 mM)). The reaction was terminated by adding 12.5 μL of HCl (5 M). The percentage of ACE-inhibition was determined by an HPLC system from Waters equipped with a 626 pump and 600S controller coupled to a 486 tunable UV detector. An aliquot of 20 μL was injected and analyzed on a reverse-phase Ultrasphere ODS analytical column (25 cm × 4.6 mm i.d., 5 μm particle size) (Beckman Coulter, Miami, FL, USA) using an isocratic elution of MeOH:ACN:0.1 % HCl (25:25:50, v/v/v) at a constant flow rate of 0.6 mL/min and HA and HHL were detected by UV at 228 nm. The average value from three determinations at each concentration, and under the same analytical conditions, was used to calculate the ACE-inhibition (%) rate as follows: % ACE-inhibition = [B − A/B − C] × 100, where A is the absorbance (Abs) of HA generated in the presence of ACE-inhibitor, B the Abs of HA generated without ACE-inhibitor, and C the Abs of HA generated without ACE. The IC_{50} value (mg/mL) was defined as the concentration of inhibitor required to reduce the HA peak by 50 % (corresponding to 50 % inhibition of ACE activity).

2.4. Determination of the in vitro antioxidant activity of tea extracts

To evaluate the antioxidant properties of bioactive natural compounds, the tea extracts under study, obtained following the methodology described in section 2.2, were evaluated using three different *in vitro* antioxidant assays.

2.4.1. Determination of DPPH-free radical scavenging activity (FRSA)

The DPPH-FRSA assay, based on both electron transfer and hydrogen atom transfer reactions, was determined according to the method of

Molyneux (2004) with some modifications (Paiva, Lima, Motta, Marcone, & Baptista, 2020).

The FRSA of each tea extract at various concentrations was determined in triplicate by measuring their ability to scavenge DPPH. The DPPH, a stable free radical, reacts with the proton donating scavenging activity of the antioxidant compounds, changing the color of the reagent's mixture from purple to bright yellow, and the intensity of this change can be measured spectrophotometrically. A 250 μL aliquot of each sample extract or BHT was added to 500 μL of DPPH (100 μM) solution. BHT was used as the reference sample at the same concentration of the tea extracts and a mixture without sample, or BHT, was used as the control. The Abs was measured at 517 nm after a post-incubation period of 30 min in darkness at room temperature. The FRSA was calculated as a percentage of DPPH decoloration using the following equation: FRSA (%) = $(1 - Abs_{sample}/Abs_{control}) \times 100$. The results were expressed as EC_{50} value (μg/mL), which is defined as the sample concentration that can quench 50 % of the DPPH free radicals. A lower EC_{50} value was indicative of a higher antioxidant activity.

2.4.2. Determination of ferric reducing antioxidant power (FRAP)

The FRAP was determined according to the method of Oyaizu (1986) with slightly modifications (Paiva, Lima, Motta, Marcone, & Baptista, 2020). The FRAP of each tea extract was evaluated based on their abilities to reduce the Fe^{3+} complex to Fe^{2+}. An increase of the Abs values indicates an increased reducing power of the samples. An aliquot of 0.4 mL of each extract sample was mixed with 0.4 mL of phosphate buffer (200 mM) at pH 6.6 plus 0.4 mL of potassium ferricyanide (1 %, w/v) and the mixture was incubated at 50 °C for 20 min. After cooling down, 0.4 mL of TCA (10 %, w/v) was added to the mixture to stop the reaction and the mixture was then centrifuged at 4000*g* for 10 min. The upper layer was then separated into aliquots of 1 mL and each one was diluted with 1 mL of deionized water plus 0.2 mL of $FeCl_3$ (0.1 % w/v). BHT was used as reference at the same concentration of the extracts. The results were expressed as EC_{50} value (μg/mL), which is the concentration that the Abs was 0.5 for reducing power and were obtained by interpolation from linear regression analysis of concentration *versus* Abs at 700 nm against a blank.

2.4.3. Determination of ferrous ion-chelating (FIC) activity

A FIC activity assay was also performed to better characterize the antioxidant activity of tea samples given that metal chelating capacity is claimed to be one of the most important mechanisms which underpin antioxidant activity (Wang et al., 2009). FIC activity was achieved according to the method of Wang et al. (2009) with some modifications (Paiva, Lima, Motta, Marcone, & Baptista, 2020). The chelating ability of each extract, at various concentrations, was evaluated by measuring the inhibition of the Fe^{2+}–ferrozine complex formation. A 100 μL aliquot of each tea extract sample (mg/mL) was mixed with 135 μL of methanol plus 5 μL $FeCl_2$ (2 mM). The reaction was started by the addition of 10 μL of ferrozine (5 mM). The Abs was measured at 562 nm after 10 min at room temperature. Methanol, instead of ferrozine solution, was used as a blank sample, which is required for error correction due to the unequal coloration of the sample solutions. Methanol, instead of a sample solution, was also used as a control. Results were expressed as relative iron chelating activity compared with the unchelated (without ferrozine) Fe^{2+} reaction, and EDTA was used as the reference standard. The FIC activity was calculated as follows: FIC activity (%) = $(A0 - (A1 - A2))/A0 \times 100$, where A0 was the Abs of the control, A1 was the Abs of the sample or standard, while A2 was the Abs of the blank.

2.5. Determination of total phenolic and total flavonoid contents of tea extracts

To evaluate the total phenolic and total flavonoid contents of bioactive natural compounds, the tea extracts under study were obtained following the methodology described in section 2.2.

2.5.1. Determination of TPC

The TPC was determined by using Folin–Ciocalteu method with some modification based on the oxidation/reduction reaction described by Waterhouse (2002). A 100 μL aliquot of each different tea extract (2 mg/mL) was mixed with 1500 μL of distilled water plus 100 μL of FCR (2 N), homogenized in a vortex for 20 s, and placed in dark. After 3 min, 300 μL of 10 % Na_2CO_3 (w/v) was added, homogenized, and incubated for 5 min at 50 °C. The Abs of samples was measured with a Shimadzu UV model 1800 at 760 nm against a blank. Gallic acid was used as a standard to produce a calibration curve at various concentrations and the results were expressed in milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g of DE). A blank sample was prepared by replacing the sample by Milli-Q-water.

2.5.2. Determination of TFC

The TFC was measured using the colorimetric method of Chang et al. (2002) with some modifications (Paiva, Lima, Motta, Marcone, & Baptista, 2020). A 100 μL aliquot of each extract sample (2 mg/mL) was mixed with 100 μL of 10 % $AlCl_3$ plus 100 μL of 10 % KCH_3CO_2, and 900 μL of distilled water. The mixture was homogenized in a vortex for 20 s and left at room temperature for 30 min. Rutin was used to produce a standard calibration curve at various concentrations and the Abs was measured at 415 nm. The results were expressed as mg of rutin equivalents per gram of dried extract (mg RE/g of DE).

2.6. Extraction methodology for crude catechin and CAF contents

The extraction of crude catechins and CAF was performed according to the method published by Baptista et al. (2014) with slight modifications. A 100 mg of tea extracts, obtained by the methodology described in section 2.2, was reconstituted in 25 mL (volumetric flask) of distilled water, and 10 mL volume was partitioned with an equal volume of chloroform to remove pigments and other non-polar plant material. Subsequently, the aqueous layer was extracted with ethyl acetate (3 × 10 mL), under the same conditions, to obtain the catechin mixture and the solution of the combined ethyl acetate extracts was evaporated in a vacuum rotary evaporator. The light-brown residue (crude catechins) was dissolved in 500 μL of water and then subjected to RP-HPLC/photodiode array detection (PDAD) analysis after being filtered through a 0.45 μm polytetrafluoroethylene membrane cartridge.

2.7. RP-HPLC analysis of catechins and CAF

The catechin profiles and CAF content of tea samples were determined by RP-HPLC/PDAD following the method of Baptista et al. (2014), which has already been validated in our previous published research study focusing on green tea extracts (Paiva, Lima, Motta, Marcone, & Baptista, 2020). Since the aromatic structural similarity of the tea catechins rendered the separation difficult, a Spherisorb ODS2 column (100 × 4.6 mm i.d.) from Waters (Milford MA, USA) was successfully used to separate the individual catechins, in particular EC and EGCG, due to its hydrophobicity, carbon number (12 %), and small particle size (3 μm). The mobile phase A was composed of acetonitrile: ethyl acetate:0.1 % orthophosphoric acid:water (4.25:1:44.75:50, v/v/v/v), and the mobile phase B was acetonitrile:water (1:1, v/v). Baseline separation was achieved with a gradient elution as follows: 100 % A for 10 min, followed by a linear gradient between phase A and phase B at an increasing rate of 2 % per min until 20 % of B was reached. This composition was maintained until the end of the run at a flow rate of 0.75 mL/min. The 5 μL injection volume was used and the total run time was approximately 40 min. The column was maintained at 35 °C and coupled to an Agilent Technologies (Palo Alto, CA, USA) Liquid Chromatograph series 1200 equipped with a PDAD fixed at 280 nm. The chromatograms were recorded according to the retention times (RT) and the quantitative analysis was achieved by the external standard method using the ChemStation Chromatography Software from Agilent

Technologies. The sample concentration was limited to the range of linearity in order to avoid peak tailing and RT shifting, which may occur when the sample amount approaches the column sample load capacity. Peak identity was assigned based on the RT following comparison with the authentic standards and/or by spiking the sample with the same standards. The presence of individual catechins was also confirmed by superimposing the peak spectrum with the corresponding authentic standard spectrum. The average of triplicate measurements was used to calculate the catechin and CAF contents and the results were expressed as milligrams per gram of sample on a dried extract basis. The total epicatechin derivatives (ECDs), total esterified catechins, and total non-esterified catechins were obtained by summation as follows: EC + EGC + EGCG + ECG, EGCG + ECG + GCG, and C + EC + EGC + GC, respectively.

2.8. Statistical analysis

All determinations were performed in triplicate and the results are expressed as the means ± standard deviations (SD). One-way analysis of variance test (ANOVA) was carried out to assess and indicate any significant differences between the mean values obtained from each sample. Correlations between the tea quantity parameters evaluated were obtained using Pearson's correlation coefficient (r) for each referred year. Significance was based on a confidence level of 95 % ($p < 0.05$). The statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. ACE-inhibitory activity

The ACE-inhibition was determined by a HPLC-UV approach and the results, described in Table 1, revealed a remarkably high activity of tea samples with IC_{50} values of 0.61 and 1.04 mg/mL, for green tea in summer and spring seasons, respectively, and 1.30 and 3.10 mg/mL for black tea in summer and spring seasons, respectively. The ACE-inhibitory activity was in agreement with the results for assessing antioxidant activity as well as total phenolic and catechin contents for the same season. Dong et al. (2011) reported that the highest ACE-inhibitory activity is associated with green tea, and the lowest activity with black tea. According to Balentine et al. (1997), this could be explained by the fermentation process, when polyphenol oxidase comes into contact with polyphenols, thereby degrading the flavanols content. Persson et al. (2006) also confirmed that an increased number of hydroxy groups and addition of a double-bound oxygen may be the reason for increasing the inhibitory effect on ACE activity. On the other hand, Hara et al. (1987) reported that EGCG has the strongest ACE-inhibitory activity among the major tea catechins and that EGCG and ECG, containing a gallate moiety has much stronger ACE-inhibitory activity than EC or EGC. These results are in according to our study that shows higher values of EGCG and ECG in green tea related to black tea, and EGCG also presented higher value in summer season explaining the strong ACE-inhibitory activity in green tea summer samples. Hara et al. (1987) attributed the strong inhibitory effect of EGCG on ACE to molecular structural interaction with the active site of ACE.

Table 1
Angiotensin I-converting enzyme (ACE) inhibition and antioxidant activities of different types of Azorean *Camellia sinensis* tea samples related to its collecting seasons.

Samples	ACE-inhibition (IC_{50}, mg/mL)	FRSA (EC_{50}, μg/mL)	FRAP (EC_{50}, μg/mL)	FIC activity (%)
A	1.04 ± 0.10^{b}	6.33 ± 0.21^{b}	7.10 ± 0.07^{a}	58.65 ± 1.10^{b}
B	0.61 ± 0.04^{a}	4.63 ± 0.03^{a}	6.50 ± 0.08^{a}	70.69 ± 0.66^{a}
C	3.10 ± 0.32^{c}	11.40 ± 0.26^{c}	16.10 ± 0.14^{c}	49.44 ± 2.00^{c}
D	1.30 ± 0.14^{b}	6.67 ± 0.03^{b}	9.30 ± 0.06^{b}	56.39 ± 1.36^{b}

Results are shown as mean ± SD (n = 3). Different superscript letters in the same column are significantly different ($p < 0.05$). Samples were grouped: A – Green tea harvested in spring, B – Green tea harvested in summer, C – Black tea harvested in spring and D – Black tea harvested in summer. Abbreviations: FRSA, free radical scavenging activity; FRAP, ferric reducing antioxidant power; FIC, ferrous ion-chelating; IC_{50} and EC_{50}, half-maximal inhibitory or effective concentration.

However, other properties of tea samples could not be directly correlated with bioactivity, but the variation of the processing conditions may also affect the chemical composition of tea leaves and consequently the ACE-inhibitory activity. Nevertheless, a true comparison of data shown here with that presented in alternative studies is quite difficult due to different methods of sample preparation and processing conditions.

The results revealed that tea as an ACE-inhibitor have the potential to be used as therapeutical candidates for prevention and/or treatment of hypertension and its related diseases. In future, this potential application for pharmaceuticals or clinical nutrition is promising and would also be a natural alternative to commercial synthetic drugs and may also be safer for consumers.

3.2. DPPH-free radical scavenging activity (FRSA)

The stable organic free radical, DPPH, has been considered a useful reagent for FRSA determination in various samples.

Table 1 presents the FRSA results of green and black tea samples, expressed as EC_{50} (μg/mL), showing higher activity in green tea samples with values of 4.63 and 6.33 μg/mL for summer and spring seasons, respectively, and lower activity for black tea samples with values of 6.67 and 11.40 μg/mL for summer and spring seasons, respectively. Furthermore, the results also highlight better values (lower EC_{50} indicates higher antioxidant activity) in the summer season for both green and black tea samples. The higher antioxidant activity observed in summer, with respect to spring, can be explained by the high rate of plant metabolism and by the greater sunlight intensity during this period. Moreover, all samples presented better antioxidant capacity than that of BHT (EC_{50} = 28.10 μg/mL). Significantly lower FRSA values (higher EC_{50}) were reported by Tong et al. (2019) for green and black tea samples (EC_{50} = 30 and 77 μg/mL, respectively), in relation to our results. According to Lee et al. (2004), tea extracts enriched with phenolic compounds showed much higher DPPH scavenging ability and the strong antioxidant activity of green tea could be mainly attributed to the high contents of EGCG, ECG, and EGC since these catechins represent approximately 80 % of total catechins in green tea. However, it is well known that the antioxidant activity of tea samples not only depends on the levels of antioxidant compounds but also on their synergistic and/or antagonistic effects.

3.3. Ferric reducing antioxidant power (FRAP)

In the reducing power assay, the presence of antioxidants in the extracts resulted in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form (Zhang et al., 2012).

Table 1 illustrates the FRAP results of green and black tea samples, expressed as EC_{50} values (μg/mL). The best results were found in green tea samples, exhibiting lower EC_{50} values of 6.50 and 7.10 μg/mL for summer and spring seasons, respectively, while the highest values in black tea samples, 9.30 and 16.10 μg/mL, were also observed in the summer and spring seasons, respectively. The best results being observed in those samples collected in the summer season for these two types of tea can be explained by the same climate conditions, already referenced for the FRSA. According to Tong et al. (2019) significantly

lower FRAP (higher EC_{50}) of 72 and 194 μg/mL, were observed for green and black tea samples, respectively, with respect to all the Azorean tea samples referenced in our study.

3.4. Ferrous ion-chelating (FIC) activity

Table 1 presents the FIC activity of green and black tea samples that exhibited the highest values in green tea from the summer season (70.69 %) followed by the spring season (58.65 %). However, they were lower than that of EDTA (96.54 %), a potent metal-ion chelator. The FIC activity results obtained from black tea samples presented lower values of 56.39 % for the summer season and 49.44 % for the spring season. These results are consistent with the FRSA and FRAP assays.

3.5. Total phenolic and total flavonoid contents

Figs. 1 and 2 present the TPC and TFC values obtained for green and black tea samples. The TPC of tea samples can be considered an indirect measure of their antioxidant activities because the basic redox mechanism of the Folin–Ciocalteu method was chosen to screen phenolic content. The TPC results, expressed in milligrams of GAE/g DE, showed that TPC values were higher in green than in black tea samples and higher in summer than in spring (340.97 and 306.19 mg GAE/g DE in green tea, respectively, while 296.13 and 215.19 mg GAE/g DE in black tea, respectively). The higher values found in samples from summer can be explained by the extended daylight hours and the intensity of sunlight that characterize this season with respect to the spring season. Rohadi et al. (2019) reported similar results in relation to our study. However, Chan et al. (2010) presented significantly lower TPC values (114–141 mg GAE/g for green tea and 60.60–84.90 mg GAE/g for black tea samples). According to Erturk et al. (2010), they also observed lower content in cool months while it being higher during the warmer months and the same authors suggested that these differences may be a result of changing temperature, irradiance, and number of daylight hours. Other authors (Mahanta & Baruah, 1992) also reported that the TPC increased with sun exposure while Harbowy et al. (1997) described that the biosynthesis of TPC is induced by stronger sunlight intensity and longer day length.

Concerning the TFC content of the tea samples, determined by an aluminum chloride colorimetric method, and expressed in milligrams of RE/g DE, the values observed were similar between the samples with exception for black tea from summer that presented the highest value of 81.60 mg of RE/g DE, followed by 67.01 mg of RE/g DE for green tea from spring. The black tea from spring presented the value of 65.51 mg of RE/g DE and the lowest value was observed for green tea from summer with 64.51 mg of RE/g DE. However, these values are higher than those reported by Tong et al. (2019), that reported 17.52 and 14.73 RE/g DE for green and black tea samples, respectively. Both Rohadi et al. (2019) and Nibir et al. (2017) also reported lower values of TFC in relation to our results. However, it should be noted that the comparison of data between several studies is difficult when different raw material, extraction protocols, analytical methods, processing conditions, and units were used, among other factors.

3.6. Catechins and CAF contents

Table 2 presents the catechin and CAF contents in green and black tea samples according to different seasons. These results indicate that the total ECDs content (EC + EGC + EGCG + ECG) was significantly higher in green tea in relation to black tea samples, presenting higher values in summer season (349.08 and 187.42 mg/g DE) for green and black tea samples, respectively. The samples from the spring season presented the values of 347.35 and 168.96 mg/g DE for green and black tea samples, respectively, and the same pattern was observed for the total esterified or gallated catechins. Moreover, the content of the esterified catechins (EGCG + ECG + GCG) was significantly higher in all samples with respect to non-esterified catechins (C + EC + EGC + GC). The two major catechins in Azorean tea samples were EGCG and ECG and this finding is in agreement with other reported studies (Obanda et al., 2001). The results also show that the individual catechin contents decreased as follows: EGCG > ECG > EC + GCG > EGC for green tea and ECG > EGCG > GCG > EC > EGC for black tea. Regarding EGCG, the highest value was found in green tea from summer (208.08 mg/g DE), followed by green tea from spring (140.91 mg/g DE), and a similar pattern was observed for black tea samples (70.81 and 67.01 mg/g DE for summer and spring, respectively). For ECG, the green tea samples presented similar results between spring and summer, 119.18 and 118.23 mg/g DE, respectively. Conversely, for black tea samples, the highest value was found in summer (95.00 mg/g DE) in relation to spring (73.47 mg/g DE). Regarding EC, the highest value was found in green tea from spring (72.61 mg/g DE) and the lowest value in black tea from summer (14.75 mg/g DE). For GCG, the highest value was found in black tea from spring (35.12 mg/g DE) and the lowest value in green tea from spring (23.70 mg/g DE). The samples from the summer season

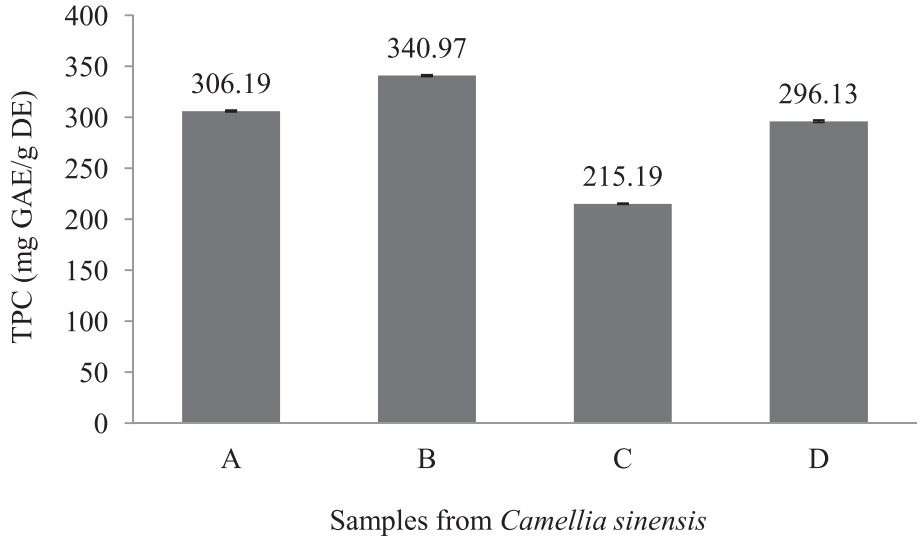

Fig. 1. Total phenolic content (TPC) of different types of Azorean *Camellia sinensis* tea samples related to its collecting seasons. Samples were grouped: A – Green tea harvested in spring, B – Green tea harvested in summer, C – Black tea harvested in spring and D – Black tea harvested in summer. GAE, gallic acid equivalents; DE, dry extract.

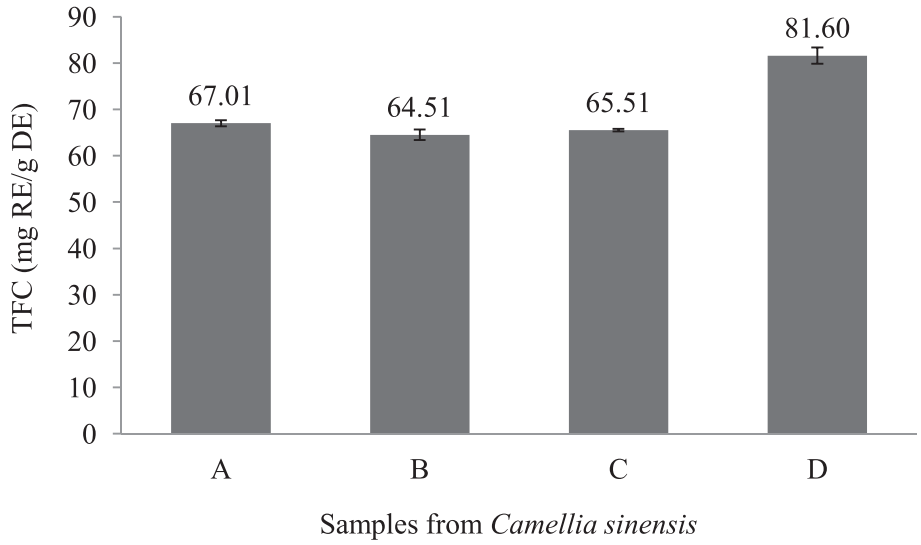

Fig. 2. Total flavonoid content (TFC) of different types of Azorean *Camellia sinensis* tea samples related to its collecting seasons. Samples were grouped: A – Green tea harvested in spring, B – Green tea harvested in summer, C – Black tea harvested in spring and D – Black tea harvested in summer. RE, rutin equivalents; DE, dry extract.

Table 2
Comparison of catechins and caffeine of different types of Azorean *Camellia sinensis* tea samples related to its collecting seasons (mg/g dry weight).

Compound	Content in *C. sinensis* green tea		Content in *C. sinensis* black tea	
	A	B	C	D
GC	6.80 ± 0.19^{b}	2.35 ± 0.11^{d}	10.53 ± 1.10^{a}	4.99 ± 0.11^{c}
EGC	14.65 ± 0.24^{a}	2.95 ± 0.25^{c}	8.13 ± 0.70^{b}	6.87 ± 0.09^{b}
C	7.16 ± 0.44^{a}	5.72 ± 0.41^{b}	5.75 ± 0.79^{b}	1.41 ± 0.13^{c}
EC	72.61 ± 0.33^{a}	19.83 ± 0.27^{b}	20.36 ± 1.13^{b}	14.75 ± 0.51^{c}
EGCG	140.91 ± 1.26^{b}	208.08 ± 8.25^{a}	67.01 ± 2.57^{c}	70.81 ± 0.14^{c}
GCG	23.70 ± 1.34^{c}	27.87 ± 1.80^{b}	35.12 ± 2.89^{a}	26.20 ± 0.03^{b}
ECG	119.18 ± 0.25^{a}	118.23 ± 7.30^{a}	73.47 ± 3.90^{c}	95.00 ± 0.69^{b}
Total ECDs	347.35 ± 1.58^{a}	349.08 ± 1.47^{a}	168.96 ± 3.17^{c}	187.42 ± 0.23^{b}
Total Est. Cat.	283.78 ± 0.33^{b}	354.18 ± 0.84^{a}	75.59 ± 4.23^{d}	92.01 ± 0.86^{c}
Total Non-Est. Cat.	101.22 ± 0.32^{a}	30.85 ± 0.81^{c}	44.77 ± 1.52^{b}	28.01 ± 0.83^{c}
CAF	18.40 ± 0.51^{a}	11.75 ± 0.14^{c}	16.12 ± 0.66^{b}	16.02 ± 0.46^{b}

Results are shown as mean ± SD (n = 3). Different superscript letters in the same row are significantly different ($p < 0.05$). Samples were grouped: A – Green tea harvested in spring, B – Green tea harvested in summer, C – Black tea harvested in spring and D – Black tea harvested in summer. Abbreviations: GC, (–)-gallocatechin; EGC, (–)-epigallocatechin; C, (+)-catechin; EC, (–)-epicatechin; EGCG, (–)-epigallocatechin-3-gallate; GCG, (–)-gallocatechin-3-gallate; ECG, (–)-epicatechin-3-gallate; Total ECDs (epicatechin derivatives), sum of EGC, EC, EGCG and ECG; Total Est. Cat. (esterified catechins), sum of EGCG, GCG and ECG; Total Non-Est. Cat. (non-esterified catechins), sum of GC, EGC, C and EC; CAF, caffeine.

presented similar values (27.87 and 26.20 mg/g DE for green and black tea samples, respectively). Regarding EGC, higher values where exhibited in the spring season in relation to summer, presenting the following ranges: 14.65–2.95 mg/g DE (green tea samples) and 8.13–6.87 mg/g DE (black tea samples), and the same pattern was also observed for C and GC. Jiang et al. (2019) reported lower values of EGCG, ECG, GCG, EC, and C in relation to ours results, but similar results were observed for EGC. However, Zheng et al. (2018) also presented lower values of EGCG, ECG, GCG, and EC, while similar values of C in both tea samples. Moreover, they also reported similar results for EGC in black tea samples and observed the highest content in green tea samples.

Regarding the CAF content, the values ranged from 18.40 to 11.75 mg/g DE in green tea samples from spring and summer, respectively, and black tea samples exhibited similar CAF values in both seasons (16.12 and 16.02 mg/g DE from spring and summer, respectively). Jiang et al. (2019) showed similar results for CAF content with respect to ours while Zhao et al. (2019) reported higher values.

The ECDs content has long been used as an important parameter to evaluate tea quality and, according to Lee et al. (2014), the combined EGCG and ECG content is a key factor affecting the antioxidant activity of tea extracts. However, it is also remarkable that the content of individual catechins in green tea is affected by agronomic conditions, particularly the plucking time. These differences in catechins may be related to differences in terms of the extraction/analysis methodologies employed as well as geographic location, different cultivars, genetic differences, processing, and storage conditions. Additionally, some authors (Tounekti et al., 2013) reported that the growing environments (including soil types, soil fertility, water stress, rainfall distribution, temperatures, sunlight intensity, and growth altitude) have an impact on the uniqueness of tea and, thus, suggested a possible explanation for the higher values of ECDs observed in Azorean green tea samples. Furthermore, as already touched upon, comparing data from several studies is difficult given that different raw materials, extraction protocols, and analytical methods are employed. Additionally, some studies (Sharma et al., 2011; Zheng et al., 2008) reported that the levels of catechins show high seasonal variations in reaction to solar irradiance, UV doses, length of daylight hours, and temperature.

3.7. Pearson correlation between parameters

A Pearson correlation was used to assess the relationship between ACE-inhibitory activity, antioxidant activity, and catechin content.

A significant correlation was observed among the methods used to determine the biological activities (Table 3). ACE-inhibition and FRSA ($r = 0.917$) were strongly correlated, and the same patterns were observed for ACE-inhibition and TPC ($r = 0.961$), ACE-inhibition and esterified catechins ($r = 0.954$), and ACE-inhibition and FIC ($r = 0.832$). A weak correlation was observed between ACE-inhibition and TFC ($r =$

Table 3
Correlation matrix of the studied parameters in Azorean *Camellia sinensis* green and black tea samples (Pearson correlations coefficients).

	ACE-inhibition	FRSA	FIC Activity	TPC	TFC	EST CAT
ACE-inhibition	1	–	–	–	–	–
FRSA	0.917	1	–	–	–	–
FIC activity	0.832	0.927	1	–	–	–
TPC	0.961	0.992	0.913	1	–	–
TFC	0.314	− 0.058	− 0.238	0.061	1	–
EST CAT	0.954	0.947	0.957	0.966	0.051	1

Aabbreviations: ACE, Angiotensin I-converting enzyme; FRSA, free radical scavenging activity; FIC, ferrous ion-chelating; TPC, total phenolic content; TFC, total flavonoid content; EST CAT, esterified catechins (epigallocatechin-3-gallate, epicatechin-3-gallate and gallocatechin-3-gallate).

0.314). The same pattern of a strong positive correlation was also observed between FRSA and FIC activity ($r = 0.927$), between FRSA and TPC ($r = 0.992$), and between FRSA and esterified catechins ($r = 0.947$) while a very weak and negative correlation was observed between FRSA and TFC ($r = -0.058$). Regarding FIC activity, the same pattern was also observed. Concerning the correlation between TPC and esterified catechins a strong correlation ($r = 0.966$) was observed along with a very weak correlation with TFC ($r = 0.061$). For TFC and esterified catechins, a very weak correlation was also seen (0.051).

These results indicate that antioxidants and polyphenols are the compounds that contribute most greatly to the ACE-inhibition in all samples, with catechins being the primary contributor, particularly in green tea samples.

4. Conclusion

The results clearly highlighted differences in the ACE-inhibitory activity in green and black tea samples harvested in different seasons (spring and summer).

The ACE-inhibitory activity, FRSA, FRAP, and FIC activity presented higher values in green tea harvested in summer while lower values in black tea harvested in spring.

The green tea samples presented a higher ACE-inhibitory activity (lower IC_{50}) with respect to black tea samples as a consequence of differences in the tea processing conditions associated with the effect of the harvested seasons.

The EGCG, ECDs, and total esterified catechins contents were higher in green tea samples harvested in summer than in spring.

The largest values for TPC were observed in green tea samples harvested in summer while lower values in black tea harvested in spring. Conversely, TFC values were higher in black tea harvested in summer and lower values in green tea harvested in summer.

There is a strong positive correlation between FRSA, FIC activity, TPC, total esterified catechins and ACE-inhibitory activity and a weak correlation between TFC and ACE-inhibitory activity.

This study revealed that Azorean *C. sinensis* green tea, as a natural product, has anti-hypertensive properties that can be exploited by the tea producers as a novel tea that presents a beneficial impact on human health.

In conclusion, the bioactivity of both green and black teas has the potential, to be used, for prevention or treatment of several diseases and would also be an innovative therapeutical alternative that may be safe and cost effectiveness for consumers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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