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Effect of pH on cream particle formation and solids extraction yield of black tea

Yuerong Liang *, Yuerong Xu

Tea Research Institute, Zhejiang University, Hangzhou 310029, People's Republic of China

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Abstract

Effect of pH on tea solids extraction yield was significant in increasing instant tea yield. Solids extraction yield was doubled when tea was extracted at pH 1.2 compared with that extracted in boiling distilled water with pH 6.8. H^+ encouraged black tea cream particle formation by either releasing more solids into the infusion or stimulating polyphenols to interact with polysaccharides and nucleophilic groups on protein in tea infusions. HPLC results revealed that theaflavins and tea catechins were leading substances affecting the formation of cream particles and infusion colour. Theaflavins and some tea catechins, such as EGCG, ECG and EGC, were dissociated or degraded under alkaline conditions and thus tea cream particles tended to dissolve and tea infusion became dark in colour. Feasibility of stepwise extraction of instant teas used for iced tea and normal drinking tea, by controlling temperature and pH, is also considered in the present paper. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Main polyphenol oxidation products in black tea infusion are thearubigins (TRs) and theaflavins (TFs), which amount to 30–60% of infusion solids and predominant chemical components of tea cream, which is a coloured precipitate in suspension, formed as a strong black tea cools down (Roberts, Cartwright, & Oldschool, 1957). TFs content is positively correlated with tea quality or market tea price (Cloughley, 1980; Hilton & Ellis, 1972). The creaming ability of black tea is also an indicator of black tea quality. However, creaming down is also a major problem in production of cold water-soluble iced tea because it makes the iced tea become a turbid liquor, which is not favoured by consumers. TFs and TRs have acidic properties (Roberts & Smith, 1961). Acidification of tea infusion by sulfuric acid results in partial precipitation of the coloured oxidation products and is therefore a means for encouraging a considerable degree of separation of the oxidation products from unchanged flavanols in black tea and for obtaining a cold water-soluble instant tea for iced tea production (Roberts, et al., 1957). Tea contains about 300–450 g kg^{-1} of extractable solids, but the yield of instant tea is only 200 g per kg tea in production scale (Chen, 1979). H^+ level affects the equilibrium concentration of TFs and more TFs are liberated when tea is extracted at low pH (Spiro & Price, 1987). As pH of an infusion is raised, the infusion becomes dark and loses its creaming ability (Harbron, 1986). Thus, behaviour of tea extracted at extreme pH levels might be of great interest in both controlling the creaming down of cold water-soluble instant tea and increasing yield of normal drinking instant tea. The present work was aimed to investigate the effect of pH on tea cream particle formation and chemical composition of tea infusion as well as solids extraction yield of black tea.

2. Materials and methods

2.1. Determination of cream particle size, volume concentration and obscuration of tea infusions

2.1.1. Method A

Sixty grams of broken black tea was brewed with 2000 ml boiling distilled water for 10 min. The hot infusion was filtered through a plug of cotton wool. When the infusion cooled down, it was transferred into eight plastic containers, each of which contained 45 ml of the tea infusion. Infusions in seven of the containers

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* Corresponding author.
E-mail address: yrliang@zju.edu.cn (Y. Liang).

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were adjusted to pH 1.2, 2, 3, 7, 9, 11 and 13 with 3 mol l^{-1} HCl or 3 mol l^{-1} NaOH, respectively, and the remaining one remained at its natural pH 4.9 as control test. pH Was determined by Mettler Toledo M_{84} pH meter, during which the infusion was swirled with a magnetic flea on a magnetic stirrer. All of the infusions were finally diluted to a final volume of 50 ml with distilled water and stood overnight in a refrigerator at 4°C. Cream particle size, volume concentration (vol. conc.), specific surface area (sp. s. a.) and liquor obstruction were determined by a S-2 Malvern Mastersizer and the background was determined with distilled water. Values of vol. conc., sp. s. a. and obscuration were directly read from the S-2 Malvern Mastersizer (Liang & Bee, 1991).

2.1.2. *Method B*

To understand the effect of pH of water used to brew tea on creaming down, cups of distilled water were adjusted to pH 1.1, 1.2, 2, 3, 4, 5, 6, 7, 9, 11 according to the above method and then heated to boiling in a microwave oven for brewing tea. Three grams of broken black tea were brewed with 100 ml of the boiling water for 10 min and the infusions were also filtered as above. Infusion pH was measured by Mettler Toledo M_{84} pH meter. Forty-five milliliters of the infusion was transferred to a glass beaker and adjusted with 3 mol l^{-1} HCl or 3 mol l^{-1} NaOH to pH 4.9, a normal pH of black tea infusion, and then was diluted to 50 ml with distilled water and stood overnight at 4°C. Cream particle size, vol. conc. and sp. s. a. of the diluted infusions were determined as method described by Liang and Bee (1991).

2.2. *Determination of tea solids extraction yields*

Quotas of 3-g broken black tea was extracted with 100 ml boiling distilled water with pH 1.1, 1.2, 2, 3, 4, 5, 6, 7, 9, 11 for 10 min as by the above method B, and the hot infusions were filtered through a plug of cotton wool. Volume of each of the resultant infusions was determined with a graduated cylinder when they cooled down to room temperature. Infusion solids concentration was determined by the drying method described by Liang and Bee (1992). Solids extraction yield (SEY) was calculated from following equation:

$$\mathrm{SEY}(\mathrm{g\ kg^{-1}}) = \frac{\text{Solids concentration (g ml}^{-1})\times\text{Infusion volume (ml)}}{\text{Dry tea weight (kg)}}$$

2.3. *HPLC analysis*

One kilogram of fresh tea leaf, which was picked from bushes of *Camellia sinensis* (L) *Var. Assamica* grown in a green house, was withered at room temperature for 14 h and the withered leaf was minced by a laboratory mincer. The minced leaf was placed in a stainless steel tray to ferment for 3 h at 26°C. Forty grams of fermented leaf were transferred to a 500-ml glass beaker and heated to 90°C in a microwave oven to stop fermentation. The leaf was then brewed with 300 ml boiling distilled water for 10 min. The hot infusion was filtered through Whatman No.541 paper. Two quotas of the filtrate (45 ml each) were transferred to two glass beakers. One of them was adjusted to pH 9.45 with 3 mol l^{-1} NaOH and the other remained at its natural pH level (pH4.9). The two solutions were then diluted to 50 ml before being injected into the HPLC column. HPLC was carried out according to the method described by Bailey, McDowell, and Nursten (1990) and Liang (1992).

All of the tests reported in the present paper were duplicated and mean values presented.

3. Results

3.1. *Effect of pH on tea cream particle formation*

Fig. 1 shows that cream particle volume concentration (vol. conc.) in tea infusions extracted by method A was decreased with increase of infusion pH from pH l. 2 to pH 9 and then went up slightly from pH 9 to pH 13. A sharp increase in vol. conc. was seen as pH decreased from 3 to 2, the latter was 0.44% (v/v), almost double that of the former. Vol. conc. was the lowest at pH 9, and it was only 9.9% of that at pH 1.2. Infusion obscuration showed the same tendency as vol. conc. did and its maximum value was seen at pH 1.2 and lowest at pH 9, but remained at 0.65–0.68 from pH 4 to pH 7. Cream particle size was also influenced by pH. Fig. 1 shows that maximum value in the curve of sp. s. a., as a function of infusion pH, appeared at natural infusion pH 4.9 and sp. s. a. went down as infusion pH was adjusted to a value of lower or higher than its natural pH.

From cream particle size distribution of infusions prepared by method A with various pHs, two types of distribution were recognized (Fig. 2). From pH 3 to pH 7, particle size distribution diagrams showed a single peak and over 70% (v/v) of particles had diameters from 0.2 to 0.6 μm. Particles below 1 μm in diameter amounted to 90% of total particle volume. When infusion pH was lower than pH 3 or higher than pH 7, particle size distribution could be divided into two fractions on the particle size scale. The first fraction was in colloidal particle size (Goodwin, 1982) and its average size was around 0.35 μm which was the same as that in infusion with natural tea infusion pH. The second fraction of particles was in a wide range of size ranging from 3 to 70 μm which was above colloidal particle size.

Particle volume ratio of first fraction to second fraction varied with pH, being for example, 8.2 at pH 1.2 and about 1.1 at pH 9. These results also suggested that the decrease of average sp. s. a. and increase of average particle size in the tea infusions with extreme pH levels were due to a high ratio of the big non-colloidal to colloidal particles (Fig. 2).

When tea was brewed in waters with pH 4–9 as method B, tea infusion pH remained at a level of normal tea infusion pH (Table 1) and colour of these tea infusions also looked the same as that of normal black tea infusions. However, if tea was extracted at pH 2 or below, the resulting infusions had a pH value close to pH of water that was used to brew tea and looked pale and cloudy in colour. When all the infusions extracted at various pHs were adjusted to pH 4.9, they were deeply red in colour, except for those extracted at pH 2 or below, which were slightly greyish. Infusion extracted at pH 9 gave the lowest liquor obscuration and particle vol. conc. The highest cream particle vol. conc. and obscuration were observed in infusion extracted at pH 1.1 (Fig .1). No distinct differences of particle size distribution could be recognized between infusions extracted at various pHs when the infusions were adjusted to

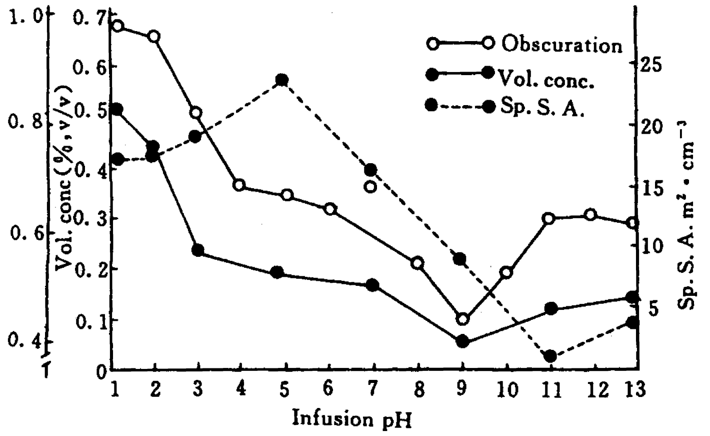

Fig. 1. Effect of pH of infusion prepared by method A on tea cream formation.

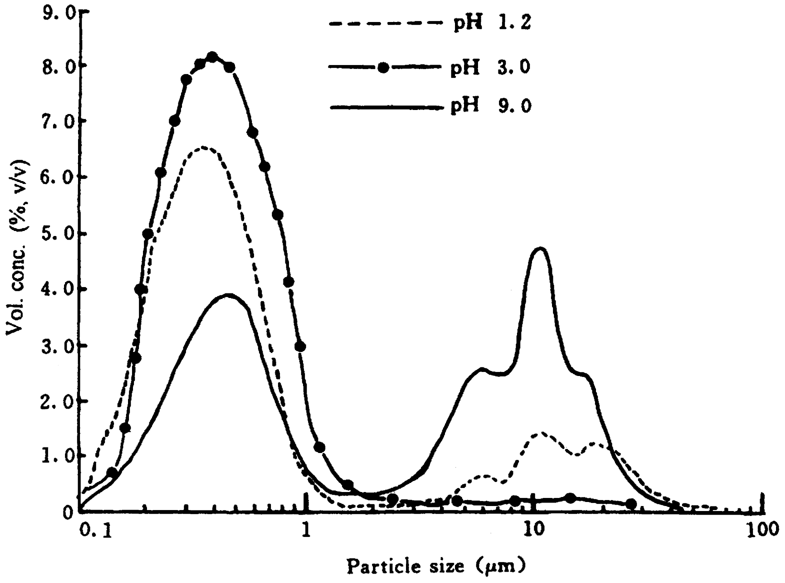

Fig. 2. Cream particle size distribution in infusions prepared by method A with various pHs.

Table 1
Relationship between pHs of infusions prepared by method B and water used to brew tea

Water pH	1.1	1.2	2	3	4	5	6	7	9	11
Infusion pH	1.14	1.23	2.82	4.67	4.91	4.93	4.93	4.93	4.95	5.45

pH 4.9 after extraction. This suggested that changes of the obscuration in infusions extracted at various pHs were induced mainly by variation of vol. conc. because the obscuration showed the same tendency as vol. conc. (Figs. 1 and 2).

3.2. *Effect of pH on tea solids extraction yield*

Results of method B showed that variation of tea solids extraction yield with pH was similar to those of tea cream particle vol. conc. and liquor obscuration with pH (Figs. 1 and 3). More tea solids were extracted when tea was extracted at extreme pH conditions, especially under strongly acidic conditions. Total solids extracted at pH 2 to pH 11 varied from 270 to 290 g kg^{-1} tea, but the figures were doubled at pH 1.1 (Fig. 3).

3.3. *Effect of pH on composition of polyphenols in black tea infusion*

Over 50 peaks were resolved in HPLC chromatograms of the tested broken black tea infusion monitored at the wavelength 280 nm (Fig. 4). Peaks 1, 10, 12, 15, 24, 27, and 29 were major contributors to 280 nm absorbance according to the peak area, especially peak 24, with over 40% of total 280 nm absorbance of infusion at pH 4.9. Peaks 15, 21, 22, 24, 29, and 35 were identified to be gallic acid, (−)-epigallocatechin (EGC), (−)-epicatechin (EC), caffeine, (−)-epigallocatechin-gallate (EGCG), (−)-epicatechin gallate (ECG) according to authentic substances, respectively. Peaks 47, 48, 49 and 50 were TF, TF3G, TF3′G and TF3, 3′DG, respectively, according to previous references (Liang, 1992; McDowell, Bailey, & Harward, 1990; Opie, Robertson, & Clifford, 1990).

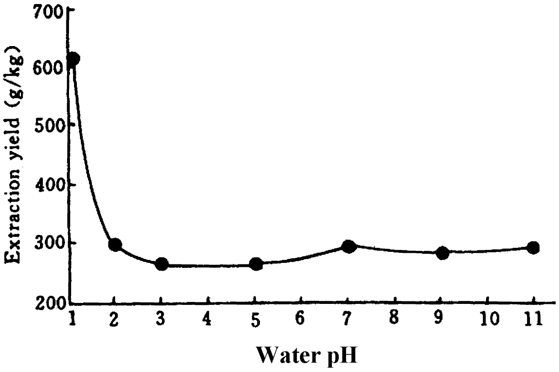

Fig. 3. Effect of pH on solids extraction yield (tea was extracted by method B).

It is shown in Table 2 that chemical composition of black tea infusion changed greatly as infusion pH was increased from pH 4.9 to pH 9.45 after extraction and filtration. Firstly, TFs, especially TF and TF3G, were seriously degraded. TF, which contributed 30.7% of total TFs absorbance at 280 nm in infusion at pH 4.9, could not be detected at 280 nm at pH 9.45. On the basis of 280 nm absorbance, 88% of TFs was lost at pH 9.45 (Table 2). Secondly, EGCG, ECG and EGC were reduced by 97, 45.5 and 3.0%, respectively, but more caffeine, gallic acid and EC were detected. The increases of gallic acid and EC may be attributed to degradation or dissociation of ECG and EGCG. The increase of caffeine might be due to the dissociation of aggregated caffeine-TFs because TFs are degraded under alkaline conditions.

4. Discussion

Four possibilities arise about the effects of pH on tea infusion cream particle formation: first, some polyphenol galloyl esters, such as (−)-epicatechin-gallate (ECG), (−)-epigallocatechin-gallate (EGCG), theaflavin-3-gallate (TF3G), theaflavin-3′-gallate (TF3′G) and, theaflavin-3,3′-digallate (TF3,3′DG) degraded under strong acidic conditions (as extracted by method A) and formed some transient intermediate species which might be captured by polysaccharides and

Table 2
HPLC results of infusions with different pHs at 280 nm

Peak	Compounds	Retention time (min)	Peak area (mm^2)	
			pH 4.90	pH 9.45
15	Gallic acid	9.392	965.41	1151
21	(−)EGC	14.184	240.69	233.40
22	(−)EC	14.834	128.42	130.27
24	Caffeine	15.562	9957	12,964
29	(−)EGCG	19.277	1281	38.94
35	(−)ECG	24.408	556.23	303.20
47	TF	38.961	235.31	None
48	TF3G	40.801	223.31	None
49	TF3′G	41.913	46.44	24.52
50	TF3,3′DG	42.294	261.23	68.83
Total of 50 peaks			24,437.65	20,542.88

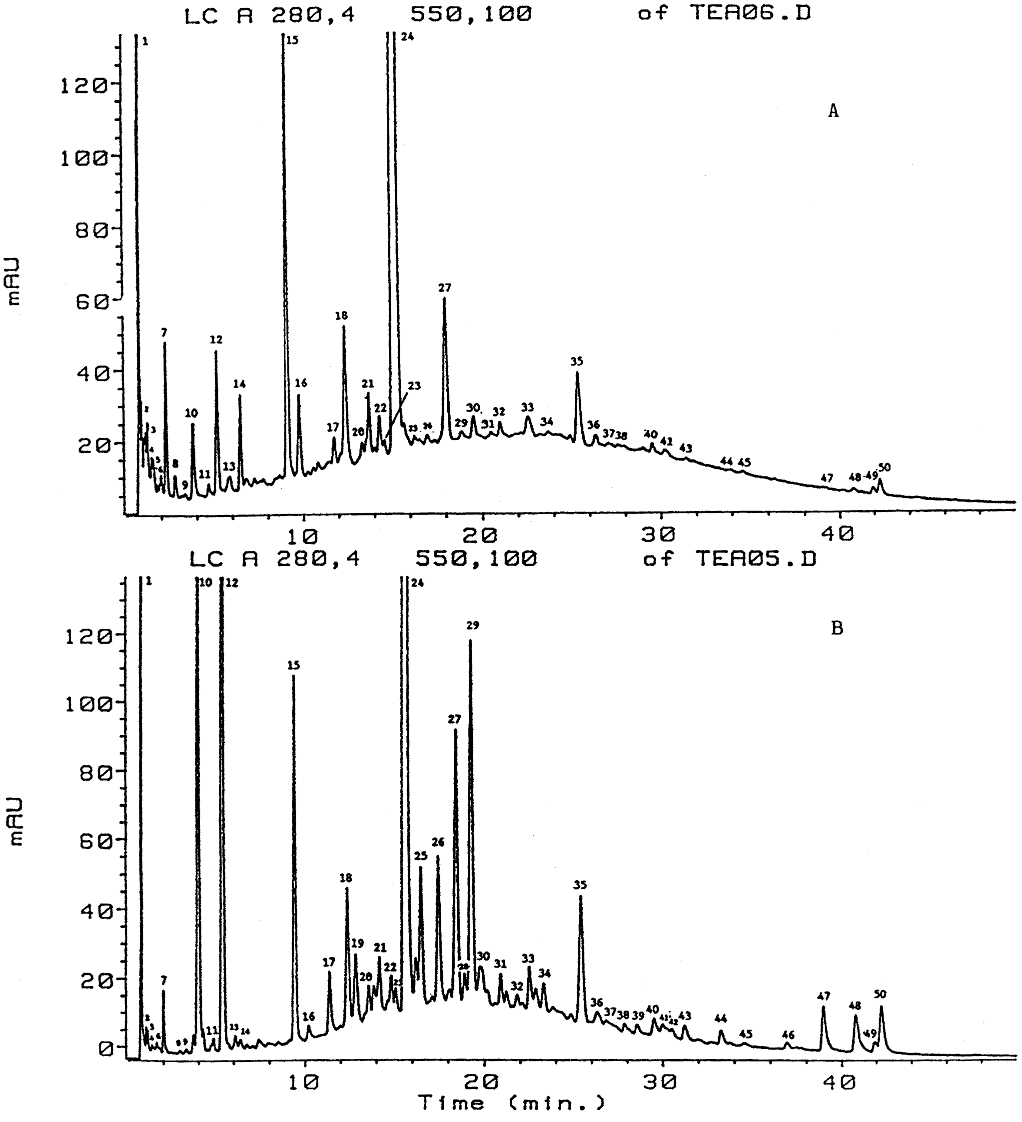

Fig. 4. HPLC diagram of tea infusion monitored at 280 nm. (A) Infusion with pH 9.45; (B) infusion with pH 4.9.

nucleophilic species, such as HS- and HN- on protein molecules, which in turn resulted in tea cream formation or liquor haze. Second, as tea was extracted under extremely acidic condition (as extracted by method B), H^+ liberated some extra substances which could not be extracted under normal brewing pH conditions (Fig. 3). These extra substances could be theaflavins (Spiro & Price, 1987) and might also include polysaccharides and proteins. In this case, cream particle formation was encouraged by increase of infusion solids concentration, though the infusions resumed their normal infusion pH after brewing (as extracted by method B). Thirdly, under alkaline conditions, contents of some tea polyphenols such as TF, TF3G, TF3′G, TF3,3′DG, EGCG, ECG and EGC decreased because of degradation or dissociation (Table 2). They are a group of major tea cream components and their decrease would result in less cream particle formation. Fourthly, partial increase of vol. conc. at pH 11 or above may be assumed to be induced by alkaline auto-oxidation of tea flavanols which had not been oxidized during the tea fermentation process. In alkaline conditions, polyphenols are readily transformed into quinones which may then interact with nucleophilic groups, such as NH_2- and SH- on proteins (Beart, Lilley, & Haslam, 1985; Cilliers & Singleton, 1990).

Degradation and dissociation of TFs are the major factor affecting changes of infusion colour at various pH levels. It was observed that, as infusion pH was raised to pH 7 or above, the infusion became dark and colloidal particles of tea cream were partially dissolved (Fig. 1). These phenomena were closely associated with the loss of tea polyphenols, such as tea flavanols, and TFs which are believed to be groups of major components in black tea cream. TFs might be dissociated and degraded under alkaline conditions (Spiro, Price, Miller,

& Aramis, 1987). The dissociation of TFs can result in formation of anion or salt forms of TFs which lose their original orange yellowish colour. Some TFs maybe degraded into TRs and the latter, which have fairly strong acid properties, are then present as anions or salts which are more deeply coloured than the original TFs. The dissociation is assumed to be reversible but the degradation irreversible. That is why the infusion colour with pH 9 or above, was partially resumed when infusion pH regained its original pH 4.9.

H^+ is the cause of the increase of tea solids extraction yield. Under strongly acidic conditions, the effect of pH on tea solids extraction yield may be attributed to the action of H^+ on tea leaf, altering leaf structure in such a way that some extra solids are liberated. H^+ might open up parts of the leaf structure which are usually inaccessible and had more water soluble components, such as TFs and TRs might be released from the leaf. The effect of H^+ on equilibrium concentration of TFs was confirmed by Spiro and Price (1987). On the other hand, some insoluble substances, such as polysaccharides, pectin and protein, may be hydrolyzed during brewing under strongly acidic conditions. Maximum solids extracted from tea with hot water was about 450 g kg^{-1} tea (Yuan, 1980), while about 600 g solids per kg of dry tea could be obtained when tea was extracted at pH 1.1 according to method B (Fig. 3). This suggested that about 150 g more solids per kg tea was released by the action of H^+. About 1% more solids was obtained when tea was extracted at pH 9 or above. TFs were dissociated under alkaline conditions (Spiro, Price, Miller, & Arami, 1987). This may be ascribed to TFs and TRs that were dissociated in such a way that they became free and more easily extracted. In this case, no insoluble components were hydrolyzed and liberated and so increase of solids extraction yield was limited. TFs and TRs are two groups of polyphenolic oxidation products in black tea infusion which have acidic properties and make tea infusion resemble a weakly acid buffer solution. It was its buffer capacity that made the infusion pH remain at normal infusion pH level, though tea was brewed in water with pHs ranging from pH 4 to pH 7 (as method B) and solids extraction yields were almost unaffected.

It is significant that H^+ was the cause of the increase of tea solids extraction yield. It is expected that instant tea yield could be increased by acidification of tea leaf during extraction but not by alkalization. The temperature at which substances (which exist in tea leaf and are involved in tea cream formation) were initially and extensively extracted into the infusion was between 50°C and 60°C and cold water-soluble instant tea could be obtained when tea was extracted at 50°C or below (Liang & Bee, 1992). This implies that stepwise extraction will be a good idea for production of instant teas for iced tea and normal drinking, respectively, for example, instant tea extracted at 50°C for iced tea and that re-extracted from the residue leaf under strongly acidic conditions for normal drinking. The acidity of the latter, of course, must be re-adjusted to the natural tea infusion pH level.

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