

Cream formation in a semifermented tea

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Abstract: The formation of cream in Paochung tea, a popular semifermented tea, which undergoes a lesser degree of enzymic oxidation during manufacture, was investigated at various extraction temperatures, extraction times, pHs and leaf/water ratios. The primary components of Paochung tea cream were catechins (30%), caffeine (20%) and protein (16%). (–)-Epigallocatechin gallate and (–)-epicatechin gallate were the major catechins precipitated during creaming, constituting 19% and 5% of the tea cream respectively. The amount of tea cream produced and its composition were influenced by extraction temperature and pH. The tea leaf/water ratio determined the amount of tea cream formed but did not affect the cream composition. Catechins were considered to be the key component in tea cream. They interacted with caffeine and protein to induce tea cream formation.

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INTRODUCTION

It has been observed that the clarity of a tea infusion changes on cooling the hot infusion down to about 20–30°C, and keeping it further in this temperature range or lower may cause the production of precipitates and/or suspensions which are known as 'tea cream'. The development of tea cream in such tea drinks as iced teas is considered an undesirable phenomenon, because it not only creates a fouling problem during the concentration process but also limits the application of tea in some drinks owing to its unattractive appearance.^{1–3} Most research related to tea cream formation has focused on black tea.^{1–5} Roberts⁶ and Smith¹ reported that the main components of black tea cream were caffeine and oxidised polyphenols such as thearubigins and theaflavins. The extent of cream formation was dependent upon the amount of thearubigins in the black tea infusion.⁷ Non-caffeine nitrogen compounds, including protein and humic acid-like substances,⁴ as well as a lipid complex comprising components such as 1-triacontanol, α -spinasterol and dihydro- α -spinasterol, have also been identified in black tea cream.⁸

The factors affecting cream formation in black tea include the extraction temperature, the pH and the concentration of the infusion.^{1–5} The extraction of black tea at a temperature below 35°C would produce an infusion incapable of creaming.³ When the pH of the solvent was maintained at 4, the maximum amount of black tea cream was formed.¹

The extent of enzymic oxidation during the manufacture of tea leaves affects the composition and

sensory properties of the tea infusion. However, little information is available concerning cream formation in semifermented teas, which are processed under a lesser degree of enzymic oxidation. It is important to understand the similarities and differences in cream composition and cream formation behaviour between semifermented and black tea. The objectives of this study were to investigate the composition of semifermented tea cream, the roles of tea components in the cream and the effects of the extraction conditions upon cream formation.

MATERIALS AND METHODS

Tea leaves

Paochung tea, harvested and processed in November 1992 at Tao Yuin county of northern Taiwan, was used for this study. For manufacturing this semifermented tea, fresh tea leaves (TTES12) were solar withered for *ca* 20 min and then indoor withered for *ca* 8 h. After panning at 270°C for 6 min, the leaves were rolled for *ca* 7 min and dried at 95°C for 10 min in an oven (primary drying). The semi-dried leaves were subjected to ball rolling and then dried again in an oven to a final moisture content of 4%. The tea leaves were stored in a freezer at –10°C to maintain their quality.

Preparation of the tea infusion

The tea leaves were ground and passed through a 140 mesh screen to remove fine particles before extraction. The ground leaves were extracted with distilled water

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with various leaf/water ratios at 90 °C for 20 min. The extract was filtered through a 140 mesh screen and then quickly cooled down to 30 °C or lower with a glass condenser using water (25 °C) as a cooling medium and held in a 2 °C water bath for 2 h to allow the development of the tea cream. The tea infusion was centrifuged at 12500 × *g* for 30 min to obtain a clarified infusion.

Analytical

The constituents of the tea cream were determined by calculating the contents in the original and clarified infusions and then making a comparison. The total solids contents of the original and clarified infusions were determined by drying 20 ml of the infusions at 90 °C.⁹ For determining the caffeine content, the sample solution was adjusted to 5–30 mg ml⁻¹ of caffeine concentration with distilled water. After adding 4% (w/v) poly(vinylpyrrolidone) (PVPP) (Sigma Co, St Louis, MO, USA) to the sample solution, the mixture was held at room temperature (25 °C) for 30 min and filtered through a Whatman No 41 filter paper to remove the polyphenols. The caffeine content of the mixture was determined by measuring its optical absorbance at 276 nm using pure caffeine as a standard.¹⁰

The total catechins content of the original and clarified infusions was determined using the HCl–vanillin method.¹¹ The catechins content of the sample solution was adjusted to 100–500 mg ml⁻¹ with distilled water. After adding 5 ml of a 4% vanillin solution and 3 ml of concentrated HCl to a 1 ml sample solution, the catechins content in the mixture was determined by measuring the optical absorbance at 500 nm using (+)-catechin (Sigma Co) as a standard. For determining the polyphenolic compounds, the tea infusion was diluted 20 times with distilled water, filtered through a 0.45 mm filter and analysed using HPLC.¹² The HPLC system (ICI Instruments, Victoria, Australia) consisted of two pumps (LC 1100) and a variable-wavelength UV detector (LC 1200). The separation was performed on an Inertsil 5 ODS-80A (250 mm × 4.6 mm) column (Vercopak, Taiwan) at 40 °C. Linear gradient elution was carried out at 1 ml min⁻¹ flow rate from 99% solvent A (0.1% phosphoric acid solution containing 0.2% acetonitrile and 5% *N,N*-dimethylformamide) and 1% solvent B (acetonitrile) after injection to 30% solvent A and 70% solvent B at 60 min. The response was determined at 280 nm. The catechins kit containing pure (+)-catechin, (–)-epigallocatechin ((–)-EGC), (–)-epicatechin ((–)-EC), (–)-epigallocatechin gallate ((–)-EGCG) and (–)-epicatechin gallate ((–)-ECG) was purchased from Nuhunakoshi Co (Tokyo, Japan). Pure gallic acid, rutin and quercetin were from Sigma Co.

For the determination of protein and pectin contents, 5 ml of tea infusion was mixed with 25 ml of 95% alcohol for 30 min and centrifuged at 3000 × *g* for 20 min, when the precipitate was redissolved in 5 ml of distilled water. The above procedures were repeated

twice. The protein content in the precipitate was determined by the Lowry method¹³ using bovine serum albumin (BSA) as a standard. The pectin content was determined by the procedure proposed by Blumenkrantz and Asoboe-Hansen¹⁴ using anhydrogalacturonic acid (AGA) as a standard.

The calcium, iron, magnesium, potassium, copper and zinc contents in the original and clarified infusions were determined by atomic absorption spectrophotometry (Smith-Hieftje 8000, Thermo Jarrell Ash Co, Massachusetts, USA). Samples were diluted 10 times with deionised water to avoid creaming prior to analysis.

For the free amino acid content determination the tea infusion was diluted 20 times with distilled water and filtered through a 0.45 mm filter. Five millilitres of filtrate was injected into a Sep-Pak C-18 cartridge (Millipore Co, Massachusetts, USA). The last 3 ml of the filtrate was collected and analysed with an amino acid analyser (Model 415, LKB Biochem Co, UK) equipped with an Ultrapac resin column (lithium form). Citrate buffer (pH 2.20–6.45) was used for elution at a flow rate of 50 ml h⁻¹. The absorbance of the infusion was measured at 570 nm.

Statistical analysis

The analysis of the variance employed an SAS program for PCs (SAS Institute, Inc, Cary, NC, USA). Duncan's multiple-range test was also applied to determine whether there were significant differences between individual treatments.

RESULTS AND DISCUSSION

Composition of Paochung tea cream

The primary components of Paochung tea cream are illustrated in Table 1. Caffeine, catechins and protein were the three major constituents. A widely supported hypothesis is that complexation is exhibited by both caffeine–polyphenols and polyphenols–protein. Complexation of polyphenols and proteins may involve two situations.¹⁵ At low protein concentrations the polyphenol associates at more sites on the protein surface to produce a monolayer which is less hydrophilic than the protein itself, and then aggregation and precipitation ensue. When the protein concentration is high, the precipitation is caused by the cross-linking of different protein molecules by the polyphenols. In the Paochung tea infusion the catechins content (1.46%) was 10 times the protein content (0.14%) (Table 1). This is thus closer to the former situation.

It is known that the primary intermolecular forces in the caffeine–polyphenols complexation are contributed by hydrogen bonding, apolar hydrophobic interactions and coordination with a metal ion.^{16,17} Meijbaum-Katzenellenbogen *et al*¹⁸ demonstrated that caffeine competes effectively with proteins for polyphenolic substrates. Thus the caffeine–catechins complexation is also likely to be important in Paochung tea cream formation.

Table 1. Comparison of selected chemical constituents of original and clarified Paochung tea infusions^a

| Constituent | Original infusion (g/100ml) | Clarified infusion (g/100ml) | Tea cream ^b (g/100ml) |
|------------------------|-----------------------------|------------------------------|----------------------------------|
| Total solids | 3.81 | 3.24 | 0.57 |
| Caffeine | 0.38 | 0.27 | 0.11 (20%) |
| Catechins ^c | 1.46 | 1.29 | 0.17 (30%) |
| Catechins ^d | | | |
| (-)-EGC | 0.027 | 0.025 | NS |
| (-)-EC | 0.068 | 0.067 | NS |
| (-)-EGCG | 0.303 | 0.197 | 0.106 |
| (-)-ECG | 0.060 | 0.031 | 0.029 |
| Protein | 0.14 | 0.05 | 0.09 (16%) |
| Pectin | 0.10 | 0.09 | 0.01 (2%) |
| Total amino acids | 0.076 | 0.070 | 0.006 |
| Theanine | 0.040 | 0.037 | 0.003 |
| Asp | 0.008 | 0.007 | 0.0003 |
| Glu | 0.007 | 0.006 | 0.001 |
| Mineral ^e | | | |
| Ca | 7.54 | 2.56 | 4.98 |
| Fe | 5.20 | 5.14 | NS |
| Mg | 4.00 | 4.00 | NS |
| K | 6.69 | 6.56 | NS |
| Cu | 0.10 | NS | 0.10 |
| Zn | 0.95 | 0.92 | NS |

^a The infusion was extracted with a 10% tea leaf/water ratio at 90°C for 20min. The clarified infusion was obtained after clarification by centrifugation. For a detailed description see Materials and Methods. Values are the averages of four determinations ($n=4$). The composition of the tea cream was determined at significance level $p<0.05$. Numbers in parentheses represent the percentages of the constituents in tea cream (g/dry basis).

^b Calculated by subtracting the value of the clarified infusion from that of the original infusion.

^c Data were determined using the HCl-vanillin method.

^d Data were determined by quantification of the HPLC method.

^e In ppm.

NS, No significant difference at $p<0.05$.

The most abundant group of substances in the Paochung tea infusion was catechins, which also accounted for approximately 30% of the total mass of the cream. The phenolic compounds in the original tea infusion and the decaffeinated tea infusion were analysed using reverse-phase HPLC (Fig 1). A comparison of changes in the peak areas of the polyphenolic constituents in the Paochung tea infusion before and after decaffeination demonstrated that those compounds with hydrophobicity higher than (-)-EGCG (eluted after (-)-EGCG) had a higher tendency to complex with other components and form cream (Table 2). The concentrations of galloyl esters in catechins, (-)-EGCG and (-)-ECG, were markedly diminished ($p<0.05$) after clarification, but the non-ester forms of catechins, (-)-EGC, (+)-C and (-)-EC, were not significantly reduced ($p>0.05$). The percentages of (-)-EGCG and (-)-ECG in the cream were 19% and 5% respectively (Table 1). In black tea the ester-form catechins can react with other catechins to produce theaflavins or thearubigins during the fermentation process.⁵ In this study the theaflavins would elute at about 59 min in Fig 1, but in the full

chromatogram there was no evidence of their presence. It is worth noting also that the baseline in Fig 1 shows little divergence from zero, indicating the absence of unresolved thearubigins.¹¹ Black tea cream contains approximately 17% caffeine, 66% thearubigins and 17% theaflavins,^{1,6} and (-)-EGCG and (-)-ECG are not the primary components. The composition of the semifermented tea cream, which does not contain thearubigins and theaflavins, is obviously different from that of the black tea cream.

The galloyl group of catechins has been identified to be an active site for binding protein through a hydrogen bond.²⁰ Martin *et al*¹⁶ suggested that a hydrophobic interaction existed between the galloyl group of polyphenols and the six-member ring of caffeine. These interaction forces contributed by the galloyl group might account for the fact that these ester-form catechins had a much greater tendency to precipitate with protein and caffeine than did the non-ester-form catechins (Table 2). However, a role for the non-ester-form catechins cannot be totally ruled out in cream formation in the Paochung tea infusion. All phenolics are able to form hydrogen bonds with caffeine and are likely to be involved in complexation and precipitation.

Millin *et al*²¹ showed that 7% of endogenous polysaccharides, especially polygalacturonic acid, took part in cream formation in black tea by forming loosely bound complexes with polyphenolic and proteinaceous materials. Although there is little information concerning the affinity between polyphenols and polysaccharides, the complexation of pectin and other constituents is less important in cream formation in Paochung tea, because its presence was relatively low (2%) in the cream.

The free amino acid contents in the original and clarified infusions were significantly different ($p<0.05$), indicating that free amino acids also participated in cream formation in Paochung tea. The major free amino acids involved in the formation of the tea cream were theanine (39%), followed by glutamic acid (Glu) (10%) and aspartic acid (Asp) (5%) (Table 1). Other free amino acids to contribute to the formation of the tea cream included asparagine (Asn), tyrosine (Tyr), valine (Val), alanine (Ala), glutamine (Gln), proline (Pro) and glycine (Gly). However, Nagalakshimi and Seshadri⁴ did not find a significant amount of theanine in the acid hydrolysate of black tea cream and concluded that free amino acids were not involved in the formation of tea cream. Nevertheless, only approximately 8% of the total free amino acids in the infusion of Paochung tea were lost after the creaming process. Therefore we suspect that free amino acids may simply co-precipitate with insoluble complexes during cream formation.

The mineral contents of Paochung tea before and after clarification are shown in Table 1. Among the six minerals tested, calcium appeared to be the only mineral to be significantly involved ($p<0.05$) in cream formation. About 66% of the calcium in the original

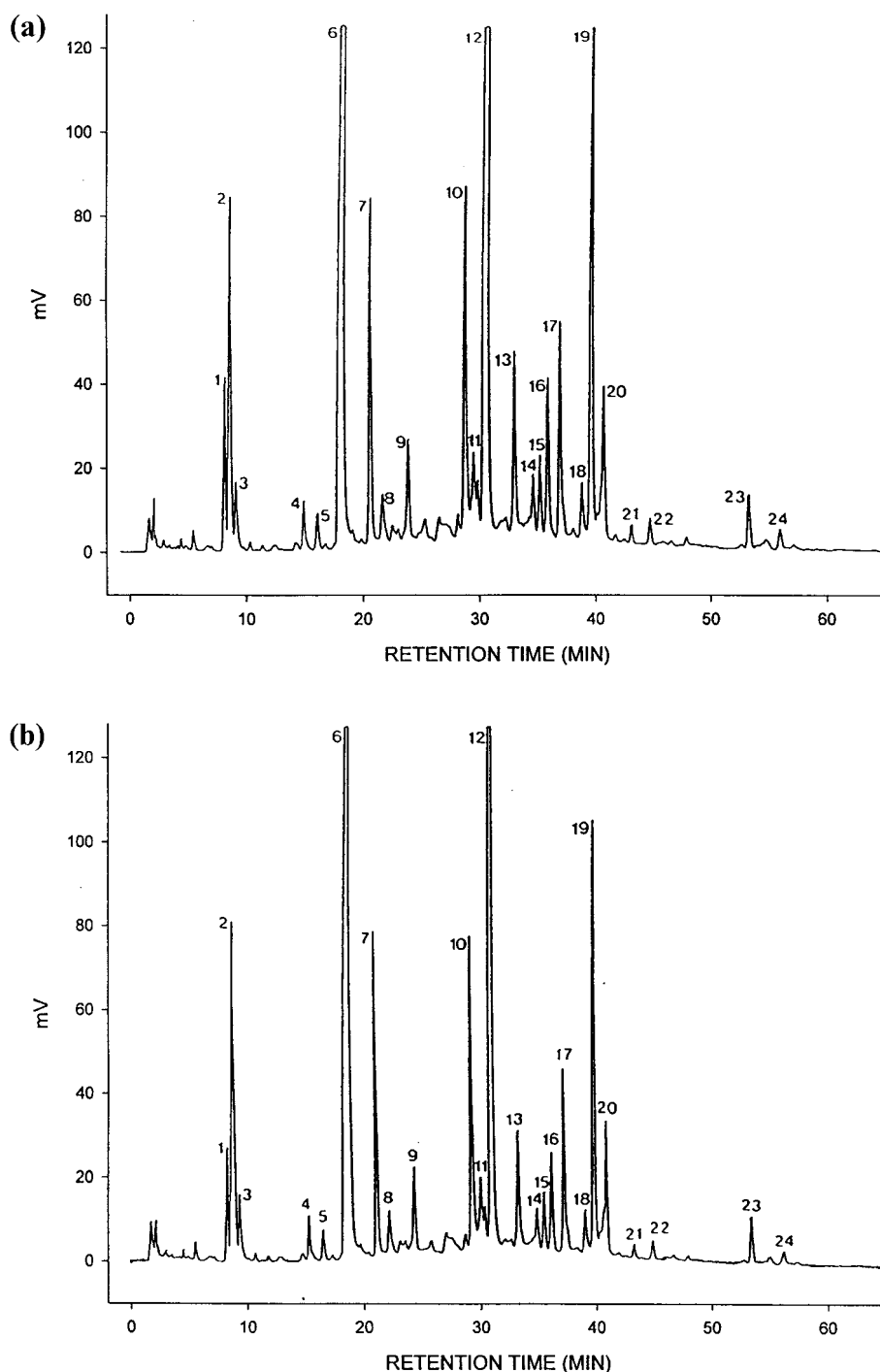


Figure 1. High-performance liquid chromatograms of polyphenols in Paochung tea infusion (a) before and (b) after clarification: peak 2, theobromine; peak 3, gallic acid; peak 6, caffeine; peak 7, (-)-epigallocatechin; peak 8, (+)-catechin; peak 10, (-)-epicatechin; peak 12, (-)-epigallocatechin gallate; peak 18, rutin; peak 19, (-)-epicatechin gallate; peak 24, quercetin. Other peaks are unidentified.

infusion participated in cream formation. Smith¹ found that both potassium and calcium existed in black tea cream. Guo and Cheng²² reported that calcium ions were more easily precipitated with polyphenols than were other cations in tea infusions. Jackson and Lee²³ stated that the insolubility of the calcium-polyphenols complex in tea was dependent upon the degree of enzymic oxidation in the fermentation process. Some research suggested that demineralisation could reduce cream formation in black tea.²⁴ However, we found that the amounts of tea cream in Paochung tea infusions extracted with tap and

deionised water were not significantly different (data not shown).

Effect of tea leaf/water ratio

The effect of the tea leaf/water ratio upon cream formation is shown in Table 3. An increase in the tea leaf/water ratio from 1.25 to 15% raised the total solids, caffeine, catechins, protein and pectin contents in the infusion. Although the amount of tea cream formed also increased with increasing leaf/water ratio, the percentage of each component in the tea cream was essentially the same. No cream formation was

| Peak | Compound | Peak area ^a | | Difference | |
|------|-------------|-------------------------|------------------------|------------|------|
| | | Original | Clarified | Area | % |
| 1 | | 421 737 ^a | 302 223 ^b | 119 514 | 28.3 |
| 2 | Theobromine | 1 024 565 ^a | 1 032 461 ^a | | |
| 3 | Gallic acid | 268 766 ^a | 273 675 ^a | | |
| 4 | | 209 110 ^a | 191 682 ^a | | |
| 5 | | 150 507 ^a | 137 664 ^a | | |
| 6 | Caffeine | 11 958 209 ^a | 8 090 541 ^b | 3 867 668 | 32.3 |
| 7 | (-)-EGC | 1 316 670 ^a | 1 239 087 ^a | | |
| 8 | (+)-C | 327 905 ^a | 312 290 ^a | | |
| 9 | | 609 212 ^a | 541 585 ^a | | |
| 10 | (-)-EC | 1 492 750 ^a | 1 402 111 ^a | | |
| 11 | | 427 212 ^a | 400 249 ^a | | |
| 12 | (-)-EGCG | 10 640 832 ^a | 7 832 818 ^b | 2 808 013 | 26.4 |
| 13 | | 868 662 ^a | 636 434 ^b | 232 228 | 26.7 |
| 14 | | 329 649 ^a | 242 614 ^b | 87 035 | 26.4 |
| 15 | | 383 010 ^a | 268 890 ^b | 114 120 | 29.8 |
| 16 | | 755 976 ^a | 519 101 ^b | 236 876 | 31.3 |
| 17 | | 958 895 ^a | 887 028 ^b | 71 867 | 7.5 |
| 18 | Rutin | 327 111 ^a | 256 750 ^b | 70 361 | 21.5 |
| 19 | (-)-ECG | 2 367 695 ^a | 1 824 376 ^b | 543 320 | 23.0 |
| 20 | | 817 665 ^a | 708 773 ^b | 108 892 | 13.3 |
| 21 | | 101 762 ^a | 60 325 ^b | 41 437 | 40.7 |
| 22 | | 133 167 ^a | 85 078 ^b | 48 089 | 36.1 |
| 23 | | 214 500 ^a | 182 075 ^b | 32 425 | 15.1 |
| 24 | Quercetin | 91 517 ^a | 68 587 ^b | 22 930 | 25.1 |

Table 2. Comparison of peak areas of polyphenols and caffeine in Paochung tea infusion before and after decaffeinating

^a Means ($n = 4$) in the same row superscripted with different letters are significantly different ($p < 0.05$).

Table 3. Effect of tea leaf/water ratio (TW) on total solids, amount of cream formation and concentrations of caffeine, catechins, protein and pectin in Paochung tea infusion and their percentages in tea cream^a

| TW (%) | Total solids (g/100ml) | Tea cream (%) ^b | Caffeine in tea infusion (g/100ml) | Caffeine | | | Catechins | | Catechins | | Pectin in tea infusion (g/100ml) | Pectin in tea cream (%) ^c |
|--------|------------------------|----------------------------|------------------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|------------------|----------------------------------|--------------------------------------|
| | | | | in tea cream (%) ^c | in tea infusion (g/100ml) | in tea cream (%) ^c | in tea infusion (g/100ml) | in tea cream (%) ^c | in tea infusion (g/100ml) | | | |
| 1.25 | 0.47 ^h | NS | — | — | — | — | — | — | — | — | — | — |
| 2 | 0.74 ^g | NS | — | — | — | — | — | — | — | — | — | — |
| 2.5 | 0.88 ^f | 6.8 ^e | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 5 | 1.97 ^e | 9.1 ^c | 0.22 ^e | 25.3 ^a | 0.76 ^e | 31.1 ^a | 0.05 ^e | 19.4 ^a | 0.05 ^e | 2.6 ^a | 2.6 ^a | 2.6 ^a |
| 8 | 3.11 ^d | 14.5 ^b | 0.33 ^d | 18.7 ^a | 1.24 ^d | 25.8 ^a | 0.11 ^d | 17.0 ^a | 0.08 ^d | 2.0 ^a | 2.0 ^a | 2.0 ^a |
| 10 | 3.84 ^c | 14.1 ^b | 0.38 ^c | 15.6 ^a | 1.50 ^c | 30.6 ^a | 0.14 ^c | 16.0 ^a | 0.10 ^c | 1.3 ^a | 1.3 ^a | 1.3 ^a |
| 12.5 | 4.69 ^b | 17.3 ^a | 0.50 ^b | 18.2 ^a | 1.79 ^b | 30.6 ^a | 0.21 ^b | 16.9 ^a | 0.13 ^b | 1.5 ^a | 1.5 ^a | 1.5 ^a |
| 15 | 5.47 ^a | 16.5 ^a | 0.58 ^a | 21.8 ^a | 2.14 ^a | 34.9 ^a | 0.25 ^a | 17.8 ^a | 0.14 ^a | 2.6 ^a | 2.6 ^a | 2.6 ^a |

^a The tea infusions were extracted at 90 °C for 20 min. The mean values ($n = 4$) in each column with the same superscript letters are not significantly different at the 5% level using Duncan's multiple-range test. NS represents no cream formation. ND means the constituents were not determined.

^b Percentage of total solids of tea infusion.

^c Percentage of tea cream by weight.

observed when the leaf/water ratio was 2% or lower, indicating that a minimum concentration was required to produce tea cream.

Effect of extraction temperature

The effect of the extraction temperature upon cream formation in Paochung tea was investigated by extracting the tea with a 12.5% leaf/water ratio at 30, 50, 70 and 90 °C for 20 min. As shown in Table 4, the total solids content and the amount of cream increased with increasing extraction temperature. The composi-

tion of the tea cream was also affected by the extraction temperature. The caffeine content remained constant. Rutter and Stainsby³ also reported that the caffeine content in black tea cream was maintained at 25–27% under various extraction conditions.

The content of catechins in the cream increased with increasing extraction temperature, while the contents of protein and pectin decreased. It was noted that there was a significant amount of tea cream in Paochung tea extracted at temperatures as low as 30 °C, but a black tea infusion extracted at 30 °C did

Table 4. Effect of extraction temperature on total solids, amount of cream formation and concentrations of caffeine, catechins, protein and pectin in Paochung tea infusion and their percentages in tea cream^a

| Extraction temp (°C) | Total solids (g/100ml) | Tea cream (%) ^b | Caffeine | | Catechins | | Protein | | Pectin | |
|----------------------|------------------------|----------------------------|------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|-----------------------------------|---------------------------------------|----------------------------------|--------------------------------------|
| | | | Caffeine in tea infusion (g/100ml) | Caffeine in cream (%) ^c | Catechins in tea infusion (g/100ml) | Catechins in cream (%) ^c | Protein in tea infusion (g/100ml) | Protein in tea cream (%) ^c | Pectin in tea infusion (g/100ml) | Pectin in tea cream (%) ^c |
| 30 | 3.37 ^d | 6.2 ^d | 0.36 ^b | 17.2 ^a | 1.30 ^d | 12.4 ^c | 0.10 ^d | 34.0 ^a | 0.04 ^c | 9.1 ^a |
| 50 | 4.04 ^c | 11.4 ^c | 0.39 ^b | 14.3 ^a | 1.48 ^c | 23.3 ^b | 0.15 ^c | 22.2 ^b | 0.06 ^b | 3.0 ^b |
| 70 | 4.24 ^b | 14.6 ^b | 0.46 ^a | 17.3 ^a | 1.63 ^b | 26.6 ^b | 0.18 ^b | 21.3 ^b | 0.06 ^b | 2.4 ^b |
| 90 | 4.73 ^a | 17.8 ^a | 0.48 ^a | 18.3 ^a | 1.79 ^a | 30.6 ^a | 0.21 ^a | 19.0 ^b | 0.13 ^a | 1.5 ^b |

^a The tea infusions were extracted with a 12.5% tea leaf/water ratio for 20min. The mean values ($n=4$) in each column with the same superscript letters are not significantly different at the 5% level using Duncan's multiple-range test.

^b Percentage of total solids of tea infusion.

^c Percentage of tea cream by weight.

Table 5. Effect of extraction time on total solids, amount of cream formation and concentrations of caffeine, catechins, protein and pectin in Paochung tea infusion and their percentages in tea cream^a

| Extraction time (min) | Total solids (g/100ml) | Tea cream (%) ^b | Caffeine | | Catechins | | Protein | | Pectin | |
|-----------------------|------------------------|----------------------------|------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|-----------------------------------|---------------------------------------|----------------------------------|--------------------------------------|
| | | | Caffeine in tea infusion (g/100ml) | Caffeine in cream (%) ^c | Catechins in tea infusion (g/100ml) | Catechins in cream (%) ^c | Protein in tea infusion (g/100ml) | Protein in tea cream (%) ^c | Pectin in tea infusion (g/100ml) | Pectin in tea cream (%) ^c |
| 5 | 4.30 ^c | 16.5 ^a | 0.48 ^a | 23.1 ^a | 1.80 ^c | 14.0 ^b | 0.18 ^b | 17.3 ^a | 0.07 ^c | 1.9 ^a |
| 10 | 4.40 ^b | 16.8 ^a | 0.44 ^a | 20.0 ^a | 1.91 ^b | 25.3 ^a | 0.20 ^b | 20.2 ^a | 0.09 ^c | 1.6 ^a |
| 20 | 4.82 ^a | 17.4 ^a | 0.48 ^a | 18.3 ^a | 2.08 ^a | 31.2 ^a | 0.24 ^a | 18.8 ^a | 0.14 ^b | 1.5 ^a |
| 60 | 4.88 ^a | 16.4 ^a | 0.47 ^a | 16.0 ^a | 2.07 ^a | 34.2 ^a | 0.25 ^a | 19.7 ^a | 0.20 ^a | 1.8 ^a |

^a The tea infusions were extracted with a 12.5% tea leaf/water ratio at 90°C. The mean values ($n=4$) in each column with the same superscript letters are not significantly different at the 5% level using Duncan's multiple-range test.

^b Percentage of total solids of tea infusion.

^c Percentage of tea cream by weight.

not form cream.³ The difference in creaming behaviour between Paochung tea and black tea was probably due to most of the polyphenols in black tea existing in their oxidised forms, having a higher degree of polymerisation and being unable to be extracted efficiently at low temperatures, whereas most of the polyphenols in Paochung tea have their simple structures unchanged and thus can be extracted at 30°C.

Effect of extraction time

Table 5 shows the effect of the extraction time upon cream formation in Paochung tea at 90°C. The total solids in the infusion increased significantly ($p < 0.05$) with increasing extraction time from 5 to 20 min, but a further increase in extraction time to 60 min did not significantly change the total solids content. An increase in the extraction time from 5 to 20 min increased the catechins and protein contents in the infusion. Increasing the extraction time further did not increase their extractability either. Therefore extraction at 90°C for 20 min was considered to be long enough to extract most of the major components that contribute to cream formation. Since the amount of the tea cream was not affected by the length of extraction time, a reduction in the extraction time even down to 5 min would not reduce cream formation in Paochung tea.

Effect of pH

The pH of the infusion affected the total solids content in the tea infusion, the amount of tea cream formation and the composition of the cream (Table 6). The total solids content had the lowest value at pH 5.5 (the pH value of the original tea infusion) owing to the low extraction rates of catechins and proteins at this pH. At low pHs (3.22 and 4.20) the catechins content in the tea infusion, as well as in the cream, was significantly higher than that at higher pHs (5.50 and 6.86). The protein content too was significantly higher, but only at the lowest pH, seeming to increase again at the highest pH. The polyphenols are believed to bind proteins primarily through the formation of multiple hydrogen bonds between their phenolic groups and the carbonyl functions of the peptide linkages.²⁵ The tea proteins are likely to have pI values close to pH 3.2–4.2, where protein–protein electrostatic repulsion would be minimised and result in maximum cream formation. However, the pH did not affect the caffeine content of the tea infusion or the percentage of caffeine in the cream. Spiro and Price^{26,27} also reported that the pH did not affect the extractability of caffeine in black tea.

Although the amount of cream formed at pH 3.22 and 4.20 was higher than that at pH 5.50 and 6.86, the extent of the difference in cream formation due to the difference in the pH of the Paochung tea infusion was

Table 6. Effects of pH of Paochung tea infusion on total solids, tea cream formation and concentrations of caffeine, catechins, protein and pectin and their percentages in tea cream^a

| pH | Total solids (g/100ml) | Tea cream (%) ^b | Caffeine in tea infusion (g/100ml) | Caffeine in tea cream (%) ^c | Catechins in tea infusion (g/100ml) | Catechins in tea cream (%) ^c | Protein in tea infusion (g/100ml) | Protein in tea cream (%) ^c | Pectin in tea infusion (g/100ml) | Pectin in tea cream (%) ^c |
|------|------------------------|----------------------------|------------------------------------|--|-------------------------------------|---|-----------------------------------|---------------------------------------|----------------------------------|--------------------------------------|
| 3.22 | 4.20 ^a | 16.1 ^a | 0.39 ^a | 22.0 ^a | 1.61 ^a | 36.8 ^a | 0.34 ^a | 33.8 ^a | ND | ND |
| 4.20 | 3.86 ^c | 15.9 ^a | 0.40 ^a | 24.5 ^a | 1.69 ^a | 37.7 ^a | 0.22 ^b | 21.3 ^{bc} | ND | ND |
| 5.50 | 3.79 ^c | 14.3 ^b | 0.40 ^a | 18.5 ^a | 1.51 ^b | 30.0 ^b | 0.14 ^c | 18.5 ^c | ND | ND |
| 6.86 | 3.98 ^b | 12.9 ^c | 0.41 ^a | 19.6 ^a | 1.42 ^c | 30.0 ^b | 0.20 ^b | 27.5 ^b | ND | ND |

^a The tea infusions were extracted with a 10% tea leaf/water ratio at 90°C for 20min. Pectin was not determined (ND) in this experiment. The mean values ($n=4$) in each column with the same superscript letters are not significantly different at the 5% level using Duncan's multiple-range test.

^b Percentage of total solids of tea infusion.

^c Percentage of tea cream by weight.

much less than that in black tea. The maximum amount of tea cream in black tea was formed at pH 3–4,¹ while no cream formation was observed at pH 6.7. It was also noted that raising the pH of the infusion significantly altered the colour and flavour of Paochung tea (data not shown). Suematsu *et al.*²⁸ stated that catechins were more unstable and became subject to greater oxidation in the high pH range during processing. Therefore adjusting the pH of the infusion may not be a feasible process for inhibiting the formation of cream in Paochung tea.

CONCLUSION

The formation of tea cream is still a significant problem in the application of Paochung tea as a cool drink, primarily because it causes an unattractive appearance and processing difficulties. The amount of cream in the tea and its composition were influenced by extraction temperature and pH. The tea leaf/water ratio was found to affect the amount of cream in the infusion but not the cream composition. The polyphenolic compounds are probably the most important components in the formation of tea cream. They form a complex with caffeine and protein to create cream. Reducing the extraction temperature and adjusting the pH reduces cream formation, but inhibition of the development of Paochung tea cream without impairing the important sensory qualities or food safety remains a challenge in the processing of this tea.

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