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Original Article

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

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ABSTRACT

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.

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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ponents of the catechins are epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), and epicatechin (EC). Medicinally, green tea has been developed into an extract and is the listed drug in Xinnaojian Tablets. Both the extract and tablets were included in the 2015 edition of Chinese Pharmacopoeia. According to the preparation of tea extracts recorded in Chinese Pharmacopoeia, green tea is extracted and filtered and the precipitant is obtained after addition of sodium hydroxide solution. Then, the pH of the precipitant is adjusted to acidic environment. The filtering solution is separated and extracted by ethyl acetate. After decompression recovery, drying and crushing, the green tea extract is obtained. The extract was believed to keep one's mind clear, uplift spirits, improve memory, prevent atherosclerosis, and prevent leukopenia caused by chemoradiotherapy (National Pharmacopoeia Commission, 2015). Although green tea extract shows obvious application advantages, it also faces the challenge of outstanding astringency.

At present, some methods are used to mask the astringency, such as microencapsulation and cyclodextrin (Boonchu & Utama-Ang, 2015; Arima, Higashi & Motoyama, 2012; Szejtli & Szente, 2005). Microencapsulation wraps the polyphenols in microparticles using polymeric materials, which can cover the taste, allow slow release, and improve drug stability (Paulo & Santos, 2017). However, the encapsulation efficiency is not satisfactory, the drug loading rate is low, and the products prepared are often mixed microcapsules and drug prototypes. These attributes lead to poor astringency masking. Cyclodextrin inclusion carries the drug using the hole of β-cyclodextrin to form a complex substance. However, the loading effect depends on the size of the holes and the size of the drug molecules. If the drug molecules are too large, they may bind to the side chains of cyclodextrins; If the drug molecules are too small, the complex compounds formed are unstable. In addition, the external hydrophilic and internal hydrophobic structure of cyclodextrin is often not conducive to the loading of water-soluble drugs. Therefore, a new masking method is needed for water-soluble substances such as tea polyphenols.

Astringency is a sensation in the mouth that is usually described as a shrinking, drying-out, stretching and wrinkling feeling (Lee & Lawless, 1991). Many foods can produce astringency, such as red wine, and this feeling can reduce acceptance. Four classical compounds can cause astringency, such as tannins, acids, metal salts and dehydrating agents (Green, 1993). Tannins are thought to be the most important compound that triggers astringency. Tannic components combine with salivary proline-rich proteins in the mouth to form a precipitate, thus reducing the lubricity of the mouth and producing the feeling of astringency (Ma et al., 2016). The proteins in saliva have many hydrophobic groups, and the tannins have both hydrophobic aromatic rings and hydrophilic hydroxyl groups, so they react with each other quickly via hydrogen bonding. Some other components can also cause astringency, such as polysaccharides and acids; Their existence can impact the perception of astringency, and some may disrupt the combination of tannins and proteins (He et al., 2015). Many factors can affect this reaction, including polyphenol types, concentration, oral temperature, salivary pH and the enzyme content in the saliva (Lee, Ismail & Vickers, 2012; Yao et al., 2010; Nayak & Carpenter, 2008; Gonzalo, Dizy & Fernández, 2014; Dinnella, et al., 2009). Among these factors, the type and concentration of polyphenols are determined by the drug itself, which cannot be changed at will. Oral temperature and enzyme concentration vary from person to person and are not easy to change. Thus, pH is the only factor that can be altered.

In this manuscript, Xinnaojian Tablets were used to study the relationship between astringency and pH. First, we explored the influence of pH on protein–polyphenol interactions and defined the optimal pH level to result in lower binding between polyphenols and proteins. Then, original and taste-masking Xinnaojian 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 Puruifa Technology Development Co., Ltd. (purity > 98%). Phosphoric acid was obtained from China Pharmaceutical Group Chemical Reagent Co., Ltd. (Beijing, China). Both methanol and acetonitrile were chromatographic grade (TEDIA, 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 protein (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 Millionth 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 determinate 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 backet 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 C_{18} 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 (X) 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 carbonyl 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 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).

$$F_0 / (F_0 - F) = 1/ (f\ K\ [Q]) + 1 / f, \quad (1)$$

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).

$$F_0 / F = 1 + K_q\ t_0\ [Q] = 1 + K_D\ [Q] \quad (2)$$

where K_D is the Stern-Volmer constant, K_q 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_D.

We can observe that the Kq 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 Kqs in the experiment were much higher than the maximum scatter collision quenching constant of various quenchers (2×10^{10} M^{-1}/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).

$$\text{Log}\ [(F_0 - F)\ /F] = \text{Log}\ Ka + n\ \text{Log}\ [Q] \quad (3)$$

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 plot 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 (ΔG), enthalpy (ΔH) and entropy (ΔS) were calculated by formulas (4)–(6):

$$\Delta G = - RT\text{Ln}\ Ka \quad (4)$$

$$\text{Ln}Ka_2 / Ka_1 = \Delta H/R\ (1/T_1 - 1/T_2) \quad (5)$$

$$\Delta S = (\Delta H - \Delta G) / T \quad (6)$$

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: ΔH = 134.72 KJ/(mol/K) and ΔS = 536.263 J/K. The ΔG 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 ΔG was lower at pH 4.9 and 6.9. Based on the results from Ross (Ross & Subramanian, 1981), the hydrophobic force positively contributed to ΔH and ΔS, the hydrogen bonds or van der Waals forces made ΔH and ΔS negative, and the electrostatic effect made $\Delta H \approx 0$ and $\Delta S > 0$. The ΔH and ΔS 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 ΔG, the better the binding ability of the system. Thus, the binding force was weaker when the pH was at 4.9 and 6.9.

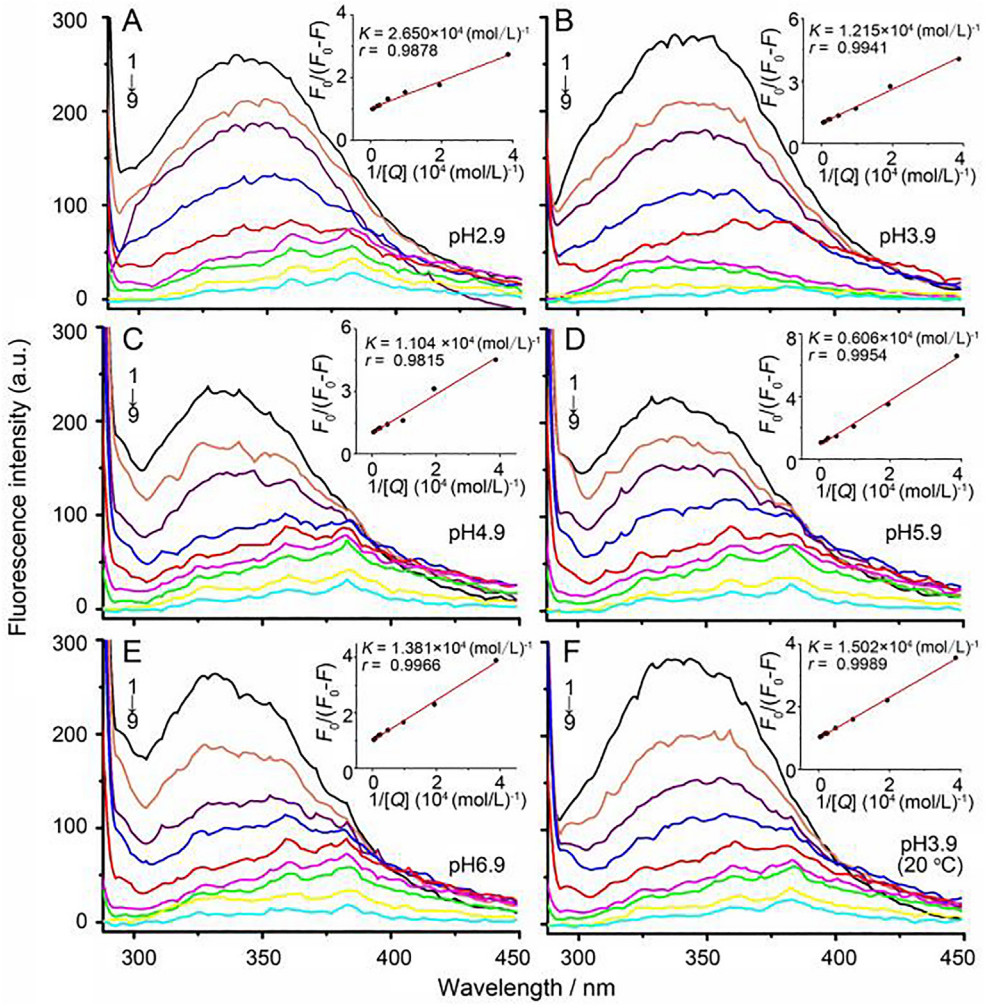

Fig. 1. Fluorescence emission spectra of tea polyphenols and β-casein at different pH. Nine curves (1–9) represent tea polyphenols concentrations of 0, 0.02586, 0.05171, 0.1034, 0.2068, 0.4137, 0.5171, 1.034, and 2.068 mmoL from top to bottom. A – E were performed at 30 °C, and F represents the system at 20 °C and pH 3.9.

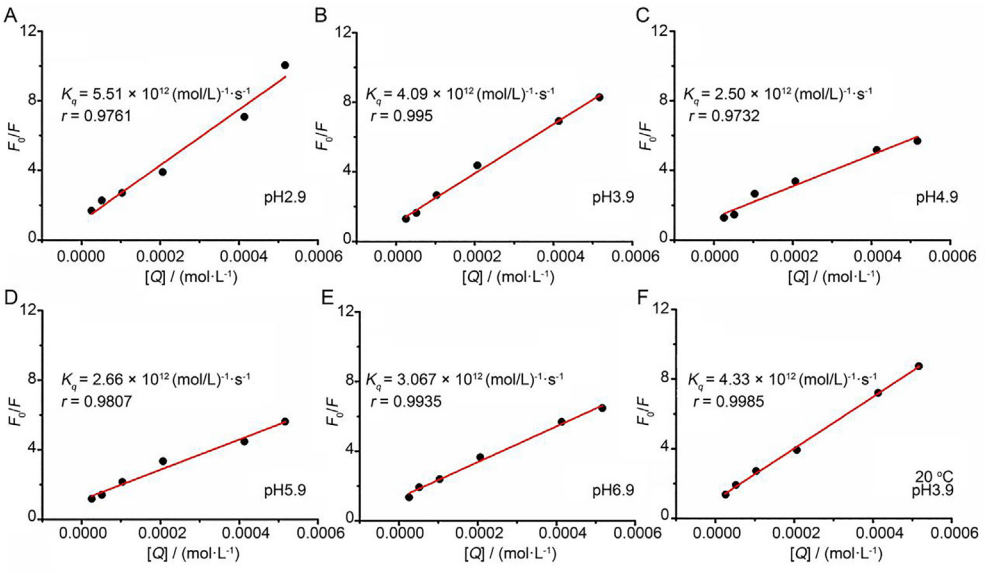

Fig. 2. Stem-Volmer plots of the fluorescence quenching constant of tea polyphenols and β-casein at different pH. A – E are the results at 30 °C, and F was performed at pH 3.9 and 20 °C.

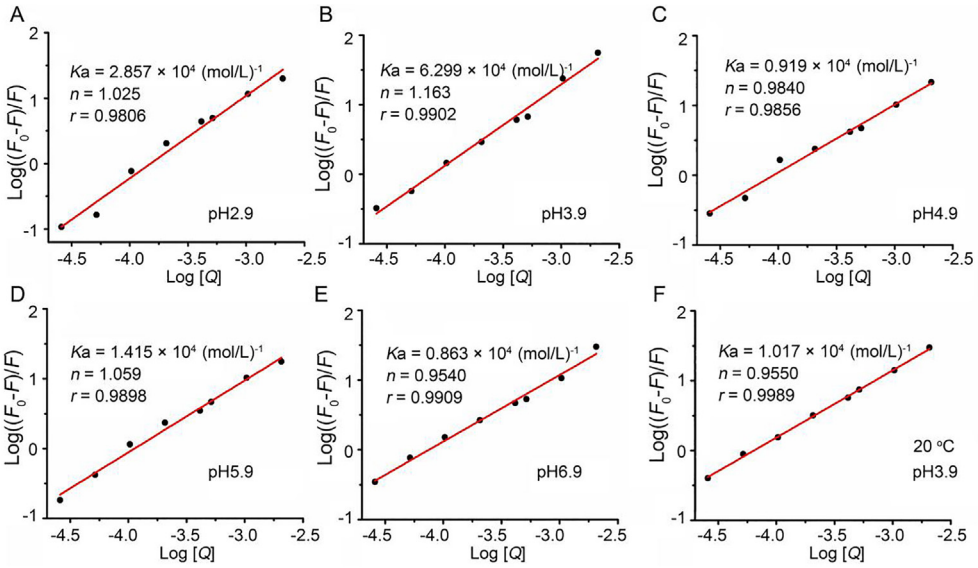

Fig. 3. Plot of Log [(F_0 - F)/F] *vs* Log [Q] for calculating the number of binding sites and the binding ability of β-casein in the tea polyphenols and β-casein system. A – E are the results at 30 °C, and F shows the results at pH 3.9 and 20 °C.

Table 1
Thermodynamic parameters of binding between tea polyphenols and β-casein.

Temperature/°C	pH	ΔG/(KJ·moL^{-1})
20	3.9	−22.489
30	2.9	−25.859
30	3.9	−27.852
30	4.9	−23.000
30	5.9	−24.089
30	6.9	−22.842

In brief, the bound water around proteins efflux and most of the proteins were detached from the saliva, and the polyphenols combinated 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 β-casein and tea polyphenol complexes during static quenching indicated that the number of β-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 Δλ = 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 β-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 β-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 β-casein system may be stable at pH 4.9 or 5.9. Combined with the fluorescence quenching results, the combination of tea polyphenols and β-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

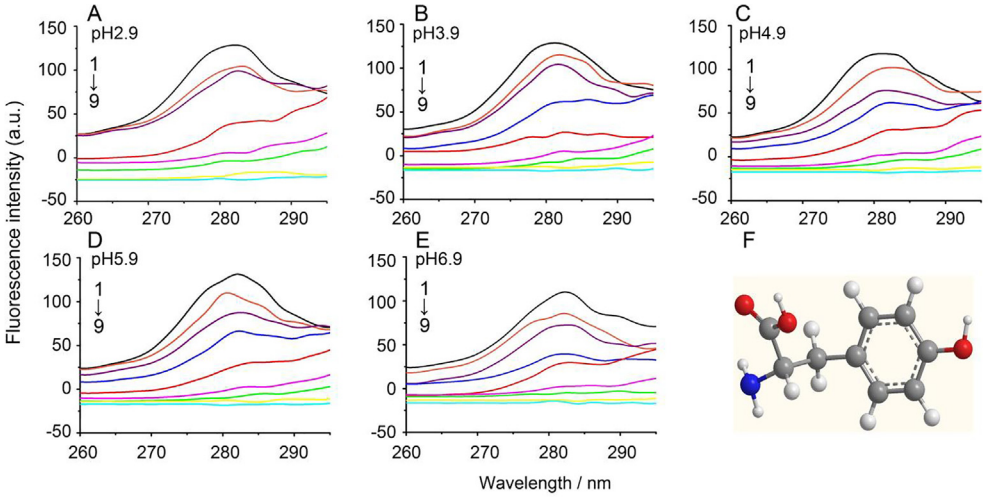

Fig. 4. Synchronous fluorescence spectrogram (Δλ = 15 nm) of β-casein and tea polyphenols reaction system at different pH. Nine curves (1–9) represent the tea polyphenols concentration of 0, 0.02586, 0.05171, 0.1034, 0.2068, 0.4137, 0.5171, 1.034, and 2.068 mmoL from top to bottom. F shows the structure of tyrosine.

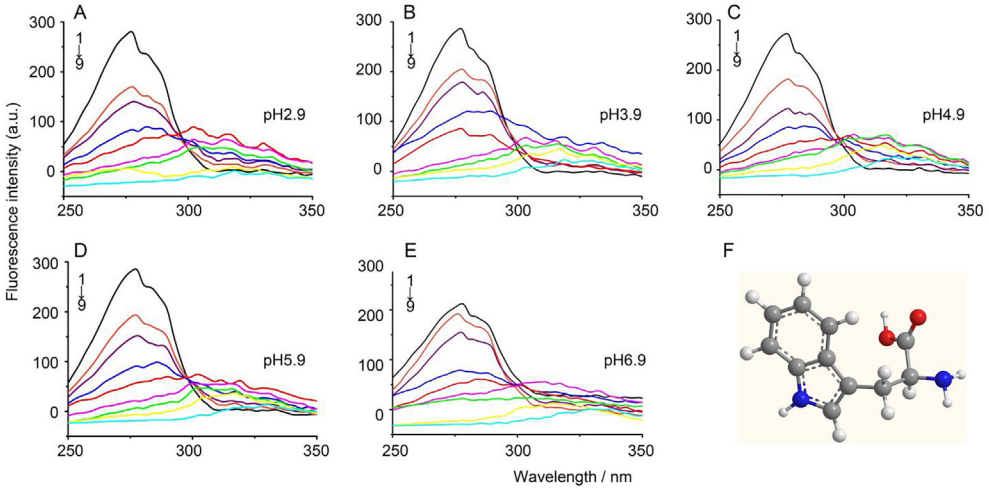

Fig. 5. Synchronous fluorescence spectrogram (Δλ = 60 nm) of β-casein and tea polyphenols reaction system at different pH. Nine curves (1–9) represent the tea polyphenols concentration of 0, 0.02586, 0.05171, 0.1034, 0.2068, 0.4137, 0.5171, 1.034, and 2.068 mmoL from top to bottom. F showed the structure of tryptophan.

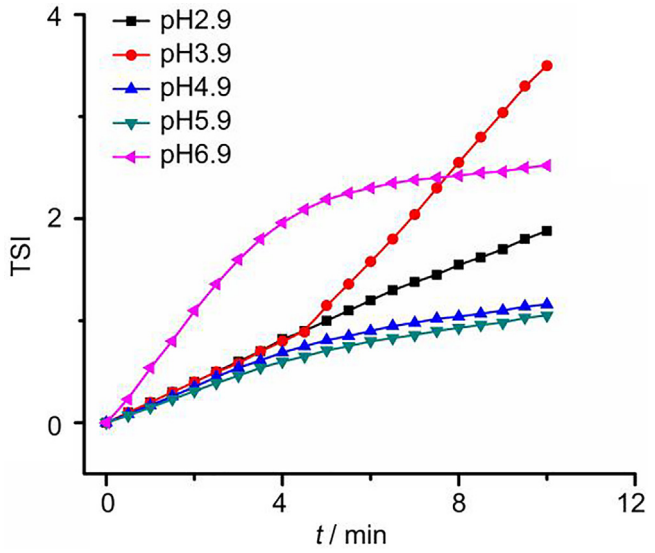

Fig. 6. Stability of tea polyphenols and β-casein complexes at different pH at 30 °C. TSI indicates Turbiscan Stability Index (TSI). A larger TSI value indicates a more unstable system.

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,

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

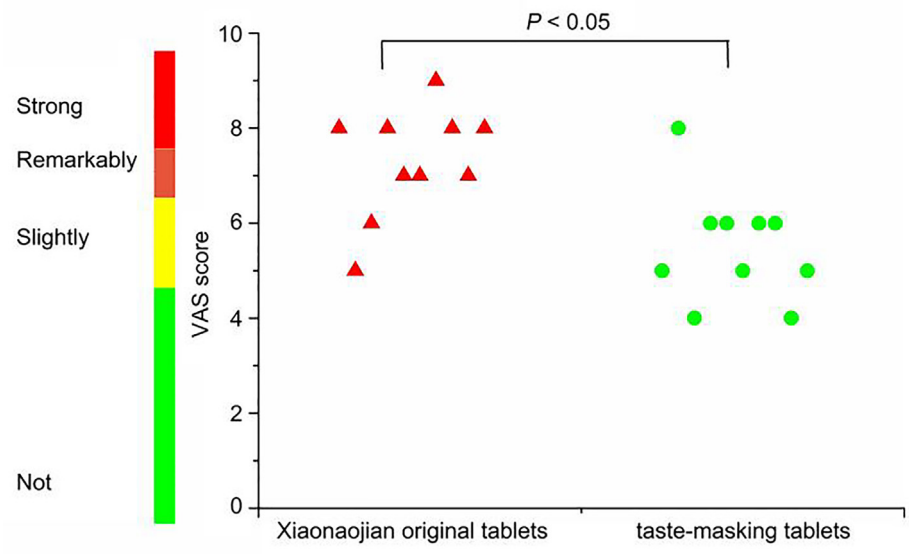

Fig. 7. Meaning of VAS and ten volunteers' astringency scores of two tablets. VAS indicates visual analogue scale.

Table 3
Standard curve of three components of tea polyphenols.

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

2019; He., 2017; Martin & Christian, 2013; Zhou., 2019). 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.

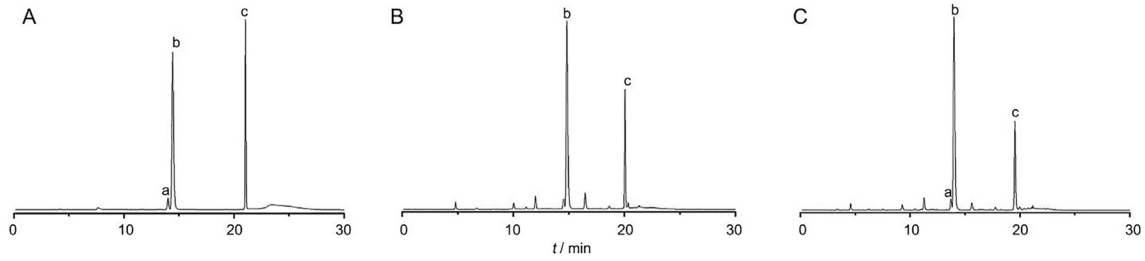

Fig. 8. Typical liquid chromatography of mix of standard samples (A), Xinnaojian taste-masking tablets (B), and Xinnaojian tablets (C). (a) EC; (b) EGCG; (c) ECG.

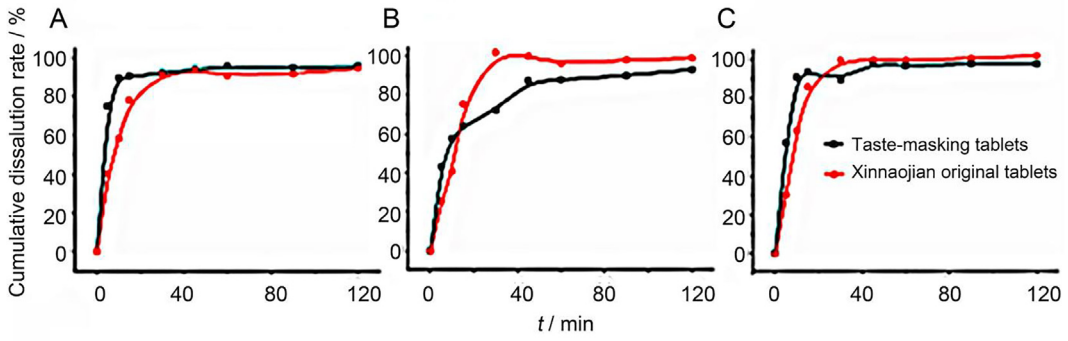

Fig. 9. Cumulative dissolution rate of Xinnaojian Taste-masking and Original Tablets. A: EC, B: EGCG, C: ECG.

However, in this paper, the obtained scores of astringency were somewhat objective because of the individuals' different acceptance and sensitivity to astringency. To make the results more convincing, an electronic tongue should be used in future experiments. Regulating pH helped to obtain a low astringency 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.

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