A Survey of Catechins Contents of Different Green Teas Currently Available in the UK market

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Abstract

The level content of the main flavanols in green tea (i.e. epi-structured catechins) in regular commercial green tea bags commonly consumed in the United Kingdom (UK)have been examined using reversed-phasehigh-pressure liquid chromatography (RP-HPLC). Tea bags 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 fourteen types of green teawere separated and determinedby HPLC analysis,i.e. (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epigallocatechin-3-gallate (EGCG) and (−)-epicatechingallate (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 96.38 to 205.03 mg/g, 1097.30 - 3421.14Trolox equivalents/g tea bags for fourteen types of green teas respectively.It was evident from the results of this study that there was significant linear and positive correlation ($r = 0.932$, $df = 13$, $p < 0.05$) is found to

exist between the total catechins contents and ORACvalues.It couldbe concluded that the developed HPLC method gave reliable results for catechins measurementscoupled to this; the well-known ORAC assay was successfully adapted to measure the antioxidant capacity of the green tea extracts throughout this study. Moreover, the higher the level of catechins the greater is the antioxidant capacity of the tea. However,the differences between the studied brands are owing to production, shelf life and storage conditions.

Introduction

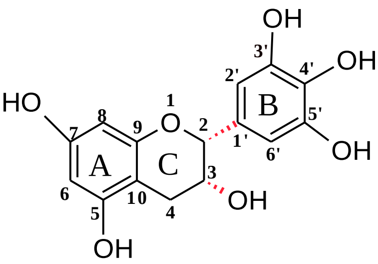
Tea has been drunk in China for over 4000 years. It was not introduced to Europe until the beginning of the seventeenth century. People enjoy tea for its taste and flavour. Today tea is the most consumed beverage in the world after water. Until relatively recently, research in teas was focused on black teas as evidenced by the amount of literature published. Since 2004, there has been a dramatic expansion of the research carried out on green teaand,this is generallybecausegreen tea is believed to provide greater benefit to human health as it naturally contains more antioxidants (primarily as catechins) compared to black tea. 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;Penget al., 2008;Belitz and Grosh, 2009;Alzubaidi, 2015):

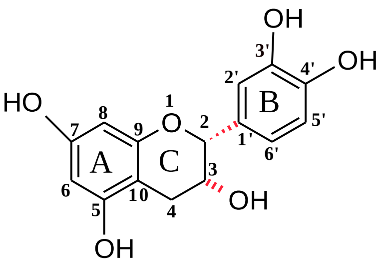
(−)-epigallocatechin-3-gallate (EGCG)
(−)-epigallocatechin (EGC)
(−)-epicatechingallate (ECG)
(−)-epicatechin (EC)
(+)-catechin (C)
(−)-gallocatechin (GC)
(−)-gallocatechingallate (GCG)

(–)-catechingallate (CG)

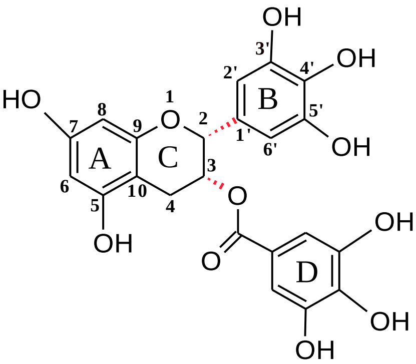
The epi-structured catechins can be found in high concentrations in both black and green teas (Williamson and Manach, 2005; Ronald, 2013)(see Figure 1.). EGCG is the major tea polyphenol, constituting 12% or more (dry matter) of fresh leaf solids. The quality of green tea is believed to correlate with the catechin levels of its fresh leaves, which decrease with the age of the leaves (Wan et al., 2008; Song et al., 2012; Al-zubaidi, 2015).

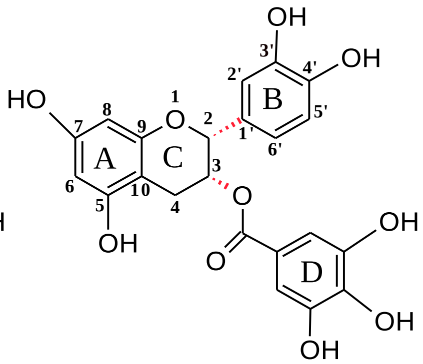
Epi-Structured





(-)-Epigallocatechin (EGC) **(-)-Epicatechin (EC)**





(-)-Epigallocatechingallate (EGCG) (-)-Epicatechingallate (ECG)

Figure1. Structures of the four catechins, generated by using ChemSketch

The principal aim of this research is to deliver the expected health benefits to the consumers,and this can be achieved by examining the main catechins (Epi-structured) contents and their profiles in (mainly) regular commercial green tea bagsdepending on availability.Results from this study will be providingsignificantdata for consumer and manufactured by demonstrating the significance of more information about thetype of green tea bags.

Materials and Methods

Teas

Fourteen different regular green tea bags were purchased fromvarious local supermarkets (Marks &Spencer, Tesco, Green Valley 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(20µl (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 level of Epi-structured catechins was determined usingthe method established by (AL-zubaidi, 2015). Briefly, each green tea bag (1.9-2.2 g) weighed and used for tea infusion.The green tea extract was prepared by infusing 2 g of thegreen tea bag in one hundred millilitresof natural mineral water in a thermos flask, the infusion temperature 100°Cwas chosen by using a hot plate for the time required (9 minutes) 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 infusionwas allowed to cool down to ~ 50°C. From this, exactly 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 filtered sample of tea infusion was collected for analysis in a 1.5 mL vial; the vial was labelled 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 analysed 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 utilised for the ORAC assay.

HPLC analysis of green tea catechins

We have developed and validated a rapid RP-HPLC method forsimultaneous separation and analysis of 8 green tea catechins using an RP-HPLC, the method was used to separate in a single run, eight catechins (AL-zubaidi, 2015). This method was employed in the present study to identify and quantify the distribution of 4 catechins present in 14 commercial green tea bags. HPLC was carried out on a Finnigan Surveyor Autosampler plus – Thermo Scientific (HemmelHempstead,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 Phenomenexwas used. The gradient system consisted of a mixture of acetonitrile and H_3PO_4: A%

(10 acetonitrile: 90 deionised water with 0.1% H_3PO_4) and B% (90 acetonitrile: 10 deionised water with 0.1% H_3PO_4), the flow rate is 0.6 mL/min.

A consistently good separation of the standard catechins from Sigma was obtained, and a typical chromatogram is given below (Figure 2). For a given HPLC system, the retention times of the peaks are characteristic of the separated catechins by which they can be identified.

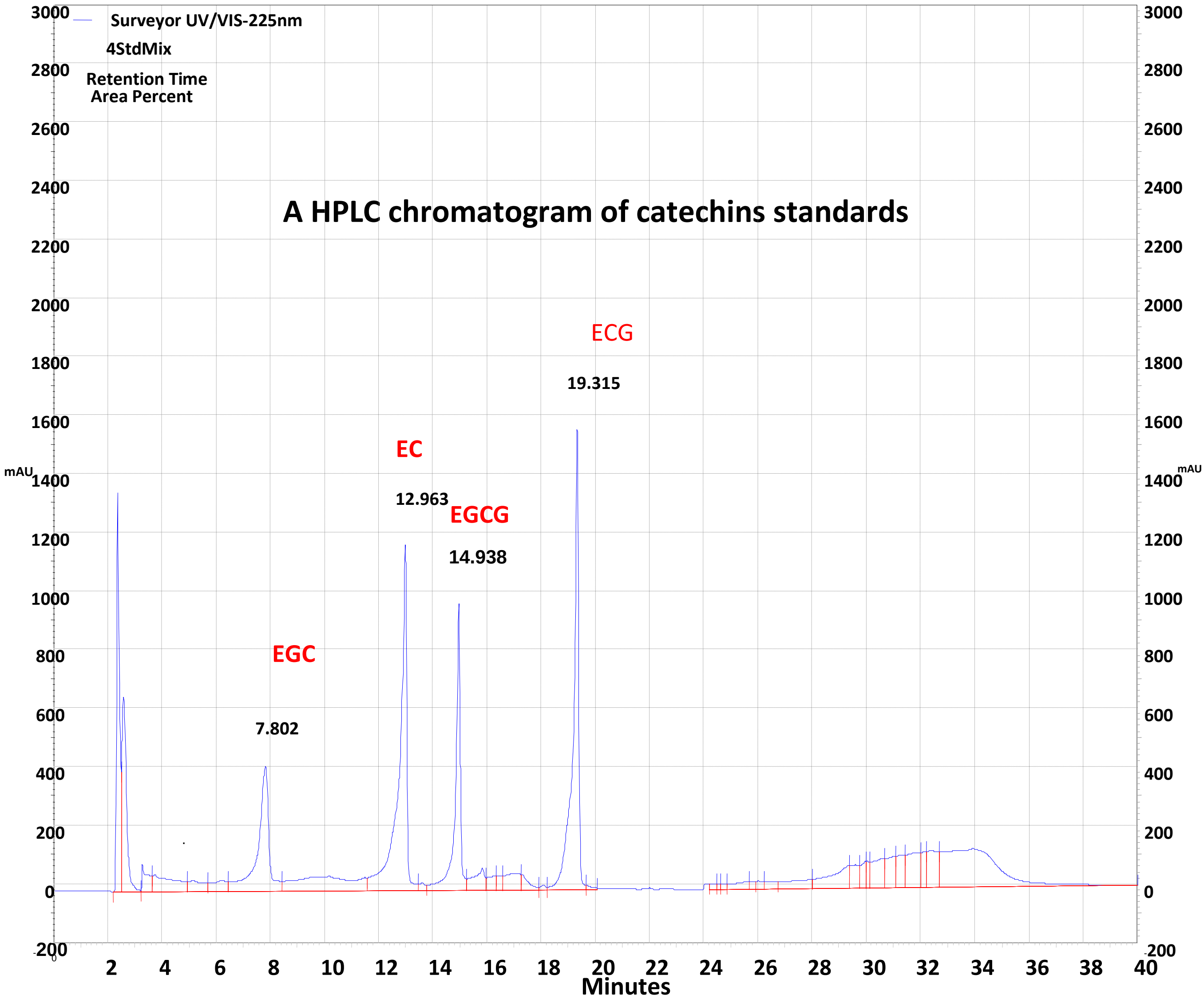


Figure 2.A HPLC chromatogram of epi-structured catechins standards: EGC, EC, EGCG and ECG.

ORAC Assay

The authors developed and validated by the published data using certified reference catechins purchased from the LGC.The Fluostar Galaxy plate reader was set up to perform a kinetic read for 45 minutes at the1-minute interval, with excitation at 485 nm, emission at 538 nm, and cut-off at 530 nm. Raw data were transferred from the Fluostar Galaxy software to a Microsoft Excel spreadsheet for further calculations.

Materials, sample preparation, assay procedure and generation of thestandard curve were as per the instruction manual ZBM0035.01 (Dec. 2010) for ORAC Antioxidant Assay Kit. The ORAC Antioxidant Assay Kit can be used to determine the total antioxidant activity in samples, by measuring the time-dependent decrease of fluorescence using a fluorescence plate reader.

Table1.Fourteen green teas evaluated in the present study.

Tea No.	Tea brand	Source
1	Organic Pure Green Tea	UK/ Marks & Spencer
2	Tesco Green Tea	UK/ Tesco
3	Chinese Green Tea with jasmine flavour	UK/ Tesco
4	Earl Grey Green Tea, Ahmed Tea London	UK/Green Valley
5	Blueberry Green Tea, Ahmed Tea London	UK/Green Valley
6	Organic Fair-trade Authentic,China Green Tea	UK/Green Valley
7	London Tea, Chinese Green Tea	UK/Green Valley
8	Ahmed Tea London Original Green Tea	UK/Green Valley
9	Ahmed Tea London Jasmine Green Tea	UK/Green Valley
10	Twining Selection Green Tea from Tesco	UK/ Tesco
11	Twinings pineapple and Green Grapefruit Tea	UK/ Tesco
12	Twinings Orange & lotus flower Green Tea	UK/ Tesco
13	Twinings Cranberry Green Tea	UK/ Tesco
14	Twinings Light & delicate Green Tea	Sainsbury’s

Statistical Analysis

The present study shows an attempt to differentiate green tea types based on epi- structured catechins content in infusions, using multivariate statistical analysis. An ANOVA was performed, using SPSS v 24, to examinethe levels of total catechins and their profile (mg/g green tea bags) as measured by HPLC.

Besides the Trolox equivalent per gram (g) original sample (mM) as measured by the ORAC assay for fourteen types of green tea bags.

Results and Discussion

Variability in catechins present in different types of green tea bags

It is known that the amount and proportion of various catechins depend on the agronomic conditions, leaf age, and degree of fermentation, which is directly correlated with the final quality of the beverage (Linet al., 1998; Khokhar and Magnusdottir, 2002).The levels of total catechins in the green teas varied from 96.38 to 205.03 mg/g and their profiles:EGCG (43.44-113.31, EGC(35.59-67.46), EC (5.62-18.57) and ECG (4.17-31.56)mg/g tea bag(Table 2).

Looking at the Ahmed Tea London Jasmine Green Tea (Tables 1 and 2; Figures 3 and 4) that has the highest level of total catechins also appears to have the most antioxidant capacity;at the other end, the tea (Twinings Orange & lotus flower 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 (96.38 – 205.03 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, 2006, and 2009; Yao et al., 2006).Profiles of the four epi-structured catechins for the fourteen types of green tea are presented in Table 2; Figures 3and 4.These epi-structured catechins divided into two groups, i.e. EGCG and ECG, EGC and EC.

EGCG and EGC appeared to play a major role in the changes of the sensory qualities of processed green tea beverages (Wang et al., 2000; AL-zubaidi, 2015).Interestingly, EGCG is the most abundant catechin in green tea; in fact, it accounts for more than halfof the total catechins in green tea. Among all the teas examined, EGCG was the highest at 113.31 mg/g tea bag followed byEGC43.44mg/g tea bag (see Tables 1 and 2; Figures 3 and 4).In general, however, EGCG is the major catechin found in green teas (Friedman et al., 2009), and it is certainly the primaryconstituent of all kinds of green teas studied. Furthermore, for these results (see Tables 1and 2; Figures; 3 and 4), there exists a trend in which the order regarding quantities of the epi-structure catechins is: EGCG > EGC > ECG > EC (44-56, 21-47, 4-15 and 5-10) % of the total catechins in green tearespectively.Again, this order of epi-structured catechinsis very similar, if not identical, to those obtained by (Khokhar and Magnusdottir, 2002; Henning et al. 2003; Friedman et al., 2005, 2006, and 2009;Yao et al., 2006 and Al-zubaidi, 2015).In contrast, the Tesco Green Tea (Tables 1and 2; Figures 3 and 4)contained higher levels of EGC (58.66 mg/g)followed by EGCG (57.94 mg/g tea bag), EC (10.22 mg/g tea bag), and ECG (9.97 mg/g tea bags)with an overalltrend of EGC > EGCG > EC > ECG.These results seem to concur with the findings of Lin et al. (1998) who reported higher levels of EGC in Japanese green teas.However, EGC was the highest at 67.46mg/g tea bagin Organic Pure Green Tea comparing with all types of green teafollowed by EC (10.22mg/g tea bag) and ECG (9.97mg/g tea bag)(see Tables 1 and 2).

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 fourteen types of green tea extract.

Tea [a]	EGC[b]	EC[b]	EGCG[b]	ECG[b]	Total catechins [b]	ORAC Value [c]
1	67.46±0.96	10.22±0.79	88.18±1.59	9.97±0.98	175.84±4.32	2381.05
2	58.66±0.07	7.65±0.28	57.94±0.08	7.09±0.01	131.34±0.14	2007.704
3	57.45±0.32	18.57±0.42	99.76±0.65	20.97±0.32	196.74±1.72	3051.914
4	57.68±5.43	10.87±2.19	61.26±4.28	7.83±1.34	137.65±13.24	2108.077
5	46.82±0.02	17.71±0.02	88.88±0.08	23.39±0.01	176.79±0.08	2421.027
6	66.78±0.06	10.31±0.01	90.76±0.13	9.04±0.00	176.89±0.20	2565.142
7	57.73±0.41	12.34±0.43	95.28±0.39	13.20±0.19	178.56±0.56	2678.845
8	54.46±0.17	15.19±0.03	110.21±0.12	20.73±0.04	200.59±0.36	3095.262
9	42.25±0.84	17.90±0.58	113.31±0.76	31.56±0.44	205.03±2.62	3421.142
10	44.09±0.50	6.80±0.27	63.66±0.50	6.13±0.31	120.68±1.58	1932.097
11	45.98±0.27	8.00±0.18	51.72±0.34	5.79±0.01	111.49±0.79	1776.343
12	35.59±0.44	5.62±0.34	50.02±0.50	5.15±0.22	96.38±1.50	1097.306
13	42.61±0.29	6.87±0.24	43.44±0.18	4.17±0.21	97.10±0.91	1214.932
14	58.63±1.00	9.35±0.54	73.24±0.76	8.24±0.39	149.46±2.69	2247.001

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

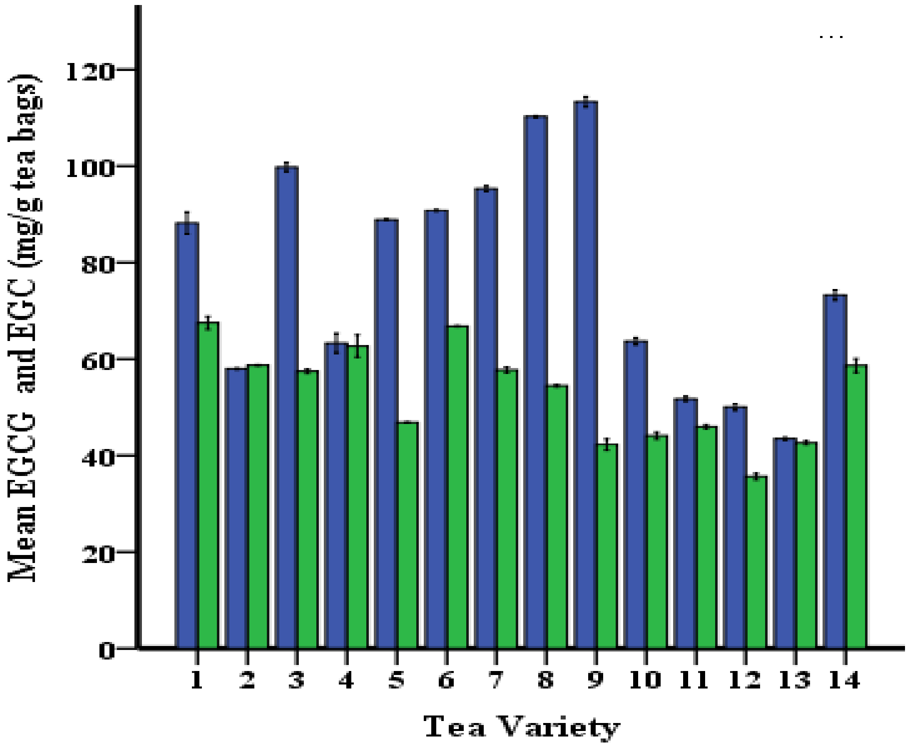


Figure 3.Bar graphs of EGCG and EGC, as measured by HPLC for fourteen types of green tea bags (n = 2; error bar = ± 2 SD).

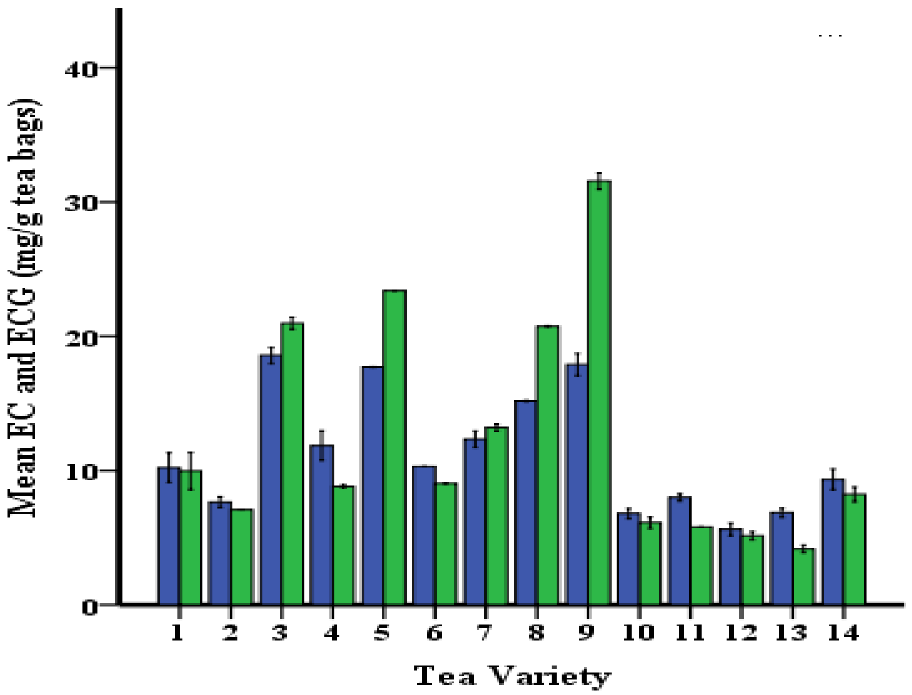


Figure 4.Bar graphs of EC and ECG, as measured by HPLC for fourteen types of green tea bags (n = 2; error bar = ± 2 SD).

Earlier, Sharma et al. (2005), researching 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. All these results further support the key observations of this study that higher infusion temperatures and longer infusion times increase the levels of green tea catechins extracted.Vuonget al., (2013) went further to demonstrate that the epi-structure catechins were stable under acidic conditions but epimerised or degraded at pH ≥ 6. The extractable solids from green tea were found to contain more epi-structured catechins at pHs 3 – 5 but more non-epi-structured catechins at pHs 6 – 7. So, according to these authors, to maximise the extraction of the epi-structure catechins and to minimise 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.

Variability in catechin content could also be due to genetic variability among the plants from which the leaves were harvestedand to soil composition. Likewise, climatic conditions, plucking season, harvesting practices, postharvest storage, sampling, and manufacturing practices (Rusaket al., 2008; Sultana et al., 2008; Friedman et al., 2009). 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).

ORAC Results

Table 2 summarises the results of the experiments,ORAC values varied from 1097.30 - 3421.14Trolox equivalents/g tea bag for fourteen types of green teas(Table 2).As published in the literature, there was asignificant positive correlation ($p < 0.05$) between the levels of green tea catechins and their oxidative capacities as measured by the ORAC assay.Figure 5shows the association between the ORAC value and thecatechin content in teas with $r = 0.93$ ($P = 0.0001$).The ORAC value of the Ahmed Tea London Jasmine Green Tea washigher than all the green tea brews, whereas the Twinings Orange & lotus flower Green Tea showed the lowest ORAC values (Table 2).

Even in the absence of any knowledge about the way catechins may act as powerful antioxidants, this correlation strongly suggests that the ORAC value is a good indicator of the antioxidant capacities of catechins. The ORAC values of these antioxidants were along with the data from other investigators (Mendilaharsu et al. 1998; Henning et al. 2003 and Al-zubaidi, 2015).In conclusion, that is, the higher the level of catechins the greater is the antioxidant capacity of the tea (Al-zubaidi, 2015).

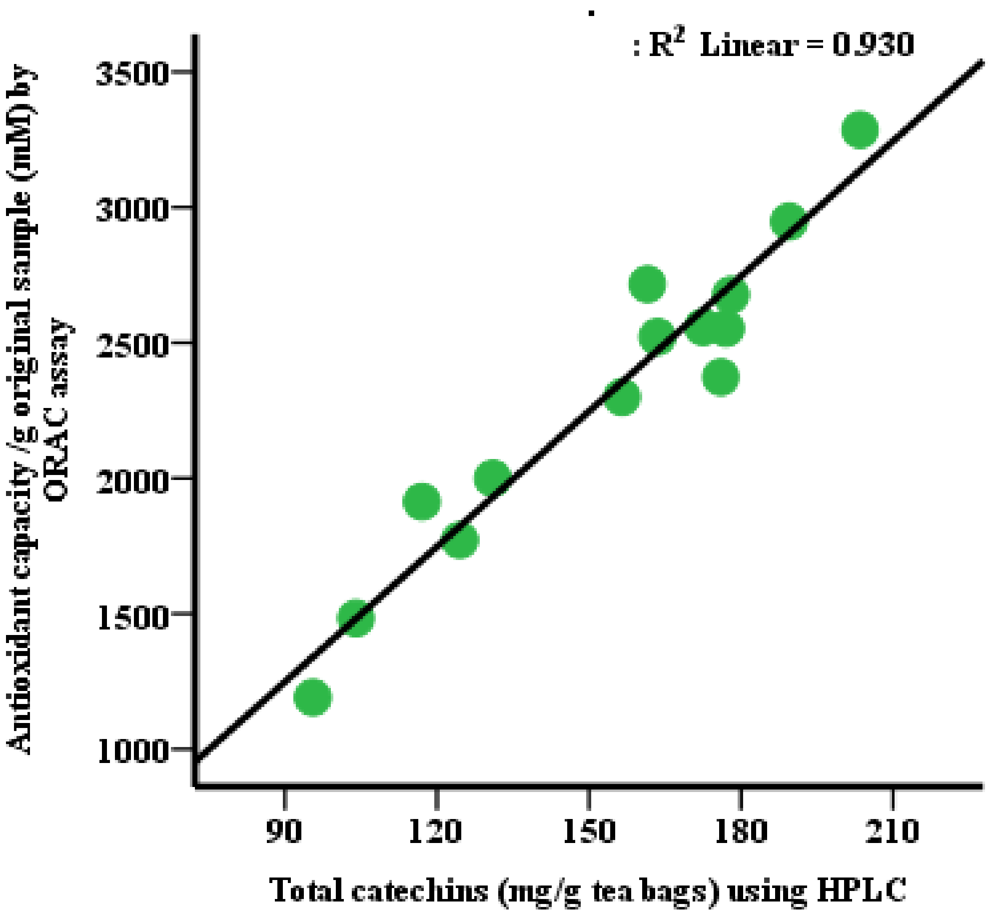


Figure 5. Correlation between total catechins (mg/g tea bag) as measured by HPLC and ORAC values (mM TE/g tea bag) by the ORAC assay.

Conclusion

This study aimsto examine the level of four catechins (Epi-structured) for the fourteen types of regular green tea bags commonly consumed in the UK were determined using RP-HPLC. Tea bags were purchased from different local supermarkets in the UK and extracted in boiling water for 9 minutes tomaximise the extraction of the epi-structure catechins and to decrease their degradation. The extracts from green teas were found to contain more epi-structured catechins at pH4. These extraction conditions help to deliver the expected health benefits to the consumers. Finally, the results of this study propose that consumers may advantage from knowing the types of green tea bags sold at retail. Further studies are needed to increase and improve our understanding of the fate as well as the availability of green tea catechins in food.

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المستخلص

اختبر مستوى الفلافونات (الكتكينات) الرئيسية (Epi-Structured catechins : EGC, EC, EGCG and ECG) في عينات أربعة عشر نوعا من أكياس الشاي الأخضر الورقية والمتاحة في أسواق لندن والتي تعتبر من أكثر أنواع الشاي استهلاكا في المملكة المتحدة باستخدام جهاز كروماتوكرافيا السائل ذو الكفاءة العالية ومتباين الطور (RP-HPLC) بعد تحضير المستخلص المائي لعينات الشاي الأخضر باستخدام المياه المعدنية الطبيعية وعلى درجة حرارة 100°م لمدة 9 دقائق وعلى pH = 4 , تم فصل الكتكينات وقياسها بواسطة جهاز HPLC ومعايرتها مع المنحنيات القياسية للكتكينات القياسية ألمجهزه من Laboratory of the Government Chemist (LGC) للتعرف على تراكيزها وكذلك تم قياس الفعالية المضادة للأكسدة في المستخلصات بطريقة اوراك Oxygen Radical Absorbance Capacity (ORAC). أظهرت النتائج تباين محتوى أنواع الشاي الأخضر من الكتكينات وبشكل معنوي $p < 0.05$ وتراوحت بين 96.38 ملغم/غم في النموذج رقم 12 و 205.03 ملغم / غم في النموذج رقم 9 وقيد يعود سبب هذه الاختلافات إلى عمليات الإنتاج أو فترة الحفظ قبل البيع أو ظروف الخزن . وكذلك أظهرت النتائج اختلافات معنوية $p < 0.05$ في قيم الفعالية المضادة للأكسدة إذ بلغت اقل قيمة 1097.3 مكافئ /غم من الشاي الأخضر في النموذج رقم 12 و 3421.14 مكافئ / غم من الشاي الأخضر في النموذج رقم 9 . بينت نتائج الدراسة الحالية بأن هناك علاقة خطية ومعنوية بين مستوى الكتكينات الكلية وقيم الفعالية المضادة للأكسدة مما يدل على كفاءة الطريقة التي طورها الباحث للقياس بجهاز HPLC حيث اقترن المستوى العالي من الكتكينات الكلية بمستوى عالي من الفعالية المضادة للأكسدة .