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Kinetics and Equilibria of Tea Infusion—Part 6: The Effects of Salts and of pH on the Concentrations and Partition Constants of Theaflavins and Caffeine in Kapchorua Pekoe Fannings

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ABSTRACT

The concentrations of theaflavins and of caffeine in sieved Kapchorua Pekoe Fannings (600–710 μm) have been determined at 80°C with a range of aqueous salt and buffer solutions of ionic strength 0·11 mol dm^{-3} and of pH 1·9 to 8·3. The caffeine concentration in the infusions and in the leaf, and its partition constant between swollen leaf and solution, was little affected by the presence of electrolytes or pH changes. The concentration of theaflavins in the infusions was not changed by the addition of salts but was considerably greater in acid media and also, temporarily, in borate buffers. The acid effect was corroborated by experiments with a Ceylon Broken Orange Pekoe blend. The most significant finding was that the concentration of theaflavins in the Kapchorua leaf rose markedly with falling pH while its partition constant decreased. These results show that hydrogen ions liberate extra theaflavin by breaking down the leaf structure and/or the bonding of some theaflavin within the leaf. The effect has significant commercial implications since the market price of tea leaf correlates with its theaflavin content.

INTRODUCTION

Tea extraction depends both upon the properties of the tea leaf and upon the properties of the infusing medium. The effects of leaf size and origin were studied in the two preceding papers of this series (Price & Spiro, 1985*a*,*b*). The present paper and the two papers to follow report on the

effects of pH and salt content of the extracting water. In homes and restaurants the tea leaf is infused in ordinary tap water which contains a range of pH and mineral contents. Not infrequently, media of more extreme pH are employed, as when lemon slices are added or a pinch of sodium bicarbonate (Beeton, 1861). The influence of the resulting pH and ionic strength changes on the rates and extents of extraction has never been investigated.

The two solubles looked at were caffeine, a weak base which protonates around pH 0 (Wood, 1903), and theaflavin, a weak acid which dissociates around pH 8 (P. D. Collier and D. R. Haisman, private communication). Both are important constituents of tea leaf. All the experiments were carried out at 80°C, a typical teapot temperature (Natarajan *et al.*, 1962). The equilibrium aspects of the extractions are examined in the present paper.

EXPERIMENTAL

Salt and buffer solutions

Chemicals were supplied by BDH unless stated otherwise, and solids were dried in a vacuum oven before use. The solutes were dissolved in distilled water, and their concentrations were adjusted so as to give an ionic strength of 0·11 M (M = mol dm^{-3}) in the extracting solutions.

Solutions of NaCl, KCl and $CaCl_2$ were made up directly. The solution of sodium benzenesulphonate had a pH at 80°C of 7·6 and its pH was adjusted to 4·8 by adding *ca* 2 cm^3 1 M HCl to 1 litre. The 0·11 M tetrabutylammonium chloride solution was prepared by neutralising Bu_4NOH solution with HCl.

Six buffer solutions were prepared to span the pH range 2·9 to 8·6. Their compositions are summarised in Table 1. The recipes were mainly taken from Bates (1973). The pH values were checked by direct measurement at 80°C with a Radiometer PHM 62 pH meter and separate glass and saturated calomel electrodes.

Equilibrium measurements

The experiments were carried out with Kapchorua Pekoe Fannings, a Kenyan black tea manufactured by the CTC process. Only the sieved size fraction from 600–710 μm was employed. Known masses of tea leaf (1–4 g) were infused at 80°C with 194·4 g of the aqueous solution, an amount that should occupy 200 cm^3 at 80°C if the density be taken as that of water.

TABLE 1
Compositions of Buffer Solutions with Ionic Strength 0·11M

Name	*Composition per litre*	*Main acid and base species*	*pH at 80°C*
Citrate	500 cm^3 0·54M citric acid + 110 cm^3 1M NaOH	H_3Cit, H_2Cit^-	2·9
Acetate	500 cm^3 0·22M HOAc + 500 cm^3 0·22M NaOAc	HOAc, OAc^-	4·7
Phosphate	500 cm^3 0·1M KH_2PO_4 + 291 cm^3 0·1M NaOH	$H_2PO_4^-$, HPO_4^{2-}	6·9
CHES[a]	500 cm^3 0·44M CHES + 500 cm^3 0·22M NaOH	$^+RNH_2R'SO_3^-$,[b] $RNHR'SO_3^-$	8·1
Ethanolamine	500 cm^3 0·44M base + 110 cm^3 1M HCl	$HO(CH_2)_2NH_3^+$, $HO(CH_2)_2NH_2$	8·2
Borate	12·4 g H_3BO_3 + 1·86 g KCl + 824 cm^3 0·1M NaOH	H_3BO_3, $H_2BO_3^-$	8·6

[a] 2-(cyclohexylamino)ethanesulphonic acid, supplied by Aldrich Chemicals.
[b] $R = C_6H_{11}$, $R' = CH_2CH_2$.

Calculations based on available density data suggest that the error involved in this assumption will be less than 1%. The infusion experiments were conducted in stoppered conical flasks that were magnetically stirred in the 80°C thermostat bath. After 30 min, 2 cm^3 samples were removed with Segma syringes fitted with stainless steel needles whose tip was protected with a glass wool filter plug to exclude tea leaves (Spiro & Selwood, 1984). To avoid analytical errors, the pHs of samples taken from buffer solution infusions or from hydrochloric acid were adjusted to 4·8 (the pH of unbuffered tea solutions) prior to analysis. For caffeine analysis a 1 cm^3 sample was diluted to 10 cm^3 with the correct amount of alkali (for the citrate buffer) or acid added to the diluting solution. The solution was then analysed by high performance liquid chromatography (HPLC) as described earlier (Price & Spiro, 1985*a*). For theaflavins (TF) analysis the alkali or acid was pipetted into the sample before analysis was carried out by the modified Flavognost method (Price & Spiro, 1985*a*; Spiro & Price, 1986). Allowance was made for the alkali/acid addition by correcting the absorbance reading by the appropriate dilution factor.

RESULTS

Table 2 lists the equilibrium concentrations (c_∞) of theaflavins and caffeine obtained by infusing 4 g Kapchorua PF leaf (600–710 μm) in a wide range

TABLE 2
Equilibrium Theaflavin and Caffeine Concentrations in Infusions of 4 g Leaf in 200 cm^3 of Various Aqueous Solutions of Ionic Strength 0·11M at 80°C

Solution	*pH*[a]	*Theaflavin* c_∞ ($\mu mol\,dm^{-3}$)	*Caffeine* c_∞ ($mmol\,dm^{-3}$)
Water	4·8	293 ± 4	2·97 ± 0·03
NaCl		298	2·84
NaO_3SPh		292	2·94
KCl		287 ± 6	2·90
Bu_4NCl		306	3·04
$CaCl_2$		284 ± 5	2·68
$CaCl_2$[b]		289	
HCl[c]	1·9	470	
Citrate buffer	3·0	401 ± 7	2·86 ± 0·03
Acetate buffer	4·7	343 ± 6	2·97 ± 0·05
Phosphate buffer	6·8	284 ± 2	2·98 ± 0·04
CHES buffer	8·0	284 ± 8	2·81 ± 0·03
Ethanolamine buffer	8·0	SEE TEXT	3·17 ± 0·04
Borate buffer	8·3	SEE TEXT	3·03 ± 0·03

[a] Final pH of equilibrium tea infusions.
[b] 0·11 mol dm^{-3} (M) $CaCl_2$ solution with an ionic strength of 0·33M.
[c] The pH was kept constant by pH-statting the solution with 1M HCl titrant and Radiometer autotitration equipment.

TABLE 3
Theaflavin and Caffeine Leaf Concentrations and Partition Constants in Different Buffer Solutions at 80°C

Constituent	*Buffer*	*pH*[a]	x_0 ($mol\,kg^{-1}$)	K' ($kg\,dm^{-3}$)	K
Theaflavin	Citrate	3·0	0·031 6	0·03	0·12
	Acetate	4·7	0·024 2	0·04	0·16
	Water	4·8	0·020 7	0·04	0·16
	Phosphate	6·8	0·016 3	0·11	0·37
	CHES	8·0	0·015 4		
Caffeine	Citrate	3·0	0·179	0·07	0·26
	Acetate	4·7	0·161	0·50	0·93
	Water	4·8	0·183	0·15	0·47
	Phosphate	6·8	0·163	0·13	0·42
	CHES	8·0	0·165	0·16	0·49
	Ethanolamine	8·0	0·168	0·64	1·03
	Borate	8·3	0·159	0·31	0·74

[a] Final pH of equilibrium tea infusions with $w = 4$ g.

of salt and buffer solutions. All figures except that for HCl are the means of at least two independent experiments. Error limits, cited only when three or more runs were carried out, are standard deviations of the means.

Further experiments were then carried out with the buffer solutions using at least five different masses of tea leaf (w) in the range 1 to 4 g. Plots of $1/c_\infty$ against $1/w$ were good straight lines as expected from the theoretical equation (Spiro & Siddique, 1981):

$$\frac{1}{c_\infty} = \frac{V}{wx_0} + \frac{1}{K'x_0} \tag{1}$$

where V is the initial volume of the solution, x_0 the concentration of the constituent in the original leaf and K' the notional partition constant of the constituent between the leaf and the solution. The values of x_0 obtained from the least-squares slopes are uncertain by *ca* $\pm 3\%$ while the K' values, derived from the small intercepts, possess error limits of at least $\pm 10\%$. These limits increase for larger K' values where the intercepts are smaller.

Because the leaf loses soluble constituents and imbibes aqueous solution, K' is related to the true dimensionless partition constant K by the equation (Price & Spiro, 1985*a*):

$$\frac{1}{K'} = \frac{A}{K_\rho} - V_n \tag{2}$$

where ρ is the density of the solution, A the swelling factor (the ratio of the mass of the swollen leaf at equilibrium to the mass of the original leaf) and V_n the net volume of liquid taken up by unit mass of the original leaf. The values chosen for these parameters were $\rho = 0{\cdot}9718\ \mathrm{kg\,dm^{-3}}$ (the density of water at 80°C), $A = 4{\cdot}25$ (M. J. Izard and D. R. Haisman, private communication) and $V_n = 2{\cdot}70\ \mathrm{dm^3\,kg^{-1}}$ (Long, 1978). The resulting values of x_0, K' and K are summarised in Table 3.

DISCUSSION

Overall assessment

In order to cover a pH range of almost seven units, buffers of quite different chemical composition had to be employed. These include solutions with large cations (ethanolamine) and large anions (citrate, CHES). It is for this reason that the salts chosen include one with a large cation (Bu_4NCl) and one with a large anion ($PhSO_3Na$). If these produced 'normal' results then any 'abnormal' result with one of the above-mentioned buffers was likely to be caused by a pH rather than a salt effect. The ionic strength was also

kept constant in all but one of the solutions in order to provide a fair basis for comparisons.

Inspection of Tables 2 and 3 shows that the equilibrium concentration of caffeine in the solution and its concentration in the leaf varied little as the medium was changed. Only in $CaCl_2$ solution was c_∞ distinctly lower. Above average caffeine concentrations were observed in Bu_4NCl and in ethanolamine buffer solutions, both of which contain large cations. Table 3 suggests that this may be due to a higher partition coefficient, although its uncertainty limits are large. There appears to be no significant trend with pH in either x_0 or K.

The c_∞ values for theaflavins (TF) also exhibit fair constancy in the salt solutions, again with a slightly higher result in Bu_4NCl but with no lowering in $CaCl_2$ media. In buffer solutions, however, quite different behaviour was observed. Here the solution concentrations increased sharply in the more acidic media. More surprisingly still, the TF concentrations, x_0, for the leaf showed a rising trend as the pH fell, being twice as large in citrate buffer of pH 3 as in CHES buffer of pH 8. The variation in the partition constants indicates a greater tendency for TF to remain in the leaf in the more acid solutions. A balance between these trends in x_0 and in K may account for the near constancy of c_∞ in the neutral and alkaline pH range. Anomalous results of another kind were found in two of the alkaline buffers, and these will be discussed first.

Theaflavin results in alkaline buffers

Three different alkaline buffers of pH $\geq$ 8 were employed because two of them gave abnormal analytical results for TF. These anomalies were not due to the high pH itself since sufficient acid was added to each sample to bring the pH to 4·8 before the analysis.

With the ethanolamine buffer, consistently low TF values were obtained. This can be ascribed to the partial suppression of the TF-Flavognost complexation reaction, an equilibrium process in which each mole of complex formed is accompanied by one mole of $HOCH_2CH_2NH_3^+$ (Spiro & Price, 1986).

In borate buffer infusions with 4 g leaf, the apparent TF concentrations rose rapidly to a high value after 4–5 min and then dropped progressively to a fifth of this figure after 30 min. It seems likely that this low figure was caused by competitive complexation for TF between buffer borate species and Flavognost, a substituted borate compound. In a trial experiment, an equilibrium tea infusion was prepared in distilled water and two samples removed. One was diluted 1:1 with water, the other with borate buffer solution. After a further 30 min at 80°C, and subsequent pH adjustment to

4·8 of the borate-containing solution, both were analysed for TF. The aqueous 'control' yielded 154 μM and the borate buffer sample 63 μM, a value 60% lower. The competitive complexation with borate appears to be a slow process for, in a similar experiment in which the diluted sample was analysed shortly after being mixed with the borate buffer, the TF concentration was 150 μM. However, these test experiments do not explain the abnormally high TF-Flavognost absorbances obtained after infusing the leaf for 4–5 min in two separate borate buffers. As normally interpreted, these absorbances gave c_∞ values of *ca* 460 μM, over 50% greater than the equilibrium concentrations in unbuffered solutions (Table 2). Perhaps borates, as well as acids (see below), can release TF from otherwise inaccessible sites in the leaf.

No abnormally high TF concentration was found with the third alkaline buffer, CHES. A plot of $1/c_\infty$ versus $1/w$ did, however, produce a small negative intercept which suggests a combination of experimental uncertainties and a large partition constant. A large value of K would be expected because, at pH 8, much of the TF is dissociated. This fact may be related to the lower x_0 value obtained for TF in both phosphate and CHES buffers compared with the unbuffered tea solution.

Theaflavin results in acid solutions

Table 3 shows that x_0 for TF rises as the pH of the buffer falls. Even the unbuffered infusion fits into this trend for the initial pH of the distilled water was *ca* 5·6. It seems unlikely that the lower values in the more alkaline buffers arose from slow initial equilibration since the rate constants in the phosphate and CHES buffers were actually larger than in unbuffered and acid solutions (Spiro & Price, 1987). Moreover, the c_∞ values for TF in the phosphate and CHES buffers were close to the values obtained with water and salt solutions (Table 2). Attention will therefore be directed to the results in the more acid media.

In citrate buffers of pH 3, c_∞ for TF was 35% greater than in unbuffered infusions while x_0 was 50% larger. To remove the possibility that citrate buffer species were responsible, an infusion experiment was carried out with an HCl solution of pH 1·9 at 80°C using autotitration to keep the pH constant. The resulting c_∞ value was 60% greater than normal. This rise in TF concentration with falling pH is unlikely to be an artifact of the analysis. Samples were always adjusted to pH 4·8 before being analysed. Moreover, when several samples from a citrate buffer tea infusion containing more than 4 g leaf were titrated with 0·1M NaOH to yield a set with pH 3, 3·5, 4 and 5, and then analysed by the Flavognost method as usual, the absorbances (corrected for the NaOH dilution) were 0·209, 0·203,

0·210 and 0·207, respectively. Thus the measured TF concentration was not influenced by the sample pH in the range 3–5.

Special experiments were devised to ascertain the source of this extra theaflavin. To test whether it was produced by acid degradation of other tea solubles, the drained liquor from an equilibrium infusion of 8 g leaf in 200 cm^3 water at 80°C was first sampled and analysed for TF (giving 586 μM) and then mixed with an equal volume of 0·1M HCl and left for a further 30 min at 80°C. Samples of this mixture, after having their pH adjusted from 1·8 to 4·8, yielded, on analysis, a TF concentration of 602 μM after correction for the dilution involved. This small increase is less than the combined experimental uncertainties. In any case, Brown *et al.* (1969) have shown that acid hydrolysis of thearubigins (TR) yields cyanidins or catechins, depending upon the conditions, but not TF. Although some components of TR may derive from TF, by and large TF and TR are formed by competitive enzyme-catalysed oxidation of the same common precursor (Brown *et al.*, 1969; Cloughley, 1980).

The spent leaf from highly acid infusions was much more fragile (slushy) than normal, and it therefore seemed likely that the additional TF arose from acid attack on insolubles in the leaf or the leaf itself. To test this, 4 g of leaf was infused with 200 cm^3 water at 80°C for 30 min. The liquor was then discarded, and the spent leaf reinfused with a fresh portion of 200 cm^3 water at 80°C for half an hour. This procedure was repeated. The third infusion was sampled and yielded a TF concentration of 46 μM. Approximately 100 cm^3 of the liquor was decanted off, 100 cm^3 of 0·1M HCl added to the remaining liquor and leaf, and the system infused for a further 30 min at 80°C. Its pH was then 2·9 and its TF concentration, 70 μM. The remaining 100 cm^3 of the third infusion will have contributed 23 μM, so that 47 μM had been created by the action of the acid solution on the leaf.

This result, and the physical appearance of the spent acid-treated leaf, suggest that the acid had opened up parts of the leaf structure that were normally inaccessible. To see if this effect could be produced without using acid, 4 g of leaf were ground up with a pestle and mortar, infused, and analysed for TF as usual. The first such experiment yielded 256 μM but with cream formation; the second, 285 μM. Neither was larger than the normal figure of 293 μM. No extra TF was therefore formed by grinding. This experiment, though not conclusive, suggests that acid releases additional TF by chemical action.

Experiments of W. M. Miller

The above experimental results are largely corroborated by earlier unpublished work in this laboratory with a different tea and a different

TABLE 4
Theaflavin Equilibrium Data obtained with Ceylon BOP Fine Leaf Blend at 80·5°C

Solution	*pH*	c_∞[a] ($\mu mol\,dm^{-3}$)	x_0 ($mol\,kg^{-1}$)	K' ($kg\,dm^{-3}$)	K
Phthalate buffer	3·1	266 ± 14			
Water	4·8	217 ± 14	0·0154	0·035	0·14
Bicarbonate buffer	8·1	194 ± 11	0·0151	0·036	0·14

[a] In infusions of 4·8 g leaf in 200 cm^3 solution.

analytical procedure (M. Spiro and W. M. Miller, unpublished work 1981). The tea employed was Ceylon Broken Orange Pekoe fine leaf blend and theaflavins were analysed by the method of Roberts & Smith (1963) as modified by Spiro & Siddique (1981). An alkaline medium (pH 8·1) was obtained with a 1 mass % aqueous solution of sodium bicarbonate (0·12M), each sample being brought back to pH 4·8 before analysis by adding a small known volume of HCl. The acid medium (pH 3·1) was a buffer solution of 0·05M potassium hydrogen phthalate + 0·0223M HCl. Here each sample was returned to pH 4·8 with NaOH solution before being analysed. The equilibrium samples were taken after stirring infusions of the leaf in 200 cm^3 solution for 20 min at 80·5°C. The usual infusion contained 4·8 g leaf. Five unbuffered infusions containing from 2 to 8 g leaf were also analysed, as were four solutions of pH 8·1 containing 2·5–8 g leaf so that values of x_0 and K could be derived. The results are summarised in Table 4.

The equilibrium TF concentration in Table 4 is seen to be significantly higher at pH 3 than in the unbuffered infusion whereas c_∞ in the alkaline medium is lower, but not significantly so. This exactly parallels the results in Table 2. However, the values of x_0 and K in Table 4 are unchanged in going from water to bicarbonate buffer although trends in these quantities appear in Table 3. As would be expected (Price & Spiro, 1985*a*), the Ceylon BOP leaf contains less theaflavins than does the Kenyan Kapchorua leaf.

CONCLUSIONS

The concentration of theaflavins in equilibrium tea infusions increases in more acid media. The concentration of TF in the leaf itself increases markedly as the pH falls. The effect is attributed to the action of hydrogen ions either in breaking down the structure of the leaf itself and/or in breaking bonds by which some of the TF is held. Further work is necessary to determine whether the extra theaflavin that is liberated has been

chemically bound or was present in previously inaccessible sites in the leaf which are unlocked by hydrogen ions. These results are commercially significant since the TF content of leaf is a good indicator of the price that a given tea fetches in the market place (Hilton & Ellis, 1972; Cloughley, 1980). There is evidence that alkaline borate media may have a similar effect on tea leaf. No abnormal pH effects were found with caffeine extraction.

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