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Effect of *Lonicera edulis* polysaccharide on reducing oral dyeing of *lonicera edulis* juice

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Abstract

Fluorescence spectroscopy, particle size determination, and potential analysis were exploited to elucidate the effect of *Lonicera edulis* polysaccharide on polyphenol protein. The results revealed that *Lonicera edulis* polysaccharides mediated the binding of polyphenols and proteins through competition and formation of ternary complexes and were also able to enhance the stability of the polyphenol-protein complex solution system. A certain electrostatic effect was also present in the process simultaneously. As confirmed by the dyeing test, to improve oral dyeing, the optimum conditions of adding polysaccharide, pectin, and casein were as follows: the dosage of the polysaccharide group was 1.2 mg/mL, coloring time was 100 min, pH value was 4.0. Pectin group added 0.8 mg/mL with coloring time 80 min, pH 5.0. The addition of casein was 1.2 mg/mL; the coloring time was 100 min with pH 5.0. The sample juice substantiated a significant improvement in the dyeing of porcine tongue mucosa. Under the optimal conditions, microscopic observation validates that the mucosal color of the porcine tongue epidermis was closer to that of unstained porcine tongue epidermis, which significantly improved astringency and oral staining.

Keywords: Lonicera edulis polysaccharide, Polyphenols, Protein, Coloring

Introduction

Lonicera caerulea (*Lonicera caerulea* L.) is also known as the mountain eggplant. The fruit is soft and juicy but difficult to store [1]; squeezing the juice is an important way for the deep processing of *Lonicera edulis* [2]. However, the interaction of the polyphenols in *Lonicera edulis* fruit juice with salivary proteins in the oral cavity form a precipitate that results in astringency, and the system is turbid, which has a significant impact on oral astringency and staining [3–6]. Non-covalent bonds [7] (H bonds, electrostatic interactions) and covalent bonds primarily affect the interaction between proteins and polyphenols. Covalent bonds are mainly based on the oxidation of proteins or polyphenols through enzymatic or non-enzymatic pathways [8]. Studies have shown that [9, 10], astringency can be reduced by polysaccharides.

The impact of these polysaccharides on the interaction of polyphenols and proteins ameliorates the pigmentation caused by the combination of fruit juice pigments and oral mucosa, which further helps to improve the taste of fruit juices [11, 12]. Existing literature supports several research methods to explore the interaction of polyphenols and proteins, including molecular docking technology [13], fluorescence spectroscopy [14–16], isothermal titration calorimetry [17, 18], etc. These methods are not only conducive to investigating small molecule compounds, the mechanism of action but also provide the platform to amend the properties and detection of polyphenol-protein conjugates. In this study, the effect and mode of action of polysaccharides on polyphenol-protein were ascertained by fluorescence spectroscopy combined with particle size and potential analysis. The pig tongue epidermal mucosa was employed to simulate the human oral epidermal mucosal environment, compared with polysaccharides, pectin, and casein were supplemented to *Lonicera edulis* juice, respectively. The effect of different addition amounts, coloring time, and pH value were

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analyzed on the dyeing effect and the effect on the juice drinking process. The staining of tongue mucosa caused by the interaction of tannin-saliva protein was ameliorated so as to reduce the coloring ability of *Lonicera edulis* juice, reduce astringency, improve juice quality, and provide a theoretical basis to enrich the production quality of juice products.

Materials and methods

Materials and reagents

Lonicera fruit polysaccharide laboratory preparation; XAD-7 macroporous resin Beijing Boao Tuoda Technology Co., Ltd.; Glucose Tianjin No. 1 Chemical Reagent Factory; Citric Acid Tianjin Kemeou Chemical Reagent Co., Ltd.; Hydrochloric Acid Tianjin Fuchen Chemical Reagent Factory; Mucin Shanghai Yuanye Biotechnology Co., Ltd.; Pig Tongue, Chaoyang Market, Harbin City; Saline Sichuan Kelun Pharmaceutical Co., Ltd.; Sodium Azide Tianjin Kemeou Chemical Reagent Co., Ltd.; Pectin Henan Wanbang Industrial Co., Ltd.; Casein Anhui Dongxin Food Ingredients Co., Ltd.

Instruments and equipment

JYL-D051 Joyoung Cooking Machine Joyoung Co., Ltd.; R-205 rotary evaporator Shanghai Shensheng Biotechnology Co., Ltd.; ZDF-6020 vacuum drying oven Shanghai Yiheng Scientific Instrument Co., Ltd.; UV-5200 ultraviolet–visible spectrophotometer Shanghai Yuanxi Instrument Co., Ltd.; RF-5301PC Fluorescence Spectrometer Shimadzu; pH Meter Tianjin Test Instrument Co., Ltd.; ESJ180-4 Electronic Balance Shanghai Guangzheng Medical Instrument Co., Ltd.; BCD-216 T XZ Refrigerator Shanghai Shensheng Biotechnology Co., Ltd.; Colorimeter Zhengzhou Great Wall Technology Industry and Trade Co., Ltd.; Microscope Shanghai Yiheng Scientific Instrument Co., Ltd.

Method

Preparation of Lonicera edulis polysaccharide

The *Lonicera edulis* powder was obtained by drying the *Lonicera edulis* jelly in a vacuum oven (55 °C)*Lonicera fructus* polysaccharides were extracted by microwave-assisted compound enzyme method, decolorized and deproteinized, then dialyzed, alcohol precipitation, freeze-dried to obtain crude polysaccharides. These were then passed through DEAE-52 cellulose column chromatography, dextran gel Sephadex G-200 column layer for analytical fractionation and purification to finally obtain *Lonicera fructus* polysaccharide [19].

Extraction of Lonicera edulis polyphenols

The method of Li et al.[20] was followed with slight modification. After homogenization of *Lonicera edulis* by ultrasonic-assisted extraction, a crude extract of *Lonicera edulis* polyphenols was extracted, followed by purification with XAD-7 type macroporous resin. The resulting filtrates were combined and freeze-dried to acquire the *Lonicera edulis* polyphenol sample [21].

Preparation of juice

Lonicera edulis polyphenol (5 mg/mL) was dissolved in the simulated fruit juice (SF) containing glucose 50 mM, citric acid 25 mM, hydrogen phosphate diamine 25 mM. The pH was adjusted to 3.5 by concentrated hydrochloric acid.

Lonicera edulis fruit juice (LF): *Lonicera edulis* fruit was pressed to obtain the puree of *Lonicera edulis*, which was diluted 20 times after suction filtration for use.

Extraction of salivary protein

Four healthy and non-smokers volunteers (male to female ratio 1:1) were selected and fasted for 2 h. Thereafter, saliva was collected between 10 to 11 o'clock, mixed, and centrifuged at 10,000 r/min for 10 min. The supernatant contained the salivary protein sample (SP).

Fluorescence spectrum measurement

Different concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 mg/mL *Lonicera fructus* polysaccharide\crude polysaccharides were added to the simulated juice, *Lonicera edulis* juice and mucin compound solution (the pH was adjusted to 6 in the acetic acid buffer solution to be close to 6). Mimicking the oral environment, the reaction was continued for 10 min at room temperature. At the excitation wavelength of 340 nm, the excitation and emission slit widths were 5 nm and 10 nm, respectively, and the measurement was conducted within the emission wavelength of 285 to 450 nm [22].

Particle size measurement

Using the principle of irregular Brownian motion of particles in the solution, dynamic light scattering technology was adopted to determine the particle size through different diffusion speeds of the particles in the solution [23]. The particle size was studies for the polysaccharide concentration of 0, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60 mg/mL p-polyphenol (0.50 mg/mL)-mucin (2.00 mg/mL) compound solution (pH adjusted to 6.0). For the influence of the size and dispersion coefficient of PDI, testing of all the samples was repeated thrice at 25 °C and 173° scattering angle.

ζ-potential analysis

ζ-potential under the action of an external electric field, the frequency or phase of the speed of light is affected by the directional movement of charged particles, and

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then the speed of ion electrophoresis is estimated [24]. A laser particle size analyzer elucidated the influence of the polysaccharide concentration of 0, 0.60, 0.80, 1.00, 1.20, 1.40, and 1.60 mg/mL on the potential of the polyphenol-mucin composite solution (pH adjusted to 6.0). The experiment was repeated at 25 °C for 3 times.

Effect of polysaccharide on polyphenol-protein in juice by SDS-PAGE

To prepare Lonicera edulis polysaccharide compound solution, crude polysaccharide, simulated juice, and Lonicera edulis juice, after 5 min of reacting with saliva protein at a ratio of 3:1 in a 37 °C water bath, were centrifuged at 10,000 r/min for 5 min, and the supernatant sample was mixed with $2 \times$ electrophoresis buffer and boiled for 5 min. Marker (4 μL) and sample (15 μL) were loaded from left to right, with the separation gel concentration of 12%, the concentration gel concentration of 5%, and the current was 30 mA. After entering the separation gel, the current was reduced to 60 mA. The gel plate was immersed in the dyeing solution for 5–10 min before being rinsed several times with distilled water and decolorized with the decolorizing solution. The gel image was captured using the gel imaging system.

Dyeing test

The fresh pig tongues with intact epidermal mucosa were washed and the sublingual meat from the tongue root to the tip of the tongue was removed. The peeled tongue mucosa was sliced into epidermal mucosa of the same shape and size and placed in 0.9% saline with a trace amount of sodium azide. The film was sealed and stored at 4 °C for future use.

This analysis was done by the CS-800 spectrophotometer. Initially, the black and white standard plates were used to calibrate the color difference meter. A layer of plastic wrap was then attached to the dyed pig tongue mucosa sample and placed at the light source for three parallel measurements. The instrument displaying the value of (L*, a*, b*) was recorded.

Where L* denotes brightness, the scale is from 0 to 100 (black to white), ΔL* is the difference in brightness (L) between the tested sample and the original juice dyed sample, the higher the ΔL value the higher the brightness; a* represents the saturation of red and green value, Δa is the difference of a value between the measured object and the standard whiteboard, the redness in the $+\Delta a$ direction is strengthened, while the greenness in the-Δa direction is strengthened, and zero indicates gray; b* signifies the saturation of the yellow-blue value, and the Δb value is the difference in b value between the measured object and the standard whiteboard, the yellowness increases in the $+\Delta b$ direction, whereas the blueness

increases in the-Δb direction and zero represents gray; respectively for L*, a*, b*, and △E*ab dyeing effect color difference analysis, where the color difference calculation formula is:

$$\Delta E*_{ab} = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{1}$$

The effect of addition amount on color difference

Polysaccharide, pectin, and casein of 0, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mg/mL were added to the *Lonicera edulis* juice. The dyeing treatment was performed under the conditions of pH 5 for 60 min. The juice staining without any ingredients served as a control. The L*, a*, b* values were measured and the △E*ab value computed.

The effect of coloring time on color difference

The stained pig tongue mucosa was placed in saliva for 20, 30, 40, 60, 90, 120 min, for staining. The juice staining without polysaccharides was considered as the control, the L*, a*, and b* values were measured, and the △E*ab value was calculated.

$$\Delta E*ab = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2} \tag{2}$$

In the formula: ΔL* represents the difference between the brightness value and the initial brightness value, Δa* designates the difference between the redness value and the initial redness value, and Δb* denotes the difference between the yellowness value and the initial yellowness value.

The effect of pH on color difference

The dyeing treatment was continued under the following pH conditions: 3.5, 4, 4.5, 5, 5.5, and 6. The juice staining without any ingredients was the control. The △E*ab was calculated by measuring the L*, a*, b* values.

Microscope analysis

The pig tongue mucosa was dyed in *Lonicera edulis* juice with polysaccharides, pectin, and casein according to the best conditions in the color difference analysis. The staining phenomenon of the pig tongue mucosal surface was then observed under a 40-fold microscope. The effect of soaking in the saliva is compared to determine its coloring ability [25].

Sensory evaluation

According to the sensory evaluation method, a sensory evaluation team was formed (10 men and women, half each), and astringency, bitterness, and sourness were distinguished by 3.0 g/L tannin, 0.1 g/L quinine, and 4.0 g/L tartaric acid solutions. The intensity range and sensory

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methods of each attribute were introduced, the fruit juice tasters were trained, tannins were used to configure the simulated juice with different astringency gradients of 0–2.0 g/L, and the members defined the strength of the astringency and repeatedly located it. In a normal temperature environment (18 ± 2) °C, the tasting reviews poured all the juice (10 mL) into the mouth, held it for 8 s, and then spitted it out, waited for about 4 min, and recorded the astringency intensity (1–10 min). This was repeated twice. Subsequently, the mouth was rinsed with deionized water, and soda biscuits were chewed to restore the sense of taste. The samples of each group were separated by 10 min.

Data statistics and analysis
All experiments were executed in parallel three times, and the results were expressed as $\pm$ standard deviation. The Origin 2018 software was adopted for graphing.

Results and analysis
Analysis of the effect of *Lonicera edulis* polysaccharide on polyphenol–protein in fruit juice
The influence of Lonicera edulis polysaccharide on the fluorescence intensity of polyphenol–protein interaction
Fluorescence spectroscopy helps to assess the type of fluorescence quenching resulting from the interaction between polyphenols and proteins, determine their binding sites and forces [26], and estimate their number of binding sites and binding constants. Finally, information such as protein structure changes is qualitatively analyzed. Mucin being the main protein component of saliva protein, it was selected for subsequent experiments. As evident from Fig. 1(A), the fluorescence spectrum peak of the polyphenol-myxoprotein complex solution was obtained at 340 nm, while the polysaccharide of *Lonicera edulis* manifested no peak. This rationalized the insignificant effect of polysaccharides on the fluorescence quenching of subsequent experiments.
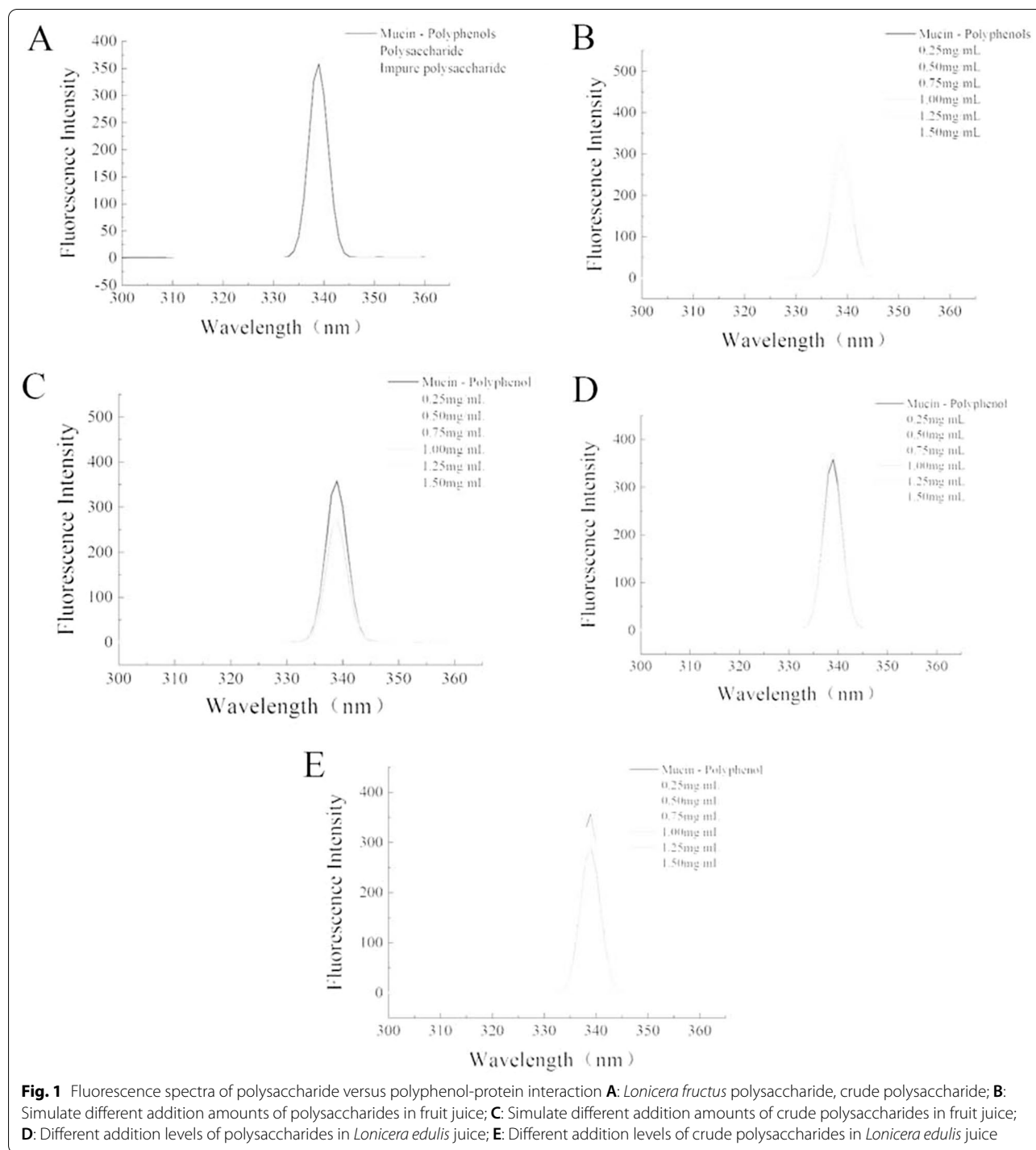
Figure 1(B) and Fig. 2(A) confirmed enhanced fluorescence quenching intensity of polyphenols-myxoprotein with the increase in the addition amount, suggesting that the low content of *Lonicera edulis* polysaccharide and myxoprotein are competitive ways to combine *Lonicera edulis* polyphenols. A continued increase in the addition of *Lonicera edulis* polysaccharide upto1.5 mg/mL witnessed a decline in the fluorescence intensity and relative fluorescence intensity, which indicated partial reaction between *Lonicera edulis* polysaccharide and *Lonicera edulis* polyphenol-myxoprotein to form *Lonicera edulis* polyphenol-myxoprotein-*Lonicera edulis* polysaccharide ternary complex [27, 28]. An obvious red-shift phenomenon noted in the characteristic emission peaks proposed that the polysaccharide of *Lonicera edulis* quenched the endogenous fluorescence of polyphenol-myosin at this time, resulting in the exposure of viscous protein chromogenic group to the hydrophilic environment and the expansion of tertiary structure [29]. Figure 1(C) and Fig. 2(B) claimed that the crude polysaccharide and purified polysaccharide of *Lonicera edulis* demonstrated the same action pathway, mainly through competition and ternary complex pathway affecting the polyphenol-mucin interaction of *Lonicera edulis*, so as to minimize the astringency and reduce the effect of *Lonicera edulis* juice on oral staining.

Figure 1(D) and Fig. 2(C) substantiated the escalation in the fluorescence quenching intensity of polyphenol-myxoprotein with the increase of the amount of *Lonicera edulis* polysaccharide added in the complex environment of *Lonicera edulis* juice. Meanwhile, an increase in the amount of *Lonicera edulis* polysaccharide reduced the relative fluorescence intensity. There were two interaction pathways. Consistent with the trend in simulated juice, a weak red-shift was also observed when the addition of polysaccharides reached 1.5 mg/mL. Comparable effects of both crude and purified polysaccharides were illustrated in Fig. 1(E) and Fig. 2(D). However, a better effect of the purified polysaccharides on the polyphenol-mucin interaction was obtained, and the polysaccharides could reach the most in the simulated fruit juice. This finding was in accordance with that of Delius et al. [30].

The influence of polysaccharides on the particle size of polyphenol–protein interaction
The particle size of the polyphenol-mucin composite solution was 235.2 nm without adding any polysaccharide, as evident from Fig. 3. A gradual increase in the particle size was observed with a concentration-dependent increase in the polysaccharide. Nonetheless, with low polysaccharide concentration, the increase in particle size was not obvious. The particle size of the composite solution increased when polysaccharides and polyphenol-mucin complexes or polyphenols formed a large grid structure through "bridging action". A continued increase in the polysaccharide manifested a large increase in the particle size of the composite solution, which may be attributed to the formation of a ternary complex between the polysaccharide and polyphenol-mucin and the thickening of the polysaccharide wall in the outer layer, which enhanced the particle size. Figure 4 confirmed a decrease in the PDI value with the increase of the concentration of *Lonicera fructus*, indicating non-uniform dispersion of the polyphenol-mucin composite solution system. The addition of polysaccharides facilitated the uniform distribution of the solution system by inhibiting the
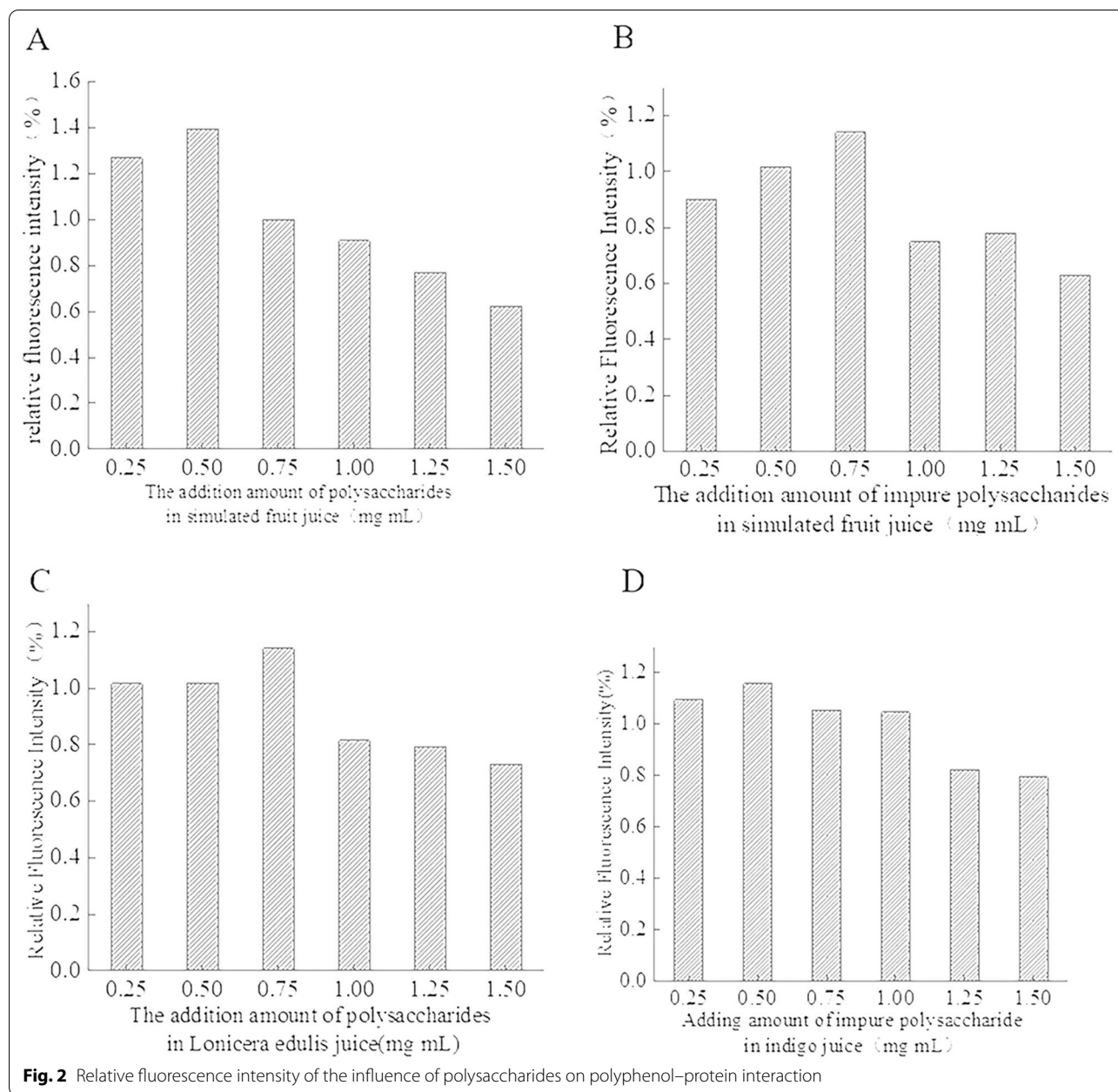
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Fig. 1 Fluorescence spectra of polysaccharide versus polyphenol-protein interaction **A**: *Lonicera fructus* polysaccharide, crude polysaccharide; **B**: Simulate different addition amounts of polysaccharides in fruit juice; **C**: Simulate different addition amounts of crude polysaccharides in fruit juice; **D**: Different addition levels of polysaccharides in *Lonicera edulis* juice; **E**: Different addition levels of crude polysaccharides in *Lonicera edulis* juice

polyphenol-mucin. The combination further stabilized the system.

ζ-*potential analysis of polysaccharide–protein interaction*
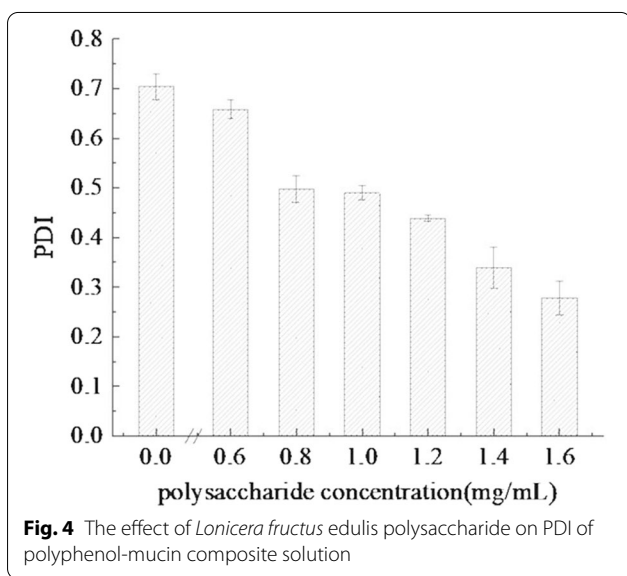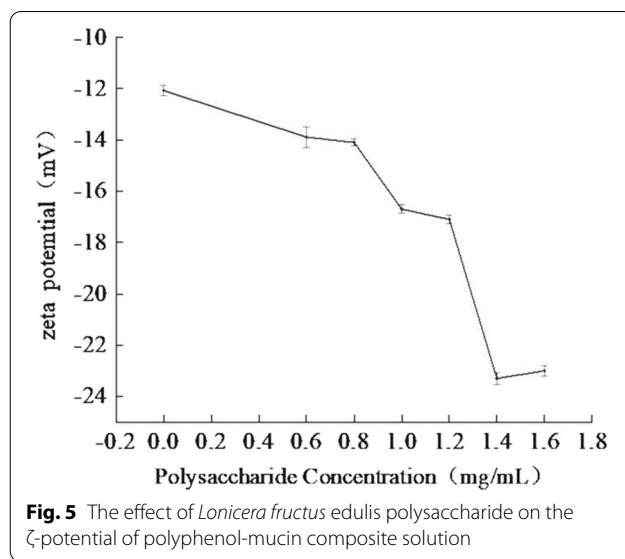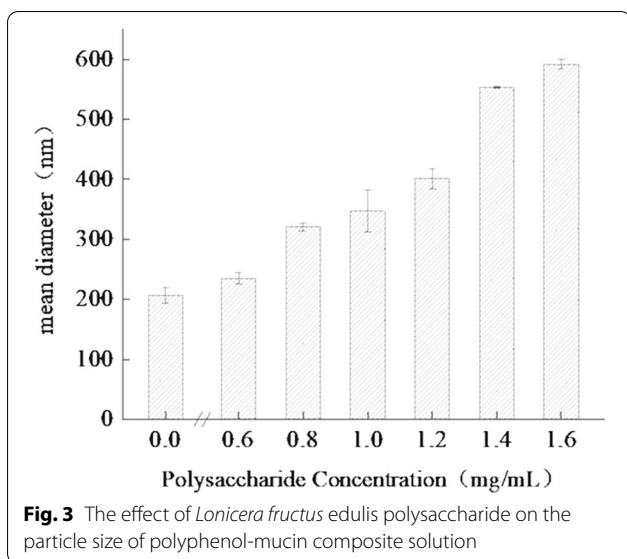Zeta potential evaluates the strength of repulsion and attraction between molecules in the system, reflects

the electrostatic force of different polysaccharide concentrations on the surface of polyphenol-mucin, and judges the dispersion stability of the system [31]. According to Fig. 5, a downward trend was revealed in the potential value of the composite solution with the

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Fig. 2 Relative fluorescence intensity of the influence of polysaccharides on polyphenol–protein interaction

increase in the concentration of polysaccharides. The synergistic effect of polysaccharide and polyphenol-protein complex might contribute to the significant decrease in the potential value. This indicated the relative stability of the *Lonicera fructus* polysaccharide and polyphenol-protein composite solution, deprotonation of some of the amino groups on the surface of the composite, a higher negative charge density of the oxygen center, and presence of electrostatic repulsion, which demonstrated a higher absolute value of the potential.

Analysis of SDS-PAGE results

The effect of polysaccharides on polyphenol–protein interaction was evaluated using molecular weight and spatial conformation based on the combination of protein and polyphenols or polysaccharides. The composite solution was centrifuged to separate and remove the precipitated protein. Therefore, the deeper the color of the electrophoresis strip, the less astringent the juice. ImageJ software was used to calculate the gray value by analyzing the intensity of the corresponding strip, as shown in Fig. 6A. Figure 6B shows
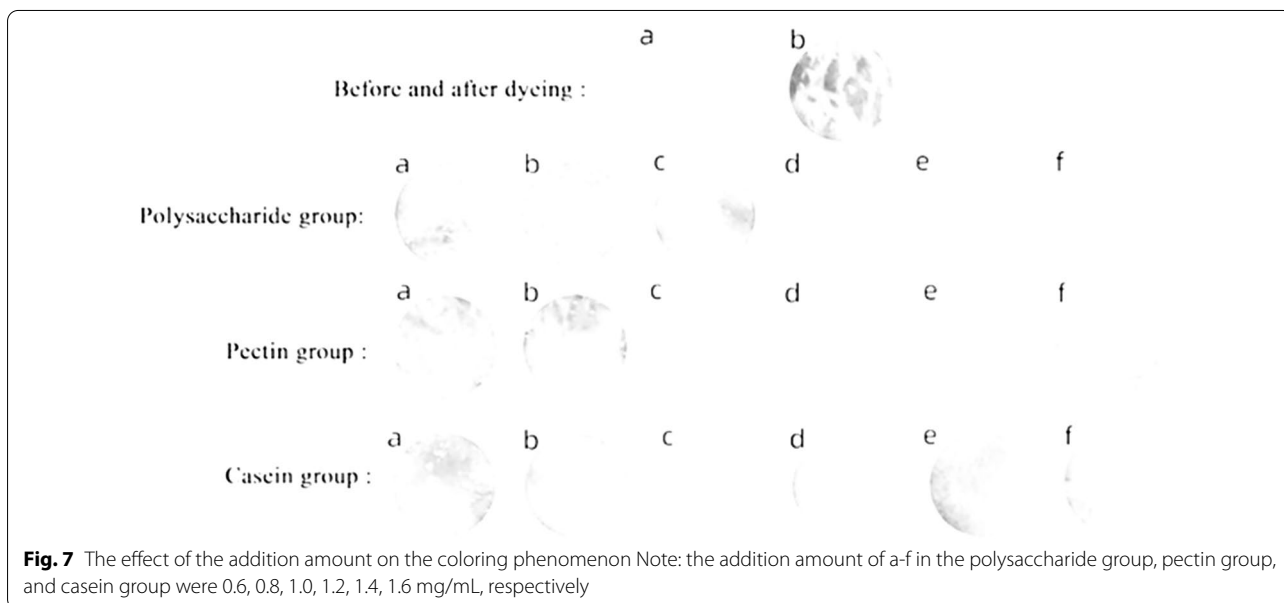
Fig. 3 The effect of *Lonicera fructus* edulis polysaccharide on the particle size of polyphenol-mucin composite solution

Fig. 5 The effect of *Lonicera fructus* edulis polysaccharide on the ζ-potential of polyphenol-mucin composite solution

Fig. 4 The effect of *Lonicera fructus* edulis polysaccharide on PDI of polyphenol-mucin composite solution

that the color of electrophoresis strips in the mixture of simulated juice and salivary protein is lighter when compared to salivary protein, indicating that the indigo polyphenols react with mucin to precipitate some proteins. However, in the polysaccharide-simulating juice-sialoprotein mixed system, the gray value of the electrophoresis band increased, indicating that the addition of polysaccharides influenced the combination of polyphenols and mucins, reducing juice astringency, and the purified polysaccharides had more obvious indigenous effects. Polysaccharides had a reduced effect on the polyphenol–protein system in the indigo juice environment. The main bands
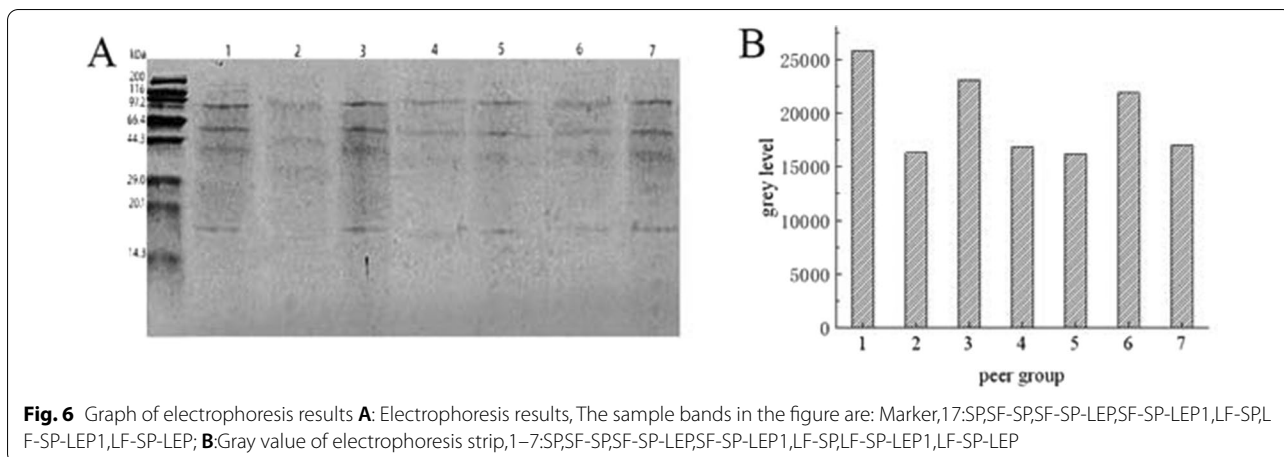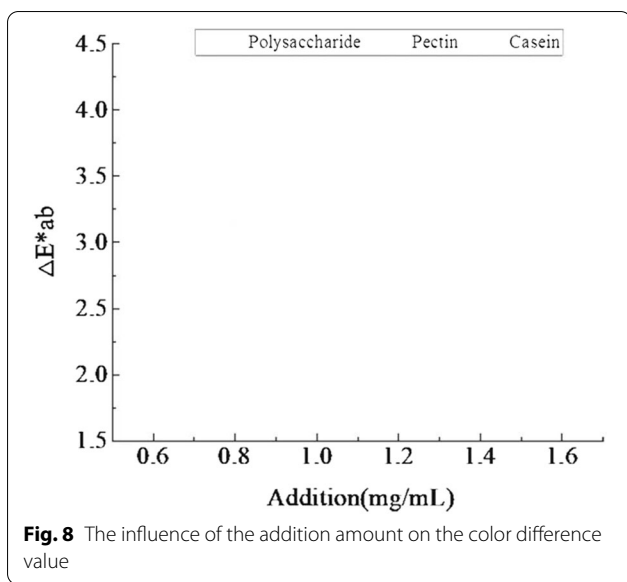
were weakened and the diffuse reflection phenomenon appeared in the polysaccharide–polyphenol–protein mixture system, indicating that a large amount of sodium dodecyl sulfate (SDS) was used in the electrophoresis process, which had the ability to destroy the non-covalent bond between molecules. It is possible to demonstrate that the polysaccharide-protein–polyphenol system complex exists as a covalent bond.

Analysis of dyeing test results
The influence of the addition amount on the chromatic aberration

Figure 7 highlighted reduction in the dyeing effect of pig tongue to varying degrees with the addition of polysaccharides, pectin, and casein. Table 1 details the strong coloring ability of *Lonicera edulis* fruit juice. With the increase of the amount of addition, the $\triangle$L* brightness change value of the three groups increased compared with the original fruit juice dyeing, indicating that the addition of polysaccharides, pectin, and casein was able to improve the staining of tongue mucosa. Figure 8 revealed that the largest brightness change value was obtained when the addition amount of the polysaccharide group reached 1.2 mg/mL. At this time, the color of the sample was brighter red and blue. When the addition amount was 1.2–1.6 mg/mL, the brightness change value decreased; when the addition amount of the pectin group reached 0.8 mg/mL, the brightness change value was the largest. At this time, the color of the sample was brighter red. When the addition amount exceeded 0.8 mg/mL, the brightness change value reduced, and the trend was flat. The casein group demonstrated the largest brightness change value when the addition amount was 1.2 mg/mL.

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Fig. 6 Graph of electrophoresis results **A**: Electrophoresis results, The sample bands in the figure are: Marker,17:SP,SF-SP,SF-SP-LEP,SF-SP-LEP1,LF-SP,LF-SP-LEP1,LF-SP-LEP; **B**:Gray value of electrophoresis strip,1–7:SP,SF-SP,SF-SP-LEP,SF-SP-LEP1,LF-SP,LF-SP-LEP1,LF-SP-LEP

Fig. 7 The effect of the addition amount on the coloring phenomenon Note: the addition amount of a-f in the polysaccharide group, pectin group, and casein group were 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 mg/mL, respectively

Table 1 The influence of the addition amount on the coloring phenomenon

Add amountmg/mL	Polysaccharide group			Pectin group			Casein group		
	$\triangle L^*$	$\triangle a^*$	$\triangle b^*$	$\triangle L^*$	$\triangle a^*$	$\triangle b^*$	$\triangle L^*$	$\triangle a^*$	$\triangle b^*$
0.6	2.12	5.46	− 0.13	2.08	5.41	− 0.23	2.12	3.14	− 1.75
0.8	1.14	4.99	− 0.53	3.5	1.53	− 1.01	2.85	2.55	− 1.82
1.0	3.16	4.16	− 1.14	2.35	2.17	− 2.42	3.22	− 1.92	− 1.93
1.2	3.27	1.35	− 1.03	2.57	− 2.31	− 2.56	3.25	− 0.89	− 1.83
1.4	3.18	− 1.55	− 1.19	3.22	− 1.93	− 1.93	2.19	− 2.32	− 2.13
1.6	3.11	2.02	− 1.08	3.36	− 2.2	− 1.38	2.18	− 2.33	− 2.54

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Fig. 8 The influence of the addition amount on the color difference value

summarizes the influence of coloring time on the coloring phenomenon. When the coloring time was 40 min, the color of the sample was darker. With the increase in the coloring time, when it reached 100 min, the maximum brightness change value of the polysaccharide group was 2.27. At this time, the sample color was brighter to greenish-blue. After exceeding 100 min, the brightness change value gradually reduced to 1.74. This may be explained by the precipitation of pigment or salivary protein-polyphenols that dissolved in saliva with the soaking time of pig tongue mucosa for too long. The pectin group revealed an initial decline and then an upward trend with the increase of the coloring time. The brightness change reached the maximum value of 2.35 when the coloring time was 80 min. At this time, the color of the sample was brighter blue. Subsequently, the brightness change value decreased to 1.83 when the coloring time was 140 min. The maximum brightness change reached 2.25 for the casein group when the coloring time was 100 min. The color of the sample was slightly brighter green. When the coloring time was 100–140 min, the brightness change value gradually lowered to 1.78. In summary, the optimal coloring time was 100 min for polysaccharides; 80 min for the pectin group; and 100 min for the casein group for the follow-up experiments (Fig. 10).

At this time, the color of the sample was brighter. When the addition amount exceeded 0.8 mg/mL, the brightness change value decreased. The difference was small when the addition amount was 0.8 ~ 1.2 mg/mL. To sum up, for follow-up experiments, 1.2 mg/mL polysaccharide, 0.8 mg/mL pectin, and 1.2 mg/mL casein were selected.

The effect of coloring time on color difference
Gradual decline in the coloring ability of the juice with an increase in the coloring time (Fig. 9) suggested amelioration of the phenomenon of pigmentation and precipitation resulting from the supplementation of polysaccharides, pectin, and casein to the juice. Table 2

The influence of pH on color difference
As evident from Fig. 11, the coloring ability of the three groups of pig tongue mucosa was much different from that before staining, indicating that the adjustment of pH facilitated significant improvement in the juice coloring ability and effective attenuation of the interaction between polyphenols and salivary proteins. Table 3

Fig. 9 The effect of coloring time on the coloring phenomenon The coloring time of polysaccharide group, pectin group, and casein group **a–f** were 40, 60, 80, 100, 120, 140 min, respectively

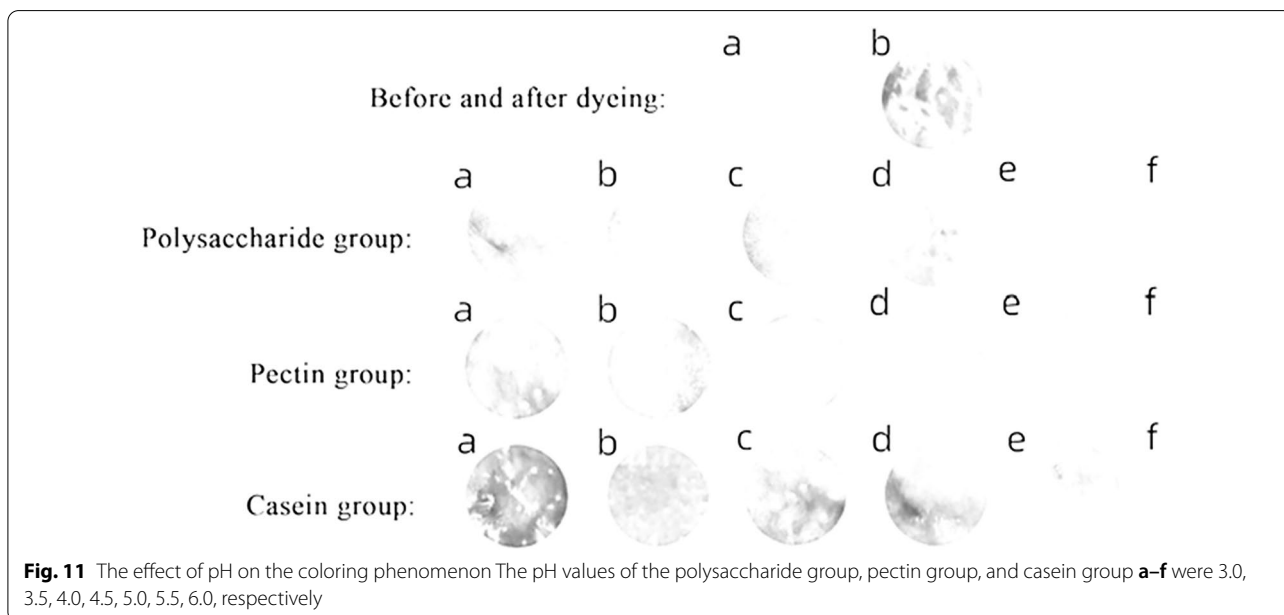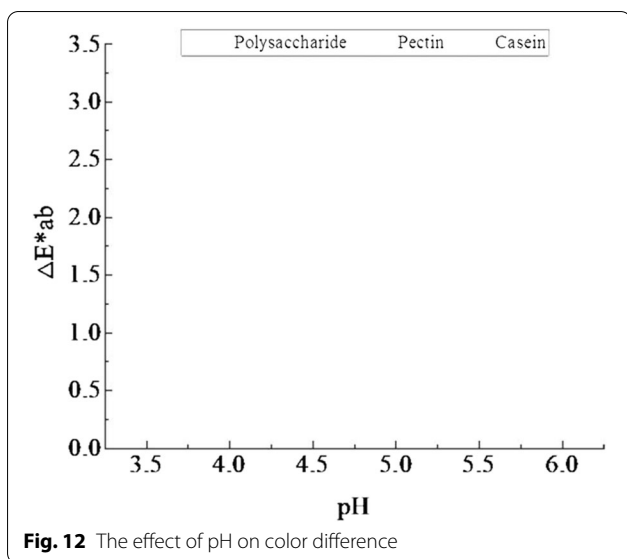**Fig. 10** The effect of coloring time on the color difference value
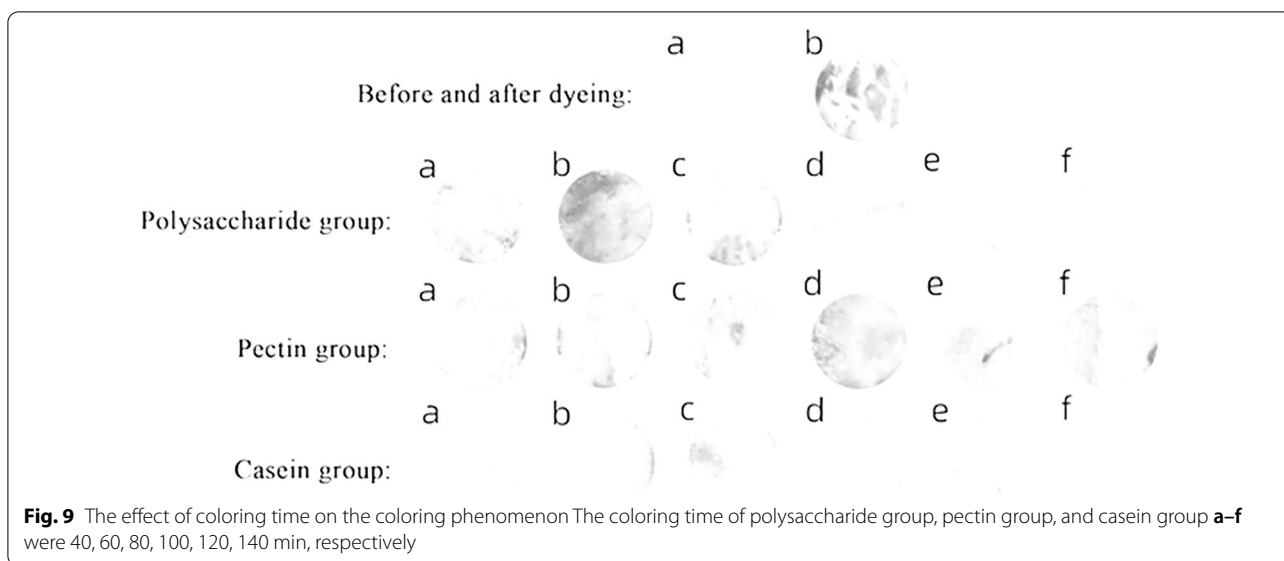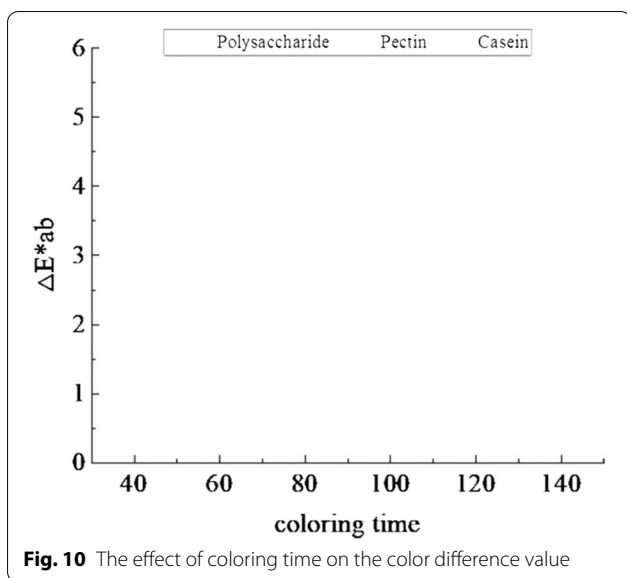
narrated the phenomenon of tongue coloration with the increase of pH. The brightness values of the polysaccharide group were 1.03, 1.04, and 1.52 at pH 4.0, 5.5, and 6.0, respectively, and the color of the sample was bright. In the case of the pectin group, the brightness value was first enhanced and then reduced with the increasing pH value. The maximum brightness change value was 0.21 at pH 5.0, and subsequently, the brightness of the sample decreased gently. The brightness value of the casein group became positive after pH 5.0. The increase in pH value led to the green color of porcine tongue mucosa staining. The continuous decrease of hydrogen ions affecting the changes of substances in the substrate juice might be responsible for this outcome. In conclusion, the optimum pH of the polysaccharide group was 4.0, the pectin group was 5.0, and the casein group was 6.0. The total color difference was the least at this time. Indigo juice had the lowest oral staining ability. This result

Table 2 The effect of coloring time on color difference

Coloring time (min)	Polysaccharide group			Pectin group			Casein group		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
40	0.12	2.46	− 1.13	0.08	2.41	− 0.23	0.1	3.14	− 1.75
60	0.14	2.99	− 0.53	0.25	2.53	− 1.01	0.45	2.55	− 1.82
80	1.16	1.16	− 2.14	2.35	0.17	− 1.42	1.85	− 1.92	− 1.93
100	2.27	− 0.98	− 0.73	2.17	− 2.31	− 2.56	2.25	− 0.89	− 0.83
120	1.78	− 1.55	− 1.19	2.02	− 1.93	− 1.93	1.59	− 2.32	− 2.13
140	1.74	1.02	− 2.08	1.86	− 2.2	− 1.38	1.78	− 2.33	− 2.54

Fig. 11 The effect of pH on the coloring phenomenon The pH values of the polysaccharide group, pectin group, and casein group **a–f** were 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, respectively

Fig. 12 The effect of pH on color difference

Fig.13 Microscopic analysis of porcine tongue mucosa before and after staining **A**: Pig tongue mucosa before staining; **B**: Dyeing of pig tongue juice and soaking in saliva; **C–E**: Juice dyeing of pig tongue mucosa with polysaccharide, pectin, and casein under optimum conditions; **F–H**: The pig tongue mucosa was dyed with polysaccharide, pectin, and casein juice under the optimum conditions, followed by soaking in saliva

is similar to that of Muhammad et al.' s study of browning during carrot juice processing, possibly due to changes in pH resulting in changes in enzyme activity (Fig. 12)[32].

Microscope analysis

Comparison of the tongue mucosa before and after staining portrayed a large difference based on the microscopic analysis. Figure 13B intuitively claimed that the original juice had a strong staining ability on the porcine tongue mucosa, and the polyphenols-sialoprotein interaction attached pigment in the juice precipitated on the porcine tongue mucosa. The coloration ability of the Fig. 13F, G, and H groups was significantly lower in comparison to that of the Fig. 13C, D, and E groups. Different from the post-treatment of saliva immersion, the lighter color may be due to the weakening of the combination of polyphenols and saliva protein in juice with polysaccharides, polyphenols, and casein, and a small amount of precipitation and pigment dissolution or shedding in saliva. The obvious impact of the polysaccharide on polyphenol–sialoprotein interaction in indigo juice was manifested

by the similarity of the F color to the color of pig tongue mucosa before staining.

Results of sensory evaluation

Table 4 delineated the sensory evaluation of the astringency of juice under the optimal conditions of three different components of polysaccharide, pectin, and casein. The intensity of astringency reduced from strong to weak

Table 3 The influence of pH on color difference

pH	Polysaccharide group			Pectin group			Casein group		
	△L*	△a*	△b*	△L*	△a*	△b*	△L*	△a*	△b*
3.5	0.33	1.98	0.36	− 0.96	0.45	0.33	− 1.49	1.67	0.73
4.0	1.03	0.08	− 1.02	− 0.82	0.38	0.41	− 1.35	− 1	− 1.02
4.5	0.85	1.78	0.26	− 0.58	0.71	− 0.58	− 1.01	2.37	− 1.64
5.0	0.86	2.01	− 0.4	0.21	0.18	− 0.03	0.92	1.58	− 0.11
5.5	1.04	− 1.09	0.26	0.16	− 0.87	− 0.51	0.89	− 1.57	1.02
6.0	1.52	− 2.16	− 0.18	0.14	− 0.36	0.26	1.38	− 0.71	− 0.74

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Table 4 Sensory evaluation results

Specimen	Astringency intensity
Raw juice	6.0
Polysaccharide in sugarcane leaves	1.9
Pectin group	2.6
Casein group	2.2

on supplementation of different substances to the juice. The order was: polysaccharide > casein > Pectin, the polysaccharide group exhibited the strongest astringency reduction effect.

Declarations

Ethical approval and consent to publication
Ethical approval was not required for this research.

Competing interests
All authors have no personal or financial conflicts interest.

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