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Research Article

Artificial Simulation of Saliva's Astringency Removal Effect on Squid

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Abstract

Astringency is a common issue in squid processing and consumption. Various techniques such as soaking in salt water, adding acids, and using enzymes have been used to eliminate astringency. However, these methods have their limitations, and the search for a better solution is ongoing. In recent years, ultrasound technology has been proposed as an effective method for removing astringency in squid. This study used four proteins, including lysozyme, bovine serum albumin, collagen, and whey protein, to simulate saliva in the human oral cavity. This study aimed to determine the removal effect of squid astringency after saliva soaking and ultrasound treatment. Physicochemical indicators such as polyphenols and flavonoids, antioxidant activity, relative polymerization degree, and solution zeta potential were used as physicochemical indicators. Sensory evaluation and volatile salt nitrogen content were used as quality indicators of squid. The results indicated that artificial simulated saliva treatment significantly reduces polyphenols and antioxidant activity in squid muscle, reduces the content of volatile base nitrogen, eliminates the astringency of squid, and improves the taste and overall quality of squid. The technique of using ultrasound technology and artificial simulated saliva is a scientific and effective method for removing astringency in squid. This method has several advantages over traditional methods, including being a non-invasive method that does not require adding any chemicals, making it an environmentally friendly solution. The use of ultrasound technology allows for removing astringency in a shorter time than traditional methods. Finally, the technique is cost-effective and easily scaled up for industrial applications. In conclusion, using ultrasound technology and artificial simulated saliva treatment is a promising method for removing astringency in squid. The technique effectively removes astringency in squid muscle, improves the taste and overall quality of the squid, and is a non-invasive, environmentally friendly, cost-effective solution that can be easily scaled up for industrial application.

Keywords

Polyphenols, Antioxidant Activity, Relative Degree of Polymerization, Zeta Potential, Metabolomics

1. Introduction

Astringency is a complex mixture of roughness, wrinkles, and dryness caused by the aggregation and precipitation of proteins in human oral saliva. There is yet to be a clear definition of the mechanism of convergence. The widely accepted theory is that specific proteins such as whey protein and mucin bind with polyphenolic substances [1] such as

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tannins, gallic acid, and flavonoids in the human oral cavity and produce soluble precipitates [2], which combine with glycoproteins to form insoluble macromolecular precipitates, reduce the smoothness of oral saliva, and through intermolecular forces and spontaneous aggregation of polyphenols, form larger verbal perceptible complexes [3].

Polyphenols are considered one of the main substances that produce astringent taste [4]. Studies have shown a linear correlation between polyphenol content and astringency perception [1]. Before reaching the critical value, the higher the polyphenol content, the stronger the astringency. Polyphenols have substantial free radical scavenging ability, namely antioxidant activity [5] and the intensity of antioxidant activity indicates the level of polyphenol content. The aggregation and sedimentation of proteins promote the production of astringency, and it is possible to estimate the relative degree of polymerization for phenolic substances. The larger the value, the greater the degree of polymerization of phenolic substances and the larger the molecular weight formed by polymerization [6].

The binding of polyphenols and salivary proteins affects the friction coefficient of saliva in the oral cavity, increasing the roughness and astringency of the oral cavity [7]. The increase in roughness indicates the formation of more or larger insoluble precipitates, namely an increase in solution turbidity and sediment particle size. The interaction between polyphenols and proteins in solution includes covalent hydrophobic interactions, hydrogen bonding, complexation reactions, covalent bonding, and changes in the net charge of protein molecules [8]. Among them, the zeta potential magnitude reflects the interaction of the charges [9].

The converging agents used in this experiment were a mixture of lysozyme rich in alkaline amino acids [10], bovine serum albumin containing 583 amino acid residues, collagen-rich in proline whey protein [11], and five proteins related to convergence to simulate human oral saliva, as well as to study the effect of acting artificial saliva [3] on the convergence of squid. We use the content of polyphenols, flavonoids, tannins, and antioxidant activity as evaluation indices to preliminarily determine the convergence strength. The experiment focuses on the content of polyphenols, the relative polymerization degree, and turbidity of the solution, i.e., the particle size zeta potential of the residue as a physicochemical indicator, to explore the interaction between polyphenols and artificial simulated saliva.

2. Materials and Methods

2.1. Reagents and Equipment

Lysozyme (LZ), bovine serum albumin (BSA), collagen (COL), whey protein (WPI), and bovine hemoglobin were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd; Tannic acid, gallic acid, and catechins were brought from Pure, while rutin was gain from Shanghai McLean Biochemical Technology Co., Ltd; The DPPH free radical scavenging ability kit was obtained from Nanjing Jiancheng Technology Co., Ltd; Squid was acquired from Zhejiang Xingye Group Co., Ltd.

Agilent1220 Infinity II HPLC, Agilent Technologies; UV-2600 UV, Shimadzu Corporation, Japan; Ultra-low temperature freeze-drying machine, Poton, Sweden; Rotating evaporator, Jiangsu Science Equipment Co., Ltd; Zetasizer Nano series particle size potentiometers, Marvin Instruments, China.

2.2. Analysis of Total Polyphenols and Polyphenol Monomer Content

Determining total polyphenol content refers to Huang's literature [12] with slight modifications. Draw a standard curve with gallic acid concentration as the x-axis and absorbance as the y-axis.

The data collection instrument for polyphenol monomer content determination mainly includes ultra-high performance liquid chromatography (Vanquish, UPLC, Thermo, USA) and high-resolution mass spectrometry (Q Active, Thermo, USA). The chromatographic conditions are as follows: Column: Waters HSS T3 (50 * 2.1 mm, 1.8 mm) μ m); Mobile phase: A phase is ultrapure water (containing 0.1% formic acid), B phase is acetonitrile (containing 0.1% formic acid); Flow rate of 0.3 mL/min; Column temperature of 40 ℃; Injection volume 2 μ L; The elution gradient and the volume ratio of ultrapure water to acetonitrile are detailed in Table 1.

Table 1. *The volume ratio of gradient elution mobile phase.*

Elution time (min)	0.1% formic acid ultrapure water	0.1% formic acid acetonitrile
0	90	10
2	90	10
6	40	60
9	40	60
9.1	90	10
12	90	10

2.3. Total Flavonoid Content

Select the commonly used sodium nitrite aluminum nitrate colorimetric method to determine flavonoids and draw a standard curve with the mass concentration of rutin as the x-axis and absorbance as the y-axis [13].

2.4. Sulfhydryl Content and Volatile Base Nitrogen

Operate according to the thiol reagent kit, take 50 μL of the prepared supernatant in 1.3.2 as the test sample, add 150 μL of methanol solution, shake for 120 minutes, and take a water bath at 37 ℃ for 15 minutes. Measure the absorbance value at the 412 nm wavelength of the UV visible spectrophotometer. The principle is that the thiol groups in the body of equatorial squid are mainly composed of glutathione thiol and protein thiol groups. These two thiol groups can react with 5,5 '-dithiobis - (2-nitrobenzoic acid, DTNB) to generate yellow 2-nitro-5-methiobenzoic acid. The product exhibits a distinct absorption peak at 412 nm, and the total thiol content can be accurately measured by adjusting the absorbance value.

Volatile base nitrogen refers to the semi-trace nitrogen determination method in GB 5009.228-2016. Process using a fully automatic nitrogen analyzer and titration with a 0.1 mol/L HCL solution. Evaluation criteria: When the volatile base nitrogen value is less than 25 mg/100 g, the freshness of squid is in a "good" state; When the volatile base nitrogen value is more excellent than 35 mg/100 g, the freshness of squid is in a "poor" state.

2.5. UV Spectroscopy Determination

The secondary structural changes of acidity-related substances in the body of equatorial squid were characterized by UV and infrared spectra, and the absorption spectra were measured using a quartz colorimetric dish. The squid sample was vacuum dried at -80 ℃ using infrared spectroscopy and measured using the potassium bromide tablet pressing method.

2.6. Antioxidant Activity

It mainly referred to the literature of Gulcin [14] and used the DPPH assay kit for determination.

2.7. Relative Polymerization Degree

Referring to the experiment of Latos Brozio [6], the squid polyphenol extract was mixed with four protein solutions in equal volumes.

2.8. Zeta Potential Analysis

Zeta potential mainly refers to Buckackova et al. [9] and Ahmad. The article by Ahmad et al. [15], with slight modifications, uses electrophoresis to determine the potential difference of the mixed solution. The sample processing steps are the same as 2.7. After shaking and mixing, measure using a potential analyzer.

2.9. Sensory Evaluation

The sensory evaluation mainly refers to Zhao, the experiment conducted by Zhao et al. [16] for sensory evaluation of squid samples and quantitative descriptive analysis of the astringency effect. The evaluation team consists of 15 people aged between 20 and 26. Using sodium glutamate (0.5 mg/mL), citric acid (0.5 mg/mL), sucrose (0.5 mg/mL), quinine sulfate (0.03 mg/mL), and sodium chloride (1.50 mg/mL) as taste attributes for freshness, sourness, sweetness, bitterness, and saltiness, respectively. Wash the surface of different de-astringent-treated squid samples and boil them in boiling water.

2.10. Data Analysis and Processing

Each experiment was repeated thrice, taking the average ± standard deviation. The data should be processed using the Social Science Statistics Package (SPSS) 19.0 software and plotted using Origin 2019.

3. Results and Discussions

3.1. Changes in Total Polyphenols, Total Flavonoids, and Polyphenol Monomer Content

Polyphenolic substances reduce astringency by forming soluble or insoluble complexes with proteins [17]. As shown in Figure 1(a), as the astringency time progresses, the content of total polyphenols gradually decreases from 3.49 mg/mL to 0.76 mg/mL. The de astringency mechanism of this experiment is consistent with the results of Torres Rochera [18], where polyphenols interact with collagen-rich in proline, leading to aggregation and sedimentation, resulting in a decrease in polyphenol content. From Figure 1(b), it can be seen that the total flavonoid content decreased, with the fastest decrease rate in the first 30 minutes and gradual slowing down after that, possibly due to a decrease in the condensable sites of the protein. Flavonoids are natural antioxidants that aggregate with proteins. This conclusion is consistent with the research findings of Sakalauskas et al. [19]. Sakalauskas et al. [19] utilized the interaction between flavonoids and amyloid proteins before and after oxidation. They concluded oxidized flavonoids have more muscular aggregation activity with amyloid proteins than unoxidized flavonoids.

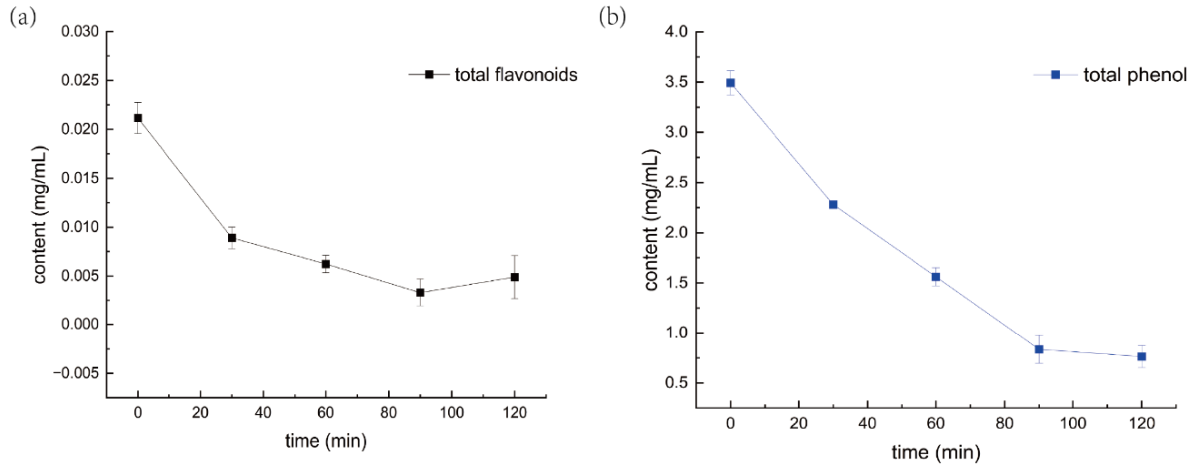

Figure 1. *Changes in total polyphenols, total flavonoids, and total thiol content.*

The content of polyphenol monomers in the sample was analyzed by liquid chromatography and mass spectrometry, and the sample was determined after ultrasonic treatment for 120 minutes in the de astringency treatment. Polyphenols can bind with proteins to form soluble complexes and can also induce the formation of protein precipitates. The article by Dalabasmaz, et al. [20], points out that the binding affinity of polyphenols depends on the molecular size of polyphenol molecules: the larger the molecular size of polyphenols, the greater the tendency to form complexes with proteins [20]. The measurement results showed that phenylpropanoid content was the highest at 80.64 ng/g, and after astringency treatment, the content decreased to 4.78 ng/g. After astringency treatment, 16 polyphenol monomers significantly decreased in content, as shown in Table 2.

Table 2. *Changes in the content of polymeric monomers in samples before and after de-astrology.*

Name	Control (ng/g)	Treatment (ng/g)
Gallic acid	0.56	0.51
Protocatechualdehyde	2.37	2.06
Vanillic acid	1.43	1.13
Caffeic acid	1.15	0.43
Syringic acid	0.44	0.23
Vanillin	7.61	7.02
p-Hydroxycinnamic Acid	1.80	0.90
Salicylic acid	1.05	0.92
Trans-Ferulic acid	0.69	0.52
Luteoloside	3.47	0.88
Luteolin	2.04	1.01
Quercetin	0.45	0.37
Hydrocinnamic acid	80.64	4.78
Trans-Cinnamic acid	2.37	0.84
Apigenin	0.57	0.44
Gossypol	2.75	0.46

3.2. Sulfhydryl Content and Volatile Base Nitrogen

According to Figure 2(a), it can be seen that during the de-astringency process, the total sulfhydryl content in the squid sample shows a trend of first increasing and then decreasing, indicating that during the de-astringency process, the protein undergoes structural degradation first, and the disulfide bond is transformed into a sulfhydryl form. Subsequently, the protein polypeptide chain undergoes aggregation and sedimentation, and the sulfhydryl group is masked by binding, resulting in a decrease in content. Duan The article et al. [21] also pointed out that the content of thiol groups can, to some extent, characterize the oxidation of proteins. According to Figure 2(b), it can be seen that the variation of volatile base nitrogen content in the treatment group is consistent with the variation of thiol content. It is in an upward stage 30 minutes before treatment, rising from the initial value of 19.67 mg/100g to 24.22 mg/100g and gradually decreasing in the subsequent treatment time. After 120 minutes of treatment, the volatile base nitrogen content is 18.39 mg/100g. This phenomenon should be because ultrasonic treatment has the effect of tenderizing meat quality. During the ultrasonic treatment, volatile salt substances produce volatilization, effectively removing harmful flavor substances in squid samples. Huang, In the study of squid food [22] also reached similar conclusions that different treatments can lead to significant differences in the volatile base nitrogen content of squid food.

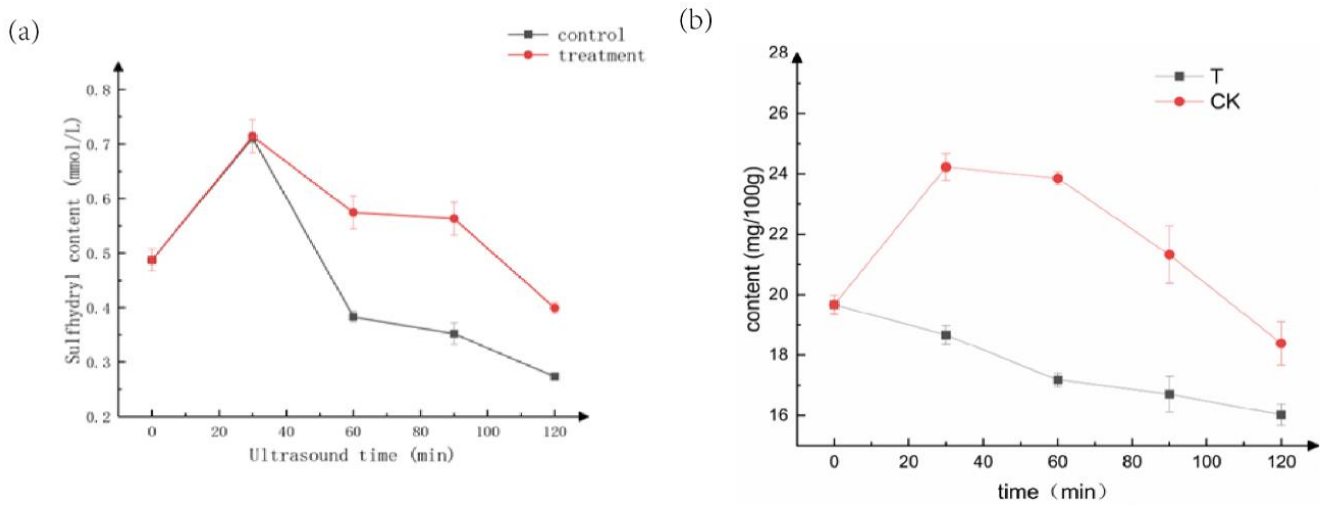

Figure 2. *Changes in nitrogen content of thin and volatile salt groups.*

3.3. UV Spectroscopy

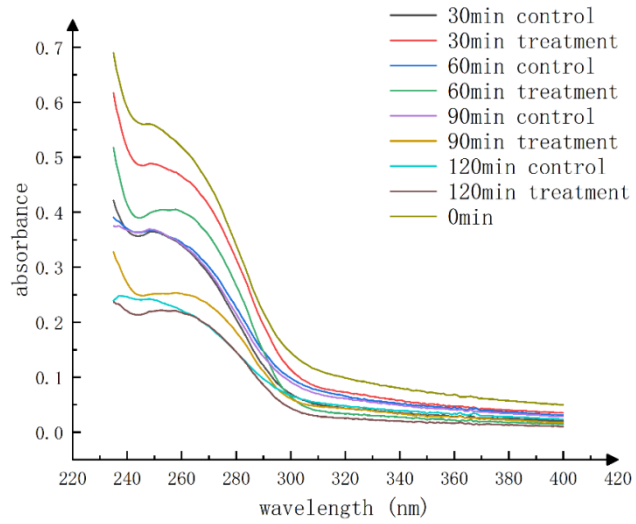

Figure 3. *Changes in UV spectrum during the de-astrogenesis process.*

As the astringency time progresses, the content of UV spectral characteristic functional groups in the equatorial squid sample significantly decreases, and the maximum absorption wavelength shifts towards the long wavelength direction. These features indicate that a chemical reaction occurred between the equatorial squid sample and the astringent, resulting in a change in the characteristic structure of the equatorial squid protein. Francisco, In an article studying the effects of temperature and pH on the secondary structure of squid [23], pointed out that UV spectroscopy was used to characterize the protein's secondary structure, and software and databases were used to estimate its secondary structure. The experimental results indicate that α- The spiral remains unchanged, β- Folding increases during high-temperature processes. Ramasamy Et al. [24] also characterized the substance extracted from squid skin using UV spectroscopy, which showed significant absorption at 220-240 nm. Combined with FT-IR spectroscopy, it was demonstrated that collagen protein with antioxidant and skincare functions was present in the extract.

3.4. Antioxidant Activity

Figure 4 shows that squid's DPPH free radical scavenging rate decreased from 1.01% to 0.47%, and the free radical scavenging rate significantly decreased, leading to a decrease in antioxidant activity. This may be due to a decrease in the content of polyphenols with antioxidant activity that can scavenge free radicals. The experimental results of et al. [9] are consistent.

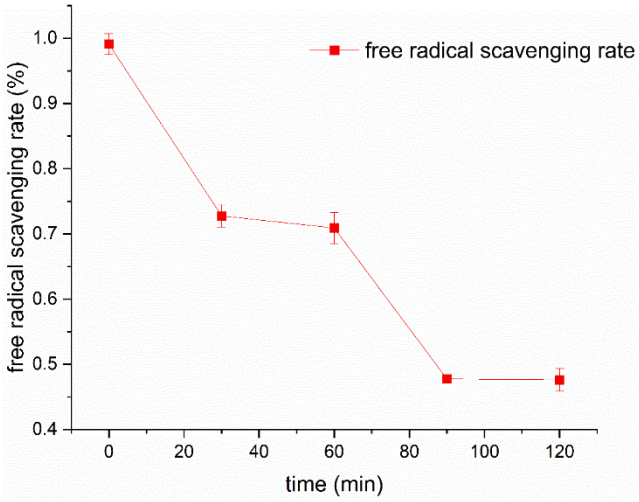

Figure 4. *DPPH radial scanning rate change charge.*

3.5. Sensory Analysis

As shown in Figure 5, the overall sensory score of the

equatorial squid sample treated with a de astringent was significantly improved. After 120 minutes of treatment, the total score was 83 points, while the other experimental group with pure water as the control only scored 44 points, indicating an unusually significant difference. In addition, the rating standard for astringency is that the stronger the astringency, the lower the score, with a maximum score of 20 points. In Figure 5, the scores of astringency sensations are listed simultaneously. It can be observed that the squid sample treated with a de-astringent agent has a shallow astringency sensation. After completing the deastringency process, the astringency sensation score attains 19 points, rendering it impossible to perceive astringency in the equatorial squid sample directly.

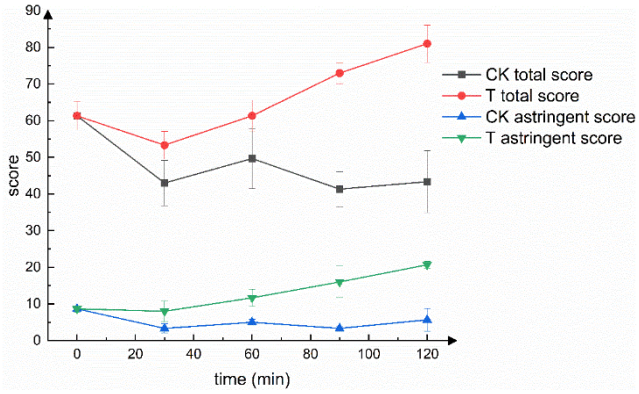

Figure 5. *Sensor score maps for different processing times.*

3.6. Relative Polymerization Degree

The antioxidant properties of polyphenols are determined by their structure, including their polymerization form [9]. The degree of polymerization of polyphenols is closely related to their antioxidant properties, anti-cancer, anti-obesity, and other properties [25]. Therefore, the degree of polymerization is one of the essential characteristics of polyphenols. As shown in Figure 6, among the four protein treatment groups, the collagen treatment group had the highest degree of polymerization of polyphenols, from 1.61 to 3.21. The relative polymerization degrees of the bovine serum albumin and lysozyme-treated groups were 1.73 and 2.11, respectively, while the relative polymerization degrees of the whey protein-treated group were 1.53. Compared with the untreated group, the polymerization degree decreased, indicating a change in the protein's tertiary structure and backbone structure and a decrease in coiling and folding.

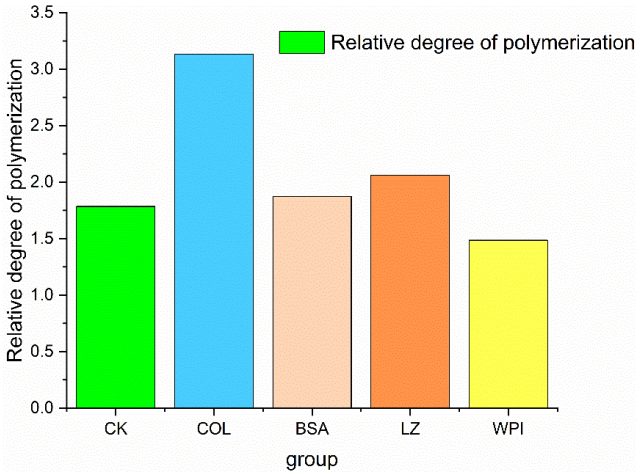

Figure 6. *The relative polymerization degree of different proteins and squid polymeric extracts.*

3.7. Relative Polymerization Degree

Ahmad et al. [26] analyzed that zeta potential represents electrostatic interactions and characterizes the interface conditions of human saliva in contact with polyphenolic substances. The astringency is closely related to the contact surface's friction, surface lubrication, and potential. Li Yan et al. [27] compared and analyzed the dynamic relationship between the potential changes of astringency-related substances and their composition through extensive targeted metabolomics techniques. Figure 7 shows that the squid sample's zeta potential is 8.64, and the potential changes significantly after treatment with different proteins. Among them, the potential slightly decreased to 7.23 when interacting with collagen. It is speculated that the COOH ions of phenolic acids in the polyphenolic extract and hydroxyproline and hydroxylysine in the collagen solution attracted each other, reducing the surface charge and thus lowering the zeta potential. The absolute value of zeta potential in the mixed solution of bovine serum albumin solution and squid polyphenol extraction solution is relatively high, and the stability coefficient of the system is low. The analysis is mainly based on the article by Bukackova et al. [9]. The mixed solution system of lysozyme and squid polyphenol extract has good stability, with a zeta potential of 4.36. The main reason is that lysozyme is an alkaline enzyme that closely binds with the COOH group of phenolic acids in squid extract, resulting in a decrease in surface charge and a decrease in the zeta potential of the system. Whey protein can bind small hydrophobic molecules, which facilitates the adsorption of whey protein on the surface of the mixed solution, decreasing charge and zeta potential.

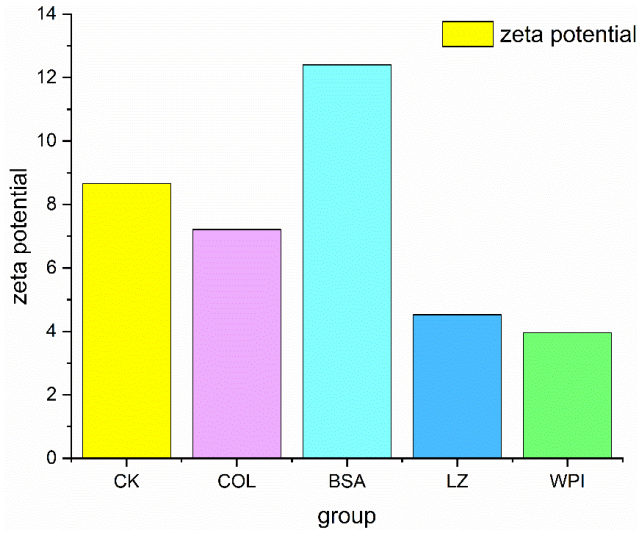

Figure 7. *Different Proteins and Squid Polyphenol Extracts zeta Potential plot.*

3.8. Effect of a Single Protein on Polyphenol Content

Targeted metabolomics was used to determine the content of various polyphenol monomers in equatorial squid samples. Four proteins were used to interact with equatorial squid, and the experimental results showed that the proteins interacted with various polyphenol monomers. The content of polyphenol monomers changed significantly, as shown in Table 3. Jia Et al. [28] used targeted metabolomics to reveal the changes in vital phenolic substances in walnuts. The article pointed out that phenolic substances such as catechins, anthocyanin trimers, and triglyceride hexahydroxydiphenol glucose can determine quality deterioration in walnuts. Therefore, phenolic substances have a significant impact on food quality.

Table 3. *Effect of different proteins on the content of polymeric monomers.*

Name	Control (ng/g)	Treatment (ng/g)
Gallic acid	0.56	0.51
Protocatechualdehyde	2.37	2.06
Vanillic acid	1.43	1.13
Caffeic acid	1.15	0.43
Syringic acid	0.44	0.23
Vanillin	7.61	7.02
p-Hydroxycinnamic Acid	1.80	0.90
Salicylic acid	1.05	0.92
Trans-Ferulic acid	0.69	0.52
Luteoloside	3.47	0.88
Luteolin	2.04	1.01
Quercetin	0.45	0.37
Hydrocinnamic acid	80.64	4.78
Trans-Cinnamic acid	2.37	0.84
Apigenin	0.57	0.44
Gossypol	2.75	0.46

4. Conclusion

This experiment used bovine serum albumin, collagen, lysozyme, and whey protein to simulate human oral saliva to remove the astringency of squid caught in equatorial waters. The findings demonstrated a significant reduction in the content of polyphenols and flavonoids, which are closely associated with astringency. The sensory evaluation experiment showed that the treated squid had almost no astringency, and the overall quality and taste were better. UV spectroscopy characterization found that the squid sample's characteristic functional group structure changed during the de-astringency treatment. Four types of proteins were used to interact with squid samples to verify the mechanism of de-astringency. Protein aggregation and sedimentation were the main ways of de-astringency. Secondly, the change in zeta potential indicated that proteins interacted with polyphenols in squid samples, resulting in a change in surface charge.

Acknowledgments

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Abbreviations

LZ: Lysozyme
BSA: bovine serum albumin
COL: collagen
WPI: whey protein
HPLC: High-performance liquid chromatography

Conflicts of Interest

The authors declare no conflicts of interest.

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