The catechins profile and antioxidant activity in different types of green tea bags

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Abstract

Tea and mainly green tea as a rich source of antioxidants has been widely known for some time. The antioxidant capacity of tea including green tea is mainly due to its catechins content. The objective of this study was to determine the relationship between the ORAC value and the catechins content. Regular and decaffeinated commercial green tea bags commonly consumed in the United Kingdom (UK) have been examined using Reversed-Phase High-pressure Liquid Chromatography (RP-HPLC). Teabags were purchased from different local supermarkets in the UK and extracted with natural mineral water at temperature 100°C for 9 minutes at the pre-adjusted pH 4. The level of four catechins (Epi-structured) for the thirteen types of green tea were separated and determined by HPLC analysis, i.e. ()-epigallocatechin (EGC), ()-epicatechin (EC), ()-epigallocatechin-3-gallate (EGCG) and ()-epicatechin gallate (ECG). The standard graphs were validated using certified reference catechins supplied by the Laboratory of the Government Chemist (LGC). The levels of total catechins and oxygen radical absorbance capacity (ORAC) values varied from 34.61to 204.55 mg/g, 830.19- 4197.81 Trolox equivalents/g tea bags for thirteen types of green teas respectively. It was clear from the results of this study that there was a significant linear and positive correlation ($r = 0.951$, $df = 12$, $p < 0.05$) is found to exist between the total catechins contents and ORAC values. It can be concluded that the results of catechins measurements coupled to this; the well-known ORAC assay was successfully modified to measure the antioxidant capacity of the green tea extracts throughout this study. Furthermore, the higher the level of catechins the higher is the antioxidant capacity of the tea. This may stimulus consumers in selecting the type of tea and tea brewing times, exhibiting more health benefits. Nevertheless, the differences between the studied brands are owing to shelf life, production and storage conditions.

محتوى الكتكينات والفعالية المضادة للاكسدة في انواع مختلفة من اكياس الشاي الاخضر

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المستخلص

يعتبر الشاي بشكل عام والشاي الأخضر بشكل خاص مصادر مهمة لمضادات الأكسدة وذلك بسبب محتواها العالي من الكتكينات ((Catechins . صممت الدراسة الحالية لدراسة العلاقة بين قيم ORAC (Oxygen Radical Absorbance Capacity) ومحتوى العينات من الكتيكينات في بعض انواع الشاي الأخضر الاكثر استهلاكا في المملكة المتحدة شملت عينات الشاي الاخضر الاعتيادي والشاي الاخضر الخالي من الكافيين Regular and decaffeinated)) وذلك باعتماد طريقة كروماتوكرافي السائل متباين الطور RP-HPLC

حضر مستخلص الشاي باستخدام (Liquid Chromatography High-Pressure Reversed-Phase) المياه المعدنية الطبيعية بتركيز ٢% وعلى درجة حرارة ١٠٠ ^{o}C لمدة ٩ دقائق و pH معدلة = ٤ .

تم فصل وقياس مستوى اربعة كتكينات (Epi- structured) شملت (EGCG,EGC,EC and ECG) في ثلاثة عشر نوعا من الشاي الاخضرالورقي(GreenTea Bag) بجهاز (HPLC) وتمت معايرتها مع الكتكينات القياسية المجهزة من شركة (LGC) (Laboratory of the Government Chemist) .

اظهرت نتائج الدراسة وجود فروقات معنوية ((p < ٠,٠٥ في المحتوى الكلي للكتكينات والقابلية المضادة للاكسدة بين انواع الشاي قيد الدراسة حيث تراوحت بين ٣٤,٦١ – ٢٠٤,٥٥ mg/g و ٨٣٠,١٩ –٤١٩٧,٨١ Trolox equivalents/g على التوالي كما وبينت النتائج ان هناك علاقة خطية موجبة ومعنوية p < ٠,٠٥)) df =١٢ و R =٠,٩٥١ بين قيم المحتوى الكلي للكتكينات والفعالية المضادة للاكسدة، ومن هذه العلاقة يمكن ان نوصي المستهلك بأختيار انواع الشاي ذات المحتوى العالي من الكتكينات وذلك لانها سوف تعطي فعالية مضادة للاكسدة اكثر من بقية الانواع.

Keywords :*Camellia sinensis* ؛**Catechins; Antioxidant Capacity; Green Tea** ؛ **decaffeinated**

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Introduction

Green tea, a non-fermented product of the leaves of the plant *Camellia sinensis*, is produced by inactivating the enzymes in the fresh tea leaves, either by firing or by steaming, to prevent enzymic oxidation. It is an excellent source of catechins, which are structurally flavanols, a group of polyphenolic antioxidants. In the main, eight types of catechins are found in green tea (Wang and Helliwell, 2000; Peng *et al.*, 2008; Belitz and Grosh, 2009; Alzubaidi, 2015).

Catechins are colorless, water-soluble compounds, which impart bitterness and astringency to green tea infusion. Almost all of the characteristics of processed tea, including its taste, color, and aroma, are associated directly or indirectly with modifications to the catechins, which account for some 20-34% of the dry weight of green tea (Belitz and Grosh, 2009). On the contrary, black tea is a fermented product made by a polyphenol oxidase-catalyzed oxidation of fresh tea catechins, which results in more complex condensed molecules that give black tea its typical color and strong, astringent flavor. Another consequence of this fermentation (oxidation) is that black tea contains much fewer catechins, around 50-60% of that in green tea. However, the epi-structure catechins comprise 30-50% of the solids in a green tea extract and close to 10% in black tea extract (Yang and Liu, 2012).

Tea is the most popular beverage besides water throughout the world. In recent years, green tea has gained increasing popularity because of its many health-enhancing and functional properties; it is now generally believed that its catechins are mainly responsible for these properties (Boskou, 2006). The latter include antioxidant, antiviral, antibacterial, anti-carcinogenic, anti-arteriosclerotic and anti-allergenic properties (Yilmaz, 2006). And green tea extracts are finding more and more potential applications in foods (Lin and Lin, 2005).

The proposed research will use regular and decaffeinated green tea bags from different commercial supply. Samples of both types of green tea will be identified and quantified. It is anticipated that the proposed research will help answer the question for instance, is there a significant difference between regular and decaffeinated green tea bags in their catechin's contents.

Materials and Methods

Teas

Thirteen different regular and decaffeinated green tea bags were purchased from various local supermarkets (Tesco and Sainsbury's) in the UK.

Chemicals

The following catechins standards were purchased from Sigma-Aldrich chemical Company Ltd: ()-Epigallocatechin (EGC): (catalogue #E3768), ()-Epicatechin (EC): (catalogue #E4018) ()-Epigallocatechin-3-gallate (EGCG): (catalogue #93894 FLUKA), ()-Epicatechingallate (ECG): (catalogue #E3893). HPLC solvents (catalog#1066-0131and catalog#1000-0280) were purchased from Fisher Scientific. Antioxidant (AOX) assay buffer (50 ml), AAPH (2, 2'-azobis-2-methyl-propanimidamide, dihydrochloride, 130 mg), trolox (20 µl (1.5 mM in AOX buffer)) and fluorescein solution (3', 6'-dihydroxy-spiro [isobenzofuran-1[3H], 9' [9H]-xanthen]-3-one; 300 µl (60 × stock)), manufactured by Zen-Bio Inc., USA as an ORAC Antioxidant Assay Kit (Cat# AOX-2) were purchased from AMS Biotechnology (Europe) Ltd.

Sample Preparation

The concentrations of Epi-structured catechins were determined using the method established by (AL-zubaidi, 2015). Briefly, each green tea bag (1.8-2.2 g) weighed and used for tea infusion. The green tea extract was prepared by infusing 2 g of the green tea bag in 100 milliliters of natural mineral water, which had been pre-heated at 100°C for 9 minutes by using a hot plate at the pre-adjusted pH 4. A magnetic stirrer was used, to deliver constant stirring of the infusion during extraction at the end of which, the teabag(s) was/were immediately removed, and the infusion was allowed to cool down to ~ 50°C. From this, precisely five mL of the infusion were collected using a ten mL disposable syringe. A filter (Acrodisc® syringe filters Nylon membrane, diam. 13 mm, pore 0.45µm) was attached to this filled syringe from which a purified sample of tea infusion was collected for analysis in a 1.5 mL vial; the vial was labeled accordingly. A small amount of infusion, i.e. 100 μL was taken from this vial and diluted to 10 mL using a grade A volumetric flask. After appropriate dilution, aliquots were taken and analyzed by RP-HPLC; the rest of the sample in the vial was stored in the freezer at −18°C for the ORAC assay later. HPLC analysis was conducted using samples of tea infusion in triplicate, while duplicate samples were utilized for the ORAC assay.

HPLC analysis of green tea catechins

A rapid RP-HPLC method has been developed and validated for simultaneous separation and analysis of eight green tea catechins using an RP-HPLC, the technique was used to separate in a single run, eight catechins (AL-zubaidi, 2015). This technique was employed in the present study to identify and quantify the distribution of 4 catechins present in 13 commercial green tea bags. HPLC was carried out on a Finnigan Surveyor Auto-sampler plus – Thermo Scientific (Hemmel Hempstead, UK) high-performance liquid chromatography (HPLC) system with reverse phase (RP) column and gradient elution was used in this research. A Kinetex 2.64 μm C18 (150 × 4.6 mm) column from Phenomenex was used. The gradient system consisted of; a mixture of acetonitrile and H_3PO_4: A% (10 acetonitrile: 90 deionized water with 0.1% H_3PO_4) and B% (90 acetonitrile: 10 deionized water with 0.1% H_3PO_4), the flow rate is 0.6 mL/min. Samples were injected on to the column via a 20 μL loop. A consistently good separation of the standard catechins from Sigma was obtained, and a typical chromatogram is given below (Figure 1). For a given HPLC system, the retention times of the peaks are characteristic of the separated catechins by which they can be identified.

Figure 1: A HPLC chromatogram of epi-structured catechins standards: EGC, EC, EGCG, and ECG.

ORAC Assay

ORAC assay was successfully adapted to measure the antioxidant capacity of the green tea extracts throughout this study. This method has validated by the published data using certified reference catechins purchased from the LGC. The ORAC assay is a kinetic assay measuring fluorescein decay and antioxidant protection over time. The antioxidant activity in teas can be normalized to equivalent Trolox units to quantify the composite antioxidant activity present, which is mostly due to the catechins.

Materials, sample preparation, assay procedure and generation of the standard curve were as per the instruction manual ZBM0035.01 (Dec. 2010) for ORAC Antioxidant Assay Kit provided by AMS Biotechnology (Europe) Ltd. The assay was conducted, courtesy of Dr. John Accord of the Department of Applied Science, LSBU, in his Molecular Biochemistry laboratory, using a Fluostar Galaxy plate reader; the fluorescence signal was measured over 30 minutes by excitation at 485 nm, emission at 538 nm and cut-off = 530 nm.

A standard curve of Trolox standards (M) was obtained with the following data.

0	6.25	12.5	25	50	100	Conc. (M)
6.694	8.66	9.06	15.69	18.57	27.48	AUC
0	1.966	2.366	8.996	11.876	20.786	NET AUC

Normalized data were used to generate Area Under the Curve (AUC) values, which were calculated using the statistical program GraphPad Prism. And net AUC was obtained by subtracting the AUC for no compound addition (i.e. with assay buffer only) from the other AUC values (see Figures 2 and 3).

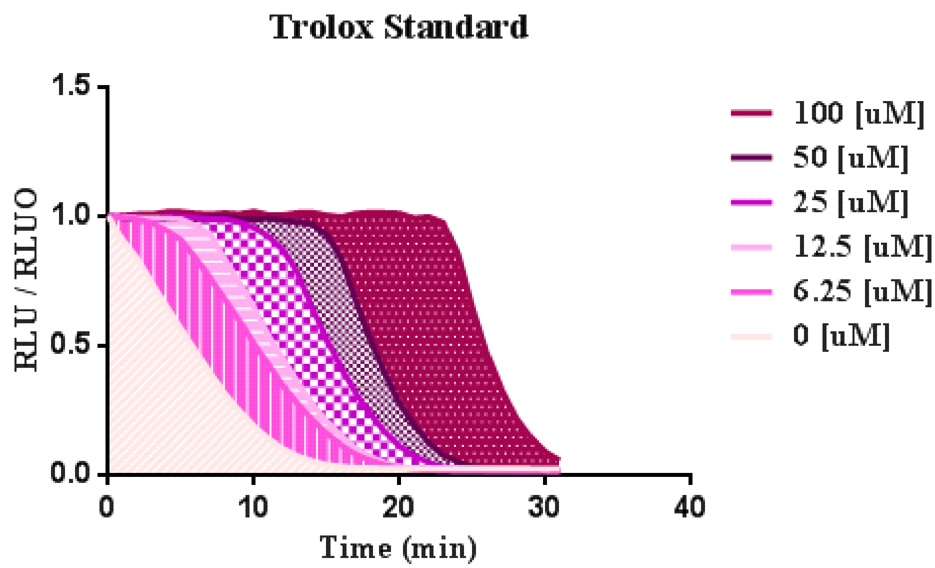

Figure 2: ORAC Assay: AUC (area under the curve) versus concentrations of standards (M), generated by using GraphPad Prism

Figure 3: Standard corrected linear regression curve of M Trolox equivalents

Table 1: Thirteen green teas evaluated in the present study

Source	Tea brand	Tea No.
Sainsbury's	Twining Pure Green Tea	1
UK/ Tesco	Twining Decaf (new Tesco)	2
UK/ Tesco	Tazo+zen green tea the vert blend with lemon	3

Sainsbury’s	Jackson's. Sencha Green Tea	4
UK/ Tesco	Jackson's. Sencha Green Tea with Mint	5
UK/ Tesco	Tazo from University Hub's	6
UK/ Tesco	Twinings Orange & lotus flower Green Tea	7
UK/ Tesco	Twinings Pomegranate Green Tea	8
UK/ Tesco	Twining's Apple & Pear Green Tea	9
UK/ Tesco	Tetley Pure Green Tea	10
UK/ Tesco	Tetley Decaf. Green Tea	11
UK/ Tesco	Clipper Pure Green Tea	12
UK/ Tesco	Clipper Decaf. Green Tea	13

Statistical Analysis

The current study shows an effort to differentiate green tea types based on epi-structured catechins content in infusions, using multivariate statistical analysis. An ANOVA was achieved, using SPSS v 23, to examine Trolox equivalent per gram (g) original sample (mM) as measured by the ORAC assay. Also the levels of total catechins and their profile (mg/g green tea bags) as measured by HPLC for thirteen types of green tea bags.

Results and Discussion

Variability in catechins present in regular green tea bags

The variability of various catechins depends on the leafage, agronomic conditions and degree of fermentation, which is directly associated with the final quality of the beverage (Lin *et al.*, 1998; Khokhar and Magnusdottir, 2002). The levels of total catechins in the regular green teas varied from 34.61to 204.55 mg/g and their profiles: EGCG (18.30-93.55), EGC (11.24-92.22), EC (2.58-13.07) and ECG (2.49-9.93) mg/g tea bag (Table 2).

Looking at the Twining Pure Green Tea (Tables 1 and 2; Figures 4 and 5) that has the highest level of total catechins also appears to have the most antioxidant capacity; at the other end, the tea (Tazo from University Hub's) that has the lowest level of total catechins also appears to have the least antioxidant capacity.

The range of total catechins levels in green teas (34.61 – 204.55 mg/g tea bag) is of a similar order to what has been reported elsewhere by (Khokhar and Magnusdottir, 2002; Henning *et al.* 2003; Friedman *et al.*, 2005, Yao *et al.*, 2006, Friedman *et al.*, 2006, and 2009, Al-zubaidi *et al.*, 2017). Profiles of the four epi-structured catechins for the ten types of green tea are presented in Table 2; Figures 4 and 5. These epi-structured catechins divided into two groups, i.e. EGCG and ECG, EGC and EC.

EGCG and EGC appeared to play a significant role in the changes in the sensory qualities of processed green tea beverages (Wang *et al.*, 2000; AL-zubaidi, 2015). EGCG is the most abundant catechin in green tea. Approximately, 50% of the total catechins in green tea. Among all the teas examined, EGCG was the highest at 93.55 mg/g teabag followed by EGC 92.22 mg/g tea bag (see Tables 1 and 2; Figures 4 and 5). In general, however, EGCG is the major catechin found in green teas (Friedman *et al.*, 2009), and it is surely the main constituent of all kinds of green teas studied. Furthermore, for these results (see Tables 1and 2; Figures; 4 and 5), there exists a trend in which the order regarding quantities of the epi-structure catechins is: EGCG > EGC > EC > ECG (42-54, 31-46, 6-19 and 4-7) % of the total catechins in green tea respectively. These results are largely in agreement with those of other researchers (Khokhar and Magnusdottir, 2002; Henning *et al.* 2003; Friedman *et al.*, 2005, Yao *et al.*, 2006; Friedman *et al.*, 2006, and 2009; Al-zubaidi, 2015 and 2017).

In contrast, the Twining Pure Green Tea (Tables 1and 2; Figures 4 and 5) contained higher levels of EGC (92.22 mg/g) followed by EGCG (89.33 mg/g teabag), EC (13.07 mg/g teabag), and ECG (9.93 mg/g tea bags) with an overall trend of EGC > EGCG > EC > ECG. These results seem to concur with the conclusions of Lin *et al.* (1998) who stated higher levels of EGC in Japanese green teas. Tables 1 and 2 illustrate the profiles and total catechins for regular and decaffeinated green tea bags.

Variability in catechins present in decaffeinated green tea bags

The levels of total catechins in the decaffeinated green teas varied from 53.82 to 116.23 mg/g and their profiles: EGCG (22.88- 59.65), EGC (16.64- 39.67), EC (6.22- 10.35) and ECG (3.95-8) mg/g tea bag (Table 2).

Looking at the Clipper Decaffeinated Green Tea (Tables 1 and 2; Figures 4and 5) that has the highest level of total catechins also appears to have the most antioxidant capacity; at the other end, the tea (Tetley Decaffeinated Green Tea) that has the lowest level of total catechins also appears to have the least antioxidant capacity.

The range of total catechins levels in green teas (53.82 – 116.23 mg/g tea bag) is of a similar order to what has been reported elsewhere by (Khokhar and Magnusdottir, 2002; Henning *et al.* 2003; Friedman *et al.*, 2005, Yao *et al.*, 2006, Friedman *et al.*, 2006, and 2009, Al-zubaidi *et al.*, 2017). Profiles of the four epi-structured catechins for the three types of decaffeinated green tea bags are presented in Table 2; Figures 4 and 5. These epi-structured catechins divided into two groups, i.e. EGCG and EGC, ECG and EC.

EGCG and EGC seemed to play a key role in the changes in the sensory qualities of processed green tea beverages (Wang *et al.*, 2000; AL-zubaidi, 2015 and 2017). EGCG is the richest catechin in green tea. Approximately, 51% of the total catechins in decaffeinated green tea. Among all the three types of teas examined, EGCG was the highest at 59.65 mg/g teabag followed by EGC 39.67 mg/g tea bag (see Tables 1 and 2; Figures 4 and 5). There exists a trend in which the order regarding quantities of the epi-structure catechins is: EGCG > EGC > EC > ECG (43-51, 31-37, 6-19 and 6-7) % of the total catechins in green tea respectively. These results are generally in agreement with those of other investigators (Khokhar and Magnusdottir, 2002; Henning *et al.* 2003; Friedman *et al.*, 2005, Yao *et al.*, 2006; Friedman *et al.*, 2006, and 2009; Al-zubaidi, 2015 and 2017). In contrast, the Twining Pure Green Tea (Tables 1and 2; Figures 4 and 5) contained higher levels of EGC (92.22 mg/g) followed by EGCG (89.33 mg/g teabag), EC (13.07 mg/g teabag), and ECG (9.93 mg/g tea bags) with an overall trend of EGC > EGCG > EC > ECG. These results seem to concur with the conclusions of Lin *et al.* (1998) who stated higher levels of EGC in Japanese green teas. Tables 1 and 2 illustrate the profiles and total catechins for regular and decaffeinated green tea bags.

Different tea varieties are harvested in a variety of ways and at various times of a year so that the plants are subjected to different environmental stress conditions (Friedman *et al.*, 2009). A more detailed and comprehensive review of the total catechin levels in decaffeinated green teas will be a worthwhile exercise.

Table 2: Profiles of the four epi-structured catechins (CATS) [()-epigallocatechin (EGC), ()-epicatechin (EC), ()-epigallocatechin-3-gallate (EGCG), ()-epicatechin-3-gallate (ECG)], total catechins and ORAC value for the thirteen types of green tea extract.

ORAC Value[s]	Total Catechins [b]	ECG [b]	EGCG [b]	EC [b]	EGC[b]	Tea[a]
4197.81	204.55±0.38	9.93±0.	89.33±0.	13.07±0.	92.22±0.	1

		00	12	00	28	
2535.43	98.37±0.03	6.01±0.01	49.36±0.02	6.22±0.00	36.78±0.00	2
2740.23	102.37±2.12	5.86±0.41	55.57±0.78	5.76±0.41	35.18±0.52	3
3847.43	186.41±0.45	9.54±0.04	93.55±0.22	10.50±0.08	72.82±0.11	4
3737.15	183.52±0.15	9.36±0.01	90.72±0.01	11.26±0.04	72.18±0.11	5
830.19	34.61±0.86	2.49±0.03	18.30±0.33	2.58±0.17	11.24±0.33	6
1955.06	87.16±0.17	3.53±0.09	36.48±0.18	6.99±0.02	40.16±0.06	7
2572.10	98.92±0.01	5.33±0.02	47.86±0.06	6.95±0.00	38.78±0.06	8
2093.30	89.27±2.27	5.15±0.32	44.10±0.50	5.90±0.49	34.11±0.96	9
3070.89	136.06±1.73	8.59±0.49	62.60±0.46	10.36±0.49	54.51±0.29	10
1935.64	53.82±0.09	3.95±0.00	22.88±0.04	10.35±0.02	16.64±0.03	11
3168.56	144.59±1.98	9.42±0.42	68.80±0.56	9.24±0.48	57.13±0.52	12
2876.40	116.23±0.48	8.00±0.07	59.65±0.08	8.91±0.12	39.67±0.21	13

[a] See Table 1 for a list of green teas.

[b]Values in mg/g ± SD (n = 2).

[c] Trolox equivalent per gram (g) original sample (mM).

It indicates that the level of catechin is significantly different ($P < 0.05$) from using multivariate analysis.

To assist in the visualization of these results, the changes in profiles of the four epi-structured catechins in the thirteen types of green tea bags (regular and decaffeinated) are presented in the following histograms (Figures 4 and 5).

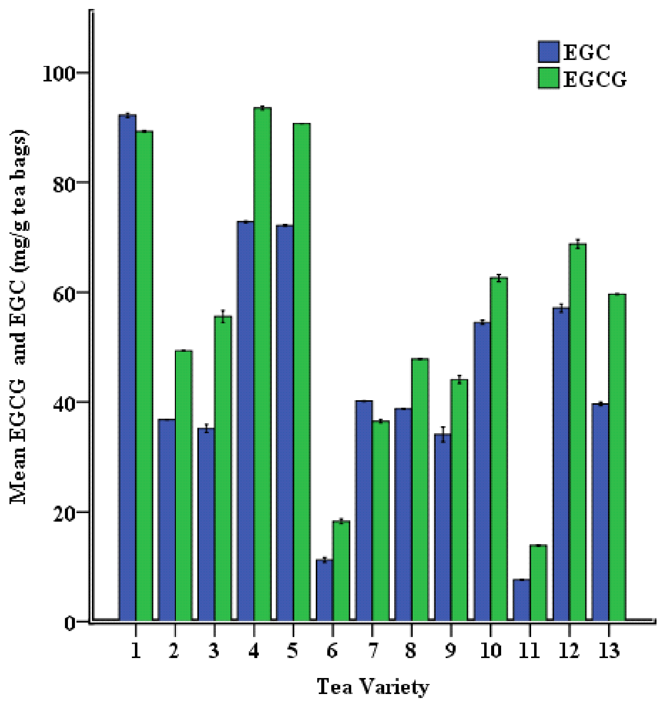

Figure 4: Bar graphs of EGCG and EGC, as measured by HPLC for thirteen types of green tea bags (n = 2; error bar = ± 2 SD).

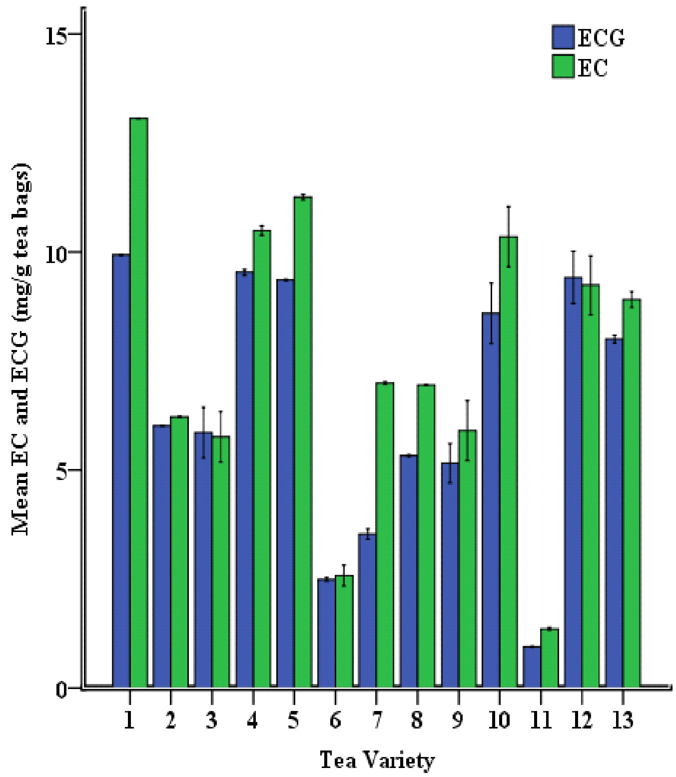

Figure 5: Bar graphs of EC and ECG, as measured by HPLC for fourteen types of green tea bags (n = 2; error bar = ± 2 SD).

Prior, Sharma *et al.* (2005), investigating the 'extractability of tea catechins as a function of manufacture procedure and temperature of infusion' found tea–infused at 100°C showed higher levels of catechins (especially EGCG and EGC) than did tea infusions made at 80°C. Vuong *et al.*, (2013) went further to demonstrate that the epi–structure catechins were stable under acidic conditions but epimerized or degraded at pH $\geq$ 6. According to these authors, to get the most out of the extraction of the epi–structure catechins and to minimize their epimerization and degradation, the pH of the infusion should be maintained between 3 and 5.3 during the aqueous brewing process. While not directly applicable here, Li *et al.* (2012) found the optimum pH for catechin stability in green tea concentrated solutions was around pH 4.

ORAC Results

Table 2 summarises the results of the experiments, ORAC values varied from 1955.06- 4197.81 and 830.19-2876.40 Trolox equivalents/g tea bag for thirteen types of regular and decaffeinated green teas respectively (Table 2). As published in the literature, there was a significant positive correlation ($p < 0.05$) between the levels of green tea catechins and their oxidative capacities as measured by the HPLC and ORAC assay correspondingly. Figure 6 shows a positive linear relationship between HPLC and ORAC. It is clear from this chart that there is a strong positive correlation ($p < 0.05$) between HPLC and the ORAC as indicated by the high value of the correlation coefficient (R^2=0.891). This is valid over the selected range of the operating conditions, namely, temperature, time, concentration, and pH would be chosen on the ground of yielding maximum levels of catechins and ORAC values.

The ORAC value of the Twining Pure Green Tea was higher (4197.81) than all the regular green tea brews, whereas the Twinings Orange & lotus flower Green Tea showed the lowest ORAC values (1955.06) (Table 2). Similarly, these results seem to concur with the findings of Al-Zubidi *et al*. (2017) who reported higher ORAC value (3421.14) in Ahmed Tea London Jasmine Green Tea. However, the Twinings Orange & lotus flower Green Tea presented the lowest ORAC values (1097.31).

Results from this preliminary study suggested that, within the experimental boundary, higher tea concentration, higher infusion temperature and lower pH significantly ($p < 0.05$) enhanced the levels of catechins in the green tea extracts. These results are mainly in agreement with those of other researchers. There is some suggestion that decaffeinated green teas may have lost a significant quantity of catechins; this needs to be further investigated. It may even be possible to distinguish regular and decaffeinated green teas purely from their different levels of catechins. The ORAC values of these antioxidants were in line with the data from other investigators (Mendilaharsu *et al*. 1998; Henning *et al*. 2003 and Al-zubaidi, 2015 and 2017). In conclusion, that is, the higher the level of catechins the greater is the antioxidant capacity of the tea (Al-zubaidi, 2015).

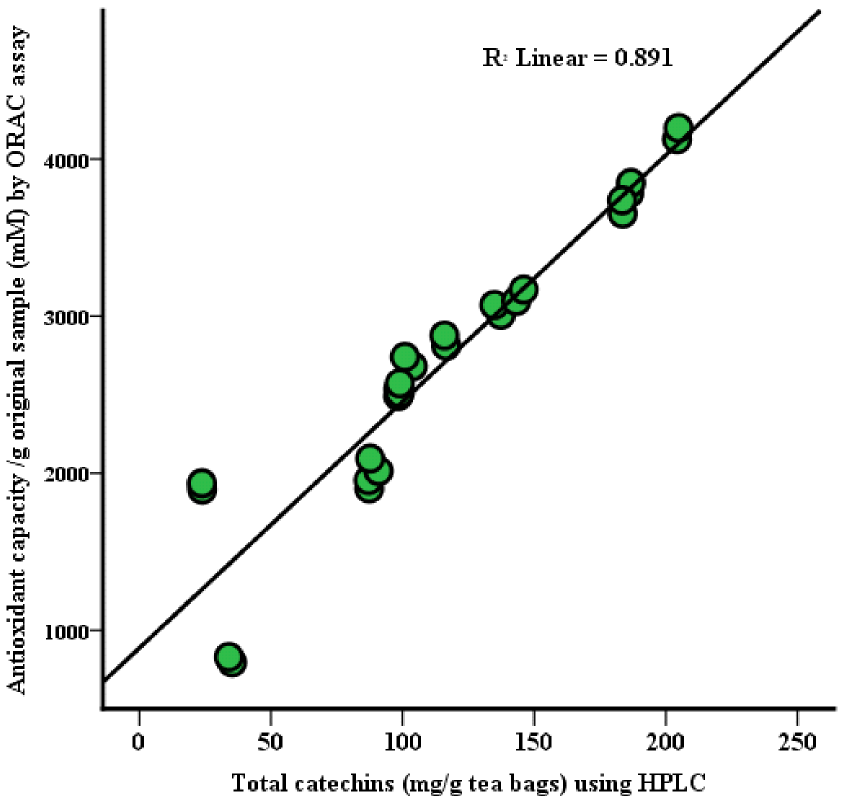

Figure 6: Correlation between total catechins (mg/g teabag) as measured by HPLC and ORAC values (mM TE/g teabag) by the ORAC assay.

Conclusion

This study aims to observe the level of four catechins (Epi-structured) for the thirteen types of regular and decaffeinated green tea bags commonly consumed in the UK were determined using RP-HPLC. The extracts from green teas were found to contain more epi-structured catechins and significantly affected by four factors, temperature, time, concentration, pH and their interaction. These extraction conditions help consumers in selecting for tea with exactly expected health benefits. Our study has shown that antioxidant capacities are positively correlated with catechins contents. The results therefore strongly suggest that ORAC value is a good indicator of the antioxidant capacities of catechins. That is, the higher the level of catechins the greater is the antioxidant capacity of the tea. Further studies are needed to distinguish regular and decaffeinated green teas purely from their different levels of catechins.

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