Original Article

A novel technology to reduce astringency of tea polyphenols extract and its mechanism

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A R T I C L E   I N F O

Article history:
Received 18 April 2020
Revised 8 December 2020
Accepted 5 March 2021
Available online 28 May 2021

Keywords: astringency β-casein fluorescence quenching tea polyphenols

A B S T R A C T

Objective: Tea polyphenols are natural extracts used widely throughout the world. However, the severe astringency of tea polyphenols has reduced patient compliance. Based on the analysis of the formation mechanism of astringency, this paper hopes to propose a new method to control the astringency of tea polyphenols and improve patient compliance without changing its effect.

Methods: Artificial saliva was used to prepare the tea polyphenols solution with different pH, using β-casein to imitate salivary protein, and preparing 1.2 mg/mL β-casein solution. A fluorescence quenching test was used to study the interaction between tea polyphenols and β-casein, combined with the stability test results of the compound, we can choose the pH with weak binding but good stability as the best pH for masking astringency. The taste-masking tablets were prepared under the best pH conditions, and the Xinnaojian Original Tablets were prepared according to the conventional preparation method. The disintegration time limit and solubility were tested respectively. The astringency of Xinnaojian original tablets and taste-masking tablets was evaluated by visual analogue scale (VAS).

Results: The result of the fluorescence quenching test prompted that the combination force was the weakest when the pH was 4.9. Further synchronous fluorescence analysis showed that an increase in pH resulted in a decrease of the binding sites between tea polyphenols and β-casein, and this decrease was closely related to changes in tryptophan residues in β-casein. Both original and taste-masking Xinnaojian Tablets were prepared. Volunteers’ VAS scores illustrated that the astringency improved significantly with the masking tablets (P < 0.05).

Conclusion: This pH-adjusting masking treatment had little effect on the recovery of polyphenols from the tablets or the dissolution of the tablets. This study provides a novel and feasible astringency masking technology for tea polyphenols and its preparation.

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1. Introduction

Tea is one of the three most consumed non-alcoholic beverages in the world. In Asian countries, such as China, Japan, Korea, and India, there are many tea consumers, and these countries have formed a unique tea culture. Because of its beneficial effects on health, tea products have emerged in many fields, such as beverage, food, cosmetics, drug and health care products. In particular, tea's potential pharmacological activities in combating antioxidant stress, inhibiting inflammatory reactions, reducing blood glucose and blood lipid levels, and providing anti-tumour and anti-atherosclerosis activities have attracted wide attention (Asensi, Ortega, Mena, Feddi, & Estrela, 2011; Kleemann et al., 2011; Kujawska et al., 2016; Mozaffari-Khosravi, Jalali, & Afkhami-Ardakani, 2009; Mukhtar & Ahmad, 2000; Singh, Akhtar, & Haqqi, 2010; Zhou, Tang, Du, Ding, & Liu, 2016). Kobayashi (Kobayashi et al., 2015) found that obesity in moderately obese patients was improved after drinking beverages rich in catechin. Tea contributed to reducing the blood pressure of prehypertensive or hypertensive individuals (Yarmolinsky, Gon, & Edwards, 2015). Some studies have found that elderly people who have tea drinking habits have a better quality of life than those who do not drink tea (Ide et al., 2014; Feng et al., 2012; Qiu, Sautter & Gu, 2012).

The health value of tea is inseparable from its chemical composition. Green tea is composed of tea polyphenols, alkaloids, amino acids, polysaccharides, volatile terpenes, vitamins, minerals and other chemical constituents (Perumalla et al., 2011). Polyphenols are considered to be the main active substances, accounting for 18%–36% of the dry weight of green tea. Among polyphenols, catechins are the most abundant ingredients, and the four major com-

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The optimal pH level to result in lower binding between polyphenols and proteins. Then, original and taste-masking Xinpiaojian Tablets were manufactured, and the astringency strength was evaluated by volunteers. Finally, the pharmaceutical properties and dissolution rates of the two tablets were compared. We hope that this research will provide a simpler method for masking the astringency of tea products. The findings will be of interest to food engineers and pharmaceutical experts.

2. Materials and methods

2.1. Materials

Tea polyphenols (No. S18152, purity ≥98%, conform to relevant quality requirements of green tea extract under Pharmacopoeia of the People's Republic of China) were from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). β-casein was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA, BioUltra, purity ≥98%, SLBN8470V). Pharmaceutical grade magnesium stearate, microcrystalline cellulose and starch were from Anhui Shanhe Pharmaceutical Excipients Co., Ltd. (Anhui, China). Epicatechin (No. PRF15120322), epigallocatechin gallate (No. 14121608) and epicatechin gallate (No. PRF8052342) were obtained from Chengdu Purufa Technology Development Co., Ltd. (Beijing, China). Both methanol and acetonitrile were chromatographic grade (TEDA, USA). Water was purified using a Milli-Q water purification system (Nanjing Yi Pu Yi Da Technology Development Co., Ltd., Nanjing, China). All other chemicals used were of analytical grade and were available locally.

2.2. Preparation of solutions

2.2.1. Preparation of β-casein solution

The complex reaction between salivary proteins and polyphenols was the function of proline-rich proteins (PRPs). The structure of β-casein is highly similar to PRPs, so it can be used to mimic salivary proteins. The protein content in saliva was approximately 0.1%–0.2%, and PRPs account for 70% of the total protein. Therefore, the concentration of β-casein solution used in this experiment was 1.2 mg/mL in ultra-pure water.

2.2.2. Preparation of tea polyphenols solution

Artificial saliva was used to prepare the tea polyphenols solution at a concentration of 3.0 mg/mL, and the pH was 3.9 at room temperature. The pH was adjusted to 2.9, 3.9, 4.9, 5.9, and 6.9 using NaOH (1 mol/L) and HCl (1 mol/L) solutions.

2.2.3. Preparation of standard solution

Standard EC, EGCG, and ECG were weighed precisely (1/10 Milligram). Bt25S Electronic Analytical Balance, Sartorius, Germany) and suitable 25% methanol was added to prepare test samples with concentrations of 59 µg/mL, 1010 µg/mL and 540 µg/mL, respectively.

2.3. Binding ability measurement

Fluorescence spectrometry is a common technique to study the interaction between biological micromolecules and small molecules, and fluorescence quenching is used frequently. Fluorescence quenching is the phenomenon in which fluorescence intensity is reduced after the reaction occurs between fluorescence materials and quenching items. Fluorescence quenching can be divided into static quenching and dynamic quenching. The protein has a fluorescence effect due to its unique amino acid residues, such as tryptophan, tyrosine and phenylalanine, and tryptophan has the...
highest quantum yield. Trp-143 is the internal fluorescence substance of β-casein and is located on the surface of the protein. When it interacts with other molecules, the conformation and fluorescence intensity of the protein may change accordingly. Suitable tea polyphenols solution was added to nine test tubes, 1 mL of β-casein solution was added, the reaction was diluted to 2 mL with ultra-pure water, and the reaction was fully mixed. Then, the mixtures were incubated in a water bath for 30 min at 30°C, and the reaction results were detected by a fluorescence spectrophotometer (Varian, USA). The emission spectra of the substances were obtained by fixing the excitation wavelength at 280 nm and scanning the emission wavelength from 287 nm to 485 nm. Catechin was chosen as the representative component because it had the highest content in tea polyphenols, and the molar mass of the tea polyphenols was 290.079 g/mol. Therefore, the final concentration of the tea polyphenols was 0, 0.0258, 0.05171, 0.1034, 0.2068, 0.4137, 0.5171, 1.103, and 2.068 mmol/L. The fluorescence intensity at λ = 340 nm was selected to estimate binding ability. The fluorescence intensity of tea polyphenols at different pH values was measured using the same procedure.

The fluorescence intensity of tea polyphenols and β-casein with different concentrations at 20°C and pH 3.9 was measured using the same procedure.

After the emission spectrum was measured, the synchronous fluorescence spectrum was recorded in the synchronous scanning mode. The wavelength was 200–400 nm, and the Δλ was 15 nm and 60 nm.

2.4. Stability of β-casein and tea polyphenol complexes

The Turbiscan stability analyser (Formulation, France) applies an 880 nm near-infrared light and reveals the stability of samples by detecting light intensity changes in transmitted light and backscattered light. This apparatus is based on the principle of multiple light scattering. The measurement probes include a light source, transmitted light detector and backscattered light detector. The probe measured the sample pool from bottom to top in one scan. The deviation of light intensity for multiple scans reflects the stability of the system, which is expressed as the Turbiscan Stability Index (TSI). A larger TSI value indicates a more unstable system.

In the reaction, 10 mL of β-casein solution was reacted with 4 mL of tea polyphenols at different pH values, and the reaction was diluted to 20 mL. The reaction solution was placed in a sample tube and scanned at λ = 880 nm near-infrared. The temperature was (25 ± 0.5) °C, and the time interval was 30 s for a total of 10 min.

2.5. Preparation of Xinnaojian original tablets and taste-masking tablets

Tea polyphenols were used to prepare the tablets, for considering the low content of tea polyphenols in green tea, which only accounted for 15%–20% of the dry weight of green tea (Shen, Hu & Yan, 2013). First, a suitable tea polyphenols solution was prepared, the water was removed by heating to 60 °C in a water bath, and the extracts were dried in vacuum drying oven (60 °C). The processed tea polyphenols powder was obtained. The tea polyphenols, microcrystalline cellulose (dried at 80–100 °C for 4 h) and starch were mixed at a ratio of 1:1:0.94, granulated with 40% ethanol, dried at low temperature, added to appropriate magnesium stearate, mixed and pressed into slices; Then, the Xinnaojian original tablets were obtained. After adjusting the pH of the tea polyphenols solution to 4.9, the taste-masking tablets were prepared using the same method for the original tablets. The tablets had an average weight of 0.4 g. Three batches each of Xinnaojian original and taste-masking tablets were prepared, and each batch contained 30 pieces. The two types of tablets had an average weight of 0.4 g.

Ten Xinnaojian original and taste-masking tablets were selected randomly, a Vernier calliper (Hangzhou Tool Measuring Tool Co., Ltd.) was used to measure the diameter and thickness of the tablets. The hardness of tablets was obtained using a YD-20 tablet hardness tester (Tian Da Da Fa Technology Co., Ltd.).

2.6. Measurement of disintegration time limit

The measurement of the disintegration time limit is to ensure that the tablets can release within specified time and to determine whether the change in pH affects the in vitro release of the taste-masking tablets. The test was performed on a ZB-1E disintegration apparatus (Tian Da Da Fa Technology Co., Ltd.) using the rotating bucket method. The tablets were placed in 800 mL of pure water at a temperature of (37 ± 0.5) °C. The disintegration of the tablets was observed and timed until they were completely dissolved. The measurement was performed six times in parallel. The final disintegration time of the Xinnaojian original and taste-masking tablets is expressed as an average.

2.7. Measurement of astringency

The astringency level was evaluated by visual analogue scale (VAS), and the definitions of the numerical scale are shown in Fig. 5. Suitable concentrations of basic solutions were prepared for detecting the sensitivity to astringent tastes before testing: acid (citric acid), sugar (sucrose), salt (sodium chloride), bitter (quinine hydrochloride), and distilled water (tannic acid). The scale and intensity of astringency were determined by the most sensitive volunteer’s feeling to different concentrations of tannic acid, and the concentrations were as follows: 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 μg/mL. The volunteers involved in this taste experiment were three male and seven female adults from Jiangxi University of Traditional Chinese Medicine and were chosen randomly. The astringency experiment was conducted by a single blind method. The experimental operators gave the participants two previously prepared Xinnaojian original and taste-masking tablets, which were identical in appearance. The volunteers placed the tablets on their tongue for approximately 30 s and scored their perceived astringency. One point means no astringency, while 10 points indicates the most astringency. After each astringency test, the volunteers had a 5 min break and drank pure water to remove the astringency in their mouth. The data of 10 volunteers’ scores for the types of tablets were organized, and the distribution of astringency was determined.

2.8. Measurement of dissolution

2.8.1. HPLC analysis conditions

The analysis of chromatographic injections was performed by high-performance liquid chromatography (Agilent 1260, USA) on an Elite C18 column (4.6 mm × 150 mm, 5 μm; filler material was Hypersil ODS2; column No. E2515597). Mobile phase A was acetonitrile-0.2% phosphoric acid (10:90), and mobile phase B was acetonitrile-0.2% phosphoric acid (80:20). The gradient elution was 0–15 min, 0–10% B; 15–19 min, 10%–45% B; 19–35 min, 45%–0% B); The injection volume was 20 μL. The flow rate was 1 mL/min; The UV detection wavelength was 280 nm; And the column temperature was 30 °C.

The standards were analysed by HPLC, and the standard curve was obtained using the concentration (μg/mL) as the abscissa and the peak area (A) as the ordinate.
2.8.2. Determination of Xinnaojian tablet dissolution

Some Xinnaojian original and taste-masking tablets were ground into powder; then 0.1 g powder was added into a 50 mL volumetric flask containing 25% methanol, sonicated for 30 min to completely dissolve the powder, and filtered. The filtrates of the two tablets were analysed according by HPLC, and the total content of the effective components in the two types of tablets was obtained.

Tablet dissolution was determined by the pulp method on a ZRS-8G intelligent dissolving test apparatus (Tianjin Tian Da Tian Fa Technology Co., Ltd., Tianjin, China). During this experiment, the rotation speed of the pulp was 50 r/min, and the dissolution medium was 900 mL of pure-water at the temperature of (37 ± 0.5) °C. Suitable tablets were placed into the dissolution cup at the same time, and 10 mL of dissolution liquid was removed at 5, 15, 30, 45, 60, 90, and 120 min and immediately replaced with 10 mL of pure water at the same temperature. The dissolved tablets at different times were passed through a 0.22 μm filter and then analysed by HPLC. The dissolution was taken as the ordinate and 1/[C] as the abscissa to make a picture, and the cumulative dissolution rate of the EC, EGCG and ECG in the tablets was taken as (Y) to show the tablet dissolution.

2.9. Statistical analysis

Statistical analyses were performed by SPSS 22.0 package (SPSS Inc., Chicago, IL, USA). The data were reported as the means ± standard deviation (SD) and as individual values in the figures. Differences were considered statistically significant at \( P < 0.05 \).

3. Results and discussion

3.1. Results of binding ability

The fluorescence intensity of β-casein and tea polyphenols at 340 nm was studied. In Fig. 1, the fluorescence intensity of the protein decreased with increasing tea polyphenols concentration at different pH values, thus, a higher degree of binding occurred. Substances containing tea polyphenols present astringency due to its effect on salivary proline-rich proteins (PRPs) in human saliva as we know it. The reaction is a hydrogen bonding effect that is mainly caused by the combination of the 5-oxazole ring carboxyl group on the proline residue of PRPs and the phenolic hydroxyl group on the polyphenol ring. The polyphenols and proteins specifically bound to form complexes with low complexation because of the limited binding sites at low concentration, thus, the astringency was lower. And as the concentration increased, the number of binding sites increased. Thus, the quenching constant was calculated from formula (1).

\[
F_0 / (F_0 - F) = 1 / (fK[Q]) + 1 / f, \tag{1}
\]

where \( F_0 \) indicates the fluorescence intensity of β-casein in the absence of quencher, and \( F \) is the fluorescence intensity of β-casein with quencher. \([Q]\) is the quencher's concentration, and \( f \) is the fluorescence quencher of the polar quencher. \( F_0/(F_0 - F) \) was taken as the ordinate and 1/[Q] as the abscissa to make a picture, and the ratio of the intercept to slope is \( K \).

The picture of \( F_0/(F_0 - F) vs 1/[Q] \) was shown in Fig. 1. The change in fluorescence intensity was assumed to originate from the combination of β-casein and tea polyphenols; thus, the quenching constant \( K \) could replace the binding constant of the complex. According to \( K \), the order of binding ability of tea polyphenols was: pH 2.9 > pH 6.9 > pH 3.9 > pH 4.9 > pH 5.9. The weakest binding was at pH 4.9 and pH 5.9.

The Stern-Volmer equation (listed as formula 2) determines whether the combination between tea polyphenols and β-casein is static quenching or dynamic quenching (Lakowicz, 1991).

\[
F_0 / F = 1 + K_0 [Q] = 1 + K_0 [Q] \tag{2}
\]

where \( K_0 \) is the Stern-Volmer constant, \( K_0 \) is the rate constant of a bimolecular quenching process, and \( t_0 \) is the average fluorescence lifetime of a fluorescent molecule in the absence of quencher (β-casein is 3.30 ns). The quenching constant reflects the touching degree and reaction speed between the quencher and fluorescent molecule. After plotting \( F_0/F vs [Q] \), a straight line with a longitudinal axis intercept of approximately 1 was obtained, and the slope was \( K_0 \).

We can observe that the \( K_0 \) at 30 °C was smaller than that at 20 °C when the system was at pH 3.9, indicating that the quenching was mainly static quenching (Fig. 2). The plots show a good linear relationship within the investigated concentrations. All the \( K_0 \)s in the experiment were much higher than the maximum scatter collision quenching constant of various quenchers (\( 2 \times 10^{-5} \) M/s), suggesting that the probable quenching mechanism was initiated by complex formation rather than by dynamic collision. (Kandagal et al., 2006; Feizi, Dehghanian & Mansouri, 2020; Siddiqui et al., 2020). The results of fluorescence measurement were used to calculate the binding constant of β-casein and tea polyphenol complexes during static quenching (Sun et al., 2011).

\[
\log [(F_0 - F)/F] = \log Ka + n \log [Q] \tag{3}
\]

where \( Ka \) is the apparent binding constant, and \( n \) is the number of β-casein binding sites. In the plot of \( \log [(F_0 - F)/F] vs \log [Q] \), Log \( Ka \) is the intercept, and \( n \) is the slope.

The plots of \( \log [(F_0 - F)/F] vs \log [Q] \) is shown (Fig. 3). The binding ability of tea polyphenols was obtained by comparing \( Ka \) and \( n \). According to the \( Ka \) value, the sequence was pH 3.9 > pH 2.9 > pH 5.9 > pH 4.9 > pH 6.9. By comparing \( n \), the order was pH 3.9 > pH 2.9 > pH 5.9 > pH 4.9 > pH 6.9. The results showed that the binding ability was weaker at pH 4.9 and 6.9. The Ka value at 30 °C was higher than that at 20 °C with pH 3.9, indicating that the combination between tea polyphenols and β-casein was a covalent form, and non-ionic forms have the primary effect.

Thermodynamic parameters can verify the binding between protein and tea polyphenols. Gibbs free energy (\( \Delta G \)), enthalpy (\( \Delta H \)) and entropy (\( \Delta S \)) were calculated by formulas (4)–(6):

\[
\Delta G = -RT \ln Ka \tag{4}
\]

\[
\ln Ka_2 / Ka_1 = \Delta H/R (1/T_1 - 1/T_2) \tag{5}
\]

\[
\Delta S = (\Delta H - \Delta G) / T \tag{6}
\]

where \( R \) is the gas constant (8.314 J/mol/K), \( T \) is temperature, and \( Ka \) is the binding constant under \( T \).

According to data from the fluorescence measurement, we calculated that the tea-protein system had the values: \( \Delta H = 134.72 \) KJ/(mol/K) and \( \Delta S = 536.263 \) J/K. The \( \Delta G \) values in the tea polyphenol and β-casein system at different pH values at 30 °C and the system at pH 3.9 at 20 °C are shown (Table 1). It’s not difficult to find that the \( \Delta G \) was lower at pH 4.9 and 6.9. Based on the results from Ross (Ross & Subramanian, 1981), the hydrophobic force positively contributed to \( \Delta H \) and \( \Delta S \), the hydrogen bonds or van der Waals forces made \( \Delta H \) and \( \Delta S \) negative, and the electrostatic effect made \( \Delta H \approx 0 \) and \( \Delta S > 0 \). The \( \Delta H \) and \( \Delta S \) values in this experiment were all higher than 0. Therefore, the reaction between tea polyphenols and β-casein was through hydrophobic force. The greater the absolute value of \( \Delta G \), the better the binding ability of the system. Thus, the binding force was weaker when the pH was at 4.9 and 6.9.
Fig. 1. Fluorescence emission spectra of tea polyphenols and β-casein at different pH. Nine curves (1–9) represent tea polyphenols concentrations of 0, 0.02586, 0.05171, 0.1034, 0.2068, 0.4137, 0.5171, 1.034, and 2.068 mmol, from top to bottom. A – E were performed at 30 °C, and F represents the system at 20 °C and pH 3.9.

Fig. 2. Stern-Volmer plots of the fluorescence quenching constant of tea polyphenols and β-casein at different pH. A – E are the results at 30 °C, and F was performed at pH 3.9 and 20 °C.
In brief, the bound water around proteins efflux and most of the proteins were detached from the saliva, and the polyphenols combined the proteins by a hydrophobic interaction. The complexes were distributed randomly in the oral mucosa and tongue, allowing a stronger intensity of astringency be produced (Jöbstl et al., 2006). The binding constant of $\beta$-casein and tea polyphenol complexes during static quenching indicated that the number of $\beta$-casein binding sites varied under different pH conditions, indicating that pH may affect the milieu of the reaction. With more phenolic hydroxyl groups of tea polyphenols, there is a stronger ability to bind proteins, and a lower pH can inhibit the ionization of polyphenols, so the polyphenols and proteins combined more easily to form a precipitate.

Synchronous fluorescence analysis (Figs. 4 and 5) showed that the tyrosine and tryptophan residues in casein played a major role during the reaction process. When $\Delta\lambda = 60$ nm, the maximum absorption wavelength of the tryptophan residue had a certain shift, which suggested that the tryptophan residue may be the main binding site of $\beta$-casein. Therefore, pH may have greater impact on this site (Zhang et al., 2015). Regulating pH helped to obtain a low astringency taste-masking tablets, which was prospective.

### 3.2. Stability of complexes

The stability of tea polyphenol and $\beta$-casein complexes was shown (Fig. 6). The system was more unstable system at larger TSI values. According to the curves in the picture, the largest TSI was at pH 6.9, and the smallest TSI was at pH 4.9 and 5.9, indicating that the tea polyphenol and $\beta$-casein system may be stable at pH 4.9 or 5.9. Combined with the fluorescence quenching results, the combination of tea polyphenols and $\beta$-casein was weaker at pH 4.9; Thus, pH 4.9 was used for subsequent experiments. The Taste-masking Tablets were adjusted to pH 4.9 to obtain the best performance.

### 3.3. Examination of tablets

We can know the characteristics of the two tablets (Table 2). The diameter and thickness are two important factors that can influence drug dosage. The hardness and disintegration time affect the biological availability. The tablets had an average diameter of approximately 11 mm and an average thickness of 4.2 mm. The taste-masking tablets had a harness of 50.1 N, while the hardness of the original tablets was 53.4 N. The tablets could be disintegrated completely within 8 min, indicating that a change of pH cannot affect the basic characteristics of the tablet.

### 3.4. Measurement of astringency

The VAS score of the two tablets also can be observe (Fig. 7). Generally, the acceptability of taste-masking tablets was significantly higher than that of the original tablets ($P < 0.05$). The data for the taste-masking tablets were primarily distributed below 6 points, while the original tablets scored higher than 7 points. This result indicated that the change in pH attenuated the astringency. However, some volunteers indicated that the two types of tablets were slightly bitter, suggesting that the pH adjustment had no obvious effect on bitterness.

### 3.5. Tablet dissolution

The standard curves of EC, EGCG and ECG (Table 3) showed that the three ingredients all had good linear relationship within the suitable range of concentrations, and $r > 0.9983$. The HPLC results (Fig. 8) indicate that the three components can be well separated by HPLC.

From the cumulative dissolution rate of EC, EGCG and ECG in Fig. 9, it was known that the cumulative dissolution of the original and taste-masking Xinnaojian tablets could be divided into two stages. Stage I was the immediate release stage in which most
components were dissolving, and stage II was the slower release stage in which the weight of the ingredients in the tablets reached a peak, and the cumulative dissolution rate of the three components was >90%. There was no significant difference between original and taste-masking tablets by comparing the cumulative dissolution rate, indicating that a pH change cannot influence the in vitro solubility of tea polyphenols.

4. Conclusion

This paper provided a novel method to reduce astringency by adjusting the pH of a tea polyphenol solution to pH 4.9 before preparing Xinnaojian tablets, thus avoiding the addition of other taste-masking excipients that increase the number of drugs and then reduce the bioavailability. The adjustment in pH significantly reduced the astringency of the tablets by comparing with the original flavour tablets. This method only requires a slight change in the preparation process and was a fairly simple astringency masking technology. Modern research trends suggest that it is a very common operation in the pharmaceutical industry to add a certain reagent to adjust the pH. Simultaneously, there are relatively complete process guidelines in the production (Cong, Yue, & Wang,
Under the requirements and guidance of the current GMP regulations, adjust pH to reduce astringency was safe and feasible. And this technology had no effect on its content, which will be interested the food and pharmaceuticals industry.

### Table 2
Tablets parameters and disintegrating limit time.

| Formula                        | Diameter/mm | Hardness/N | Thickness/mm | Disintegration time/min |
|--------------------------------|-------------|------------|--------------|-------------------------|
| Taste-masking tablets          | 11.11 ± 0.02| 4.22 ± 0.03| 50.1         | 6 ± 0.25                |
| Xinnaojian original tablets    | 11.10 ± 0.01| 4.26 ± 0.03| 53.4         | 7 ± 0.45                |

**Fig. 7.** Meaning of VAS and ten volunteers’ astringency scores of two tablets. VAS indicates visual analogue scale.

### Table 3
Standard curve of three components of tea polyphenols.

| Target ingredients | Regression curve | r    | Linearity range/ (\(\mu g\) mL\(^{-1}\)) |
|--------------------|------------------|------|------------------------------------------|
| EC                 | \(Y = 16.34X - 2.750\) | 0.9983 | 0.885–14.16                             |
| EGCG               | \(Y = 29.04X - 44.848\) | 0.9999 | 5.05–151.5                               |
| ECG                | \(Y = 40.296X - 3.8075\) | 0.9999 | 1.62–64.80                               |

**Fig. 8.** Typical liquid chromatography of mix of standard samples (A), Xinnaojian taste-masking tablets (B), and Xinnaojian tablets (C). (a) EC; (b) EGCG; (c) ECG.

**Fig. 9.** Cumulative dissolution rate of Xinnaojian Taste-masking and Original Tablets. A: EC, B: EGCG, C: ECG.
However, in this paper, the obtained scores of astrignency were somewhat objective because of the individuals’ different acceptance and sensitivity to astrignency. To make the results more convincing, an electronic tongue should be used in future experiments. Regulating pH helped to obtain a low astrignency extract, but to determine if the low pH affected pharmacological activity, a fingerprint analysis was performed. The results showed that pH modification had little effect on the chemical composition of tea polyphenols. To determine whether pH modification had an effect on blood pressure, anti-atherosclerosis and other functions, further in vivo research needs to be.

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.

Acknowledgement

This research was supported by the National Natural Science Foundation of China. (Nos. 81673615, 81403115).

References

Arima, H., Higashi, T., & Motoyama, K. (2012). Improvement of the bitter taste of drugs by complexation with cyclodextrins: Applications, evaluations and mechanisms. Therapeutic Delivery, 3(3), 633–644.
Asensi, M., Ortega, A., Mena, S., Feddi, F., & Estrela, J. M. (2011). Natural polyphenols in cancer therapy. Critical Reviews in Clinical Laboratory Sciences, 48(5-6), 197–216.
Boonchu, T., & Utama-ng, N. (2015). Optimization of extraction and microencapsulation of bioactive compounds from red grape (Vitis vinifera L.) pomace. Journal of Food Science and Technology, 52(2), 783–792.
Cong, R. L., Yue, Y. Y., & Wang, X. C. (2019). Experimental research on industrial production of baijincan. Journal of The Minzu University China (Natural Sciences Edition), 28(1), 5–11.
Dinnella, C., Recchia, A., Fia, G., Bertuccioli, M., & Monteleone, E. (2009). Saliva characteristics and individual sensitivity to phenolic astrignent stimuli. Chemical Senses, 34(4), 295–304.
Feizi-Dehbayee, M., Dehghanian, E., & Mansouri-Torshizi, H. (2021). A novel palladium (II) antimutagen agent: Synthesis, characterization, DFT perspective. Spectroscopic Spectrochimica Acta. Part A, Molecular and Biomolecular. Spectroscopy, 249, 119215.
Feng, L., Li, J., Ng, T. P., Lee, T. S., Kua, E. H., & Zeng, Y. (2012). Tea drinking and cognitive function in oldest-old Chinese. The Journal of Nutrition, Health & Aging, 16(9), 754–758.
Gonzalo-Dago, A., Dizy, M., & Fernández-Zurbano, P. (2014). Contribution of low molecular weight phenols to bitter taste and mouthfeel properties in red wines. Food Chemistry, 154, 187–198.
Green, B. G. (1993). Oral astrignency: A tacit component of flavor. Acta Psychologica, 84(1), 119–125.
He, M., Tian, H., Luo, X., Qi, X., & Chen, X. (2015). Molecular process in research on fruit astrignency. Molecules, 20(1), 1434–1451.
He, S. S. (2017). The secondary development application study of Jianghuang Tablet Master thesis. Guangzhou University of Chinese Medicine.
Ide, K., Yamada, H., Takuma, N., Park, M., Wakamiya, N., Nakase, J., et al. (2014). Green tea consumption affects cognitive dysfunction in the elderly: A pilot study. Nutrients, 6(10), 4032–4042.
Jöbstl, E., Howse, J. R., Fairlough, J. P. A., & Williamson, M. P. (2006). Nonsense cross-linking of casein by epigallocatechin gallate characterized by single molecule force microscopy. Journal of Agricultural and Food Chemistry, 54(12), 4077–4081.
Kandagal, P. B., Ashoka, S., Seetharamappa, J., Shaikh, S. M. T., Jadegoud, Y., & Ijare, O. B. (2006). Study of the interaction of an anticancer drug with human and bovine serum albumin: Spectroscopic approach. Journal of Pharmaceutical and Biomedical Analysis, 41(2), 193–199.
Kleemann, R., Verschuren, L., Morrison, M., Zadelaar, S., van Erk, M. J., Wielinga, P. Y., et al. (2011). Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models. Atherosclerosis, 218(1), 44–52.
Kobayashi, M., Kawano, T., Ukawa, Y., Sagesaka, Y. M., & Fukuhara, I. (2016). Green tea beverages enriched with catechins with a galloyl moiety reduce body fat in moderately obese adults: A randomized double-blind placebo-controlled trial. Food & Function, 7(1), 498–507.
Kujawski, M., Ewertsowska, M., Ignatowicz, E., Adamska, T., Szafer, H., Gramza-Michalowska, A., et al. (2016). Evaluation of safety and antioxidant activity of yellow tea (Camellia sinensis) extract for application in food. Journal of Medicinal Food, 19(3), 330–336.
Lakowicz, J. R. (1991). Principles of fluorescence spectroscopy. Die Naturwissenschaften, 78(10), 456–457.
Lee, C. A., Ismail, B., & Vickers, Z. M. (2012). The role of salivary proteins in the mechanism of astrignency. Journal of Food Science, 77(4), C381–C387.
Lee, C. B., & Lawless, H. T. (1991). Time-course of astringent sensations. Chemical Senses, 16(2), 225–238.
Ma, S., Lee, H., Liang, Y., & Zhou, F. (2016). Astringent mouthfeel as a consequence of lubrication failure. Angewandte Chemie (International ed. in English), 55(19), 5793–5797.
Martin, F., & Christian, F. (2013). pH measurement in pharmaceutical production. Process, 1, 52–53.
Mozaffari-Khosravi, H., Jalali, B. A., & Afkhami-Ardakani, M. (2009). Effect of sour tea (Hibiscus sabdariffa) on blood glucose, lipid profile, and lipoproteins in diabetics. Iranian Journal of Endocrinology & Metabolism, 10(6), 589–597.
National Pharmacopoeia Commission. (2015). Pharmacopoeia of the People’s Republic of China, 4th ed. Beijing.
Mulkir, H., & Ahmad, N. (2000). Tea polyphenols: Prevention of cancer and optimizing health. The American Journal of Clinical Nutrition, 71(6), 1688S–702S.
Nayak, A., & Carpenter, G. H. (2008). A physiological model of tea-induced astrignency. Physiology & Behavior, 95(3), 290–294.
Paulo, F., & Santos, L. (2017). Design of experiments for microencapsulation applications: A review. Materials Science & Engineering, C, Materials for Biological Applications, 77, 1327–1340.
Perumalla, A. V. S., & Hettiarachchy, N. S. (2011). Green tea and grape seed extracts - Potential applications in food safety and quality. Food Research International, 44(4), 827–839.
Qiu, L. L., Saatter, J., & Gu, D. (2012). Associations between frequency of tea consumption and health and mortality: Evidence from old Chinese. British Journal of Nutrition, 108(9), 1686–1697.
Ross, P. D., & Subramanian, S. (1981). Thermodynamics of protein association reactions: Forces contributing to stability. Biochemistry, 20(11), 3096–3102.
Shen, M., Hu, J. B., & Yan, C. (2013). Research progress on analytical methods of tea polyphenols in green tea. Drug Evaluation Research, 36(5), 389–393.
Siddiqui, S., Ameen, F., Kausar, T., Nayeem, S. M., Ur Rehman, S., & Tabish, M. (2021). Biophysical insight into the binding mechanism of doxofylline to bovine serum albumin: An in vitro and in silico approach. Spectrochimica Acta. Part A, Molecular and Biomolecular. Spectroscopy, 249, 119296.
Singh, R., Akhtar, N., & Haq, M. T. (2016). Green tea polyphenol epigallocatechin-3-gallate: Inflammation and arthritis. Life Sciences, 86(25–26), 907–918.
Sun, Y., Wei, S., Yin, C., Liu, L., Hu, C., Zhao, Y., et al. (2011). Synthesis and spectroscopic characterization of 4-butoxy-xy-n-ocadetyl 1,8-sulphahalimide as a new fluorescent probe for the determination of proteins. Bioorganic & Medicinal Chemistry Letters, 21(12), 3798–3804.
Szejtli, J., & Szente, L. (2005). Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins. European Journal of Pharmaceutics and Biopharmaceutics, 61(1), 115–125.
Yao, J. W., Lin, C. J., Chen, G. Y., Lin, F., & Tao, T. (2010). The interactions of epigallocatechin-3-gallate with human whole saliva and parotid saliva. Archives of Oral Biology, 55(7), 470–478.
Yarmolinsky, J., Gon, G., & Edwards, P. (2015). Effect of tea on blood pressure for secondary prevention of cardiovascular disease: A systematic review and meta-analysis of randomized controlled trials. Nutrition Reviews, 73(4), 236–246.
Zhang, L., Liu, B., Li, Z., & Guo, Y. (2015). Comparative studies on the interaction of ceftizime with bovine serum albumin by fluorescence quenching spectroscopy and synchronous fluorescence spectroscopy. Luminescence, 30(5), 686–692.
Zhou, H. W. (2019). Study on pH value control in the production of acrylonitrile. China Chemical Trade, 11(13), 224.
Zhou, Y., Tang, J., Du, Y., Ding, J., & Liu, J. Y. (2016). The green tea polyphenol EGCG potentiates the antiapoptrotic activity of sunitinib in human cancer cells. Tumour Biology, 37(7), 8535–8566.