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Epimerisation of catechins in green tea infusions

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Abstract

Tea catechins undergo many chemical changes such as oxidation and epimerisation during the course of the manufacturing and brewing processes. In the production of green tea, the oxidation is limited by inactivating the enzymic activity in freshly picked tea leaves by firing or steaming. As a result, epimerisation of the catechins is thought to be one of the most important reactions in the manufacture of green tea. The epimerisation of catechins in green tea infusions using both purified water and tap water at different temperatures has been investigated by HPLC and is reported in the present paper. Individual catechins can undergo epimerisation at high temperatures; however, in green tea infusions, the predominant change appears to be epimerisation from the epistructure to the nonepistructure. It has been found that this epimerisation takes place more easily in tap water than in purified water. The complexity of the ions present in the tap water together with the difference in pH between tap and purified water are thought to be the main factors influencing this observation. In the infusion brewed with tap water, the catechins are easily epimerised and then rapidly degraded. Stability studies on catechins in green tea strong infusions, which were prepared with a mixture of ethanol and purified water, in the ratio of tea leaves to solvent 1:5 (w/v), have shown that epimerisation can be observed at 40°C after a few days storage. Therefore, it is thought that not only temperature, but also heating time influences the epimerisation of catechins in green tea infusions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Green tea; Catechins; Epimerisation; HPLC

1. Introduction

Tea is now consumed throughout the world and is the most popular beverage after water. It has been shown that green tea contains various components with antioxidative and anticarcinogenic properties (Dreostic, Wargovich & Yang, 1997; Jankun, Salman, Swiercz & Skrzypczak-Jankun, 1997; Wiseman, Balentine & Frei, 1997; Yang, 1997). Of these components, catechins, which make up about 20% of the dry weight of green tea, are thought to be the most important.

Green tea catechins are, structurally, primarily flavanols. The main catechins in green tea are (−)-epigallocatechin gallate ((−)-EGCG), (−)-epigallocatechin ((−)-EGC), (−)-epicatechin gallate ((−)-ECG) and (−)-epicatechin ((−)-EC). The structures of the original catechins and their epimers are illustrated in Fig. 1. Tea catechins undergo many chemical changes during the course of the manufacturing and brewing processes. Studies have been undertaken on the oxidative conversion of catechins to theaflavins and thearubigins during black tea manufacture (Bajaj, Anan, Tsushida & Ikegaya, 1987; Davis, Cai & Davies, 1995; Roberts, 1962). In the production of green tea, this oxidation is limited by inactivating the enzymes in freshly picked tea leaves through firing or steaming. It has been shown that epimerisation, i.e. the conversion of the tea catechins to their corresponding isomers, can occur during the production of tea and tea drinks (Kiatgrajai, Wellons, Gollob & White, 1982; Komatsu, Suematsu, Hisanobu, Saigo, Matsuda & Hara,1993; Nakagawa, 1967; Suematsu, Hisanobu, Saigo, Matsuda, Hara & Komatsu, 1992). It was recognised that catechins undergo epimerisation at the C-2 position in hot aqueous solution (Kiatgrajai et al., 1982; Nakagawa, 1967). This was confirmed by Seto, Nakamura, Nanjo and Hara (1997) through ^{1}H- and ^{13}C-NMR, and optical rotation analyses. Epimerisation at the C-3 position only occurs when oxidative degallation is taking place (Coggon, Moss, Graham & Sanderson, 1973). Usually people make a cup of tea with tap water.

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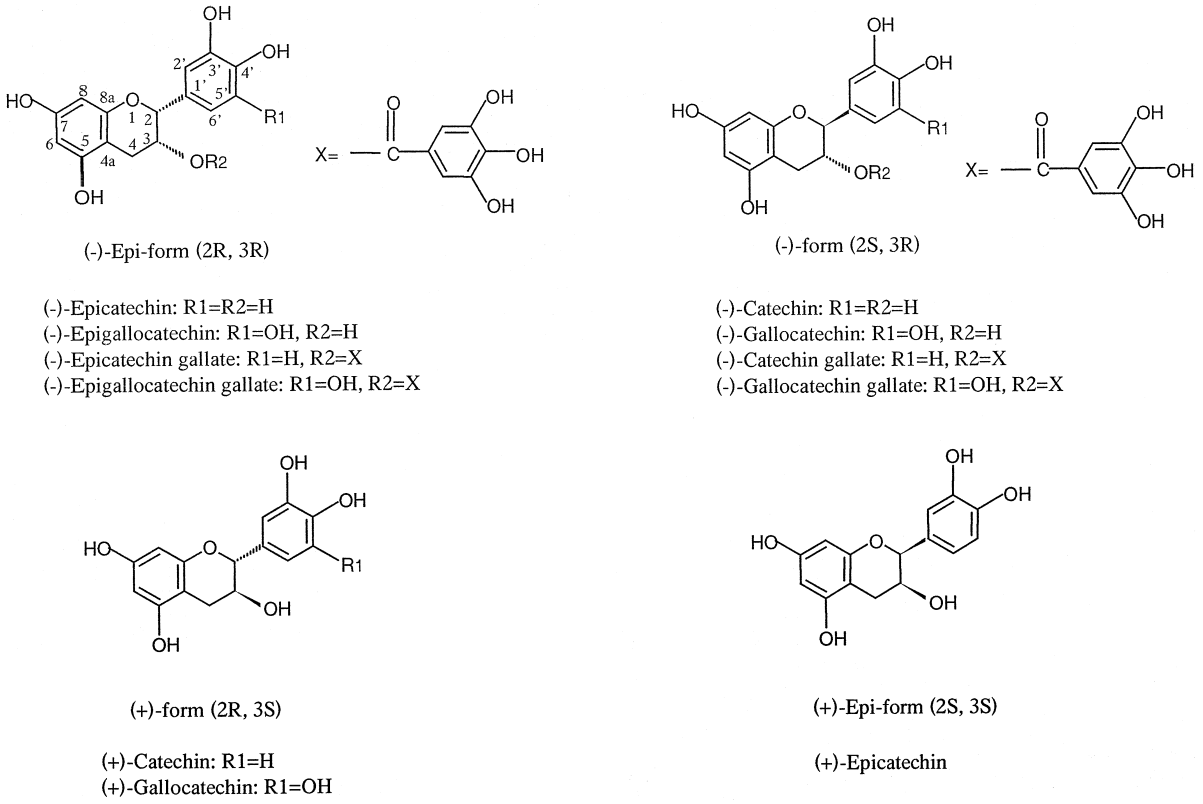

Fig. 1. Chemical structures of tea catechins and their epimers.

It is important, therefore, to understand the fate of catechins in a tap water base. However, to our knowledge all of the results on tea catechins were obtained using purified water or a mixture of organic reagents and purified water or a buffer solution based on purified water. As a result, little attention has been given to tracing the changes which occur when brewing tea with tap water.

In the present paper, we report our findings on the epimerisation of catechins in green tea infusions made with both purified and tap water at different temperatures. The factors influencing the stability of catechins in the infusions are discussed.

2. Materials and methods

2.1. Samples

Gunpowder tea, originally produced in China, was purchased from a local teashop in Hitchin, Hertfordshire, UK. Green tea infusions were prepared with the gunpowder tea according to the conventional tea brewing method: taking 1.00 g of sample and infusing with 100 ml of boiling purified water or tap water for 5 min, and then, after filtering, the infusions were cooled quickly to ambient temperature using running water.

Compositional information for tap water in the Hitchin area is shown in Table 1. The purified water used in the experiment was prepared using a water purification system (Purite Ltd, England) and had a resistivity over 17.5 MΩ cm.

2.2. Standards

(+)-Catechin ((+)-C), (−)-catechin ((−)-C), (−)-EC, (−)-EGC, (−)-EGCG, (−)-ECG, (+)-gallocatechin ((+)-GC) and (−)-catechin gallate ((−)-CG) were purchased from Sigma Chemical Co. (−)-Gallocatechin gallate ((-)-GCG) was separated and purified from green tea by the Tea Research Institute, Chinese Academy of Agricultural Sciences. All other reagents were standard items from reputable commercial sources. All solvents used for extraction were of analytical grade.

2.3. Preparation of the epimers of tea catechins

About 2 mM of each catechin standard was prepared with purified water and heated at 20, 40, 80 and 100°C for 20 min. The heated solutions were cooled to ambient temperature, immediately, under running water. The tea infusions made with purified and tap water were heated for 20 min at the same temperatures as the catechin standards, and cooled to ambient temperature, immediately, under running water. All samples were centrifuged at 13,000 rpm for 10 min prior to HPLC analysis.

Table 1
Compositional information for tap water in the Hitchin area[a]

Parameter	Units	Content	Mean
Conductivity	μS/cm	505–881	564
pH	pH units	7.1–7.9	7.2
Aluminium	μg Al/l	< 2–9	4
Nitrate	mg NO_3/l	17–42	31
Nitrite	mg NO_2/l	< 0.008–0.073	0.012
Ammonium	mg NH_4/l	< 0.04	< 0.04
Iron	μg Fe/l	< 15–151	19
Manganese	μg Mn/l	< 2	< 2
Copper	μg Cu/l	6–47	27
Zinc	μg Zn/l	< 6–33	20
Lead	μg Pb/l	< 1	< 1
Calcium	mg Ca/l	109–122	116
Chloride	mg Cl/l	29–57	43
Magnesium	mg Mg/l	1.9–5.2	3.6
Sodium	mg Na/l	8–13	11
Potassium	mg K/l	1.2–2.3	1.8
Total hardness	mg Ca/l	125	125
Total phosphorus	μg P/l	< 50	< 50
Fluoride	μg F/l	< 80–100	90
Oxidisibility	mg O_2/l	< 0.5–1.7	1.1

[a] The information was kindly supplied by Three Valleys Water plc, UK.

2.4. Analytical determinations

Each catechin and tea sample was analysed by the following HPLC method. An HP 1100 series liquid chromatograph system, comprising vacuum degasser, quaternary pump, auto-sampler, thermostatted column compartment, and diode array detector, was used. The column was a C18 reversed phase Kingsorb 5 μm (150×4.6 mm). Solvents for the separation were 0.1% orthophosphoric acid in water (v/v) (eluent A) and 0.1% orthophosphoric acid in methanol (v/v) (eluent B). The gradient was as follows: 0–5 min, 20% B; 5–7 min, linear gradient from 20 to 24% B; 7–10 min, 24% B; 10–20 min, linear gradient from 24 to 40% B; 20–25 min, linear gradient from 40 to 50% B; 25–30 min, linear gradient from 50 to 20% B; and 30–35 min, 20% B. The flow rate was 1.0 ml/min. The column was operated at 30°C. The sample injection volume was 20 μl. UV spectra were recorded from 200 to 400 nm, and peak areas were measured at 210 nm. The UV spectra obtained for each peak, after subtraction of the corresponding UV base spectrum, were computer normalised and the plots were superimposed. Peaks were considered to be chromatographically pure when there was exact coincidence to their corresponding UV spectra. Chromatographic peaks in the samples were identified by comparing their retention time and UV spectra with those of the reference standards. Working standard solutions (5–30 μl) were injected into the HPLC, and peak area responses were obtained. A standard graph for each component was prepared by plotting concentration versus area. Quantification was carried out from integrated peak areas of the sample against the corresponding standard graph.

3. Results and discussion

3.1. Epimerisation of catechin standards

About 2 mM of each catechin standard was prepared with purified water and heated at 20, 40, 80 and 100°C for 20 min. The degree of conversion for individual catechins was determined by analysing the content of the original catechin (unconverted) and its epimer (converted) in the reaction mixture. The results showed that, after 20 min, no epimerisation was observed if the samples were treated at 40°C or below. However, epimerisation between the epi- and nonepistructure was observed for all individual catechin standards at temperatures above 80°C. This is consistent with the result reported by Komatsu et al. (1993). Table 2 shows the degree of conversion for each individual catechin standard at 100°C. This shows that catechins with 2R,3R configuration, i.e. 2,3-*cis* form give higher levels of conversion to their corresponding epimer than catechins with 2S,3R configuration, i.e. 2,3-*trans* form. This is thought to be due to the difference in stereochemistry: *trans*-forms, in general, being more stable than *cis*-forms. Addition of the percentage of converted and unconverted catechins does not give 100%, as reported earlier by Seto et al. (1997). This is thought to be due to other reactions which are also taking place, such as oxidation or degradation. The quantity of catechins which cannot be accounted for is listed under "others" in Table 2. By introducing nitrogen gas into the (−)-EGCG solution whilst heating at 100°C the conversion product (−)-GCG increased by up to 40%. Originally the purpose of using nitrogen was to displace oxygen to suppress the possibility of oxidation during the heating process. However, it was found that by using nitrogen during the 20 min heating period the pH of the solution increased by about 1.5 units. Therefore it is thought that the 40% increase produced by using nitrogen during the heating of (−)-EGCG at 100°C might be due either to the suppression of oxidation of (−)-EGCG or to the increased pH of the solution. Factors influencing the stability of catechins will be discussed later. (−)-EC and (−)-EGC are precursors of theaflavin, one of the important oxidation derived components in black tea (Nakabayashi, 1991). For these two catechins, the higher percentage in "others" is thought to be largely due to their oxidation to form dimers, or the intermediary products of theaflavin. However, why (+)-C had a higher percentage in "others" requires further investigation.

3.2. Epimerisation of catechins in tea infusions

Gunpowder tea leaves were brewed separately with boiling purified water and boiling tap water, and the contents of catechins in the infusions were compared. The results are given in Fig 2. These show that, by using boiling tap water, the content of the nonepi-structured catechins, namely, (−)-GC, (−)-C, (−)-GCG, and (−)-CG increased while the content of the epi-structured catechins, namely, (−)-EGCG, (−)-EGC, (−)-EC, and (−)-ECG decreased when compared to using boiling purified water. This indicates that, during the tea brewing process, the epimerisation of the catechins occurs more readily when using boiling tap water. The two kinds of infusions after being cooled to ambient temperature (20°C) were heated in a water bath at 40, 80 and 100°C for 20 min. The results are presented in Fig. 3A and 3B. For the infusion brewed with purified water, the content of the nonepistructured catechins did not increase during the heating process at 40°C for 20 min when compared to the sample kept at ambient temperature, indicating that no further epimerisation had occurred. However, further epimerisation was observed in the sample brewed with purified water at temperatures above 80°C (Fig. 3A). It can be seen from Fig. 3B that the epimerisation takes place more easily in tap water than in purified water: the decrease in epicatechins and the corresponding increase in nonepicatechins can occur even at 40°C. From the results obtained with pure catechins, all of the catechins are epimerised when they are heated. However, because the content of the epi-structured catechins is usually greater than that of nonepistructured catechins in a tea infusion and the degree of conversion for epistructured catechins is greater than that for nonepistructured catechins, the content of epistructured catechins is reduced towards the equilibrium of epi- and nonepistructures. As a result, the major change was epimerisation from epi-structured catechins to nonepistructured catechins in tea infusions during this heat treatment.

Table 2
Degree of conversion for individual catechin standards at 100°C

Starting compound	Configuration	Epimers	Converted (%)	Unconverted (%)	Others (%)
(−)-EGCG	2R,3R	(−)-GCG	34.6	58.9	6.5
(−)-EGC	2R,3R	(−)-GC	34.3	7.7	58.0
(−)-ECG	2R,3R	(−)-CG	28.5	62.7	8.8
(−)-EC	2R,3R	(−)-C	42.4	16.9	40.7
(−)-GCG	2R,3R	(−)-EGCG	27.6	56.0	16.4
(+)-GC	2R,3S	(+)-EGC	19.0	70.0	11.0
(+)-C	2R,3S	(+)-EC	19.3	23.6	57.1

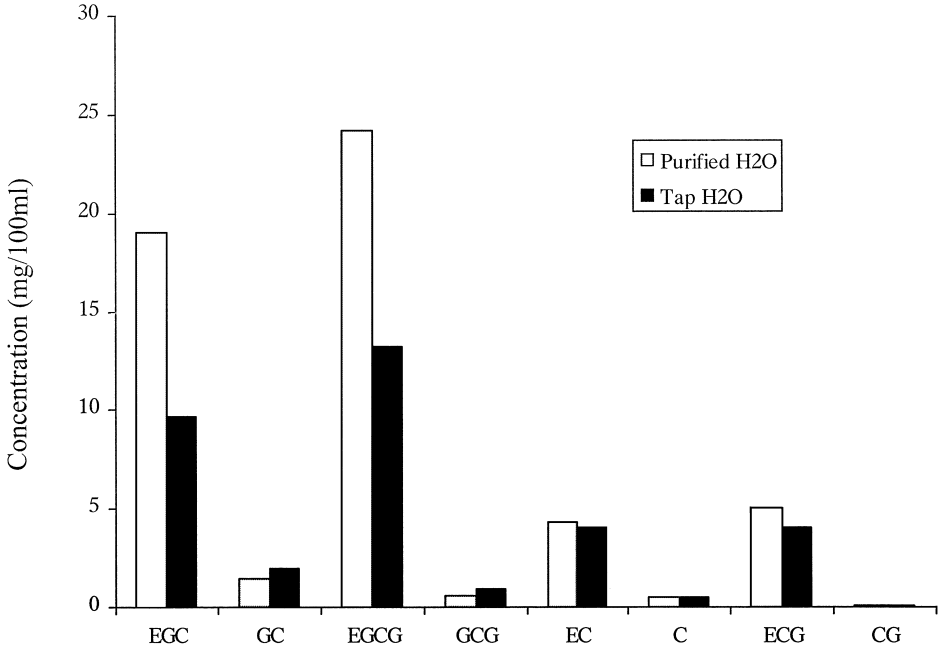

Fig. 2. Catechins in green tea infusions brewed with purified water and tap water.

3.3. Effect of time on the epimerisation of catechins

Infusions of gunpowder tea brewed with purified water and tap water were cooled to ambient temperature immediately and then heated at 100°C for different periods of time. The changes to the catechins during the heating process are shown in Table 3. These results show that the conversion from (−)-EGC and (−)-EGCG to their corresponding epimers, (−)-GC and (−)-GCG in the infusion brewed with purified water increased during the first 3 hours heating and then decreased slightly, while, for (−)-EC and (−)-ECG, their corresponding epimers continuously increased during the course of 5 h heating. However, in the infusion brewed with tap water, (−)-GC, (−)-GCG and (−)-CG reached a maximum level after 20 min of heating, and (−)-C reached a maximum after 1 h of heating, and then each decreased with heating time (Table 3). This indicates that after the catechins reached the maximum level of epimerisation, the predominant change becomes the degradation or oxidation of the catechins. Furthermore, it was found that the catechins were highly unstable in the infusion brewed with tap water. Even if it was kept at ambient temperature for a few hours, the content of (−)-EGC and (−)-EGCG decreased greatly without epimerisation occurring.

Komatsu et al. (1993) have reported that the initiation temperature for the epimerisation of epistructured catechins on an Arrhenius plot was about 82°C. The results described above on green tea infusions brewed with purified water appear to support their finding. Nevertheless, as reported above, in tap water, epimerisation of catechins can be observed even at 40°C.

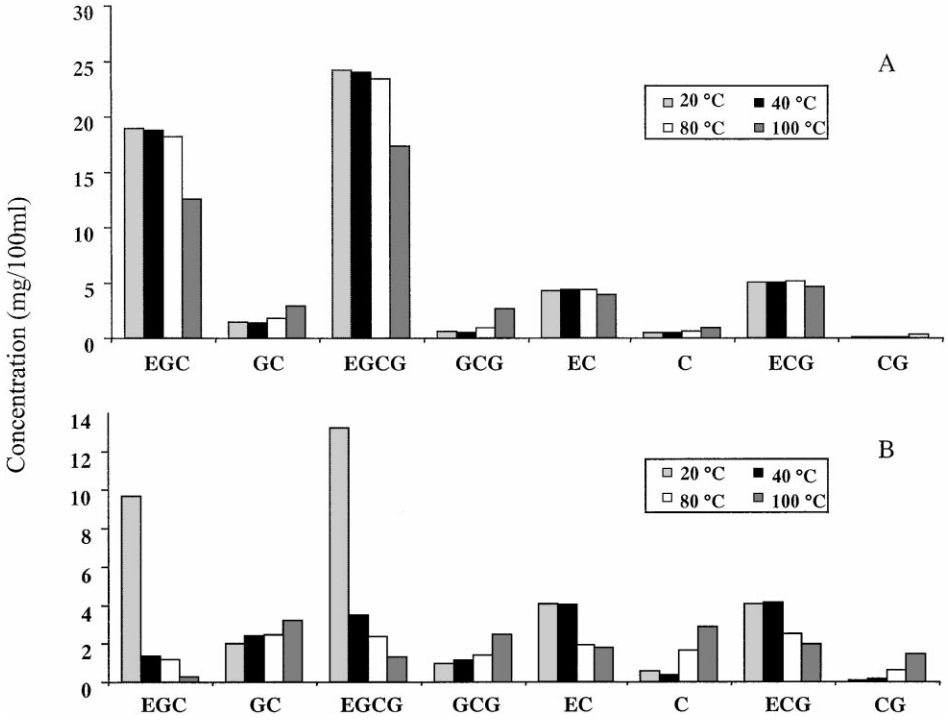

Fig 3. Epimerisation of catechins in the tea infusions at different temperatures. A: purified water; B: tap water.

Table 3
Content of catechins in green tea infusion when heated at 100°C (mg/100 ml)

	Purified water					Tap water				
Compound	0 h	20 min	1 h	3 h	5 h	0 h	20 min	1 h	3 h	5 h
(−)-EGC	19.0	14.6	12.6	8.40	5.34	9.68	0.28	0.26	0.16	0.15
(−)-GC+ (+)-GC	1.45	2.94	3.61	5.66	5.36	2.00	3.23	1.32	0.16	0.09
(−)-EGCG	24.2	19.3	17.4	12.1	8.44	13.2	1.34	0.61	0.23	0.15
(−)-GCG	0.61	2.63	3.58	6.34	6.03	0.98	2.44	1.05	0.34	0.16
(−)-EC	4.34	3.99	3.87	2.90	2.35	4.05	1.77	1.40	1.04	0.82
(−)-C+(+)-C	0.51	0.93	1.03	1.71	1.88	0.56	2.87	2.88	2.16	1.77
(−)-ECG	5.02	4.65	4.52	3.38	2.73	4.06	1.98	1.51	1.02	0.67
(−)-CG	0.06	0.36	0.43	0.99	1.14	0.09	1.44	1.34	0.95	0.66

We have also monitored the changes of catechins in green tea strong infusions, which were prepared with a mixture of ethanol and purified water in the ratio of leaves to solvent 1:5 (w/v), at 5, 25 and 40°C over a 6 months shelf life study. Interestingly, epimerisation was still clearly observed in samples kept at 40°C (Table 4). Nonepistructured catechins continued to increase with a corresponding decrease in epistructured catechins during the entire shelf life. These changes were very similar to those found in normal green tea infusions which had been heated at temperatures over 80°C for 20 min, although the conversion rate was not as high as that in the normal tea infusion. Therefore, the epimerisation of catechins in green tea infusions is thought to be influenced, not only by temperature, but also by heating time. This finding appears to disagree with the conclusion reported by Komatsu et al. (1993), who supposed that the stability of catechins in tea infusions was influenced by temperature rather than heating time. No epimerisation has been observed in the samples of strong infusions stored at 5 and 25°C.

3.4. Factors influencing the stability of catechins in tea infusions

Figs. 4 and 5 show the content of (−)-EGC and (−)-EGCG in the tea infusions made with water treated in the following ways: (A) tap water (pH 7.1); (B) acidified tap water (adjusted with 1.0 M HCl to pH 5.9); (C) purified water (pH 5.9); and (D) alkalised purified water (adjusted with 0.1 M NaOH to pH 7.1). The infusions, when made, were either cooled immediately to ambient temperature or heated at 100°C for 20 min. The influence of the different treatments on the content of either (−)-EGC or (−)-EGCG in the tea infusions can be derived from Figs. 4 and 5. The difference between (D) alkalised purified water (pH 7.1) and (A) tap water (pH 7.1) defined as DA represents the effect of the ions in tap

Table 4
Stability of catechins in strong infusion of gunpowder tea stored at 40°C (mg/100 ml)

Compound	0 day	10 days	30 days	60 days	120 days	180 days
(−)-EGC	363	362	335	317	266	173
(−)-GC+ (+)-GC	50.1	54.7	62.5	68.5	104	101
(−)-EGCG	726	721	675	627	610	441
(−)-GCG	14.4	23.1	30.9	80.5	94.1	108
(−)-EC	87.3	88.4	77.6	71.5	62.3	44.8
(−)-C+(+)-C	18.7	21.3	20.8	31.1	32.1	32.4
(−)-ECG	180	174	170	156	143	108
(−)-CG	13.8	14.1	14.9	15.9	17.8	19.0

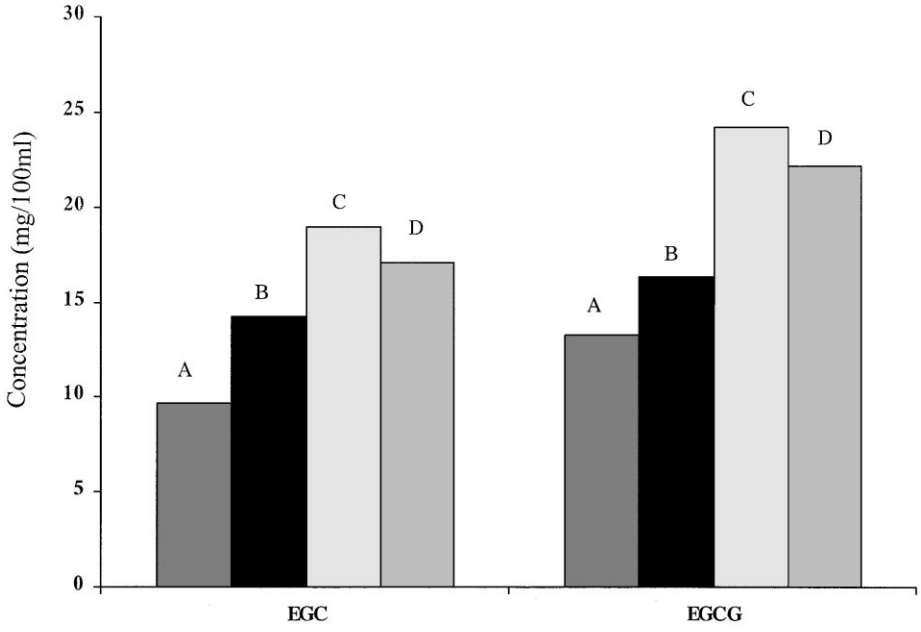

Fig. 4. Factors influencing the stability of catechins at ambient temperature (20°C). A: tap water; B: acidified tap water; C: purified water; D: alkalised purified water.

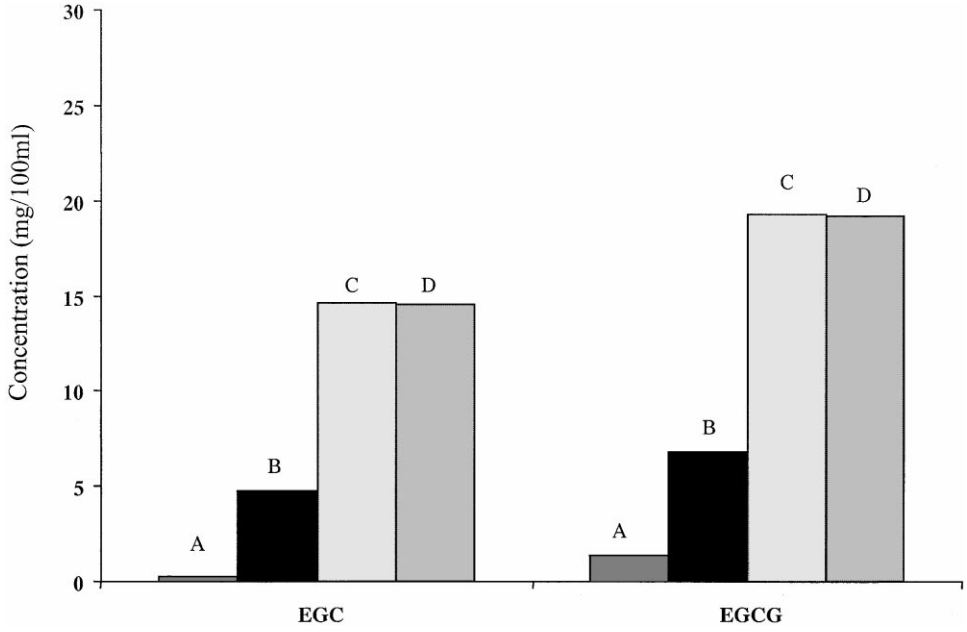

Fig. 5. Factors influencing the stability of catechins at 100°C. A: tap water; B: acidified tap water; C: purified water; D: alkalised purified water.

water at pH 7.1. Likewise CB, BA, and CD represent the effect of the ions in tap water at 5.9, the effect of pH with tap water ions and the effect of pH without tap water ions, respectively. From the figures, it can be seen that DA > CB > BA > CD. This implies that the stability of these catechins is affected more by the effect of the ions present in the water than by the pH of the water and that, for the same ionic environment, the catechins are less stable at higher pH values. (−)-EGC and (−)-EGCG are also less stable at higher temperatures for a given set of conditions, their stability in tap water at higher temperatures being significantly reduced. There should be little difference in dissolved oxygen content between purified water and tap water. It has been reported that metal ions, for example, iron and copper, activate oxygen in water, forming complexes which catalyse the oxidation of catechins (Chen, You & Chen, 1997). We have investigated the effect of iron, copper, calcium, and magnesium on the stability of the catechins in tea infusions by dosing purified water adjusted to pH 7.1 with 1.0 M HCl separately with the maximum levels reported for these metal ions in Hitchin tap water. The results showed that there was no effect on the stability and epimerisation of the catechins. Chen et al. (1997) found that the concentrations of Fe (II), Fe (III), Cu (II), and Ca (II) necessary to cause a significant decrease in polyphenols in oolong tea infusions were 20, 20, 5 and 200 ppm, respectively. These concentrations were more than 130 and 100 times higher for iron and copper, respectively, than the maximum concentrations in Hitchin tap water, but only 1.6 times higher for calcium. Therefore, the effect of ions on the stability of tea catechins remains unclear and further study appears to be necessary.

Results from the present study indicate that the decrease in epistructured catechins is not mirrored by a similar increase in the corresponding epimers after heat treatment. This is because there are several competing reactions occurring including epimerisation, oxidation and degradation making prediction of the reaction of catechins more complex. This is consistent with the result given by Coggon et al. (1973), in which they have found that epimerisation takes place under oxidative conditions, in competition with other reactions, such as the formation of bisflavanols and thearubigins.

It should be pointed out that, using the stated HPLC elution system, (−)-GC and (+)-GC, (−)-C and (+)-C could not be separated. However, the increase of the relevant peaks after heating may be regarded as (−)-GC and (−)-C. Saijo and Takeda (1999) also held this view when they analysed catechins in various kinds of green tea using HPLC.

4. Conclusions

Individual catechins undergo epimerisation at high temperatures. However, in green tea infusions the predominant change appears to be epimerisation from the epistructure to the nonepistructure. It has been found that this epimerisation takes place more easily in tap water than in purified water. The complexity of the ions in tap water and the different pH between tap and purified water are thought to be the main reasons for the different conversion rates for individual catechins. In the infusion brewed with tap water, the catechins are easily epimerised and then rapidly degraded. Stability

studies on catechins in green tea strong infusion have shown that epimerisation can be observed at 40°C over prolonged storage. Therefore, it is thought that, not only temperature, but also heating time influences the epimerisation of catechins in green tea infusions.

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