

Black tea stain formed on the surface of teacups and pots. Part 2 – Study of the structure change caused by aging and calcium addition

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Abstract

The black tea stain formed on the surface of teacups consists of oxidized tea polyphenols together with several percent calcium silicates, as shown in our preceding paper. The previous study introduced the hypothesis that the calcium bridging in the polyphenols and the oxidative polymerization promote the stain formation and development. The present infrared spectroscopy (IR) study showed that the increase in the stain formation following the addition of calcium ions is caused by the calcium bridging between the polyphenol molecules. Mass spectroscopy (MALDI-TOF-MS) study provided sufficient evidence that exposing the stain to air also enhances the polymerization of polyphenols. Those structure changes made the stain much less soluble in water. The effectiveness of calcium-chelating agents on the removal of the stain was in accordance with the order of chelating formation constants.

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1. Introduction

The chemical composition and formation mechanism of the black tea stains that are deposited on teacups and teapots have not been of interest to researchers or detergent manufacturers for a long time, probably because the stains could be easily removed by bleaching or scrubbing. Our earlier study (Tanizawa, Abe, & Yamada, *in press*) was concerned mainly with the chemical composition of the stain. The study showed that the stain consists of oxidized tea polyphenols together with several percent of calcium silicates, and that the composition of the tea stain is quite different from that of the tea scum (Spiro & Jaganyi, 1993, 1994a, 1994b; Spiro, Chong, & Jaganyi, 1996) and the tea cream (Roberts, 1963; Rutter & Stainsby, 1975). The adsorbed tea stain on the surface of a teacup is often hard to remove when the teacup is used repeatedly. In our earlier paper we introduced the hypothesis that the cal-

cium bridging in the polyphenols and the oxidative polymerization play an important role in the stain formation and its stabilization. Removal of such stable stains by usual detergents has been a goal for detergent manufacturers.

Phenomena analogous to the calcium bridging and the oxidative polymerization of the polyphenols have been known in other fields. For example, our *in vitro* study concerning the salivary proteins in the oral cavity (Tanizawa, Johna, Yamamoto, & Nishikawa, 2004) demonstrated that calcium ions enhance the pellicle formation on the tooth enamel surface by virtue of the calcium bridging between the proteins. Another example is the study of the custom of stained teeth (Ishikawa, 1968; Nordbo, Eriksen, Rolla, Attramadal, & Solheim, 1982), known as “Ohaguro,” which means the teeth dyed in black, which was practiced mainly by Japanese women until the early 20th century. The dye is a mixture of powdered gallnuts containing polyphenols and iron shavings dissolved in vinegar. When the dye was applied on the teeth and air-dried repeatedly, dark-coloured materials were produced by the oxidation of polyphenols.

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Understanding the mechanism of black tea stain formation and the structural changes that accompany it is required to find effective means for removing the stain without bleaching or scrubbing. The present study mainly dealt with the effect of both the calcium addition to the tea infusion and the exposition of the stain to air on the changes in the structure and the soluble property. The standard reagents such as catechin and catechol, which are supposed to be model materials to the tea polyphenols, were also analysed by Fourier transform infrared spectroscopy (FT-IR) and matrix-assisted laser desorption ionization technique with a time-of-flight mass spectrometer (MALDI-TOF-MS) to elucidate the mechanism by which the stain was stabilized. We also investigated the effectiveness of calcium chelating agents on the removal of stains.

2. Materials and methods

2.1. Preparation of the black tea infusion

We used Iipton Yellow Label Tea in teabags. Porcelain tiles (SPKC-100/L01, 100 × 100 mm and 20 × 100 mm) were obtained from INAX (Japan) and were used for the surface to be stained. In the standard procedure, two teabags were infused at 95 °C for 2 min in 500 ml of pure water or Tokyo tap water in a wide 1-l glass beaker. The Tokyo tap water used in this work was soft water; 0.52 mmol/l of calcium, i.e., 2.91 mg/100 ml CaO, 0.71 mmol/l of magnesium, 0.3 mmol/l of silicate, and 2 mmol/l of carbonate. The pure water contained only a trace of calcium, magnesium, and silicate less than 0.01 mmol/l. In the experiments using synthetic hard water, CaCl₂ (Wako Pure Chemical Industries, Japan) was added to the tea infusion such that the concentration of calcium ions was 5.0 mmol/l at maximum.

2.2. Generation of the stain on the tile surface

The teabags were removed from the infusion, and then a porcelain tile was immersed such that the lower half surface was sunk below the waterline. The beaker was not covered; it was allowed to stand for 24 h at room temperature. The tile was withdrawn, and the surface was rinsed with distilled water and dried in air. The above standard procedure was repeated a given number of times using the same tile to accumulate the deposit on the same position of the tile surface. We then analysed the stained tiles.

2.3. Modification of the stain structure by aging in air and acid treatment

The stain formed on the tile was allowed to stand in air at room temperature for specific periods of time. In addition, the scrubbed stain (10 mg) was treated with HCl (0.1 mol/l, 2 ml) and left to stand for 1 h at 100 °C. The dissolved parts were dried at 60 °C under decompression and analysed.

2.4. Preparation of reference substances for IR and MS analyses

A complex of catechol with calcium ions was prepared by dissolving catechol (Tokyo Kasei Kogyo Co., Japan) and calcium chloride at a molar ratio of 2:1, then dried at room temperature under decompression. Polymerized catechin was prepared by adding catechin (10 mg, Tokyo Kasei Kogyo Co.) to Tokyo tap water (2 ml) and stirring the mixture. Water was evaporated in a 60 °C water bath. This dispersion–evaporation procedure was repeated several times.

2.5. Analyses of the stain by IR and MS

The stains formed on the tiles were scrubbed off using plastic spoon and dried in air, and the IR spectra were recorded using a conventional potassium bromide disc method. Mass spectra were acquired by means of the MALDI-TOF-MS in the following procedure. Distilled water (500 µl) was added to the dried stain samples (5 mg), and the samples were then mixed using an ultra sonicator (solution A). To make a second solution, distilled water (500 µl) and acetonitrile (500 µl) were added to α -cyano-4-hydroxycinnamic acid (α -CHCA, Sigma Aldrich, 97%, 10 mg) as a matrix agent, and the solution was mixed using an ultra sonicator (solution B). Solutions A and B were mixed at a volume ratio of 10:1 to make solution C. The solution C (1 µl) was dropped onto a TOF-MS plate and dried in air. Then, the mass spectra were acquired on a spectrometer (Voyager DE, PE BIOSYSTEMS).

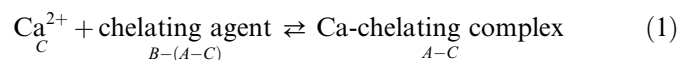
2.6. Removal of the stain by chelating agents

A simple model detergent simulating a commercially available one was prepared such that sodium sulfate (0.083%), sodium percarbonate (0.015%), sodium carbonate (0.015%), and polyoxyethylene-polyoxypropylene alkyl ether (0.0053%) were dissolved in distilled water. Then, to the model detergent solutions, chelating agents were added such that the concentration of each chelating agent was 0.025%. The chelating agents were citric acid, NTA (nitrilotriacetic acid), and EDTA (ethylenediaminetetraacetic acid tetrasodium salt). The tiles with 10-month stains were immersed in these solutions and allowed to stand for given time periods, after which the tiles were withdrawn and dried in air.

2.7. Determination of the chelation formation constant for calcium ions

The aqueous solutions containing the chelating agents and calcium chloride were prepared with distilled water. After the equilibrium shown by Eq. (1) was reached, the free calcium ions were determined by colorimetric analysis using the pontachrome violet SW as an indicator agent (Satsuki, Nagoh, & Yoshimura, 1998a, 1998b). Then, the

values of the formation constant were calculated using Eq. (2).



$$K = [A - C] / [C][B - [A - C]] \quad (2)$$

where K is the formation constant, A is the initial concentration of calcium ions (mol/l), B is the initial concentration of the chelating agent (mol/l), and C is the concentration of free calcium ions (mol/l).

3. Results

3.1. The structure change of the stain by aging in air

The IR spectra of the stains stored in air for one day, five days, and one month were recorded in the 800–4000 cm^{-1} spectral region, as shown in Fig. 1. The spectrum of the one-day stain was characteristic of typical polyphenols, showing the existence of OH in the region of 3300–3500 cm^{-1} , C=C at 1450–1600 cm^{-1} , CO at 1200–1300 and 1600 cm^{-1} , respectively. The peak at 1000–1150 cm^{-1} due mainly to ethers (–COC–), however, appeared and increased with the storage time, indicating that polymerization of polyphenols caused by oxidative-condensation reactions proceeded. The spectra of the five-day and one-month stains were quite different from that of the one-day stain. There was no remarkable change in the signal at 1600 cm^{-1} , which means that no other structure change occurred besides the ether group formation.

To identify the structure of the aged products, we performed mass spectrometric analysis. MALDI-TOF-MS spectra of the stains kept in air for one day and one month are shown in Fig. 2, together with the background spectrum of the α -CHCA matrix. As a large number of signals were observed and the matrix signals were overlaid on those of the stain, it was almost impossible to assign each signal of the fragment ions. The spectrum of the one-month stain was different from that of the one-day stain; the one-month stain uniquely displayed several small peaks at

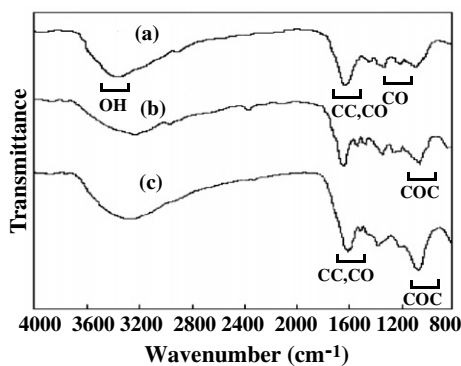


Fig. 1. Infrared spectra changes of tea stain formed in tap water. The stains were stored in air for 1 day (a) 5 days (b), and 1 month (c).

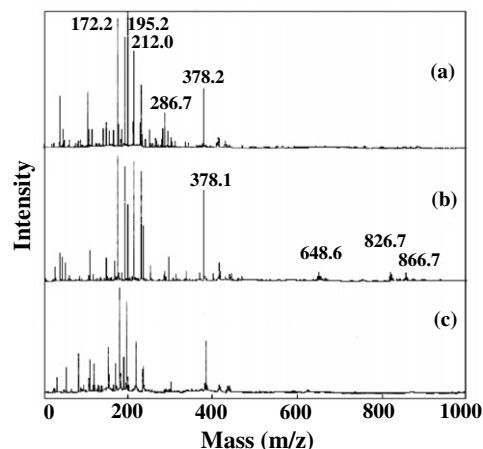


Fig. 2. Mass spectra changes of stain formed in tap water. The stains were stored in air for 1 day (a) and 1 month (b) and α -CHCA as the matrix material (c).

around m/z 650–870 which were not observed for the one-day stain. MALDI, generally known as a soft ionization technique, produces almost exclusively intact pseudo-molecular ion species without ion fragmentation. Therefore, those data are supposed to give information about molecular weight of the organic polymers depending on the degree of polymerization. Thus, MALDI-TOF-MS together with the IR data have provided sufficient evidence that exposing the stain to air enhances the polymerization of polyphenols.

To clarify the relationship between the degree of polymerization and the soluble property of the stain, we performed a cleaning test in which a glass beaker containing distilled water was placed in a supersonic washer, and then the stain-adsorbed tile was partly immersed in distilled water and treated for 5 s. Fig. 3 shows that the one-day stain was removed almost completely, but the one-month stain remained on the tile surface. Those results, though obtained in a simplified condition, are in line with the phenomenon that is often observed when teacups are used repeatedly.

3.2. The effect of calcium ions on stain formation

In our preceding study (Tanizawa et al., in press), scanning electron microscope–energy dispersive spectroscopy

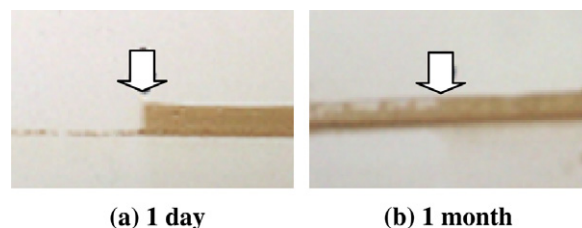


Fig. 3. Cleaning test of stains formed in tap water. The stains were stored in air for 1 day (a) and 1 month (b) then treated by supersonic washer. Left half was immersed in distilled water, and treated for 5 s.

(SEM–EDS) analysis of stained tile showed that calcium was detected not only in the aggregates of the calcium silicates but also in the underlying organic materials. This result suggests that calcium ions play an essential role in the formation of the stain. To confirm the roles of calcium ions, we investigated their effect on stain formation. We added calcium chloride (Wako Pure Chemical, Japan) to the black tea infusion such that the concentrations of calcium ions ranged from 0.5 to 5.0 mmol/l. Fig. 4 shows that the calcium ions greatly increased the amount of the stain. These effects may be ascribed to the combination of calcium ions and various polyphenolic compounds such as tannins. Cleaning tests were performed in the same manner as that shown in Fig. 3, and the results are shown in Fig. 5. The difficulty in removing the stain was in accordance with the amount of existing calcium ions. Thus, the calcium addition increased the amount of the stain, and in addition it made the stain stable against removal, probably because of the calcium bridging. A more detailed mechanism will be discussed later.

3.3. Removal of the stain by chelating agents

The calcium bridging, besides the aging in air, has been found to be a critical factor in making stains stable against removal. Thus, we investigated the possibility of breaking the calcium bridging using chelating agents. We examined three agents: citric acid, nitrilotriacetic acid (NTA), and ethylenediaminetetraacetic acid tetrasodium salt (EDTA).

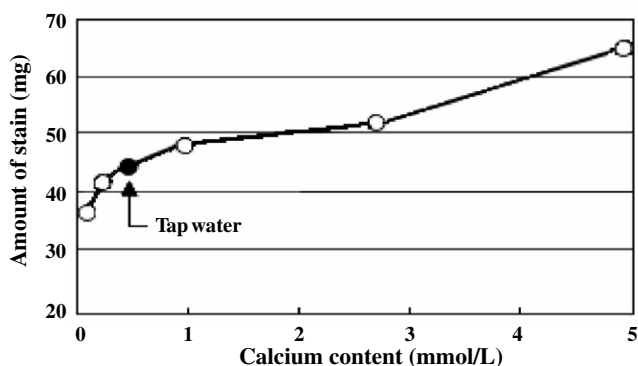


Fig. 4. Effect of calcium content in tea infusion on the formation of stain. Brewed in tap water (●) and artificial water (○).

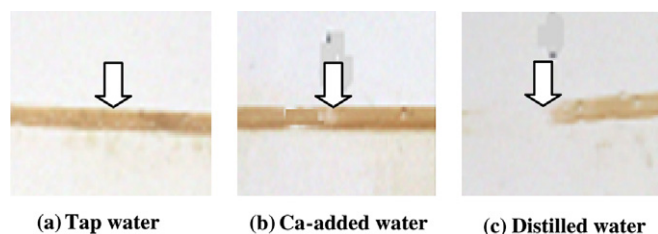


Fig. 5. Cleaning test of stains formed in tap water (a) Ca-added water; 1 mmol/l (b) and distilled water. (c) Left half was immersed in distilled water and treated for 5 s by supersonic washer.

Table 1
Chelating formation constants for calcium ion (log K , k:l/mol)

Citric acid	NTA	EDTA	Catechin
3.7	6.4	10.4	3.6

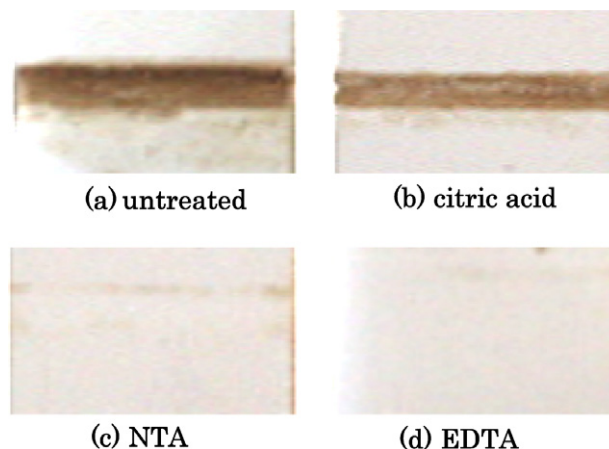


Fig. 6. Cleaning test of stain formed in tap water. The stained tiles were immersed in the solutions of chelating agents for 40 min.

First, we measured the chelating formation constants of these chelating agents and catechin as a model material of the stain; the results are shown in Table 1. The formation constants for NTA and EDTA agreed with the reported values. Among these agents, EDTA showed the largest value, followed by NTA, and then citric acid and catechin. This result indicates that the catechin-calcium complex might be decomposed by these chelating agents. As shown in Fig. 6, the removal experiments performed in distilled water system have revealed that the effectiveness of the calcium chelating agents on the removal of the stain was in accordance with the order of the chelating formation constants; citric acid had little or no effect on the removal of the stain, and EDTA was the most effective agent. These effects of the chelating agents observed in the distilled water system were not, however, reproduced in the Tokyo tap water system, probably because of interference by calcium, magnesium, and other ions contained in the tap water.

4. Discussion

In our earlier work (Tanizawa et al., *in press*) we introduced the hypothesis that oxidative polymerization and calcium bridging in polyphenols caused stain formation and enhanced the stabilization of the net structure. In the present work, MALDI-TOF-MS provided sufficient evidence that exposing the stain to air enhanced the polymerization of polyphenols, and the calcium addition to the tea infusion also increased the amount of the stain on the tile. The mechanism of the polymerization and the calcium bridging seems to be worth investigating and discussing in understanding the details of tea stains. For this

purpose, the catechin and the catechol were investigated as the simple model materials of polyphenols.

The polymerized catechin was prepared by adding catechin to Tokyo tap water and evaporating repeatedly. Then, those materials were characterized by IR and MALDI-TOF-MS methods in the same manner as that used for characterizing the tea stain. IR spectra changes of the catechin following the dissolution-drying treatment are shown in Fig. 7. The broad peak at around 1100 cm^{-1} was probably due to the increased phenol ether with the treating times, and it was quite different from the sharp peaks that are normally observed for the polyphenols like catechin. These spectra changes were similar to those observed in the tea stain. The mass spectra changes of the catechin after the dissolution-drying treatment are shown in Fig. 8. The small peaks at around m/z 650–870 were observed for the treated catechins but not for the original catechin. Those results suggested that the phenol ethers were produced by the condensation following the oxidation reaction. By analogy with those results of the standard catechin, similar phenomena are likely to have progressed in the case of the black tea stain; the structure changes of the stain shown in Figs. 1 and 2 were caused by the polymerization of the

polyphenols by aerobic oxidation, though naturally the polyphenols had been partly oxidized when the black tea leaf was prepared (Menet, Sang, Yang, Ho, & Rosen, 2004; Su, Leung, Huang, & Chen, 2003; Willson & Clifford, 1992).

The stain under consideration consists of tea polyphenols together with calcium silicates, as shown in our preceding work. Our experiments have shown that the calcium addition to the tea infusion solely enhanced the formation of stains, even in the absence of silicate ions. Thus, we believe our earlier hypothesis that the calcium bridging between polyphenols is involved in the stability of a stain is reasonable. Incidentally, regarding the calcium bridging, catechol is known to form stable complexes by its two ligands with a variety of metal ions, and it is used as an analytical reagent for detecting metal ions. The complex formation of catechol and its derivatives with metal ions has been investigated recently (Namasivayam & Sumithra, 2004; Turkel, Berker, & Ozer, 2004). Catechol is also considered to be a simple model of the polyphenol structure. Therefore, investigation of the structure change of catechol caused by the formation of the calcium complex should be useful in characterizing tea stains. The complex was prepared by dissolving catechol and calcium chloride with a molar ratio of 2:1. The IR spectra of catechol and its complex with calcium are shown in Fig. 9. The difference in the spectra at around 1600 cm^{-1} was remarkable; the unification of two peaks was observed for the calcium-treated catechol. This was probably caused by the calcium chelation with two hydroxyl groups within a molecule or the calcium bridging formations between plural molecules. On the other hand, as shown in Fig. 10, a peak at 1600 cm^{-1} split into two peaks when the tea stain was treated by hydrochloric acid (0.1 mol/l). By an analogy with the IR spectrum change of catechol, an opposite reaction to the calcium complex formation would have occurred. In this case, the calcium bridging between plural molecules had already existed within the stain structure, and the acid released calcium ions from the complex to recover the original polyphenols. The hypothetical chemi-

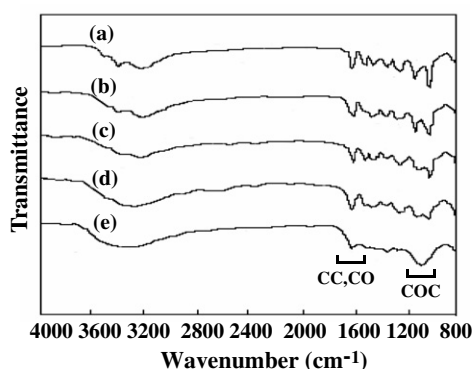


Fig. 7. Infrared spectra change of standard catechin (a) by dissolution-drying treatment one (b) five (c) seven (d) and ten (e) times.

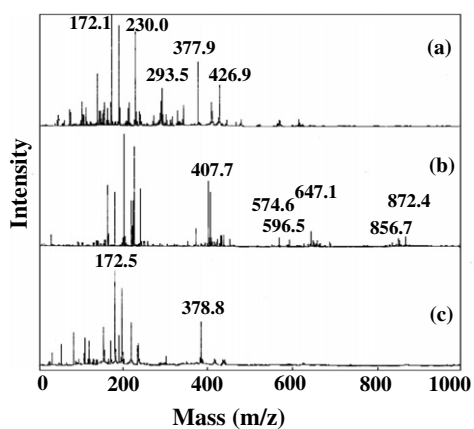


Fig. 8. Mass spectra of standard catechin (a), treated catechin 10 times (b) and α -CHCA as matrix material (c).

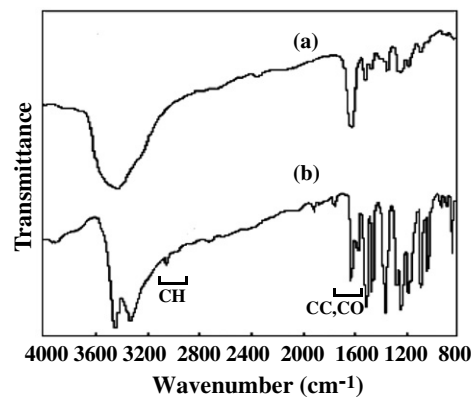


Fig. 9. Infrared spectra of calcium-treated catechol (a) and untreated catechol (b).

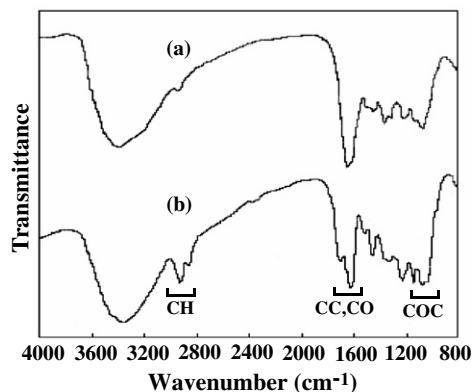


Fig. 10. Infrared spectra of tea stain formed in tap water (a) and tea stain treated with 0.1 N HCl (b).

cal reaction of the catechol complex formation have been simplified and summarized in Fig. 11.

The chelating agents such as EDTA, NTA, and citric acid were investigated, and the calculated chelating formation constants are shown in Table 1 and the results of cleaning tests in Fig. 6. The effectiveness of the agents as tea stain removers was strongly dependent on the chelating formation constants of the agents. The chelating agents appear to have affected the calcium bridging of the polyphenols to displace calcium ions and to have increased the solubility of the calcium-bridged polyphenols.

A formation model of the black tea stain based on the former work and the present results is illustrated in Fig. 12. Soon after black tea is brewed, water starts to evaporate and the waterline sinks. In the neighborhood of the waterline, various solutes in the tea infusion are concentrated, and this phenomenon is one of the driving forces of the stain formation. We believe the siloxane bonds on the glaze of porcelain tile surface are the binding sites for the polyphenols. The hydroxyl group of the polyphenols interacts directly with the siloxane sites by virtue of the hydrogen bonding. The stains on the surface of tiles are, however, not simply the deposit of tea infusion. The adsorbed polyphenols interact with calcium ions and are oxidized to form a stable stain, whereas the polyphenols are adsorbed weakly on the tile surface below the waterline without being oxidized. The addition of extra calcium ions enhances the calcium bridging, and the aging in air accelerates the oxidation of the stain. The concentrated calcium and silicate ions react in the course of evaporation of water to form calcium silicate aggregates. Magnesium and sodium ions in tea infusion do not form water-insoluble materials. In the tea infusion prepared with distilled water,

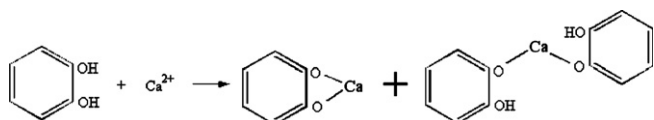


Fig. 11. Hypothetical reaction mechanism of catechol complex formation with calcium ion.

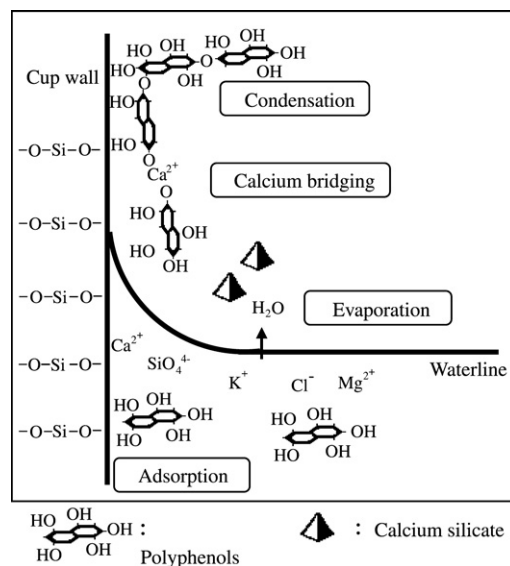


Fig. 12. Schematic simplified illustration of formation of black tea stain on cup wall.

a comparably soluble stain is formed, because only a few inorganic ions are contained to enhance the formation of the stain.

5. Conclusion

The black tea stain we studied consisted of oxidized tea polyphenols together with calcium silicates. The hypothesis that both the calcium bridging in the polyphenols and the oxidative polymerization promote the stain formation and its development proved to be reasonable according to IR and MS studies. On the basis of the former and present results, the model structure and the formation mechanism of the stain were proposed. The effectiveness of the calcium chelating agents on the removal of the stain was in accordance with the order of the chelating formation constants for calcium ions. This study is one step in the comprehension of the whole stain structure. As the organic matrix is especially complex, further studies will be required to provide a more detailed picture of the stain, and to develop the means of cleaning the stain without scrubbing or bleaching.

References

- Ishikawa, T. (1968). Study on the effects of Ohaguro on tooth enamel. *Shikwa Gakuho*, 68, 1450–1470.
- Namasivayam, C., & Sumithra, S. (2004). Adsorptive removal of catechol on waste Fe(III)/Cr(III) hydroxide; equilibrium and kinetics study. *Industrial & Engineering Chemistry Research*, 43, 7581–7587.
- Menet, M. C., Sang, S., Yang, C. S., Ho, C. T., & Rosen, R. T. (2004). Analysis of theaflavins and thearubigins from black tea extract by MALDI-TOF mass spectrometry. *Journal of Agricultural and Food Chemistry*, 52, 2455–2461S.
- Nordbo, H., Eriksen, H. M., Rolla, G., Attramadal, A., & Solheim, H. (1982). Iron staining of the acquired enamel pellicle after exposure to tannic acid or chlorhexidine: Preliminary report. *Scandinavian Journal of Dental Research*, 90, 117–123.

- Roberts, E. A. H. (1963). The phenolic substances of manufactured tea. X. The creaming down of tea liquors. *Journal of the Science of Food and Agriculture*, *14*, 700–710.
- Rutter, P., & Stainsby, G. (1975). The solubility of tea cream. *Journal of the Science of Food and Agriculture*, *26*, 455–463.
- Satsuki, T., Nagoh, Y., & Yoshimura, H. (1998a). Effect of calcium ions on detergency. Part 1. Development of a new method for determining free calcium ions in a washing liquor. *Tenside Surfactants Detergents*, *35*, 60–64.
- Satsuki, T., Nagoh, Y., & Yoshimura, H. (1998b). Effect of calcium ions on detergency. Part 2. Interactions between a surfactant, a calcium-sequestering builder and calcium ions. *Tenside Surfactants Detergents*, *35*, 112–118.
- Spiro, M., & Jaganyi, D. (1993). What causes scum on tea? *Nature*, *364*, 581.
- Spiro, M., & Jaganyi, D. (1994a). Kinetics and equilibria of tea infusion. Part 10. The composition and structure of tea scum. *Food Chemistry*, *49*, 351–357.
- Spiro, M., & Jaganyi, D. (1994b). Kinetics and equilibria of tea infusion. Part 11. The kinetics of the formation of tea scum. *Food Chemistry*, *49*, 359–365.
- Spiro, M., Chong, Y. Y., & Jaganyi, D. (1996). Kinetics and equilibria of tea infusion. Part 13. Further studies on tea scum: the effects of calcium carbonate, lemon and sugar. *Food Chemistry*, *57*, 295–298.
- Su, Y. L., Leung, L. K., Huang, Y., & Chen, Z. Y. (2003). Stability of tea theaflavins and catechins. *Food Chemistry*, *83*, 189–195.
- Tanizawa, Y., Abe, T., Yamada, K. (in press). Black tea stain formed on surface of teacup and pot; Part 1. *In vitro* study on the chemical composition and structure.
- Tanizawa, Y., Johna, N., Yamamoto, Y., & Nishikawa, N. (2004). Salivary films on hydroxyapatite studied by in vitro system for investigating effect of metal ions and by quartz-crystal microbalance system for monitoring layer-by-layer film formation. *Journal of Cosmetic Science*, *55*, 163–176.
- Turkel, N., Berker, M., & Ozer, U. (2004). Potentiometric and spectroscopic studies on aluminum(III) complexes of some catechol derivatives. *Chemical & Pharmaceutical Bulletin (Tokyo)*, *52*, 929–934.
- Willson, K. C., & Clifford, M. N. (1992). *Tea: Cultivation to consumption*. London: Chapman & Hall, pp. 555–601.