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Variations in the amounts of individual flavanols in a range of green teas

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The concentrations of the major tea flavanols in nine green teas from China and Japan have been investigated. Results show that epi-gallocatechin (EGC) is the most prevalent flavanol and not epi-gallocatechin gallate (EGCG) as previously thought. Studies of the equilibrium behaviour of extraction of tea flavanols and caffeine into aqueous media at 80°C and 60°C for one of the teas, Sen Cha Uji Tsuyu, indicate that extraction efficiencies are similar to that for extraction of soluble components from black teas. These studies also support the idea of EGC being the flavanol with the highest concentration. This is of relevance to the current interest in tea flavanols as antimutagenic agents in the treatment of cancers. From the flavanol content results there appears to be a correlation between the amount of flavanol and the price of the tea. It is possible that this might be used to aid the pricing of green teas at auction.

INTRODUCTION

Green tea is widely used, particularly in parts of Asia. A hot aqueous infusion of the processed leaves forms the main staple beverage for many millions of people. Consequently it is important not only economically as a bulk commodity but also from a dietary point of view. Freshly picked tea leaf contains a group of small molecular weight polyphenolic compounds known as tea flavanols. These have similar structures (Roberts, 1962) all based on a catechol moiety as shown in Fig. 1.

The main research interest in tea flavanols has been because they are precursors of other polyphenolic compounds made during the manufacture of black tea (Harler, 1963), which is more important commercially than green tea. In black tea manufacture the flavanols are enzymatically oxidised to form highly reactive orthoquinones. These intermediates react further to form theaflavins and thearubigins (Hilton, 1970; Hilton & Ellis, 1972). These two series of compounds are important to the quality of the final black tea product (Hilton & Ellis, 1972); hence there has been a large effort expended in studying this reaction which is usually referred to as tea fermentation. Green tea differs from black tea in that the enzymes responsible for the fermentation, polyphenoloxidases, are deactivated by steaming prior to maceration of the picked leaves. The flavanols and enzymes are spatially separated in the fresh leaf (Harler, 1963). After maceration during green tea manufacture, the leaf is then dried, graded and packaged. Consequently in a green tea there are appreciable concentrations of the flavanols; levels may be 30% in total by dry weight (Millen *et al.*, 1969). These are soluble to some extent in water and so appear in the final beverage in significant amounts.

The amounts of flavanols that are ingested by habitual green tea drinkers have become a new major focus of research in the last few years. There have been a number of reports of low incidences of certain types of cancer amongst populations where green tea is drunk in high amounts (Charles, 1991). For example, a survey carried out in Japan found that the incidence of death from cancers, especially stomach cancers, was markedly lower in men and women from Shizouka province in Japan, where green tea is a staple food product, compared with the rest of the Japanese population (Wang *et al.*, 1989). Recent epidemiological studies have indicated that two of the major flavanols in green tea, epi-gallocatechin gallate (EGCG) and epi-catechin gallate (ECG), strongly inhibit mutations of species of bacteria (Kada *et al.*, 1985). This antimutagenic activity has been linked to green tea flavanols' ability to scavenge free radicals such as $HO_2^{\cdot}$ and $O_2^{\cdot}$ (Ruch *et al.*, 1989). Other claims have been made for the activity of green tea flavanols in connection with their inhibition activity against tumours from PAHs (polyaromatic hydrocarbons), dental caries, and reverse transcriptase human immunodeficiency virus (HIV) (Nakane & Ono, 1989; Sakanaka *et al.*, 1989).

The purpose of the present work is to investigate the equilibrium and kinetic behaviour of aqueous extraction of green tea solubles, particularly flavanols. There

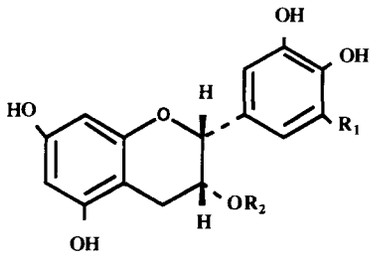

Fig. 1. Structure of the major tea flavanols: epi-catechin (EC)—R1 = H, R2 = H; epi-gallocatechin (EGC)—R1 = OH, R2 = H; epi-catechin gallate (ECG)—R1 = H, R2 = 3,4,5-trihydroxybenzoyl; epi-gallocatechin gallate (EGCG)—R1 = OH, R2 = 3,4,5-trihydroxybenzoyl.

has been no previous work on the extraction equilibrium of individual flavanols from green tea products. In addition, there have been very few previous reports giving accurately measured amounts of individual flavanol present in green tea (Tereda *et al.*, 1987). The current study aims to measure the amounts of the major flavanol species in a range of commercial green teas and look at the variations. The increasing interest in tea flavanols and their proposed use in therapeutic preparations make this work on aspects of the extraction process quite timely.

MATERIALS AND METHODS

Analysis of tea flavanols in a variety of teas

Nine different green teas were obtained commercially. The amounts of the tea flavanols in an infusion were determined by taking a representative 4 g sample of the tea and equilibrating it with 250ml distilled water in a conical flask thermostatted at 80°C for 1 h. Temperature control was better than ±0·5°C. The solutions were stirred using submersible stirring pads (Kartell, Milan). After this time a sample was taken, filtered through a 0·45 μm filter (Bonnet Equipment, Sydney) and diluted accurately by a factor of 10. It was then filtered again prior to injection and analysed for flavanols present by high performance liquid chromatography. The HPLC method was one modified from work by Hoeffler and Coggon (1976) and is described below:

—Column used: C18 μ-Bondapal column (3·9 × 300 mm
—Pump: Waters 510; flow rate 1·2 ml/min
—Detector: Water UV–visible spectrophotometer model 450 set at 280 nm and 0·01 AUFS
—Mobile Phase: acetic acid : methanol : dimethylformamide : water (1 : 2 : 40 : 157)
—Injection volume : 50 μl

To quantify the amounts of the flavanols, standards of one of them, epi-catechin (EC), were prepared from purified crude green tea extracts by chromatographic methods (Price & Spitzer, 1992) and also from a sample obtained commercially (Sigma). The concentration range used was 0·02–0·14 mM and these were run in conjunction with the tea samples. The calculated amount of EC in a sample was then used to quantify the other three major flavanols by using the ratio of the extinction coefficient for the flavanol at 280 nm compared with that for EC. Literature extinction coefficients were used (Vuatez *et al.*, 1959; Wilkins *et al.*, 1971) but these values were checked for EC and for epi-gallocatechin (EGC) using purified samples (Price & Spitzer, 1992). Comparison with the literature extinction coefficients was extremely good (better than ±2%), giving the authors confidence in their method of analysis.

Determination of equilibrium coefficient for extraction of green tea flavanols

The equilibrium coefficients for the extraction of the four major flavanols from a green tea were determined at two temperatures, 60°C and 80°C. The two-phase model and methods of Spiro and co-workers were employed (Spiro & Siddique, 1981; Price & Spiro, 1985) to facilitate this. The tea chosen was Sen Cha Uji No Tsuyu from Japan. It was selected because it was found to be particularly rich in flavanols, thus aiding analysis. The tea was first sieved on an Endecotts sieve shaker with a set of standard sieves. The major fraction was the one in the range 2000–1700 μm. This fraction was utilised in the subsequent investigation. A series of flasks containing 250 ml of water was thermostatted at the desired temperature, either 60°C or 80°C, in a water bath. Varying amounts of green tea from 2 to 10 g were added to the flasks. These were then stirred and left to equilibrate. For the 80°C runs this took 1 h whilst the 60°C experiments were left for 6 h. Both these times were tested by preliminary experiments. Samples were then taken from each of the flasks, filtered, diluted and analysed for flavanol content using HPLC as described above.

RESULTS AND DISCUSSION

Amounts of flavanols in a variety of commercial green teas

The concentrations found in the equilibrium solutions from the nine teas investigated were converted to a percent dry weight basis for ease of comparison. These are tabulated in Table 1.

Seven of the teas were from China, a number from Fuijian province. Two of them were, however, Japanese green teas (Sen Cha Uji Tsuyu and Daigo Sen Cha). Consequently a variety of different growing areas were taken into account by this investigation. The most striking point of the results is that the highest flavanol levels are for EGC and EGCG in all the teas studied. This is in contrast to earlier findings (Millen *et al.*, 1969) which suggested that EGCG is usually by far the most abundant flavanol present. The present results indicate that EGC is just as important. It should be noted, however, that Millen and co-workers used a

Table 1. Variations in individual flavanol concentration in nine commercial green teas extracted using water at 80°C

Name of tea	All values in % w/w: EC	EGC	EGCG	ECG	Flavanols
Loong Ching green tea	1·61	4·04	3·95	0·91	10·51
Green tea—Grand Tea Co.	0·87	3·60	2·79	0·56	7·82
Special Chunmee	1·18	3·93	3·47	0·77	9·35
Jasmine tea—Fujian	1·18	3·45	3·33	0·62	8·58
Sen Cha Uji Tsuyu	1·95	5·44	3·57	0·73	11·69
Daigo Sen Cha	1·80	4·61	2·19	0·82	9·42
Lung Ching China tea	2·00	5·02	2·70	0·50	10·22
Jasmine tea 'Bulk'	0·96	2·75	3·95	0·74	8·40

much more exhaustive extraction procedure to obtain the flavanols. Possible implications of this will be discussed later. Other recent data for hot aqueous extraction of a small number of Japanese green teas indicate that EGC concentrations are comparable to those for EGCG (Terada *et al.*, 1987).

Another feature of the results is that the total flavanol concentration obtained by aqueous infusion is in the range 8–12% w/w, which is smaller that the amounts quoted previously for green tea flavanols (up to 30% on a dry weight basis). From the range of teas investigated there is no explanation for this. It might again be attributed to the method of extraction; however, the equilibrium work using Sen Cha Uji Tsuyu seems to refute this, as is described later.

Previous work for a range of black teas has shown that there is a definite correlation between the amount of the polyphenolic theaflavins present in a tea and the price the tea fetches on the auction room floor (Hilton & Ellis, 1972; Cloughley, 1980). Given the present results for a range of green teas obtained from commercial sources, it was of interest to see if a similar correlation might exist between the price of green tea and the amounts of flavanols present in it. Figure 2 shows a plot of the price paid for the tea (AU$/kg) versus the total flavanol content as measured above. There is a clear correlation between the two variables (r^2 = 0·77). Similar correlations may be obtained for the individual flavanol amounts. For example, with EGC a correlation coefficient (r^2) of 0·83 is obtained. This is an interesting finding and raises the possibility of flavanol analyses being used as a quality indicator in green tea marketing. This conclusion is only preliminary and it would be interesting to conduct further work to substantiate it.

Two-phase equilibrium results

Equilibrium infusions of the green tea, Sen Cha Uji Tsuyu, using tea : water ratios from 10:250 to 2:250 for two temperatures (60°C and 80°C) were analysed for the flavanol concentrations as described above. As caffeine was resolved and eluted in the same chromatogram as the flavanols, the concentration of caffeine in each was also determined. This served as a useful comparison. Caffeine is an important constituent in both green and black tea. There is, in addition, equilibrium information for caffeine extraction from a number of black teas in the literature (Spiro &

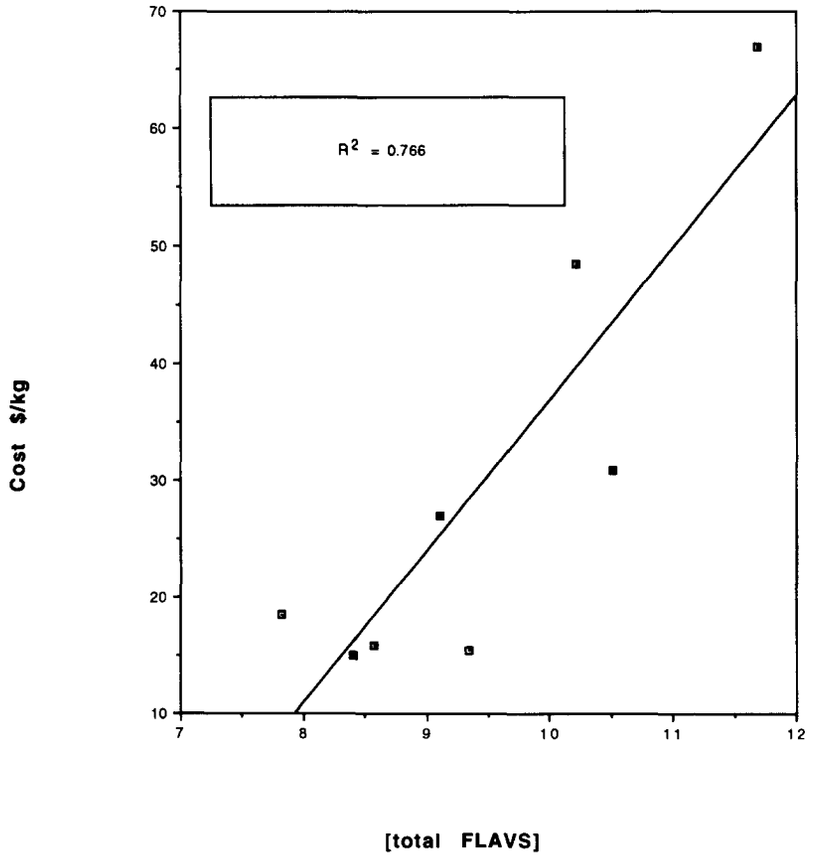

Fig. 2. Correlation between price of individual green teas and total flavonol concentration.

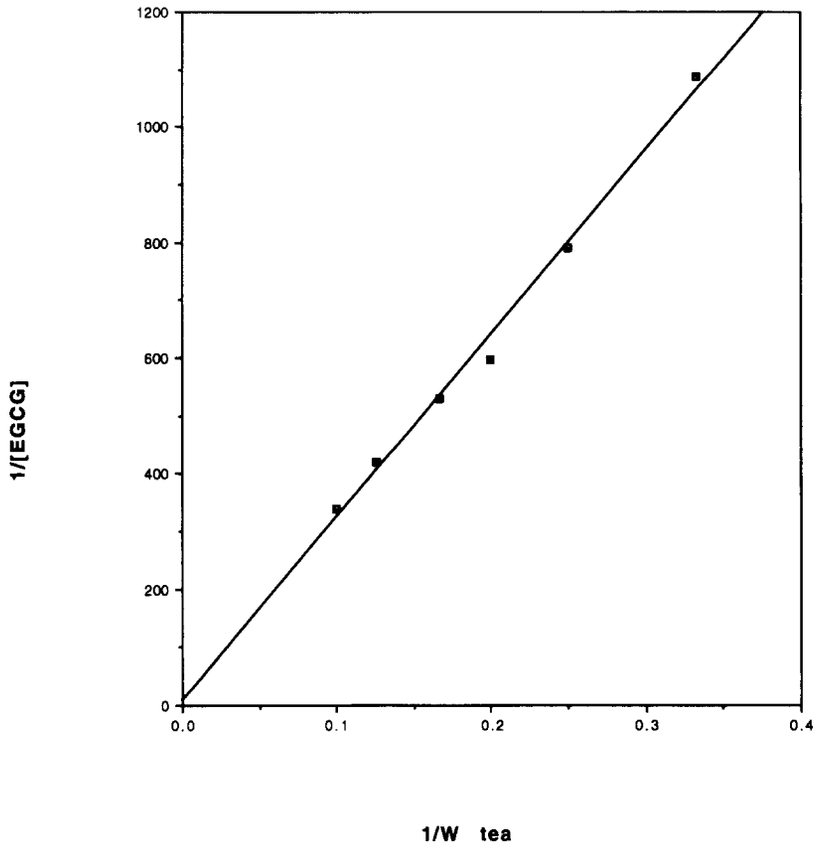

Fig. 3. Equilibrium plot for EGCG at 80°C: 1/weight of tea vs 1/[EGCG].

Siddique, 1981; Price & Spiro, 1985). The various concentrations obtained were fitted to Spiro's two-phase equilibrium model (Spiro & Siddique, 1981). This predicts that the reciprocal of the equilibrium concentrations of a component, $1/c$, that has infused from w grammes of tea into V cm^3 of aqueous media will be linearly related to $1/w$ according to the following equation:

$$1/c = V/wx_0 + 1/K'x_0 \quad (1)$$

where x_0 is the amount of the component in the 'as received' tea and K' is a fictional partitional coefficient between the leaf and the aqueous media. It is fictional because it assumes the leaf has retained its original mass. Changes in mass due to loss of solubles and water inhibition may, however, be allowed for if necessary (Spiro & Siddique, 1981). This method thus allows one to estimate the true amount of a component in a tea and the efficiency of the extraction process. An example of this type of analysis is shown in Fig. 3 for the extraction of EGCG at 80°C. A good linear plot is obtained with a small intercept. From the coefficient of the line, both x_0 and K' may be estimated.

Table 2 summarises the analyses for the equilibrium extraction of the four major flavanols and caffeine from the Sen Cha tea at 60°C and 80°C. The values of K' are difficult to analyse in any detail. The smallness of the intercepts obtained and the length of the extrapo-

Table 2. Equilibrium parameters for extraction of flavanols and caffeine from Sen Cha green tea at 60 and 80°C

Constituent	60°C			80°C		
	x_0 (mol/kg)	x_0 (wt%)[a]	K' (kg/litre)	x_0 (mol/kg)	x_0 (wt%)	K' (kg/litre)
EC	0·069	2·0 (±0·1)	0·19 (0·13–0·35)[b]	0·047	1·4 (±0·1)	>0·09[c]
EGC	0·230	7·1 (±0·9)	0·64 (>0·12)	0·23	7·1 (±1·7)	0·17 (0·1–0·9)
EGCG	0·084	3·9 (±0·1)	0·15 (0·1–0·24)	0·087	4·0 (±0·2)	0·23 (0·15–0·6)
ECG	0·021	0·94 (±0·07)	0·06 (±0·04)	0·019	0·82 (±0·05)	0·16 (0·15–0·12)
Caffeine	0·190	3·70 (±0·06)	0·23 (>0·10)	0·205	4·0 (±0·3)	0·14 (0·1–0·98)

[a] Values in parentheses indicate uncertainty of amount of component in the tea.
[b] Range of uncertainty in K' given in parentheses. Estimated from standard deviation of the y-intercept in plots using eqn (1). Where only one value is given, this is a *lower* bound for the value of K'. This is due to the error (SD) in the y-intercept being greater than the value itself. See text.
[c] For this component the intercept from the plot was negative. Limit of K' estimated from the range of the intercept using the uncertainty from the least squares determination.

lations lead to very large uncertainties in their values. In some cases the standard deviation in the determined y-intercept value is comparable to (or larger than) the value itself. The table, therefore, quotes ranges for the K' values determined from the standard deviation in the intercept value. In cases where the lower limit of the intercept becomes negative this leads to a negative K' which is nonsensical. The reliability of the K' is related directly to the magnitude of x_0: a large x_0 leads to a greater uncertainty in K'. Given these errors it is difficult to look for differences in the values of various flavanols or to ascertain any temperature dependency. It is also for this reason that no attempt has been made to convert K' values to true partition coefficients (K) that take into account discharge of solubles and leaf swelling (Long, 1978; Spiro & Siddique, 1981). True partition coefficients for components from black teas have been found to be 2–3 times larger than the corresponding K' values (Spiro & Siddique, 1981) calculated using appropriate solubles discharge and swelling data. However, it may be noted that the K' values for the flavanols and caffeine from Sen Cha green tea at 80°C are not dissimilar to the partition coefficients for theaflavins, thearubigins and caffeine in black tea extraction (K' = 0·05–0·3) at the same temperature.

The data for the amount of flavanol in the tea are more interesting. The values of x_0 for each species are independent of temperature within experimental uncertainty. This is as one would hope, since x_0 represents the amount of a component within the leaf as received and should be independent of extraction procedure. The only exceptions to this are the x_0 values for EC where that at 80°C is substantially smaller. If the values of x_0 in Table 2 at 80°C are compared with the amounts of flavanols estimated directly from the infusion concentrations (see Table 1), there is general agreement within experimental uncertainty. The values from the two-phase model are mostly larger than those from Table 1. This is to be expected given that the calculations using the two-phase model take into account a partitioning of the component between the tea matrix and the aqueous solution. The total flavanol content of the Sen Cha tea from this method is estimated to be 14·3 ± 3·1%. This compares with 11·7% from the infusion method.

The x_0 value for caffeine (about 3·9%) is consistent with values calculated for a range of black teas (3–5%) for both whole teas and sieved fractions (Spiro & Siddique, 1981; Price & Spiro, 1985). One would expect a similar value for the caffeine content in the different types of tea, given that they all come from the same species of plant. It also suggests, of course, that caffeine content in the final product are relatively unaffected by the manufacturing process, differences between individual tea samples being much greater. The reasonable values obtained for caffeine in this tea give the authors some confidence in their chromatographic method of analysis and in the flavanol concentrations determined.

The values of x_0 for the amounts of the four flavanols in Sen Cha tea are consistent with the values found from the infusion concentration measurements. The calculations of x_0 take into account partition of the flavanols and are thus independent of extraction method. This suggests that the wt% values for flavanols calculated in the nine varieties of green tea are reasonable estimates. All the teas looked at were large leaf varieties, so consequently they might be assumed to have similar partitioning behaviour. This supports the idea that EGC is as prevalent in green tea samples as EGCG.

CONCLUSIONS

Nine different whole large leaf green teas have been investigated for their flavonol content. In all cases the levels of both EGC and EGCG were the highest. Previous work indicated that EGCG was the most prevalent flavanol component (Millen *et al.*, 1969). This might be due in part to the use of modern chromatographic methods with greater accuracy. This finding is relevant to the recent surge of interest in green teas in relation to cancers. EGCG has been deemed to have most antimutagenic activity. If there is a large difference in the activity of the four major flavanols, any future commercial extraction of flavanols from green tea products to produce therapeutic drugs would need to consider the amounts of the different flavanols present.

Detailed equilibrium experiments using one of the teas, Sen Cha Uji Tsuyu (Japan), support the fact that EGC is often the dominant flavanol present. In this case there is 75% more EGC than EGCG. These experiments also indicate that the efficiency of a hot aqueous extraction for these flavanol components from green tea is not dissimilar to that for major flavour and colour components from black teas.

Preliminary conclusions for these nine teas suggest that there may be a correlation between the price of a green tea and the flavanol content. This is similar to conclusions reached about theaflavin content in black teas (Hilton & Ellis, 1972). It may be possible to utilise this correlation with the flavanols in the pricing of green teas at auction. In the present case, however, the teas were bought commercially and the prices do not represent auction floor proceeds. Consequently the suggestion is only tentative and it would be interesting to carry out further studies.

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