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Synergistic, antagonistic and additive interactions of green tea polyphenols

Marta Colon[1] · Cristina Nerín[1]

Abstract Green tea is a natural source of polyphenols where their catechins and flavonols are the major components. Their antioxidant activities are the most important biological effect and often the object of study. DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical assay has been carried out to measure the individual scavenging activities expressed as percentage of DPPH inhibition for each tea polyphenol, and (−)-gallocatechin gallate (GCG) (74.04 ± 0.38 %) and (−)-epigallocatechin gallate (EGCG) (69.51 ± 0.27 %) were the most active compounds. Synergistic, antagonistic and additive interactions among catechin derivatives as well as combined with green tea extract have been analyzed, where GCG and EGCG provided most of the synergistic effects. Flavonols such as quercetin (54.61 ± 0.21 %) and kaempferol (24.06 ± 0.02 %) also showed free radicals' scavenging activity. Interactions between flavonols and individual catechins as well as their mixtures in the tea extract have been investigated. The results suggested that the presence of tea flavonols in tea extract provided additive interactions and the tea catechins were responsible for synergism in green tea. This work can be the starting point of the research about supplemented green tea from its own catechins to increase the total antioxidant capacity of the green tea.

✉ Cristina Nerín
cnerin@unizar.es

[1] Department of Analytical Chemistry, Aragon Institute of Engineering Research I3A, CPS-University of Zaragoza, Torres Quevedo Building, María de Luna St. 3, 50018 Saragossa, Spain

Introduction

Green tea is a complex mixture containing a wide range of flavonoid compounds. Flavonoids are subdivided in six classes: flavones, flavanones, isoflavones, flavonols, flavanols and anthocyanins varying their structures around the heterocyclic oxygen ring [1]. The major flavonoids found in green tea are flavan-3-ol derivatives, which are also known as catechins, and flavonol derivatives. Catechin accounts for up 30 % of water-soluble solids of the dry weight of green tea [2, 3]. Myricetin, quercetin and kaempferol are the main derivatives of the flavonol group. Flavonols are structurally more stable than catechins, and it has been demonstrated that tea is an important source of these compounds [4, 5]. The most important role of flavonoids is their capacity to act as antioxidants, which affects oxygen free radicals and lipid peroxidation. Consequently, flavonoids are involved in the prevention of carcinogenic, mutagenic and arteriosclerotic diseases [6–12].

The antioxidant capacity of green tea has been studied in depth by chemical-based assays. Among them, we can mention the popular DPPH free radical assay [13], the oxygen radical absorbance capacity (ORAC) assay [14], the methodologies based on the reduction in metal ions such as the ferric reducing antioxidant power (FRAP) assay [15] and cupric ion-reducing antioxidant capacity (CUPRAC) assay [16] as well as the electron spin resonance (ESR) assay, which measures the activity of tea toward superoxide ion [17]. In food contact materials, the antioxidant capacity of green tea has been even measured by the free radical assay [18, 19], and this methodology has been applied to determine the antioxidant capacity directly in the active plastic material [20].

It is known that the chemical structure of green tea polyphenols influences their antioxidant properties. Recent studies have demonstrated, by Trolox equivalent

antioxidant capacity (TEAC) method [21], the influence of stereochemistry on the antioxidant properties of catechin epimers as well as the relationship between the structures of several flavonoids and their ORAC values [22]. Furthermore, structurally diverse tea polyphenols might provide similar, or different but complementary, effects with respect to their antioxidant activities [23]. Previous works have investigated about the antioxidant capacity of food mixtures [24] or even combinations between green tea and other antioxidant compounds [25]. However, the synergistic, antagonistic or additive interactions among the own polyphenols of green tea have not been researched yet.

The aims of this work were (1) to evaluate the antioxidant capacity of the individual catechins as well as that of the green tea extract which contains the mixture of all tea polyphenols, (2) to study the synergistic, antagonistic and additive interactions resulting from the combination of catechins, and consequently, to discuss which catechins were the responsible for enhancing the antioxidant capacity of the green tea and (3) to evaluate the influence of minor tea polyphenols such as quercetin and kaempferol on the green tea extract. To summarize, the main target of this work was to know what tea constituents provided the synergistic interactions and, consequently, what tea constituents were responsible for the antioxidant capacity of green tea.

Materials and methods

Reagents and solutions

Quercetin [>98 % (HPLC), Cas 117-39-5] (Q); kaempferol [>90 % (HPLC), CAS 520-18-3] (K); (+)-catechin [>99.0 % (HPLC), CAS 154-23-4] (C); (−)-epicatechin [>95.0 % (HPLC), CAS 490-46-0] (EC); (−)-epicatechin gallate [>98 % (HPLC), CAS 1257-08-5] (ECG); (−)-catechin gallate [>98 % (HPLC), CAS 130405-40-2] (CG); (−)-epigallocatechin [> 95.0 % (HPLC), CAS 970-74-1] (EGC); (−)-gallocatechin [>98 % (HPLC), CAS 3371-27-5] (GC); (−)-gallocatechin gallate [>98 % (HPLC), CAS 4233-96-9] (GCG); (−)-epigallocatechin gallate [>95.0 % (HPLC), CAS 989-51-5] (EGCG); and 2,2-diphenyl-1-picryl-hydrazyl (free radical, CAS 1898-66-4) (DPPH) were all supplied by Sigma-Aldrich Química S.A. Methanol (high-performance liquid chromatography (HPLC) grade CAS 67-56-1) was provided by Scharlab (Mollet del Vallés, Spain).

An individual solution of C (0.276 mM), EC (0.276 mM), GC (0.261 mM), EGC (0.261 mM), CG (0.182 mM), ECG (0.182 mM), GCG (0.174 mM), EGCG (0.174 mM), quercetin (0.265 mM) and kaempferol (0.279 mM) standards was prepared. All the stock solutions

were prepared in methanol and were stored in the fridge, and they were used during 3 weeks. Green tea extract solution was also prepared in methanol with a concentration of 100 μg per gram. The green tea solution was filtered using a 0.22-μm Nylon syringe filter (KX Syringe Filter, 25 mm, 0.22 μm Nylon, Kinesis, UK). A 0.1-mM solution of DPPH free radical was prepared in methanol. The DPPH solution was stored at −20 °C in hermetically sealed amber glass bottle, and it was prepared daily.

Green tea extract

Green tea extract Sunphenon 90 MB (GTE) was from TAIYO Europe (Filderstadt, Germany), and the extract contained around 75 % total catechins (w/w, HPLC determination provided by the supplier Company).

DPPH radical assay

The DPPH radical assay is based on the scavenging of DPPH radical through the action of an antioxidant that decolorizes the DPPH solution. To calculate the antioxidant capacity of each individual standard as well as the green tea extract, a 100 μL of standard or green tea solution was added to 3.9 mL of a 0.1-mM DPPH solution. To evaluate the synergistic effects (SEs) between catechins as well as the mixtures between catechins and Q or K, a total of 100 μL from each standard in equimolecular proportions (1:1 v/v ratio) were added to 3.9 mL of a 0.1-mM DPPH solution. The antioxidant effects of Q and K into the spiked GTE were also calculated. For that, a total of 100 μl from mixtures between GTE and Q or K were prepared as follows: 1:1, 1:2, 1:3, 1:9, 2:1, 3:1 and 9:1. All the mixtures were shaken vigorously for 10 s, and the capped cuvette was placed at room temperature in the dark for 15 min. The reaction time recommended in the original assay proposed by Brand-Williams et al. [26] was 30 min, but several researchers have reduced this time to the range of 3–16 min [27–29]. After the reaction between DPPH and the polyphenols, the absorbance of the mixture was measured at 515 nm with a UV-1700 PharmaSpec UV–Vis spectrophotometer (Shimadzu, Japan). All the measurements were taken in triplicate.

Radical scavenging activity of the mixture samples was expressed as the inhibition percentage of free radical by the sample and was calculated using the following equation:

Scavenging effect (% Inhibition)
$$= \left[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \right] \times 100,$$

where A_{control} is the absorbance value of the control (3.9 mL DPPH plus 100 μL methanol) and A_{sample} is the absorbance value of the sample (3.9 mL DPPH plus 100 μL sample).

Calculation of synergistic effects (SE) of antioxidant mixtures

The experimental scavenging capacity (ESC) of antioxidant mixtures was calculated using the equation described by Mensor et al. [30]:

$$\% \ ESC = 100 - \left[\left[\left(A_{sample} - A_{blank} \right) \times 100 \right] / A_{control} \right],$$

where A_{sample} was the absorbance value of the sample (3.9 mL DPPH plus 100 µL sample), A_{blank} was the absorbance value of antioxidant solution in methanol and $A_{control}$ was the absorbance value of control (3.9 mL DPPH plus 100 µL methanol). The theoretical scavenging capacity (TSC) was the sum of the scavenging capacities of each antioxidant, calculated using the individual scavenging capacity in the following equation (Fuhrman et al.) [31]:

$$\% \ TSC = 100 - [(100 - ESC_A) \times (100 - ESC_B / 100)],$$

where ESC_A and ESC_B represented the percentage ESC of the individual antioxidant. The SE was calculated using the following equation (Fuhrman et al.) [31]:

$$SE = ESC/TSC,$$

where SE was considered when SE was higher than 1 (SE > 1), antagonistic effect was considered when SE was lower than 1 (SE < 1) and additive effect was considered when SE was approximately 1. Synergistic, antagonistic or additive effect was assigned depending on the significant differences ($p < 0.01$) between the experimental scavenging value and the theoretical scavenging value.

Statistical analysis

All analyses were performed in triplicate. The data were expressed as means ± standards errors, and one-way analysis of variance (ANOVA) was performed to test for significant differences among the means. Differences among means at $p < 0.01$ were considered significant.

Results and discussion

Scavenger capacity of individual green tea catechins and green tea extract

The scavenging activities of the eight catechin standards are shown in Fig. 1a. The radical scavenging effect is expressed as percentage of inhibition (% Inhibition) of DPPH radical by each catechin. To calculate the changes in the % Inhibition of catechins, three different concentrations were used. The DPPH radical assay was performed during 30 min and the scavenging inhibition was calculated in different time periods of 10 s, 3, 5, 10, 15 and 30 min.

After 15 min, the signal of % Inhibition of catechins was completely stabilized and this value was used to compare results. For all standards, the graphs showed that the stronger activity was reached at the higher concentration measured. Figure 1a shows that GCG and EGCG, which are the gallocatechin gallate isomers, exhibited the highest % Inhibition. For a concentration of 0.174 mM, GCG and EGCG reached a scavenging activity of 82.23 ± 0.58 and 74.26 ± 0.28 %, respectively. The variation in % Inhibition between 15 and 30 min was below to 1 %. The high scavenging activity demonstrated that GCG and EGCG were very efficient antioxidants and both stereoisomers exhibited similar % Inhibition values. After GCG and EGCG, ECG exhibited the higher scavenging activity (50.22 ± 0.25 %). The maximum percentage was reached at 0.182 mM. However, CG, which is its corresponding stereoisomer, showed a lower inhibition (25.01 ± 0.34 %) at the same concentration (0.182 mM). The maximum % Inhibition for C (49.70 ± 0.43 %) and EC (23.78 ± 0.45 %) was reached at 0.276 mM. With respect to GC and EGC, the inhibition value (0.260 mM) was 47.08 ± 0.25 and 4.47 ± 0.27 %, respectively. EGC showed the lowest % Inhibition of DPPH radical. The reaction between EGC and DPPH radical did not seem to be dose dependent, because the scavenging activities did not vary significantly between different concentrations. In fact, the variation in scavenging activity was lower than 3 %. The rest of catechin standards showed a large increase in the scavenging capacity in a dose-dependent manner. Specifically, GCG exhibited the most important variation in the scavenging activity, around up 50 % between the major (0.174 mM) and the minor (0.044 mM) concentration used for its DPPH radical assay. The variation for C and GC was around 20 %. On the other hand, EC, CG, ECG and ECGC showed a different behavior. In the case of the minimum and intermediate concentration, the radical reaction did not depend on the concentration of catechin solutions. However, at the maximum concentrations, the scavenger analysis was strongly dose dependent for EC, CG, ECG and EGCG.

After the analysis of each individual catechin standard, the scavenging activity of GTE was evaluated. Three concentrations, from 25 to 100 µg of extract per gram of methanol, were measured. Figure 1b shows the scavenging capacity of GTE after reaction with DPPH radical. As catechin standards, GTE exhibited the major scavenger activity for the maximum concentration, namely 34.86 ± 0.18 % of DPPH inhibition at 100 µg GTE/g methanol after 15 min. The radical reaction between GTE and DPPH seems to be dose dependent on the GTE concentration, because the scavenging activity significantly varied respect to the concentration evaluated.

The main difference between GTE and catechin standards was the total % Inhibition reached. At 15 min of
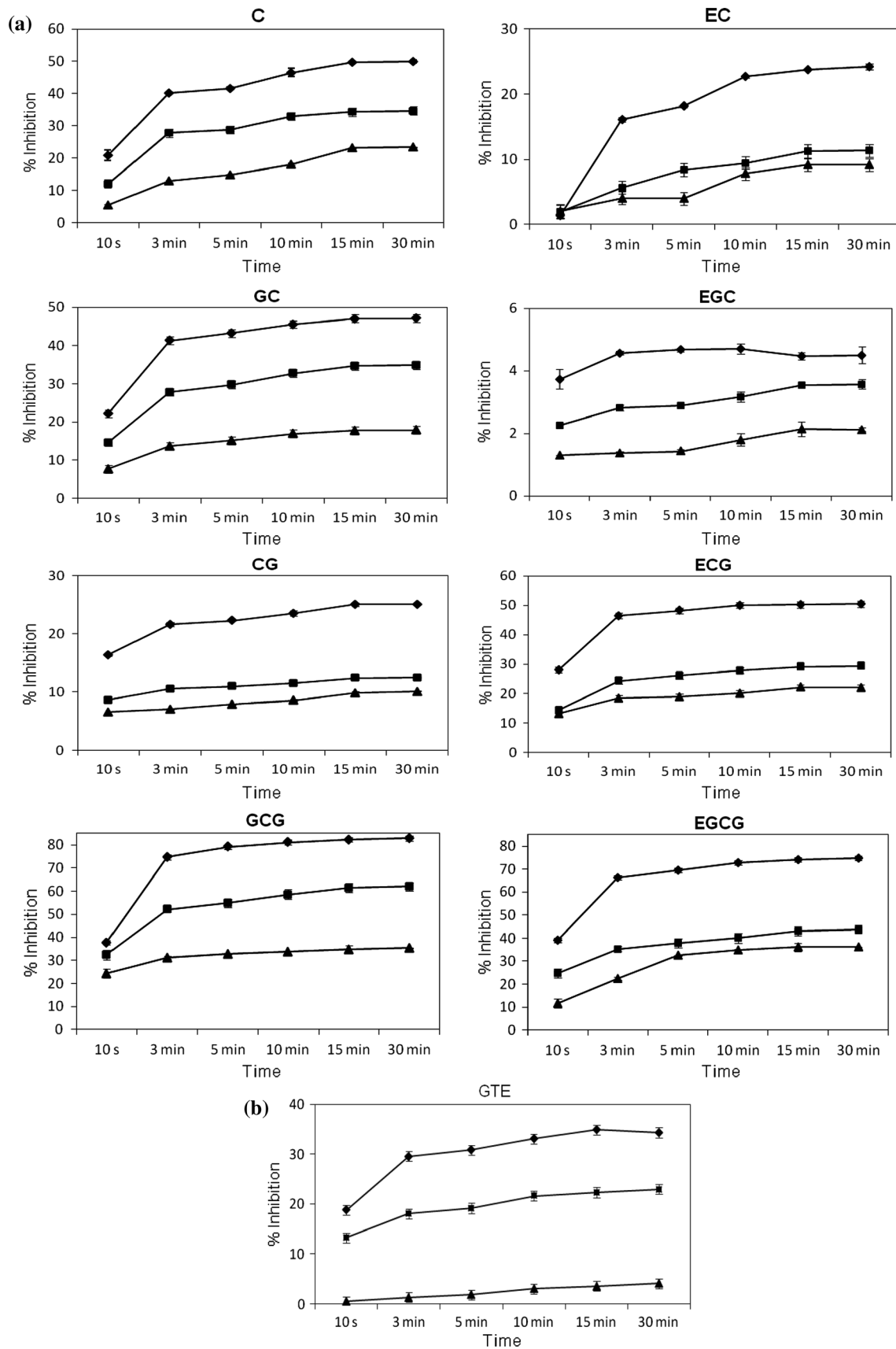
(a)

(b)

◄ **Fig. 1** **a** Scavenging activity of individual tea catechins expressed as percentage of inhibition of DPPH radical (% Inhibition). *Error bars* represent means ($n = 3$) ± standard deviation (C and EC: *filled triangle* = 0.069 mM, *filled square* = 0.138 mM, *filled diamond* = 0.276 mM; GC and EGC: *filled triangle* = 0.065 mM, *filled square* = 0.131 mM, *filled diamond* = 0.261 mM; CG and ECG: *filled triangle* = 0.046 mM, *filled square* = 0.091 mM, *filled diamond* = 0.182 mM; GCG and EGCG: *filled triangle* = 0.044 mM, *filled square* = 0.087 mM, *filled diamond* = 0.174 mM). **b** Scavenging activity of green tea extract (*filled triangle* = 25 µg GTE/g methanol solution, *filled square* = 50 µg GTE/g methanol solution, *filled diamond* = 100 µg GTE/g methanol solution). *Error bars* represent means ($n = 3$) ± standard deviation

DPPH reaction, GCG (82.23 %), EGCG (74.26 %), ECG (50.22 %), C (49.70 %) and GC (47.08 %) obtained higher inhibition values than GTE (34.86 %). On the other hand, CG (25.01 %), EC (23.78 %) and EGC (4.47 %) provided lower scavenging activities than GTE. These results suggested that the possible interactions between individual catechins could influence the total scavenger capacity of GTE. Previous studies carried out on essential oils showed that their antioxidant capacity could be achieved by the sum of the individual antioxidant capacities of the individual components [32]. Taking into account this information, a full study was proposed, which considers all the catechin mixtures, to investigate which were the tea catechins that provided SEs.

Synergistic effects of catechin standard mixtures and their mixtures with green tea

To research the antioxidant interactions between tea catechins, individual tea standards were mixed in pairs and DPPH radical assay was carried out to evaluate their synergistic properties. Mixtures were prepared in pairs to study the effect of each catechin respect to the others. The mixtures were prepared in the same concentrations (1:1, v/v) to compare the results in the same proportions. If two catechins were mixed in different concentrations and one of them provided a high individual antioxidant capacity, the results obtained from the mixture could come only from the most active catechin and not from the mixture. The ESC was recorded, and they were compared to the TSC calculated from the individual scavenging capacities. Figure 2a shows the SE value obtained for the different mixtures. Asterisks denoted catechin mixtures that exhibited a significant difference between ESC and TSC ($p < 0.01$). Catechin mixtures were prepared in pairs of stereoisomers C + EC, CG + ECG, GC + EGC and GCG + EGCG as well as in pairs of catechin standards regardless stereochemistry (the rest of the possible combinations listed in Fig. 2a). First, the stereoisomer mixtures showed that C + EC and GC + EGC exhibited additive interactions with SE values of 0.73 ± 0.05 and 0.75 ± 0.03, respectively, while the

ESC values of GCG + EGCG and CG + ECG were significantly higher than TSC values and a synergistic interaction was considered for these pairs of catechins. The SE value obtained for GCG + EGCG was 1.09 ± 0.03 and the SE value for CG + ECG was 1.12 ± 0.01. With respect to the rest of catechin samples, the group of C mixtures provided additive as well as antagonistic interactions. The SE values of C with GC (0.76 ± 0.02), EGC (0.72 ± 0.01), CG (0.71 ± 0.06) and ECG (0.89 ± 0.02) were lower than 1, and these differences were found to be statistically significant. These mixtures exhibited antagonistic interactions. The mixtures of C with gallocatechin gallate derivates (GCG and EGCG) exhibited additive interactions due to the fact that experimental values were not significantly different to the theoretical ones. In the case of its stereoisomer, the mixtures with EC presented an identical behavior. The SE values of EC + GC (0.51 ± 0.01); EC + EGC (0.46 ± 0.01); EC + CG (0.46 ± 0.02); and EC + ECG (0.52 ± 0.03) were significantly lower than 1, and these results concluded that an interaction happened among these catechins, thus displaying antagonism. The mixtures of EC with gallocatechin gallate derivatives were additive. With respect to the mixtures with GC, the GC + GCG (1.28 ± 0.01) and GC + EGCG (1.05 ± 0.04) pairs exhibited statistically significant differences and synergism among these catechins was observed. However, the GC + CG and GC + ECG mixtures did not show significant differences between experimental and theoretical scavenger values. In the case of EGC, the mixtures with GCG (1.07 ± 0.04) and EGCG (1.03 ± 0.02) showed SE values significantly higher than 1 and a synergistic interaction was associated. Concerning the catechin gallate stereoisomers, the mixtures with EC provided additive interactions because the experimental results were similar to the theoretical ones for each individual catechin. Finally, the SE values of GCG + CG (1.08 ± 0.01) and GCG + ECG (1.10 ± 0.01) mixtures as well as EGCG + CG (1.03 ± 0.01) and EGCG + ECG (1.05 ± 0.03) mixtures were significantly higher than 1, and consequently, all these mixtures exhibited synergistic interactions.

Figure 2b shows the percentage of each type of interaction associated with each catechin compound. As can be seen, the pairs of stereoisomers (C and EC, GC and EGC, CG and ECG, GCG and EGCG) exhibited the same percentages. In the case of C and EC, synergistic interactions were not observed, antagonistic effects occurred in 57 % and additive effects occurred in 43 %. The opposite behavior was observed for GCG and EGCG. Antagonistic interactions were not found, and the major synergistic interactions (57 %) were associated with these stereoisomers. GCG and EGCG provided 43 % of additive interactions. GC and EGC stereoisomers showed the same percentage of synergistic and antagonistic interactions (29 %) and a
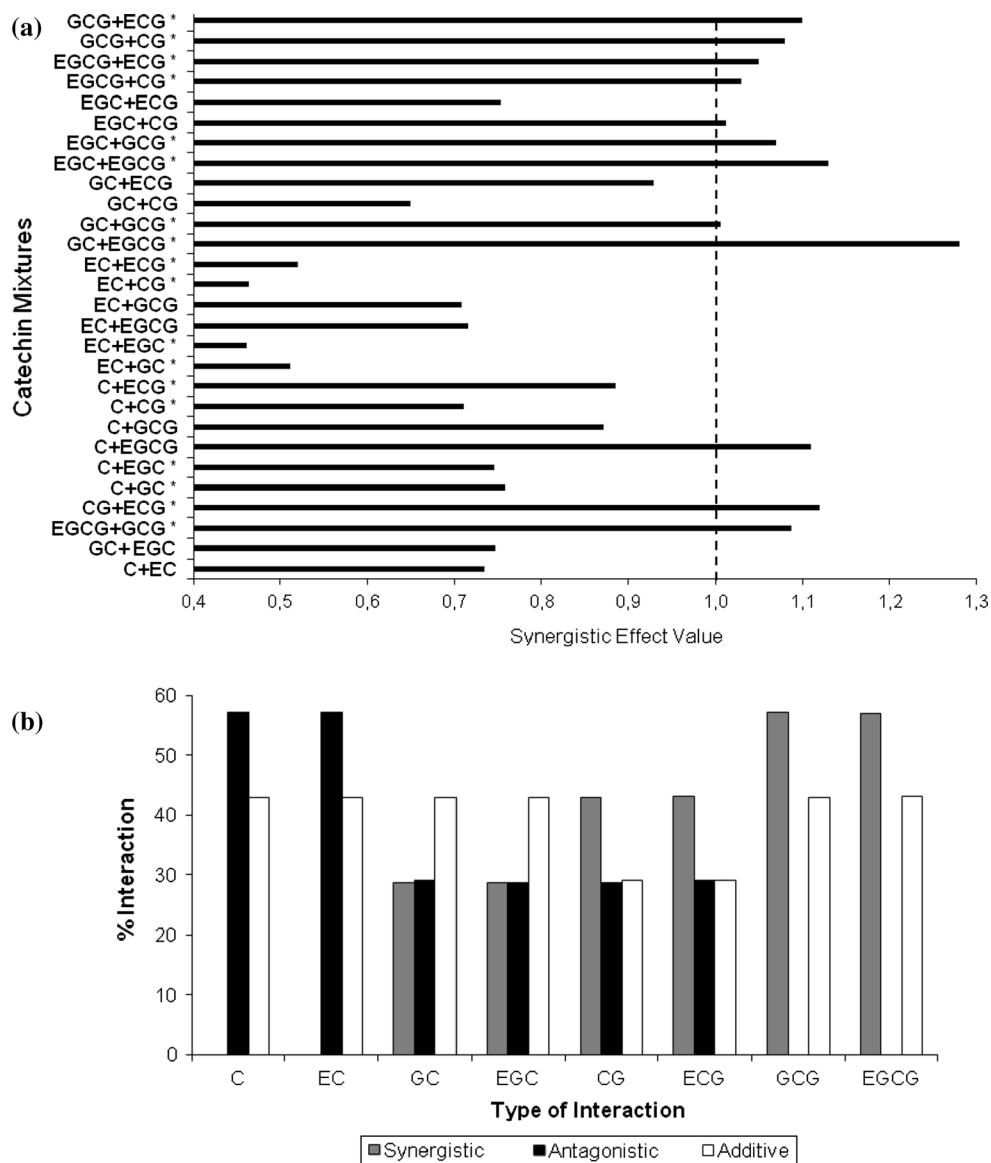
Fig. 2 a Synergistic effect values calculated for catechin mixtures. *Dashed line* represents SE value equal to 1. *Asterisks* denote a significant difference ($p < 0.01$) between ESC and TSC values. **b** Percentage of synergistic, antagonistic and additive interactions observed in each tea catechin

higher additive effect (43 %). In the case of CG and ECG, SEs (43 %) were higher than antagonistic (29 %) and additive (29 %) effects. From these results, it can be concluded that the most abundant SEs were associated with the mixtures containing GCG (57 %), EGCG (57 %), CG (43 %) and ECG (43 %). SEs associated with GC (29 %) and EGC (29 %) correspond only to the mixtures with GCG and EGCG (Fig. 2a). These results also indicated that the combination of several antioxidants did not guarantee that the interactions among them were additive.

Further, the structure of CG, ECG, GCG and EGCG, namely galloylated catechins, shares a common fragment, which is represented in Fig. 3 (fragment X), and it is known as galloyl fragment. Many studies have reported

that the scavenging effects of galloylated catechins were stronger than those of nongalloylated catechins (C, EC, GC and EGC). On the other hand, the scavenging effects of GC and EGC, which have trihydroxyl groups in the B ring (Fig. 3), were stronger than those of C and EC, which have dihydroxyl groups in B group [33–37]. Therefore, the presence of the galloyl fragment at the C rings (Fig. 3) and the additional hydroxyl group at the B ring played the most important role in the antioxidant capacity of the tea catechins. These previous works can confirm why the most abundant synergisms were associated with GCG, EGCG, GC and EGC derivatives. In case of GCG or EGCG, the 57 % of synergism was justified by the presence of the galloyl fragment in the C ring as well as a trihydroxyl group in
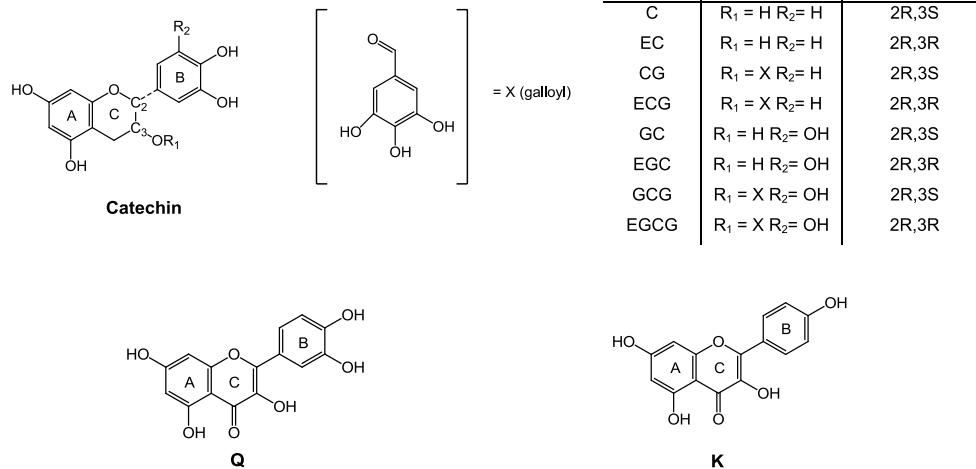
Fig. 3 Structure of tea catechins (**a**), quercetin (**b**) and kaempferol (**c**)

Table 1 Synergistic effect of mixtures between green tea extract and individual tea catechins ($n = 3$)

Mixture (1:1)	SE	Effect
GTE + C	1.02 ± 0.03	Additive
GTE + EC	1.02 ± 0.02	Additive
GTE + CG	$1.01^* \pm 0.04$	Synergistic
GTE + ECG	$1.05^* \pm 0.09$	Synergistic
GTE + GC	0.96 ± 0.11	Additive
GTE + EGC	0.89 ± 0.08	Additive
GTE + GCG	$1.55^* \pm 0.12$	Synergistic
GTE + EGCG	$1.52^* \pm 0.01$	Synergistic

Asterisks denoted a significant difference between experimental and theoretical scavenging value (* $p < 0.01$)

the B ring. The synergism observed for the mixtures containing CG and ECG (43 %) was associated with the presence of the galloyl fragment, but the presence of a dihydroxyl group in the B ring provided less SE. with respect to the nongalloylated catechins, GC and EGC exhibited SE only in the presence of GCG and EGCG, but C and EC did not exhibit any synergistic association. Any nongalloylated catechin contains the galloyl fragment in the C ring, but the number of hydroxyl groups in the B ring differs between these pairs of catechins (Fig. 3). For this reason, SE was observed for GC and EGC mixtures but not for C and EC mixtures.

To complete this study, an experiment based on the SE values obtained from the mixtures of green tea extract and each individual catechin was performed. Here, the GTE–catechin mixtures have been prepared in equimolecular proportions to evaluate the antioxidant effect of each catechin over the same proportion of green tea extract. At this point, recall that green tea extract contains the mixture of eight catechins, and therefore, this experiment permitted us to evaluate the real effect of each catechin with respect to others contained in green tea. Table 1 shows the SE values obtained for each mixture and the type of interaction associated. As expected, the mixtures among GTE and GCG (1.55 ± 0.12), EGCG (1.52 ± 0.01) and CG (1.01 ± 0.04) and ECG (1.05 ± 0.09) provided synergistic interactions. The rest of combinations provided additive interactions. Again, the results concluded that GCG, EGCG, CG and ECG had a SE on both mixtures with individual catechins as all catechins combined in the green tea extract.

If the antioxidant capacity of a mixture of compounds is intimately connected to the different interactions that take place, then the total antioxidant capacity of this mixture can increase or decrease depending on the possible synergistic or antagonistic interactions. Therefore, if GCG, EGCG, CG and ECG provided most of the SEs, their presence in the tea composition can increase the total antioxidant capacity as the results have demonstrated.

Scavenger capacity of tea flavonols: quercetin and kaempferol

Quercetin (Q) and kaempferol (K), which are classified as flavonol derivatives, make up to 2–3 % of the soluble solids of tea [38, 39]. Previous studies have demonstrated that these compounds were potent antioxidants due to the combination of a keto group conjugated to a double bond in the C ring, together with adjacent hydroxyl groups in the B ring [40, 41]. Specifically, Q contains two hydroxyl groups in the B ring with respect to the one hydroxyl group
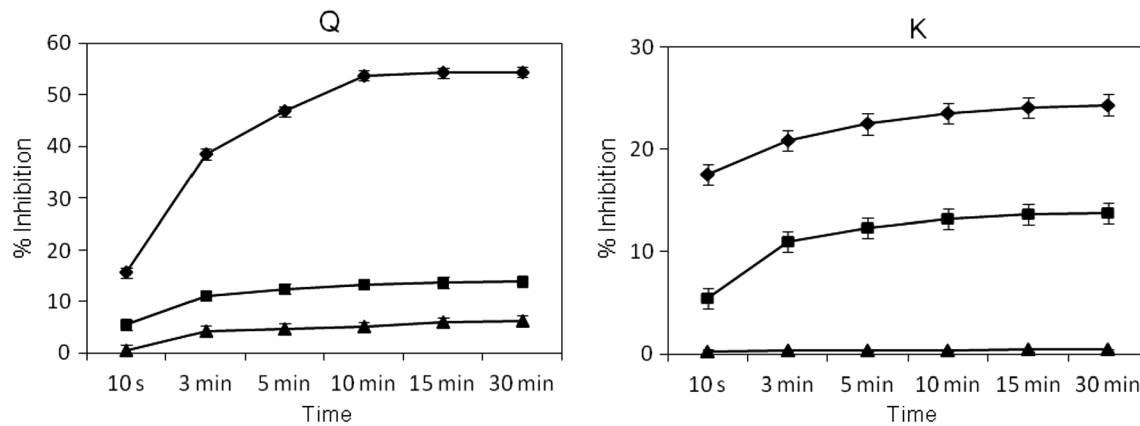
Fig. 4 Scavenging activity of quercetin and kaempferol. *Error bars* represent means ($n = 3$) $\pm$ standard deviation (Q: *filled triangle* $= 0.066$ mM, *filled square* $= 0.133$ mM, *filled dia-* mond $= 0.265$ mM; K: *filled triangle* $= 0.070$ mM, *filled square* $= 0.140$ mM, *filled diamond* $= 0.279$ mM)

of K (Fig. 3). The scavenging capacities of Q and K have been measured by the DPPH radical assay and are shown in Fig. 4. The radical scavenging effect is expressed as percentage of inhibition (% Inhibition) of DPPH radical by each flavonol. To evaluate the changes in the % Inhibition of flavonols, three different concentrations of Q or K were used. Both showed the maximum percentage when the maximum concentration of them was measured. After 15 min, the DPPH signal was completely stabilized for both experiments. The scavenging activity was 54.61 ± 0.21 % for Q (0.265 mM) and $24.06 \pm 0, 02$ % for K (0.279 mM). In the case of quercetin, the radical reaction appeared to be dose dependent because the % Inhibition varied significantly among different concentrations measured. For kaempferol, the lowest concentration did not afford scavenger activity. With respect to the maximum and the intermediate concentration, the radical reaction also appeared to be dose dependent. These results suggested that Q was better antioxidant than K. This behavior has been observed in other antioxidant assays such as TEAC, FRAP, hypochlorite scavenging assay and deoxyribose assay [41]. The higher antioxidant capacity of Q can be related to the presence of two hydroxyl groups in the B ring (Fig. 3). The low antioxidant capacity of K can be attributed to the presence of a single hydroxyl group in the B ring, which apparently has a little contribution in its scavenger ability (Fig. 3). These conclusions have been already reported in the bibliography [41, 42].

With respect to the scavenging value obtained for the catechin standards (from 4.47 to 82.23 %) (Fig. 1a) as well as GTE (34.86 %) (Fig. 1b), Q (54.61 %) and K (24.06 %) (Fig. 4), intermediate scavenger values were achieved. These results suggested that Q and K can present antioxidant interactions with tea catechins.

Synergistic effects of Q and K with catechins and green tea extract

Catechins, quercetin and kaempferol are typical green tea polyphenols and well-known as antioxidants. Tea catechins form at least 20 % of the dry weight of green tea, while tea flavonols make up to 2–3 % of the water-soluble solids of green tea [38]. The aim of this part of the research was to study the antioxidant interactions between these polyphenols in two different experiments. First, mixtures of individual catechins and Q or K were prepared. Second, mixtures of Q and K with green tea extract were evaluated. In this case, mixtures of Q and K with GTE were carried out to discuss the results obtained when several polyphenols were mixed and when these polyphenols were mixed in different proportions.

Several polyphenols mixtures were evaluated to calculate their SE values. Table 2a shows the SE values of the mixtures between quercetin and the individual catechins as well as the mixtures between kaempferol and the individual catechins. All the mixtures were prepared in equimolecular proportions (1:1, v/v) to compare the results in the same conditions. In Table 1, the mixtures Q + catechins did not show significant differences, and therefore, the associated interactions were additive except for Q + EGCG combination. In this case, the SE value was 1.14 ± 0.03 and the ESC value was significantly higher than the TSC value. The antioxidant interaction associated with Q + EGCG mixture was a synergistic interaction. This effect can be related to their chemical structures as has been previously explained. This synergistic interaction can be favorable by the presence of the galloyl fragment in the C ring and the trihydroxy group in the B ring of EGCG in combination with the dihydroxy group in the B ring of quercetin (Fig. 3). On

Table 2 Synergistic effect of quercetin (Q) and kaempferol (K), (a) with the different tea catechins ($n = 3$), (b) in green tea extract (GTE) mixtures ($n = 3$)

Mixture (1:1)	SE	Effect	Mixture (1:1)	SE	Effect
(a)					
Q + C	1.08 ± 0.12	Additive	K + C	1.08 ± 0.04	Additive
Q + EC	1.08 ± 0.03	Additive	K + EC	1.09 ± 0.06	Additive
Q + CG	0.86 ± 0.04	Additive	K + CG	0.75 ± 0.13	Additive
Q + ECG	0.76 ± 0.09	Additive	K + ECG	0.76 ± 0.10	Additive
Q + GC	0.85 ± 0.01	Additive	K + GC	0.78 ± 0.08	Additive
Q + EGC	0.79 ± 0.01	Additive	K + EGC	0.76 ± 0.01	Additive
Q + GCG	0.84 ± 0.02	Additive	K + GCG	0.73 ± 0.03	Additive
Q + EGCG	1.14* ± 0.03	Synergistic	K + EGCG	1.00 ± 0.05	Additive

Mixture Q:GTE	SE	Effect	Mixture K:GTE	SE	Effect
(b)					
1:1	1.01 ± 0.02	Additive	1:1	0.98 ± 0.03	Additive
2:1	0.99 ± 0.07	Additive	2:1	0.89 ± 0.09	Additive
3:1	0.97 ± 0.01	Additive	3:1	0.86 ± 0.01	Additive
9:1	0.94 ± 0.03	Additive	9:1	0.73 ± 0.01	Additive
1:2	1.05* ± 0.01	Synergistic	1:2	0.93 ± 0.02	Additive
1:3	1.09* ± 0.04	Synergistic	1:3	1.04* ± 0.03	Synergistic
1:9	1.15* ± 0.06	Synergistic	1:9	1.05* ± 0.01	Synergistic

Asterisks denoted a significant difference between experimental and theoretical scavenging values (* $p < 0.01$)

the other hand, the mixtures K+ catechins showed additive interactions in all the cases (Table 1).

After the study of Q and K with the individual tea catechins, mixtures between GTE and Q or K were prepared. Table 2b presents the SE values obtained for the different mixtures. In this case, the mixtures were prepared in different proportions to study the antioxidant effect of Q and K in GTE. The study of Q and GTE mixtures showed synergistic interactions for mixtures Q:GTE as follows: 1:2 (1.05 ± 0.01); 1:3 (1.09 ± 0.04); and 1:9 (1.15 ± 0.06) For K:GTE mixtures, SEs were only observed for 1:3 (1.04 ± 0.03) and 1:9 (1.05 ± 0.019). All these results suggested that combination of green tea with Q and K did not show antagonistic interactions and the presence of these flavonols in green tea did not decrease the total antioxidant capacity. However, it can be observed that the synergistic interactions took place when the concentration of the green tea, which contains the mixture of the eight catechins, was higher than the concentration of Q or K. Therefore, these results suggested that catechins were responsible for synergism in green tea extract, and the GTE antioxidant capacity was related to the synergistic interactions taking place in the presence of catechins. This study reaffirmed the conclusions achieved in the previous section.

To summarize, green tea polyphenols showed synergistic, antagonistic and additive interactions among them, affecting the total antioxidant capacity of green tea extract. Further, catechins were responsible for synergism in GTE,

and specifically, GCG, EGCG, CG and ECG provided most of the synergistic interactions. These conclusions can be the beginning of an important research based on the supplemented antioxidant food. The addition of the tea catechins in the own green tea composition could increase the antioxidant properties of the whole green tea, and therefore, a supplemented green tea could be prepared with better antioxidant properties. The highlight of this kind of supplemented food is that they are enriched with their own antioxidant constituents which are from the nature and safe for human health. Further, this kind of supplemented extract could be applied to the food industry, especially the active packaging industry to prepare food packages with better antioxidant properties and increase their added value.

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