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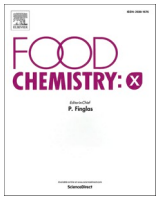

Biochemical insights into tea foam: A comparative study across six categories

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ABSTRACT

Tea foam properties, crucial indicators of tea quality, have gained renewed interest due to their potential applications in innovative beverages and foods. This study investigated the foaming properties and chemical foundations of six major tea categories through morphological observations and biochemical analyses. White tea exhibited the highest foaming ability at 56.28%, while black tea showed the best foam stability at 84.01%. Conversely, green tea had the lowest foaming ability (10.83%) and foam stability (54.24%). These superior foaming characteristics are attributed to the relatively low lipid content and acidic pH values. Surprisingly, no significant correlation was found between tea saponin content and foaming properties. Instead, specific amino acids (including Tyr, Gaba, Phe, Ile, and Leu) and catechins (GA and CG) were identified as potential contributors. These results deepen our understanding of tea foam formation and offer insights into utilizing tea-derived plant-based foams in food products.

1. Introduction

Foams are crucial components of diverse beverage products, crucially influencing their sensory quality (Pérez-Magariño et al., 2015). Previous research has delved into understanding the foaming properties and major constituents, particularly in beverages such as wine and coffee (Cilindre, Liger-Belair, Villaume, Jeandet, & Marchal, 2010; Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015; Piazza, Gigli, & Bulbarello, 2008). However, comprehending the foam system remains challenging due to the inherent instability driven by surface tension at the interface of air bubbles and the continuous medium (Amagliani, Silva, Saffon, & Dombrowski, 2021). Several factors, including surfactant concentration (Xiong, Ho, Bhandari, & Bansal, 2020; Osorio et al., 2014), temperature (Lajnaf, Zouari, Trigui, Attia, & Ayadi, 2020), pH level (Van de Vondel, Janssen, Wouters, & Delcour, 2023), and ionic strength (Yang et al., 2024), have been identified as significant influencers of both foam formation and stability. Furthermore, there has been increasing exploration into plant-based foams derived from sources like soy, beans, and sesame, driven by health, ethical, and religious considerations. These alternatives are gaining prominence as viable substitutes for animal-based foams such as cow milk foam in coffee, egg white foam in cake, and cream in beverages (Cano-Medina et al., 2011; Ruíz-Henestrosa et al., 2007; Shao et al., 2024; Yang et al., 2023; Zakidou & Paraskevopoulou, 2021).

Tea is a typical plant-derived beverage with foaming properties. The ancient tea treatise "The Classic of Tea" revered tea foam as a hallmark of its quality, emphasizing its texture, whiteness, and longevity (Lu, 2011). Even today, tea enthusiasts engage in ceremonial practices involving the meticulous stirring of tea soup to generate foam, underscoring the enduring fascination with tea's sensory attributes (Yao, 2019). Despite centuries of observation and appreciation, the scientific understanding of tea foam remains elusive. Early studies suggested that tea saponin, a natural surfactant, contributes significantly to foaming and emulsifying properties in tea leaves (Huang et al., 2024; He et al., 2023). Other research has focused on proteins for their surface-active properties during the foaming process (Vanrell et al., 2007; Xiong et al., 2020). Moreover, the complex biochemical composition of tea infusion, including amino acids, caffeine, and tea polyphenols, may also influence foaming characteristics (Liang, Fu, Yin, & Xu, 2022; Ferrari, Ravera, De Angelis, Liverani, & Navarini, 2010).

Tea is classified to six categories based on the degree of polyphenol oxidation and distinct processing procedures, ranging from lowest to highest oxidation: green tea, yellow tea, white tea, black tea, oolong tea, and dark tea (Li, Zhang, Wan, Zhan, & Ho, 2022). Each tea category harbors unique intrinsic components. However, the foaming properties across these categories have not been comprehensively evaluated, nor

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have the key biochemical factors influencing tea foam properties been thoroughly investigated. This study addresses these gaps by exploring the foaming properties of the six distinct categories of tea and examining their underlying biochemical foundations. By analyzing both macroscopical and microcosmic morphologies and quantifying key biochemical constituents, we aim to elucidate the mechanisms governing foam formation across diverse tea categories.

2. Materials and methods

2.1. Materials

Six different categories of Chinese representative tea (Matcha green tea, Huoshan yellow tea, Bai Mudan white tea, Wuyi oolong tea, Congou black tea, and Puer dark tea) were obtained from Jiangxi Tongzhou Green Food Development Co., Ltd. and ground to powders (diameter<0.45 mm) in June 2023.

2.2. Measurement of foam morphology

A sample of (1.0 ± 0.05)g ground powder from each type of tea was brewed with 100 mL of 100 °C ultrapure water for 15 min. The mixture was then stirred by a milk frother (Mongdio, China) at the speed of 8500 r·min^{-1} for 1 min. Then, photos from both the front and upper sides were taken, and the height of the foams was measured at 0 h. 0.5 h, 1 h, 2 h, 4 h, and 6 h directly in the sample. The volume of the foam was calculated by the formula volume = surface area × height. Foaming ability (FA) was calculated using eq. FA = initial foam volume/100 × 100%; Foam stability (FS) was calculated using eq. FS = foam volume at 0.5 h/ initial foam volume×100%.

The RGB and the color description of each type of tea foam were determined at 0 h after foaming according to Robert Ridgway's Color Standards and Color Nomenclature (Christensen, Miller, & Tuthill, 1994) by the tool in https://colornamer.robertcooper.me/.

For the measurement of the foam morphology data and microscope pictures of white tea and black tea foam, (0.6 ± 0.05)g ground tea powder was brewed with 60 mL 100 °C ultrapure water for 15 min and stirred at the speed of 5000 r/min for 2.5 min by a DAF100 dynamic foam analyzer (Kruss, German) and the dynamic change of the foam morphology was kept recording for 0.5 h.

2.3. Separation of tea foam and tea soup of white tea and black tea

A sample of (1.0 ± 0.05)g ground powder of each type of tea was brewed with 100 mL 100 °C ultrapure water for 15 min and stirred by a milk frother (Mongdio, China) at the speed of 8500 r·min^{-1} for 1 min. The foam and soup were separated and centrifuged to defoam and filter the tea powder. The supernatants were preserved for further experiments.

2.4. Measurement of pH values

A sample of 1.0 g ground powder of each type of tea was brewed with 100 mL 100 °C ultrapure water for 15 min and then centrifuged for 10 min (3500 r/min). After cooling down to room temperature, the pH values of the supernatant liquid were measured by a PHS-2F pH meter (Inesa, China).

2.5. Determination of soluble sugar, soluble protein, and free amino acids

A sample of 0.3 g tea powder was extracted with 45 mL boiling water for 45 min in a 100 °C water bath. After centrifuging, the supernatant was diluted to 50 mL and the sample solution was obtained (Li et al., 2024; Li et al., 2024).

The soluble sugar content was determined by the method of anthrone-sulfuric acid colorimetric (Wang et al., 2022), 8 mL of 2 mg/mL anthrone-sulfuric acid solution was added to 1 mL sample solution in an ice bath, and the mixture was immediately shaken and boiled in a 100 °C water bath for 7 min. Subsequently, it was promptly cooled in an ice bath for 10 min, and the soluble sugar content was calculated based on the standard curve under 620 nm.

The soluble protein was characterized by the Coomassie Brilliant Blue method, 0.1 mL of the sample solution was mixed with 3 mL of Coomassie Brilliant Blue solution. After 6 min, the absorbance was measured at 595 nm with the blank serving as a control, and the protein content was calculated based on a standard curve made by bovine serum albumin.

The free amino acids were determined by the ninhydrin colorimetry method from the national standard GB/T 8314–2013, 1 mL of the sample solution was added into a 25 mL flask, and 0.5 mL of pH 8.04 phosphate buffer and 0.5 mL of 2% ninhydrin solution were added. The mixture was heated in a boiling water bath for 15 min and after cooling, it was diluted to 25 mL. After 10 min, the absorbance was measured at 570 nm. The amino acid content of the sample solution was determined based on the standard curve.

2.6. Determination of water extract, tea polyphenols, catechins, caffeine, and free amino acids

The water extract content was determined using the full amount method at 120 °C ± 2 °C according to the national standard GB/T 8305–2013. Tea polyphenols were determined by the Folin-Ciocalteu method (the national standard GB/T8314–2013).

For catechins and caffeine determination, the sample solution was filtered through a 0.22 μm membrane and then subjected to chromatographic analysis under the following conditions: a C18 chromatographic column was used with mobile phase A consisting of 30 mL acetonitrile (HPLC grade, Astoon, USA), 5 mL acetic acid (analytical reagent, Hushi, China), and 965 mL water, filtered through a water membrane, while mobile phase B comprised 300 mL acetonitrile, 5 mL acetic acid, and 695 mL water, filtered through an organic membrane. Gradient elution was performed, with phase B transitioning from 20% to 80% over 40 min. The flow rate was set at 1.0 mL/min, detection occurred at a wavelength of 280 nm, and the temperature was maintained at 25 °C. Each sample injection volume was 10 μL.

For free amino acids, a 10 μL sample solution was combined with 1 mL boric acid solution (0.4 mol/L, pH 10.2), 790 μL distilled water, and a 200 μL derivatization buffer consisting of 0.08 g O-phthalaldehyde, 1 mL acetonitrile solution, 125 μL 3-mercaptopropionic acid (99%, Ourchem, China), and 7 mL boric acid solution. After filtration through a 0.22 μm membrane, the resulting mixture was subjected to detection. A C18 chromatographic column was used, and mobile phase A (40 mM/L Na_2HPO_4, pH 7.8) and mobile phase B with 45% methanol (HPLC grade, Aladdin, China) and 45% acetonitrile (HPLC grade, Astoon, USA) were prepared. The linear gradient elution was performed: from 0 to 18 min, transitioning from 95% to 20% A; from 18 to 23 min, from 20% to 0% A; and from 23 to 30 min, from 0% to 95% A (Cheng, Wu, Liu, Wang, & Xu, 2022).

2.7. Determination of soluble pectin

A sample of 1.0 g ground tea powder was mixed with 25 mL of 95% ethanol (analytical reagent, Hushi, China) and heated in a water bath at 90 °C for 30 min, then cooled to room temperature. Afterward, it was centrifuged at 3000 rpm for 5 min, and the supernatant was discarded. The precipitate was then re-extracted three times and dried in a 60 °C oven for 3 h to obtain the alcohol-insoluble fraction. 100 mg of alcohol-insoluble fraction was taken and added to 15 mL of distilled water in a 50 °C water bath for 30 min to dissolve the pectin. After cooling to room temperature, it was centrifuged at 3000 rpm for 5 min, and the supernatant was collected. The precipitate was re-extracted two times, and the combined extracts constituted the water-soluble pectin extract.

Following the method of Blumenkrantz and Asboe-Hansen (1973), 1 mL of the extract was mixed with 6 mL of 0.48% sodium tetraborate-sulfuric acid solution, boiled for 10 min, and cooled to room temperature. Then, 100 μL of 0.15% meta-hydroxy diphenyl solution was added. For the control sample, 100 μL of 0.5% NaOH solution was added. The mixture was immediately stirred and left at room temperature for 40 min. The absorbance was measured at a wavelength of 530 nm. D-galacturonic acid was used to construct the standard curve for calculating the soluble pectin content.

2.8. Determination of tea saponins

A sample of 1.0 g ground tea sample powder was mixed with 20 mL of 80% ethanol and subjected to reflux extraction at 80 °C for 2 h in a water bath. The extract was filtered immediately and the residue was washed with 80% ethanol. The combined filtrate was then adjusted to 50 mL with 80% ethanol. 2 mL of the supernatant was diluted to 10 mL with methanol, and thoroughly mixed and filtered through a 0.22 μm organic membrane. The HPLC conditions were as follows: mobile phase consisting of methanol-water (*v*/v = 9:1), isocratic elution, flow rate of 0.5 mL/min, detection wavelength at 210 nm, injection volume of 10 μL, and column temperature maintained at 25 °C (Nie, Huang, & Yang, 2022).

2.9. Determination of lipid contents

A sample of 0.300 g of ground tea sample was mixed with 4 mL of water and 5 mL of HCl (analytical reagent, Hushi, China) in a test tube, followed by digestion in a 70 to 80 °C water bath with stirring by a glass rod every 5–10 min until complete. After digestion, 5 mL of ethanol (analytical reagent, Hushi, China) was added, and the mixture was transferred to a 100 mL mixing cylinder with stopper. It was washed multiple times with a total of 15 mL of anhydrous ether (analytical reagent, Hushi, China), and the washings were combined. After shaking for 1 min and settling for 20 min, the upper supernatant was transferred to a pre-weighed flask. An additional 5 mL of anhydrous ether was added to the cylinder and the procedure was repeated. Finally, the ether in the flask was evaporated using rotary evaporation, and the flask was dried at 100 °C for 1 h, then cooled and reweighed to determine the lipid content.

2.10. Statistical analysis

Tbtools (Chen et al., 2020) was applied in the heat map and clustering analysis. Correlation analysis and its visualization were performed by the psych package in the Spearman method and pheatmap package in R studio. SPSS (26.0) was used for the independent-sample *t*-test and one-way analysis of variance (ANOVA) with Tukey's method. Graphs were generated using Excel and GraphPad Prism 6.0. A 95% confidence interval ($P < 0.05$) was considered statistically significant.

3. Results and discussion

3.1. Macroscopical morphology of six categories of tea foam

As shown in Figs. 1A~F and Table S1, different categories of tea had distinctive macroscopical morphology with different RGBs. Green tea foam exhibited a color of light grape green with RGB (172,167,112), characterized by an uneven distribution of foam sizes, showing fine and dense bubbles in the center accompanied by larger bubbles around the

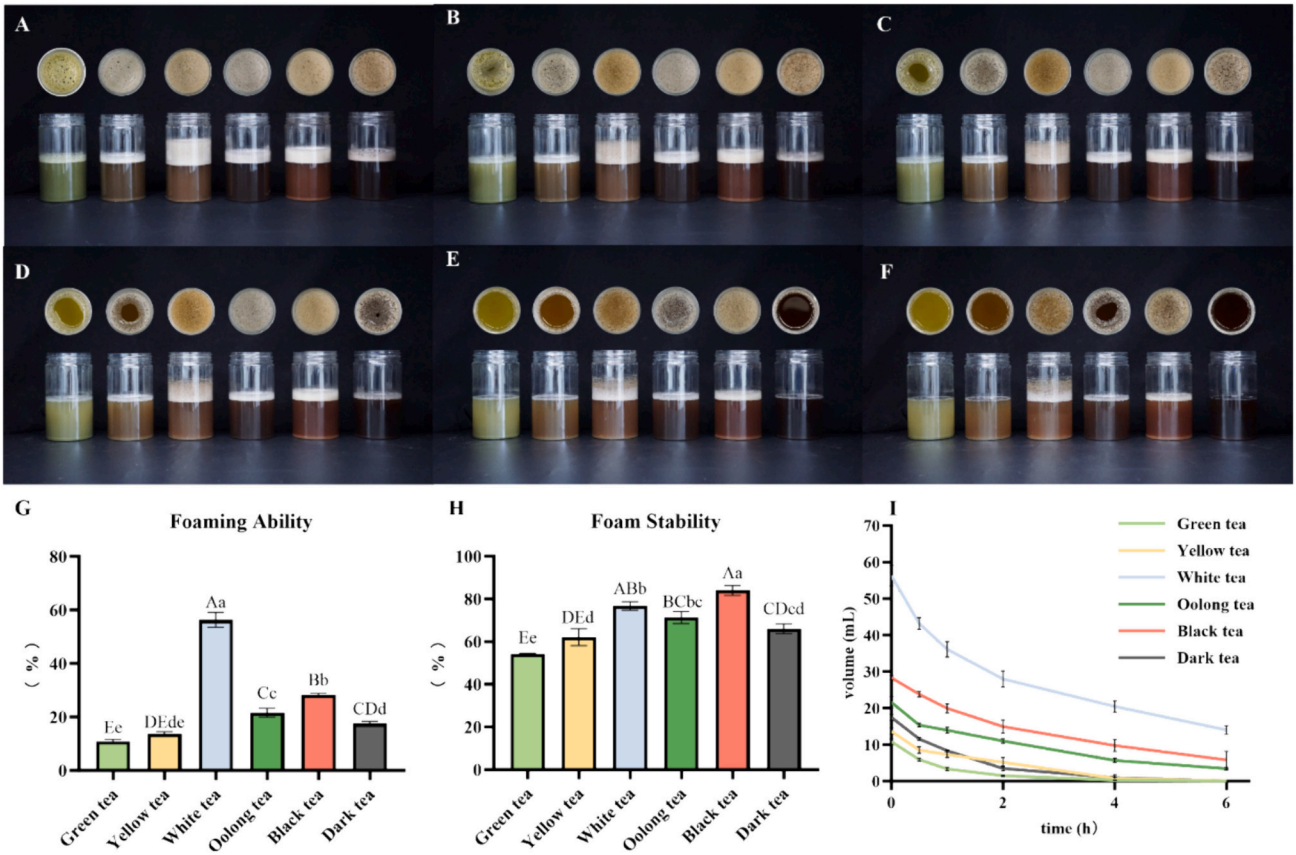


Fig. 1. The foam morphology of six categories of tea after foaming at 0 h, 0.5 h, 1 h, 2 h, 4 h, and 6 h (A ~ F). Foaming ability of six categories of tea (G). Foam stability of six categories of tea (H). Changes in foam volume within 6 h after foaming (I). Different uppercase and lowercase letters represent significant differences at $P < 0.01$ and $P < 0.05$, respectively.

periphery, with a thin foam layer. Yellow tea foam displayed a color of light olive-gray with RGB (170,164,149), demonstrating a relatively uniform distribution of foam sizes with a thin foam layer. White tea foam appeared cinnamon-drab with RGB (164,148,119), exhibiting a fine sponge-like structure with an even distribution of foam sizes and a thicker foam layer. Oolong tea foam showed a color of pale quaker drab with RGB (158,152,141), featuring a finer texture with a relatively uniform distribution of sizes and a slightly thicker foam layer. Black tea foam presented a color light drab with RGB (167,150,120), showing a fine texture with a uniform distribution of sizes and a small amount of larger bubbles in the center, with a thicker foam layer. Dark tea foam displayed a buffy olive color with RGB (148,126,95), exhibiting a

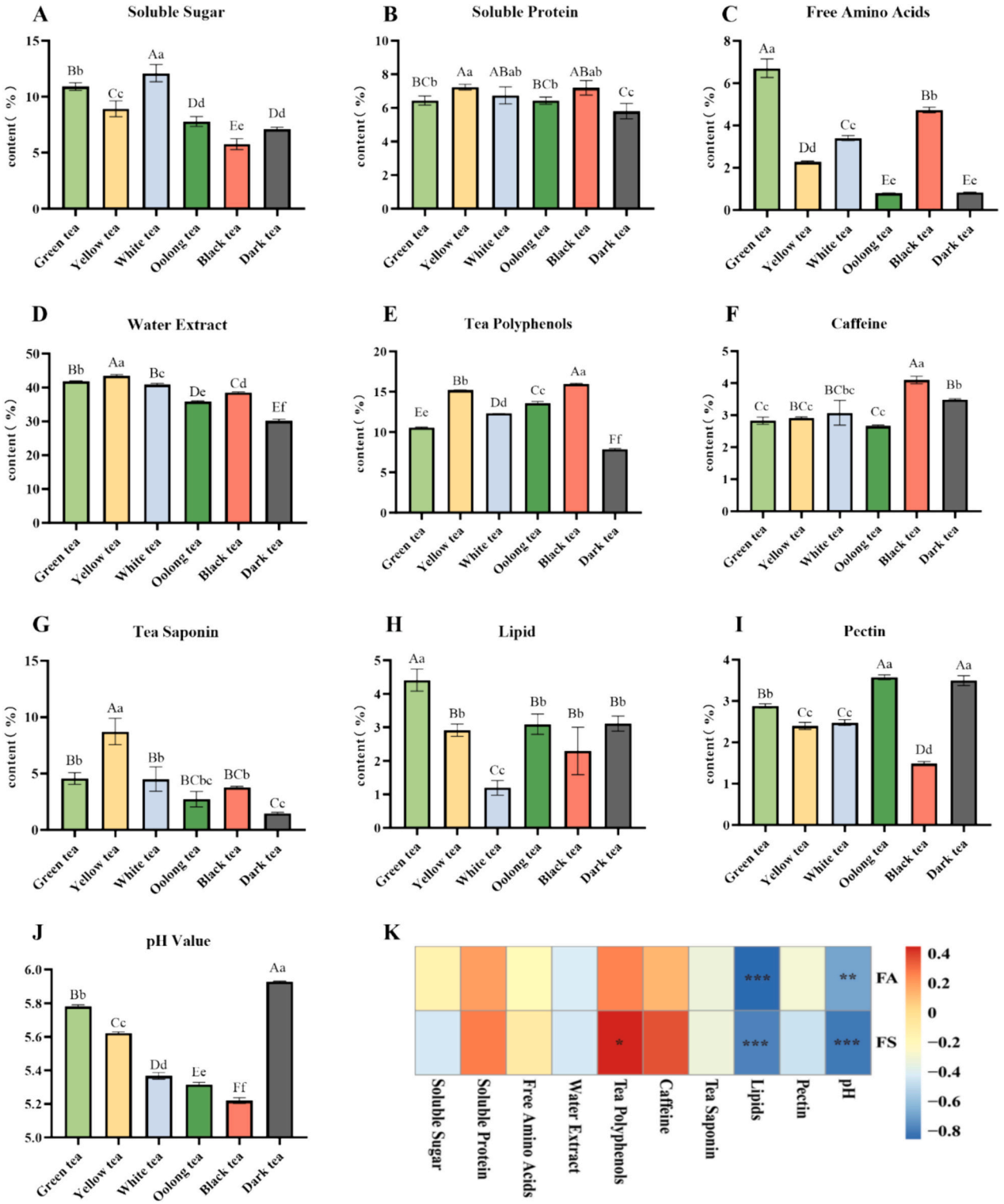


Fig. 2. Main biochemical substances content of six categories of tea (A ~ I). pH value of six categories of tea infusion (J). Correlation analysis map of main biochemical substances content and pH with FA and FS among six categories of tea (K). Different uppercase and lowercase letters represent significant differences at $P < 0.01$ and $P < 0.05$, respectively. An asterisk (*) and three asterisks (***) represent significant differences at $P < 0.05$ and $P < 0.001$, respectively.

slightly coarser texture with larger bubbles around the periphery and a thin foam layer.

The foaming abilities (FA) were observed in the order of white tea > black tea > oolong tea > yellow tea > dark tea > green tea (Fig. 1G). White tea exhibited the highest FA of 56.28%, which was 5.20 times higher than that of green tea (10.83%), and it showed significant differences with the other categories of tea ($P < 0.01$). Black tea followed with an FA of 28.32%, which was 2.61 times of green tea.

Fig. 1H revealed that after 30 min of foaming, green tea foam showed the lowest foam stability (FS) of 54.24%, and the tea soup appeared. In contrast, black tea had the highest FS of 84.01%, with significant differences from the other categories of tea ($P < 0.05$). The FS of white tea was 76.77%, followed by oolong tea (71.29%).

As shown in Fig. 1I, green tea, yellow tea, and dark tea underwent a rapid reduction in volume, decreasing to <40% of their initial volume after 2 h, and further dwindling to under 1 mL after 4 h. Oolong tea exhibited an inflection point at 0.5 h, after which the rate of volume reduction remained relatively low and stable. Although the initial foam volume of dark tea was higher than that of yellow tea, it experienced a rapid decline in volume after 2 h of foaming (3.47 mL), whereas yellow tea maintained a volume of 5.17 mL. Within 6 h, the foam volume followed the order: white tea > black tea > oolong tea, with white tea initially exhibiting the largest volume (56.28 mL). However, as time progressed, the difference in foam volume between white tea and black tea gradually diminished.

3.2. Main biochemical substances content and pH of six categories of tea

The content of nine biochemical substances and the pH value of solutions from six categories of tea were identified (Fig. 2). White tea exhibited the highest soluble sugar content at 12.10%, while black tea had the lowest at 5.76%, with significant differences ($P < 0.01$) among most tea samples. Yellow tea had the highest protein content at 7.23%, followed closely by black tea (7.20%), whereas dark tea had the lowest at 5.80%. Green tea had the highest free amino acid content (6.71%), significantly surpassing oolong tea and dark tea (8.4 and 8.07 times higher, respectively). Water extract content generally decreased with the degree of oxidation, with yellow tea having the highest content at 43.49%, and dark tea the lowest at 30.17%. Tea polyphenol content varied significantly among all categories ($P < 0.01$), with dark tea having the lowest content at 7.88%. Caffeine content ranged from 2.66% in oolong tea to 4.10% in black tea. Yellow tea had the highest tea

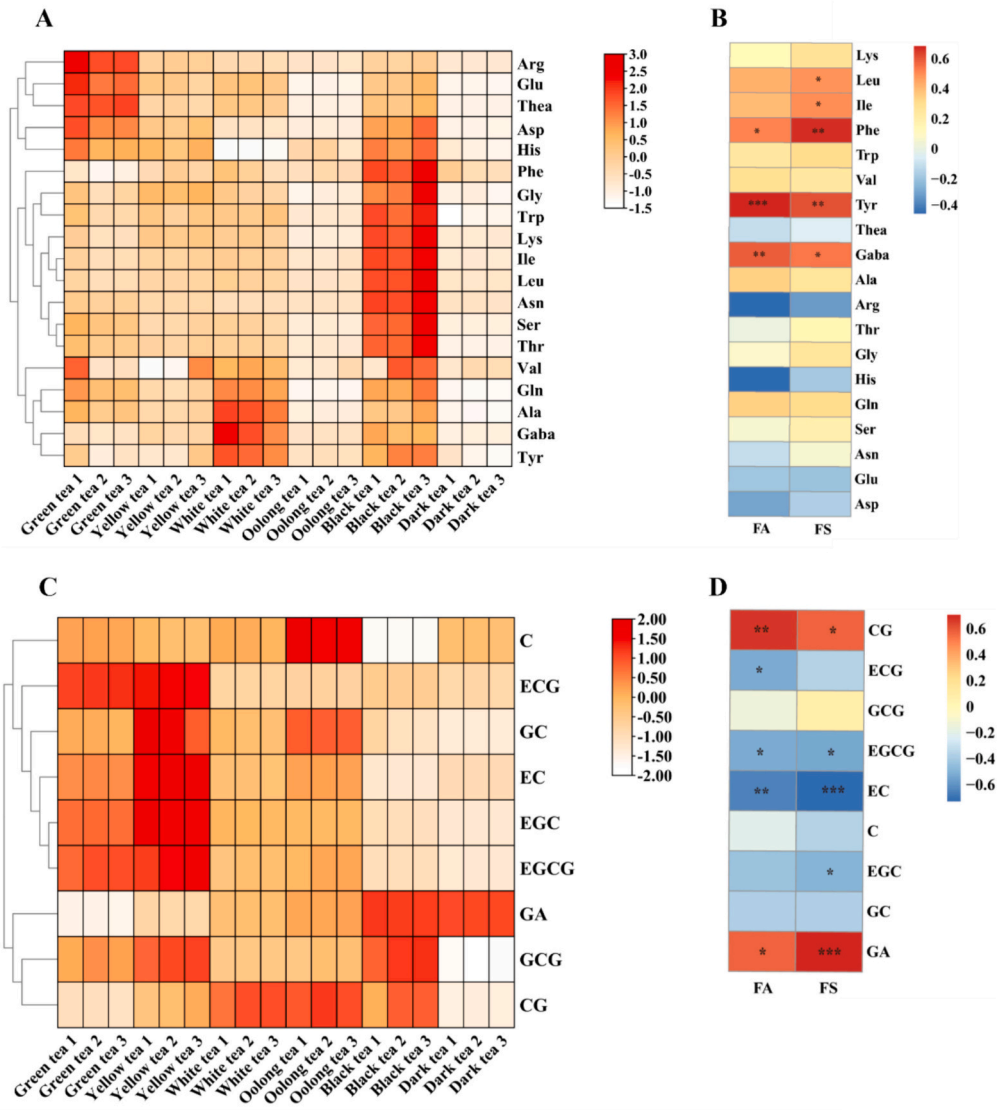


Fig. 3. Clustering heat map of amino acid components among six categories of tea (A). Correlation analysis map of amino acids content with FA and FS (B). Clustering heat map of catechin components among six categories of tea (C). Correlation analysis map of catechins content with FA and FS among six categories of tea (D). An asterisk (*), two asterisks (**), and three asterisks (***) represent significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

saponin content at 8.73%, while dark tea had the lowest at 1.46%. Lipid content ranged from 1.20% in white tea to 4.41% in green tea. The average content of soluble pectin was 2.71%, with oolong tea having the highest content at 3.58%. All tea categories exhibited weak acidity. The pH value decreased significantly with increasing oxidation degree, except for dark tea, which had the highest pH at 5.93, followed by green tea (5.78) and yellow tea.

Correlation analysis revealed significant negative correlations ($P < 0.01$) between pH values and lipid content with both foaming ability (FA) and foam stability (FS). Tea polyphenols exhibited a significant positive correlation with FS. Although not statistically significant, soluble protein and caffeine showed relatively strong positive correlations with both FA and FS compared to other components.

The hierarchical clustering heat map of amino acid components in six categories (Fig. 3A) revealed three major clusters. The first cluster comprised Arg, Glu, Thea, Asp, and His, predominantly enriched in green tea. The second cluster included Phe, Gly, Trp, Lys, Ile, Leu, Asn, Ser, and Thr, mainly enriched in black tea. The third cluster consisted of Val, Gln, Ala, Gaba, and Tyr, primarily enriched in white tea. Table S2 showed that Asn, Ser, Gly, Thr, Trp, Phe, Ile, Leu, and Lys in black tea were significantly higher than in the other tea categories ($P < 0.01$). Correlation analysis results of FA and FS with amino acids (Fig. 3B) indicated significant positive correlations of Leu and Ile from the second cluster with FS ($P < 0.05$). Phe had a significant positive correlation with both FA ($P < 0.05$) and FS ($P < 0.01$). Tyr and Gaba from the third cluster significantly positively correlated to both FS ($P < 0.01$ and $P < 0.05$, respectively) and FA ($P < 0.001$ and $P < 0.01$, respectively).

Fig. 3C revealed that catechin components among the six major categories of tea clustered into two groups. C, ECG, GC, EC, EGC, and EGCG formed one group, predominantly found in green and yellow tea; while GA, GCG, and CG formed another group, concentrated in black tea, white tea, and oolong tea. The content of GA and GCG in black tea was significantly higher than in other categories of tea ($P < 0.01$), as shown in Table S3. Yellow tea exhibited significantly higher levels of EGC, EC, EGCG, and ECG compared to other categories ($P < 0.01$). Correlation analysis between FA and FS with catechins (Fig. 3D) indicated a significant positive correlation of CG with FA and FC. Conversely, EGCG and EC showed significant negative correlations with FA and FC, while ECG exhibited a significant negative correlation with FA, and EGC demonstrated a significant negative correlation with FS.

3.3. Microcosmic morphology and key biochemicals distribution in tea soup and tea foam of white and black tea

To better understand the microcosmic morphology of tea foam and the distribution of key biochemicals in tea soup and tea foam, we further characterized and compared the foam properties of white tea and black tea, with the former exhibiting the highest FA, and the latter demonstrating the highest FS with relatively fine foam.

Results of foam structural parameters (Figs. 4A~G) revealed that the maximum foam volume of white tea was 143.3 mm^{-2}, which is 2.7 times greater than that of black tea (52.9 mm^{-2}), with volume expansion ratios of 4.4 and 1.6, respectively. After the foaming process, white tea and black tea showed similar average bubble area and quantity initially. However, after 30 min, the average bubble area of white tea reached 157,842 um^2 compared to 18,210 um^2 for black tea, resulting in only 6.34 bubbles per square millimeter for white tea and 54.92 bubbles for black tea, indicating an 8.7-fold difference. The half-life of white tea foam was 532.62 s, while that of black tea was 1741.50 s.

Additionally, as Figs. 4H~I showed, after 3 min, both white tea and black tea exhibited dramatic decreases in foam quantity, with white tea showing an exponential decline within 3 to 15 min, indicating a rapid reduction. Moreover, the slope of the average bubble area of white tea increase rate was initially higher compared to black tea.

Fig. 4J~O demonstrated that within the same field of view between 600 s and 2000 s, the number of white tea bubbles increased with varying sizes, including large, medium, and small bubbles, while black tea showed relatively universal small bubbles.

Overall, these results suggested that white tea exhibited a larger initial foaming capacity but underwent rapid depletion, leading to unstable foam conditions, whereas black tea produces finer and more enduring foam, indicating greater stability in foam properties.

As shown in Fig. 5, the soluble protein content exhibited no significant variations between the tea soup and tea foam in both white tea and black tea. However, the free amino acid content in black tea foam (0.51 mg/mL) was significantly higher than that in the tea soup ($P < 0.05$). Additionally, the content of free amino acids in white tea foam was found to be higher than that in tea soup. Cluster heatmap analysis revealed that in both black tea and white tea, the content of Val in tea foam was higher compared to that in tea soup.

The content of tea polyphenols varied between the tea soup and tea foam of white tea and black tea. In white tea, the tea soup exhibited a significantly higher content of tea polyphenols than the foam, whereas in black tea, the foam showed a higher tea polyphenol content than the soup. Similarly, the total catechins content followed a similar trend as that of tea polyphenols, although no significant differences were observed. The cluster heatmap analysis indicated significant differences in the distribution of catechins between the tea soup and foam in both white tea and black tea. Specifically, in white tea, the content of EGCG in the tea soup was significantly higher than that in the foam, while in black tea, the content of GCG and CG in the foam was significantly higher than that in the tea soup.

4. Discussion

4.1. The foam morphology varied in six categories of tea

The morphology of tea foam has traditionally served as an indicator of tea quality. Furthermore, in the context of tea ceremonies, the characteristics of tea foam, including its color, foaming ability, foam stability, and texture, are particularly emphasized. Our study revealed that the morphology of tea foam varies across the six major categories of tea. Notably, oolong tea exhibited a darker color in the tea soup, while its foam appeared lighter and whiter. Similarly, yellow tea foam shared a resemblance with oolong tea foam. Black tea and white tea produced foam with a slightly yellowish hue, while green tea foam was greener. In contrast, dark tea generated brownish foam.

In terms of foaming properties, white tea and black tea exhibited higher foaming abilities, with white tea having the best foaming ability (FA) and black tea having the best foam stability (FS). Oolong tea also demonstrated good foaming properties, while green tea, yellow tea, and dark tea showed relatively poor foaming abilities. Withering is a continuous water-loss process in the first stage after postharvest of white tea, black tea, and oolong tea (Wang et al., 2019; Jiang et al., 2019), involveing the prolonged spreading of freshly harvested leaves. During withering, macromolecules hydrolyzed into smaller molecules, proteins underwent hydrolysis, and free amino acids increased (Zhou et al., 2020). It is speculated that the longer withering time formed the material basis for good foam formation.

After foaming for 2 h, the volume of white tea foam decreased to <50% of its original volume, and the foam size rapidly increased within the first 500 s after foaming. In contrast, the foam of black tea could maintain a relatively delicate state for a longer period. Therefore, in practical applications, white tea foam would be preferred for achieving a rapid foam volume increase, while black tea would be chosen for its finer and more enduring foam.

Our results indicate that tea foam, particularly from white tea and black tea, could serve as a potential plant-based alternative for foam-inclusive foods. The interest in plant-based foams is growing due to lifestyle preferences, ethical considerations, and religious restrictions (Zakidou & Paraskevopoulou, 2021). Lu, Lee, and Yang (2023) developed plant-based liquid egg analogues using ingredients including soy

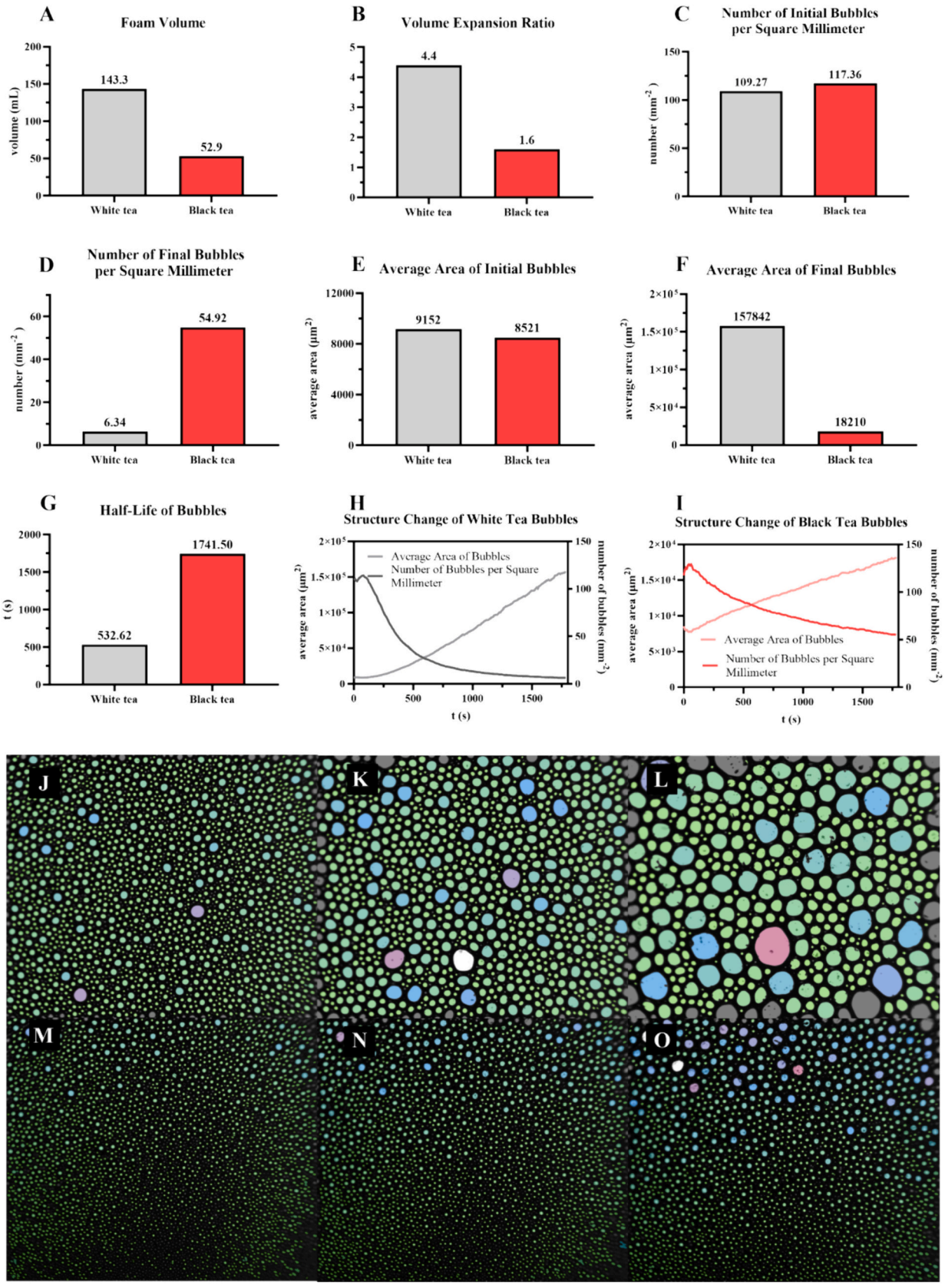
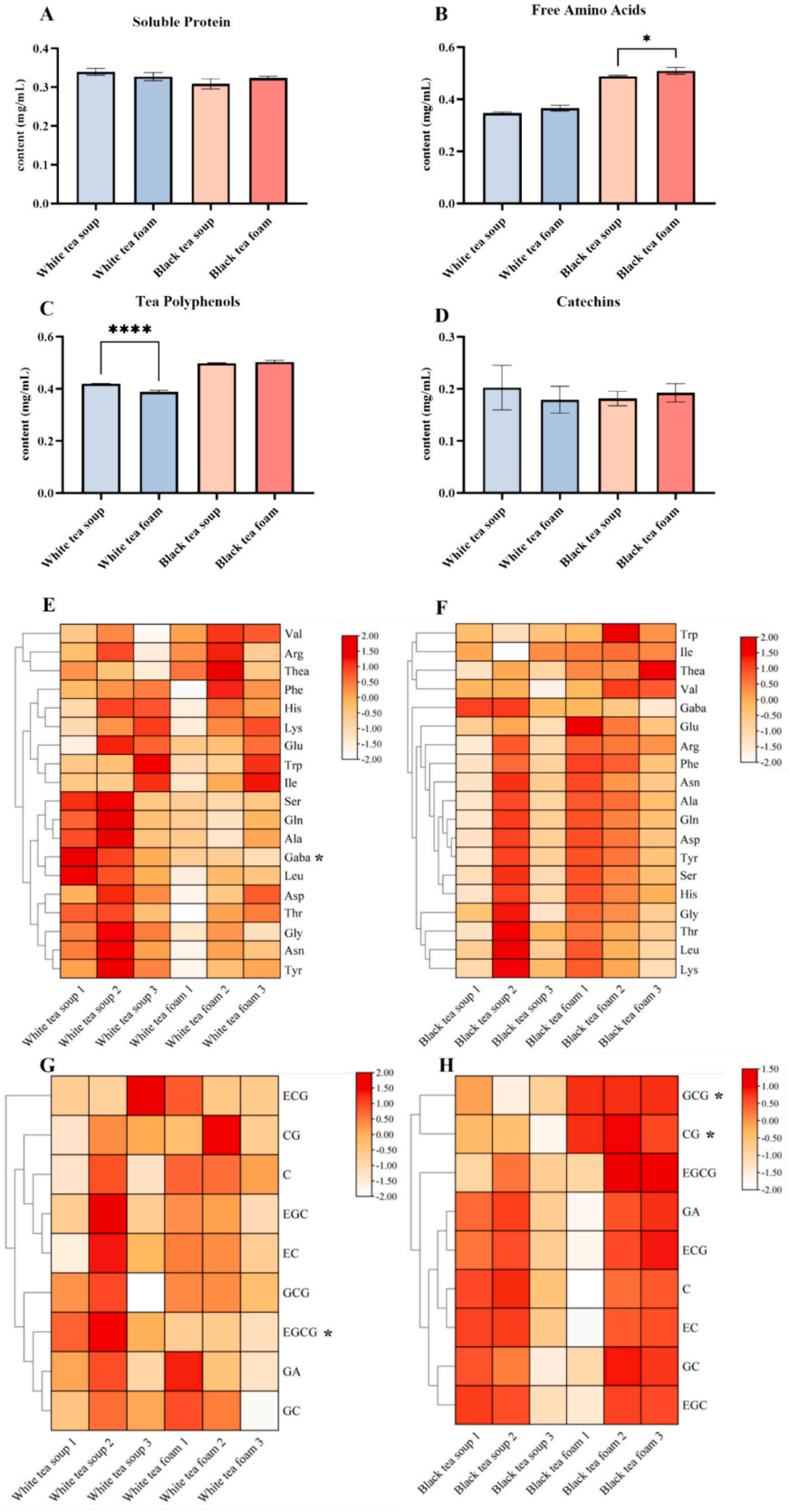


Fig. 4. The foaming properties of white tea and black tea. The initial foam volume (A). The ratio of foam volume to liquid volume at the max foam volume (B). Number of initial bubbles per square millimeter (C). Number of final bubbles per square millimeter 30 min after foaming (D). The average area of initial bubbles (E). The average area of final bubbles is 30 min after foaming (F). The time consumed for a 50% reduction in bubble count (G). Changes in the average area of bubbles and the number of bubbles per square millimeter of white tea and black tea within 30 min (H ~ I). Microcosmic morphology of white tea foam at 600 s, 1000 s, and 2000 s (J ~ L). Microcosmic morphology of black tea foam at 600 s, 1000 s, and 2000 s (M ~ O).



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protein isolate, chickpea flour, potato starch, and sunflower oil, and the FA was around 75% at 3.5 min after whipping and the FS was around 50% at 60 min. However, comparing results across different studies can be challenging due to differences in plant species, environmental conditions, extraction processes, and the methods and conditions used for generating and analyzing foams(Amagliani et al., 2021). The six categories of tea undergo unique processing procedures using raw materials of varying maturity. In the tea industry, green tea and black are typically produced from tender fresh leaves, whereas oolong tea and dark tea are often processed with relatively mature fresh leaves to meet specific processing and flavor requirements. The different maturity levels of fresh leaves result in distinct biochemical profiles, which might influence their foaming abilities.

4.2. Tea saponin content was not the primary determinant affecting FA or FS

Tea saponin, classified as a triterpenoid saponin, is known for its amphiphilic properties, contributing to its reported good foam characteristics (Chen, Yang, Chang, Ciou, & Huang, 2010; Tang et al., 2021). Sapainins from other plants such as saffron, soapnut, and *Furcraea foetida* also showed notable foaming potential(Esmaeelian, Jahani, Feizy, & Einafshar, 2024; Randriamamonjy et al., 2022; Yekeen, Malik, Idris, Reepei, & Ganie, 2020). In contrast, our study revealed that the content of tea saponin did not exhibit a strong correlation with foam ability (FA) and foam stability (FS). Tea infusion constitutes a complex system wherein various components can interact with each other. Research indicated that protein-tea saponin complexes could more effectively reduce surface tension, enhance foam liquid film strength, and increase foam volume compared to tea saponin monomers at equivalent concentrations (Huang et al., 2023). Additionally, non-purified extracts demonstrated superior foaming ability compared to purified tea saponins derived from *Camellia oleifera* cake (Li, Zhu, et al., 2024).

pH was found to influence the self-assembly of saponins and affect the morphology of the complexes (Liao et al., 2021). As pH decreased, the ZETA potential and surface tension of tea saponin decreased, resulting in stronger foaming ability and denser, more uniform foam structure (Li, Zhu, et al., 2024). Besides, saponin and protein could combine and form an interfacial film with stability, flexibility, and rigidity(Li et al., 2023). We inferred that the content of tea saponin was not the primary factor influencing FA or FS, as pH and other substances could alter the properties of tea saponin, thereby further impacting foaming characteristics.

4.3. Lipids have negative effects on the FA and FS

Lipids, typically employed as anti-foaming agents, are detrimental to the formation and stability of protein systems. They could weaken the interactions between adsorbed proteins and form unstable oil bridges between adjacent foaming surfaces, thereby leading to film rupture and affecting the properties of protein-stabilized foam (Yang, Berton-Carabin, Nikiforidis, Van Der Linden, & Sagis, 2022; Zhang et al., 2024; Zhang, Wang, Li, Li, & Qi, 2024). The addition of even a small amount of egg yolk, which contains a high level of lipids, can significantly reduce the volume of egg white foam (Sun et al., 2023). Similarly, our results indicated a significant negative correlation between lipid content and the foaming ability and foam stability of tea leaves.

In our study, the content in green tea was the highest (4.41%). Oolong tea and yellow tea also had a relatively high content with 3.10% and 2.92% respectively. Tea leaves contain various categories of lipids, including free fatty acids, glycerols, phospholipids, glycolipids, sterols, waxes, carotenes, terpenes, hydrocarbons, tocols, chlorophyll pigments, and their derivatives (Huang et al., 2024). The content of chlorophyll detected in fresh tea leaves was 5.8% (Li et al., 2017). Green and yellow teas contain relatively high levels of chlorophyll, but after processing steps such as rolling, oxidation, and drying in other categories of tea, the chlorophyll content decreases to trace amounts (Li et al., 2017). Furthermore, reports have shown that during the processing of black tea, the content of glycoglycerolipids was significantly decreased, and lipoxygenase (LOX) was activated and accelerated, leading to lipid degradation (Takeo & Tsushida, 1980). Li et al. (Li et al., 2017; Li et al., 2021) identified a total of 283 individual lipid species in green tea through lipidomics, while only 192 lipid species were found in black tea. Our results also demonstrated that black tea had a relatively low lipid content of 2.30%. Overall, the lipid content decreased with increasing oxidation levels of tea. Notably, dark tea had the second highest lipid content, partly due to the increase in lipid content with leaf maturity (Ravichandran and Parthiban, 2000), as the raw materials for dark tea processing are often more mature than those for other teas. Additionally, an extraordinary accumulation of hydroxy fatty acids (FAHFA) was detected after fermentation (Li et al., 2023), and dark tea is the only type of tea involving microbial fermentation.

4.4. Saccharides and their derivatives may have contributed to foam quality

Although soluble sugars or pectin did not exhibit a significant correlation with foaming ability and foam stability in our study, saccharides are deemed crucial taste components in tea quality assessment, believed to contribute positively to a mellow and thick taste (Deng et al., 2022). White tea exhibited the highest content of soluble sugar among the six categories of tea, with its concentration in tea soup significantly surpassing that in tea foam. Due to polysaccharides' hydrophilic nature, they are difficult to aggregate at the air-water interface and are retained in the aqueous phase, yet they stabilize protein foam systems by increasing solution viscosity (Martínez, Farías, & Pilosof, 2011). Studies showed that the addition of sugar reduced bubble size and achieved a more uniform distribution of foam systems (Ochi, Katsuta, Maruyama, Kubo, & Ueda, 2000). A 3% sugar-added egg white solution exhibited increased hydrophobicity at the surface, reduced surface tension, and enhanced foam capacity and stability (Sun et al., 2022). The addition of sugars (trehalose and sucrose) reduces protein damage and preserves protein foam (Clarkson, Cui, & Darton, 2000). Withering is a crucial process in white tea production. During the prolonged withering period, the soluble sugar content can significantly increase (Zhou et al., 2022).

The Maillard reaction is one of the primary chemical reactions during tea processing (Yang et al., 2022), involving sugars and amino acids or proteins as substrates, leading to the formation of a series of complex products such as Amadori rearrangement products, Strecker degradation products, and polymeric melanoidins (Hodge, 1953; Shakoor, Zhang, Xie, & Yang, 2022). Numerous studies have shown that the Maillard reaction could enhance the water solubility of proteins and improve foamability and stability by unfolding and disordering the protein structure (Wen et al., 2020; Zhang, Wang, et al., 2024; Zhang et al., 2020). It has been observed that the content of Maillard reaction product 2 (MRP2) in black tea was higher than in other categories of tea (Wang et al., 2024). Besides, during the drying process of black tea, the content of soluble sugars and pectin decreases significantly (Wen, Cui, Dong, & Zhang, 2021). Our findings revealed that among the six major categories of tea, black tea exhibited the lowest soluble sugar content, while its foam stability was the highest. Besides, the bubble size of black tea was obviously less than white tea. This observation suggests that the Maillard reaction, which potentially consumed soluble sugar and its resulting products might have a positive influence on foam properties.

4.5. Contributions of protein, amino acids, and different pH

The content of soluble protein in six categories of tea varied from 5.80% to 7.23%, and it had a positive correlation with FC and FS. Xiong et al. (2020) observed that increasing whey protein content from 1.5% to 4% significantly enhanced foam stability. Higher protein concentrations often correlate with better foaming performance and increased

foam stability (Vanrell et al., 2007). Ren et al. (2019) extracted water-insoluble proteins from three categories of tea and found that the foaming capacity of oolong tea and black tea was significantly higher than that of green tea. The differences might be due to different protein categories and content in different categories of tea.

Although no significant differences were detected, the content of protein in white tea' soup was lower than that in the foam, while black tea foam's protein content was higher than that in its tea soup. Sun et al. (2023) found pure egg white foam's protein content was higher than that in the liquid, however, upon the addition of low-density lipoprotein, the protein content in the foam began to decrease below that of the liquid. The results suggested the protein could be competitively displaced from the a/w interface by other substances.

There was limited reporting on the influence of amino acids on FA and FS, with a few studies suggesting that amino acids might have enhanced foam quality (Martínez-Lapuente, Guadalupe, Ayestarán and Pérez-Magariño, 2015). Our study showed there were no significant correlations between the total free amino acids and FA or FS. However, Gaba, Tyr, Phe, Ile, and Leu had significant positive correlations with foam quality. Green tea had the highest content of free amino acids, however, it was concentrated in a few categories, such as Thea, Glu, and Arg. Black tea had the second-highest content, with levels of nine amino acids significantly higher than those in other categories of tea. Besides, the content of black tea's amino acids in tea foam was significantly higher than that in tea soup, indicating those amino acids might have positive effects on foam properties. White tea exhibited significantly higher GABA content than other teas ($P < 0.01$), attributed to the prolonged withering period, which led to continuous GABA accumulation (Zhou et al., 2022).

In the foaming system, pH value was regarded to affect the foaming abilities by changing electrostatic interactions among amino acids or proteins (Dachmann, Nobis, Kulozik, & Dombrowski, 2020). Studies showed altering the pH of the solution away from the isoelectric point of proteins resulted in an increase in foam capacity (Ruíz-Henestrosa et al., 2007; Tang et al., 2022). However, Dachmann et al. (2020) found the foaming stability reached the highest level when the pH was around the potato protein isolate's effective isoelectric point. Interestingly, the pH of tea soup showed a downward trend with the increase of tea's oxidation degree except for dark tea. Dark tea is the only type of tea in which microbes were profoundly involved in piling fermentation (Lin et al., 2021), and the process might changed the biochemical profile and caused a relatively high pH value. Ren et al. (2019) found the solubility of tea water-insoluble proteins increased when the pH increased from 4 to 6. However, our results revealed significant negative correlations between foam quality and pH values, with black tea having reletively small and stable bubbles with the lowest pH value. Higher tea FS and FA were associated with lower pH values within the range of 5.22 to 5.93.

4.6. *Effects of tea polyphenols and catechins on foaming properties*

Tea polyphenols, which are primary functional components in tea, are mainly comprised of catechins, anthocyanins, flavonoids, and phenolic acids (Qi et al., 2023). Tea polyphenols display amphiphilic properties, with their hydroxyl groups serving as both hydrophilic segments and hydrogen bond donors/acceptors, while the aromatic benzene units function as hydrophobic segments (Xu et al., 2023). Our results exhibited positive correlations between tea polyphenol content with FS ($P < 0.05$) and FA. Diaz et al. (2022) found that modified whey protein–polyphenol particles' FA and FS were significantly contributed compared with unmodified whey protein particles. The interactions between polyphenols and proteins were able to enhance the foaming properties of proteins, manifested in reducing bubble size and increasing FA and FS (Kim, Wang, & Selomulya, 2024).

Catechins constitute the principal tea polyphenols, comprising approximately 60% to 80% of its composition (Qi et al., 2023). In our study, both CG and GA showed significant positive correlations with FA and FS ($P < 0.01$), however, EGC, EC, EGCG, and ECG showed negative correlations with FA or FS. This result might be attributed to the distinct chemical structures of various catechin components (Braicu, Ladomery, Chedea, Irimie, & Berindan-Neagoe, 2013).

The catechins' content in white tea and black tea's soup and foam was found to be inconsistent. For black tea, GCG and CG in tea foam were significantly higher than those in tea soup, which might be a potential reason for black tea foam's better FS. In contrast, no catechins in white tea had a significantly higher content in tea foam than in tea soup.

In summary, tea polyphenols, especially catechins, significantly impact tea foaming properties. Specifically, catechins like CG and GA exhibit positive correlations with FA and FS, while others demonstrate negative correlations. The complex interplay of their chemical structures underscores the necessity for further investigation into the effects of different monomers on foaming properties.

5. Conclusion

In this study, we revealed the foaming properties of six categories of tea, emphasizing the pivotal roles of pH values and underlying biochemical factors, such as lipid content, in determining foam characteristics. Our results showed that white tea had the highest FA and black tea exhibited the highest FS. Both FA and FS were enhanced at lower lipid content and lower pH values. Moreover, a higher content of tea polyphenols correlated with enhanced FS. Contrary to expectations, we found no evidence linking tea saponin content to FA or FS, likely due to the significant roles of pH and proteins in modifying saponin properties. Specific amino acids, including Tyr, Gaba, Phe, Ile, and Leu, were positively associated with foam properties. Catechins such as GA and CG were identified as potential contributors to foam properties, while ECG, EGCG, EC, and EGC might have adverse effects. Our work provides comprehensive insights into the formation of tea foam, highlighting the need for further research to investigate the impact of different biochemical groups in tea and their interactions on the foaming properties.

CRediT authorship contribution statement

Zixin Ni: Writing – original draft, Investigation, Data curation. **Wei Chen:** Investigation. **Hongjing Pan:** Investigation. **Dengchao Xie:** Investigation. **Yuefei Wang:** Writing – review & editing. **Jihong Zhou:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101596.

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