Research Article

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Effect of ultrasound combined with pineapple protease treatment on the tenderness of dried shrimp

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Abstract

BACKGROUND: In order to improve the tenderness of dried shrimp products as well as to reduce the hardness of the meat during the drying process, shrimp were treated with ultrasound combined with pineapple protease and the tenderization condition was optimized by measuring the texture and shear force of dried shrimp. In addition, the sulfhydryl content, myofibril fragmentation index (MFI) and microstructure were also examined to clarify the mechanisms of shrimp tenderization.

RESULTS: The results showed UB1 group with ultrasonic power of 100 W, heating temperature of 50 °C and pineapple protease concentration of 20 U mL^{-1} were the optimum tenderization conditions, where shrimp showed the lowest hardness (490.76 g) and shear force (2006.35 gf). Microstructure as well as sodium dodecyl sulfate–polyacrylamide gel electrophoresis results suggested that during the tenderization process the muscle segments of shrimps were broken, degradation of myofibrillar proteins occurred, and MFI values and total sulfhydryl content increased significantly ($P < 0.05$) (MFI value = 193.6 and total sulfhydryl content = 93.93 mmol mg^{-1} protein for UB 1 group).

CONCLUSION: Ultrasound combined with bromelain could be used as a simple and effective tenderization method for the production of tender dried shrimp. The best conditions were 100 W ultrasonic power, 50 °C ultrasonic temperature, and 20 U mL^{-1} bromelain.

Supporting information may be found in the online version of this article.

Keywords: bromelain; microstructure; *Penaeus vannamei*; tenderization; texture; ultrasonic

INTRODUCTION

White shrimp (*Penaeus vannamei*) is one of the most valuable aquaculture commodities globally because of its high nutritional and economic value.[1] In China, the production of *P. vannamei* has increased significantly over the last 3 years, and reached 2.09 million tons in 2022.[2] The proportion of dried shrimp in the shrimp products market has increased year by year, as they are easy to transport, eat and store, and the unique flavor produced after drying is also one of the main reasons for their popularity.[3] However, shrimps often undergo physical or chemical changes such as protein crosslinking and serious water loss during the heating process, which not only causes the meat to become tougher and less tasty, but also reduces its digestibility.[4,5] Dried shrimp are unpopular with the elderly, children, and others with weak chewing abilities, because they are hard and difficult to chew.[6-8] Many previous studies have shown that meat jerky with high hardness tends to be difficult to digest, whereas digestibility significantly increases after tenderization.[9-12] Therefore, it is essential to explore methods of tenderizing dried shrimp to improve its chewiness and digestibility.

There are many intrinsic factors that affect the tenderness of meat products, such as collagen content, and the structure of myofibrils and muscle segments.[13,14] Therefore, using a suitable pretreatment method to change the muscle structure has a significant influence on improving the tenderness of meat products.[15,16] Until now, chemical, physical and enzymatic methods have been used in the meat processing industry to achieve the desired tenderness of meat products.[17] Pineapple protease is an exogenous enzyme obtained from plants, which has the function of breaking down myofibrils and connective tissues to improve the tenderness of meat products.[18] Ketnawa and Rawdkuen[19] found that marination of meat in bromelain solution significantly increased muscle tenderness. Besides, more

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aldehydes and ketones were produced during the tenderizing process, which improved the meat flavor.[17] Currently, enzymatic tenderization is often performed by immersion or injection to allow the enzyme solution to enter the meat.[20] However, both methods are unsatisfactory because of time cost, damage to the tissue, and excessive tenderization of the meat surface.[21,22] In addition, our previous study showed that the pigment of shrimp was seriously reduced after prolonged immersion in the enzyme solution, which made their color very unacceptable and influenced their appearance. Therefore, it is necessary to explore a novel, efficient and non-destructive method to accelerate the penetration of enzymes to achieve uniform tenderization of the meat.

Ultrasonic tenderization is considered to be a highly safe, non-destructive method and has been used to improve the quality of meat products,[23] as well as in the processing of fruits and vegetables.[24,25] This technology proved to enhance meat tenderness by disintegrating muscle fibers, myofibrillar proteins (MPs), and intramuscular connective tissue, as evidenced by Noor *et al.*[26] Many studies have also shown that low-power ultrasound could accelerate the marination of meat products by promoting molecular diffusion,[21] while high-power ultrasound could increase the activity of protein hydrolytic enzymes.[21,27] Many studies have also demonstrated that during ultrasonic pretreatment of samples the shear force generated by the collapse of bubbles during cavitation leads to tissue rupture and the formation of a large number of microchannels.[28,29] Additionally, Fernandes *et al.*[30] demonstrated that with the use of ultrasound the diffusion rate of water increased and the overall drying time was reduced. Barekat and Soltanizadeh pointed out that the combined use of ultrasound and papain was beneficial in improving the functional properties of bull muscles.[31] Therefore, the combination of enzymatic tenderization and ultrasonic technology provides a new approach to tenderizing shrimp meat. However, few studies have focused on the effect of ultrasound combined with enzyme treatments on aquatic products.

Therefore, the purpose of this study was to optimize the tenderization conditions of shrimp treated with ultrasonic synergistic bromelain by determining the texture and shear force of dried shrimp. Meanwhile, changes in sulfhydryl content, myofibrillar fragmentation index (MFI) and microstructure of dried shrimp during the tenderization process were determined to clarify the mechanism of shrimp tenderization.

MATERIALS AND METHODS

Sample preparation

Fresh shrimp (*Penaeus vannamei*), with an average weight of 12.56 ± 2.25 g, were purchased from the local market in Ningbo of China and transported to the laboratory in ice within 1 h. Shrimp meat was obtained after the head and shell were removed; each 100 g of shrimp meat was then vacuum packed in polytetrafluoroethylene bags and stored in a −40 °C freezer until use. Food-grade bromelain (6000 U g^{-1}, EC 3.4.22.3) was purchased from Zhejiang Yinuo Biotechnology Co., Ltd (Hangzhou, Zhejiang, China).

Experiment design

The frozen shrimp meat was thawed by immersing it in an ice–water mixture for 2 h. Following the thawing process, the samples were placed in a bromelain solution with concentrations ranging from 10 to 30 U mL^{-1}, containing 2% salt, maintaining a sample-to-solution ratio of 1:1 (w/v). Simultaneously, the meat was subjected to ultrasonic treatment for 5 min using a range of ultrasonic powers (50–250 W) and temperatures (30–70 °C). The ultrasonic processor was a rectangular vessel with an effective volume of 10 L, equipped with a 40 kHz transducer at the bottom. During sonication, the temperature was maintained by adjusting the sonicator's settings. Subsequent to the ultrasonic treatment, the meat was uniformly spread onto a wire mesh and subjected to drying using a hot-air dryer. The drying process was executed at a temperature of 100 °C until the moisture content reached the target range of 50 ± 3%.

In designing the experimental runs, a single-factor experiment was conducted, focusing on key performance indicators such as shear force, texture profile analysis (TPA), and color, to preliminarily determine the optimal conditions for the primary factors, including ultrasonic treatment (power and temperature) and bromelain concentration. The optimal conditions for each single-factor group as well as the control group (no treatment) were selected for subsequent analysis of the tenderization mechanism. Further insights into the tenderization mechanism of shrimp meat were gained by examining the protein pattern, total sulfhydryl content, surface hydrophobicity, MFI, and microstructure of the selected experimental groups.

Texture profile analysis

TPA was evaluated using a texture analyzer (TA.XT Plus, Stable Micro Systems Ltd, Godalming, UK) according to the method of Chen *et al.*[32] The second (thickest) segment of dried shrimp meat was compressed by a cylindrical probe (P/36R) to 30% of its original height, measured at a pre-test speed of 2 mm s^{-1}, 1 mm s^{-1} of test speed and 1 mm s^{-1} of post-test speed, with compressed times of 2 s, and time interval of 5 s. Data for hardness, springiness, chewiness, cohesiveness, and gumminess were obtained directly using Texture Expert software. The experiments were carried out with five replicates.

Shear force

Shear force was measured using a method described previously, with slight modifications.[32,33] An RTA meat texture analyzer (Tengba Instrument Technology Co. Ltd, Shanghai, China) with Warner–Bratzler shear apparatus was used to detect the shear force of dried shrimp muscle. The first major peak (usually the highest, representing the maximum shear force required to shear the muscle sample) was recorded. Texture of dried and shelled samples was determined at the junction of the third and fourth abdominal segments and by compressing the samples perpendicular to the muscle fiber axis; at least five replicates were measured for each group.

Color

The color of the dried shrimp was assessed using a colorimeter (CR-400, Konica Minolta, Inc., Tokyo, Japan). The instrument was calibrated with a standard white plate before measurement. For each group, the color was measured on the second abdominal section of dried shrimp samples.[34] Color values were reported using the CIE-Lab scale as L^* (brightness), a^* (+a, redness; −a, greenness), b^* (+b, yellowness; −b, blueness). The experiments were carried out in six replications.

The hue angle ($H°$) was calculated using the following equation[35]:

$$H° = \text{acrtan}\left(b^*/a^*\right) \times \left[360°/(2\times 3.14)\right]$$

Color difference (ΔE) was calculated using the following equation[34]:

$$\Delta E = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2\right)]^{1/2}$$

where Δ represents the value of the measurement group minus the control group.

Extraction of myofibrillar proteins

MPs were prepared as described by Yang *et al.*,[36] with minor modifications. Briefly, 4 g of tenderized shrimp meat was homogenized with a pre-cooled 4× volume of Tris–HCl buffer (consisting of 2.42 g Tris and 5.84 g NaCl), with the volume adjusted to 1000 mL using ultrapure water. HCl was utilized to achieve a pH of 7.5. The precipitate was collected by centrifugation at 4000 × *g* for 5 min at 4 °C and washed three times; finally, the MPs were obtained after filtering through four layers of gauze. All the above steps are performed at low temperature (0–4 °C). The concentration of MP was measured using the Biuret method[37] and the solution was diluted with the same Tris–HCl buffer for subsequent analysis.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was carried out, essentially as described by Laemmli,[38] on 12% separating gels and 4% stacking gel. 200 mL of 2 mg mL^{-1} MP solution was mixed with 50 mL protein loading buffer containing 25% Tris–HCl (1 mg mL^{-1}, pH 6.8), 50% (v/v) glycerol, 5% (w/v) *β*-mercaptoethanol, 5% (w/v) SDS, and 0.05% (w/v) bromophenol blue, and, after boiling at 100 °C for 5 min, 10 μL protein solution was loaded onto the gel. After electrophoresis, the gels were stained using 1 mg mL^{-1} Coomassie brilliant blue R-250 for 45 min and decolorized with ethanol–acetic acid solution until the protein bands were clear.

Total sulfhydryl content

Total sulfhydryl content was determined according to the method of Kang *et al.*[39] The 500 μL MP samples (protein concentration adjusted to 5 mg mL^{-1}) were added to 2.5 mL Tris–glycine buffer (pH 8.0) containing 8 mol L^{-1} urea, 0.086 mol L^{-1} Tris, 0.09 mol L^{-1} glycine, 0.005 mol L^{-1} EDTA (ethylenediaminetetraacetic acid), and 0.02 mL of 4 mol L^{-1} Ellman's reagent (5′,5-dithiobis(2-nitrobenzoic acid), DTNB). The reaction was performed in the dark for 30 min at room temperature and the absorbance was measured at 412 nm. Total sulfhydryl group contents were calculated as follows:

$$\text{SH content}\left(\mu\text{mol SH g}^{-1}\text{ protein}\right) = (73.53 \times A_{412} \times D)/C$$

where A_{412} is the absorbance at 412 nm, C is the concentration (mg protein mL^{-1}), D is the dilution factor, and the factor 73.53 is derived from $10^6/(1.36 \times 10^4)$.

Surface hydrophobicity

Briefly, 1.0 mL MP (2 mg mL^{-1}) solution was mixed with 200 μL bromophenol blue sodium (BPB) (1 mg mL^{-1}), and the buffer solution was used as a reference. All samples were stirred for 10 min, then centrifuged at 2000 × *g* for 15 min. The absorbance of the supernatant (diluted 10 times) was measured at 595 nm. The sample with the same treatment in the absence of protein was used as the control. Surface hydrophobicity is expressed by the content of BPB bound and calculated from the following equation:

$$\text{BPB bound}\,(\mu\text{g}) = 200 \times (A_c - A_s)/A_c$$

where A_c and A_s represent the absorbance values of control and sample at 595 nm, respectively.

Myofibrillar fragmentation index

The MFI of shrimps was determined with reference to Li *et al.*, with major modifications.[40] 4 g tenderized minced meat was homogeneous with 40 mL of 20 mmol L^{-1} potassium phosphate (PBS) buffer (pH 7.0) containing 0.1 mol L^{-1} KCl, 1 mmol L^{-1} $MgCl_2$, and 1 mmol L^{-1} EGTA (ethylene glycol tetraacetic acid). The precipitate was obtained after 10 000 × g centrifugation for 15 min at 4 °C. The precipitate was then resuspended in 40 mL of the same PBS buffer, stirred and centrifuged again, and the supernatant was discarded. The precipitate was resuspended in 10 mL buffer and filtered through a two-layer cloth filter. The suspension was then diluted with PBS buffer to a protein concentration of 0.5 ± 0.05 mg mL^{-1}. MFI is the absorbance value of the myofibrillar suspension, which was measured at 540 nm and multiplied by 200.

Scanning electron microscopy (SEM)

Sample preparation was carried out according to the method of Lan *et al.*,[41] with a slight modification. The tenderized and dried samples were cut into 5 cm × 5 cm × 1 cm pieces in the cross-sectional direction. They were then fixed with 2.5% glutaraldehyde at 4 °C for 24 h and washed with 0.1 mol L^{-1} phosphate buffer solution (pH 7.2) three times. Subsequently, the samples were dehydrated with a series of concentrations of ethanol and *tert*-butanol solutions. The treated samples were freeze-dried and coated with gold, and then observed by SEM (S-3400 N, Hitachi Ltd, Tokyo, Japan).

Histological observations

Microstructural analysis of shrimps was performed according to the method described by Li *et al.*[42] The meat, after tenderizing and drying, was fixed in 10% formaldehyde solution for 24 h, followed by paraffin embedding, cutting, dewaxing, staining with hematoxylin and eosin, and then observation by light microscopy.

Statistical analysis

All measurements were performed with at least three replicate samples. The results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $P < 0.05$. Pearson correlation coefficients were calculated to assess the relationships between physical indicators and biological parameters. All data analyses and calculations were executed using Origin Pro 9.0 (OriginLab Inc., Northampton, MA, USA).

RESULTS AND DISCUSSION

Single-factor experiment

TPA

TPA is a double compression test employed to assess the textural properties of foods and establish connections with perceived sensory characteristics. The variations in texture properties are

illustrated in Table 1. A single treatment involving ultrasound and bromelain exhibited a slight decrease in this value, likely due to the rapid disintegration and fragmentation of muscle, although it did not have a significant effect.[22,43] As the ultrasonic power increased, there was a gradual decrease in the hardness values. Specifically, the group subjected to 100 W of ultrasonic energy exhibited a hardness measurement of 490.76 ± 49.43 g, significantly lower than that the group that did not undergo ultrasound treatment ($P < 0.05$). This aligns with the findings of Jayasooriya *et al*., who observed a progressive reduction in the hardness of bovine muscles with an increasing duration of ultrasound treatment.[22] Similar trends were observed concerning temperature and enzyme concentration. These trends suggest that the mechanical effects and cavitation initiated by ultrasound lead to tissue degradation, creating more space between myofibrils and facilitating bromelain penetration. Simultaneously, under optimal temperature conditions, the enzyme demonstrates maximal efficiency in breaking down MPs and collagen into smaller molecules.[43,44] However, excessively high temperatures (e.g., 70 °C) may lead to enzyme inactivation, loss of content during the heating process, and an increase in the soluble collagen content within the muscles.[45,46] In terms of springiness, both pineapple protease and ultrasonic treatment resulted in a decrease in elasticity, which may be related to muscle internal bond breakage due to cavitation effects.[47]

Chewiness refers to the force required to chew a sample effectively for swallowing.[48] Consistent with the observed trend in hardness, both ultrasound and enzymatic treatments led to a decrease in the force required for chewing. Moreover, chewiness is associated with moisture content, and the reduction in chewiness can be attributed to the increase in moisture content.[47] Cohesiveness represents the strength of internal connections within the muscle. The decrease in cohesiveness might be due to proteolysis as a result of the breaking of weak bonds between proteins. Previous studies have found a significant decrease in adhesion after bull meat immersion in papain solution for 20 min with ultrasonic treatment.[47] However, there was no significant difference ($P > 0.05$) in the cohesiveness among the groups in this experiment (Table 1), which may be due to the shorter treatment time as well as different enzyme activities. Gumminess is determined by multiplying hardness by cohesiveness.[48] Therefore, adhesion, cohesion, and hardness values are directly related.

Shear force

Shear force serves as a direct and crucial indicator of the tenderness of meat and meat products, with lower shear force values corresponding to higher tenderness.[49] In comparison to untreated samples, dried shrimps treated with ultrasound and enzymes showed reductions in shear force values. The combined treatment notably improved tenderness. As illustrated in Fig. 1(A), among the ultrasonic-treated shrimp muscles the dried shrimp with 100 W power showed the lowest shear force value of 2006.35 ± 45.75 gf, which was consistent with the hardness value. These findings are consistent with those reported by Peña-Gonzalez *et al*., who observed lower shear force values in sonicated meat compared to the control.[50] However, it is worth noting that excessive sonication power did not yield further benefits for meat tenderization. The reduced shear force values can be attributed to the hydrodynamic shear forces and localized high temperatures generated by ultrasonically induced cavitation, resulting in structural changes in the food structure.[51] Moreover, at a temperature of 60 °C (Fig. 1(B)), more significant reductions in shear force were observed. This can be linked to the elevated temperature, which reduces shear force through protein denaturation and an increase in protease activity.[47] Meat tenderness may be improved through the fragmentation of MPs due to the heightened activities of exogenous proteolytic enzymes, as indicated by the shear force value observed during the 25 U mL^{-1} treatment (Fig. 1(C)).

Table 1. Texture properties of dried shrimp meat over single-factor tests

Factor	Level	Hardness (g)	Springiness	Chewiness	Cohesiveness	Gumminess
Ultrasonic power (W)	0	848.26 ± 41.50a	0.71 ± 0.04ab	440.76 ± 18.04a	0.73 ± 0.02ab	620.25 ± 10.01a
	50	763.62 ± 30.09b	0.77 ± 0.03a	421.35 ± 15.70a	0.69 ± 0.01b	511.84 ± 57.56b
	100	490.76 ± 49.43e	0.62 ± 0.07b	217.65 ± 7.90d	0.72 ± 0.02ab	400.21 ± 38.01c
	150	548.69 ± 10.66de	0.74 ± 0.01a	297.19 ± 5.94c	0.73 ± 0.02ab	401.91 ± 1.8c
	200	595.51 ± 38.52cd	0.77 ± 0.04a	339.96 ± 5.85b	0.76 ± 0.01a	405.56 ± 40.38c
	250	654.14 ± 19.10c	0.77 ± 0.00a	365.38 ± 30.38b	0.71 ± 0.03ab	463.23 ± 61.14bc
Ultrasonic temperature (°C)	0	789.96 ± 16.31a	0.74 ± 0.02ab	409.22 ± 8.51a	0.7 ± 0.02a	555.27 ± 25.38a
	30	609.19 ± 28.27c	0.75 ± 0.04ab	317.50 ± 27.76b	0.69 ± 0.05a	421.91 ± 13.65c
	40	515.86 ± 15.29d	0.71 ± 0.02bc	261.20 ± 22.48c	0.71 ± 0.03a	365.29 ± 24.79d
	50	504.03 ± 3.72d	0.67 ± 0.00c	232.45 ± 6.55c	0.69 ± 0.02a	349.42 ± 12.58d
	60	456.10 ± 13.97e	0.71 ± 0.03bc	235.18 ± 29.88c	0.72 ± 0.04a	328.41 ± 29.92d
	70	657.53 ± 0.08b	0.77 ± 0.01a	368.95 ± 20.73a	0.73 ± 0.03a	478.21 ± 19.29b
Bromelain concentration (U mL^{-1})	0	858.25 ± 40.59a	0.71 ± 0.03a	446.6 ± 19.09a	0.73 ± 0.03a	629.63 ± 4.36a
	10	634.44 ± 25.79c	0.69 ± 0.03a	309.58 ± 13.23bc	0.71 ± 0.03a	448.70 ± 2.31c
	15	552.90 ± 30.01d	0.73 ± 0.05a	287.13 ± 8.65cd	0.7 ± 0.03a	368.85 ± 52.1d
	20	504.03 ± 3.72d	0.67 ± 0.00a	232.45 ± 6.55de	0.69 ± 0.02a	349.42 ± 12.58de
	25	394.90 ± 18.68e	0.68 ± 0.01a	188.87 ± 8.98e	0.70 ± 0.01a	276.36 ± 10.89e
	30	759.88 ± 40.34b	0.68 ± 0.06a	358.28 ± 60.87b	0.70 ± 0.02a	528.58 ± 45.56b

Note: Data are given as mean values ± standard deviation. Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

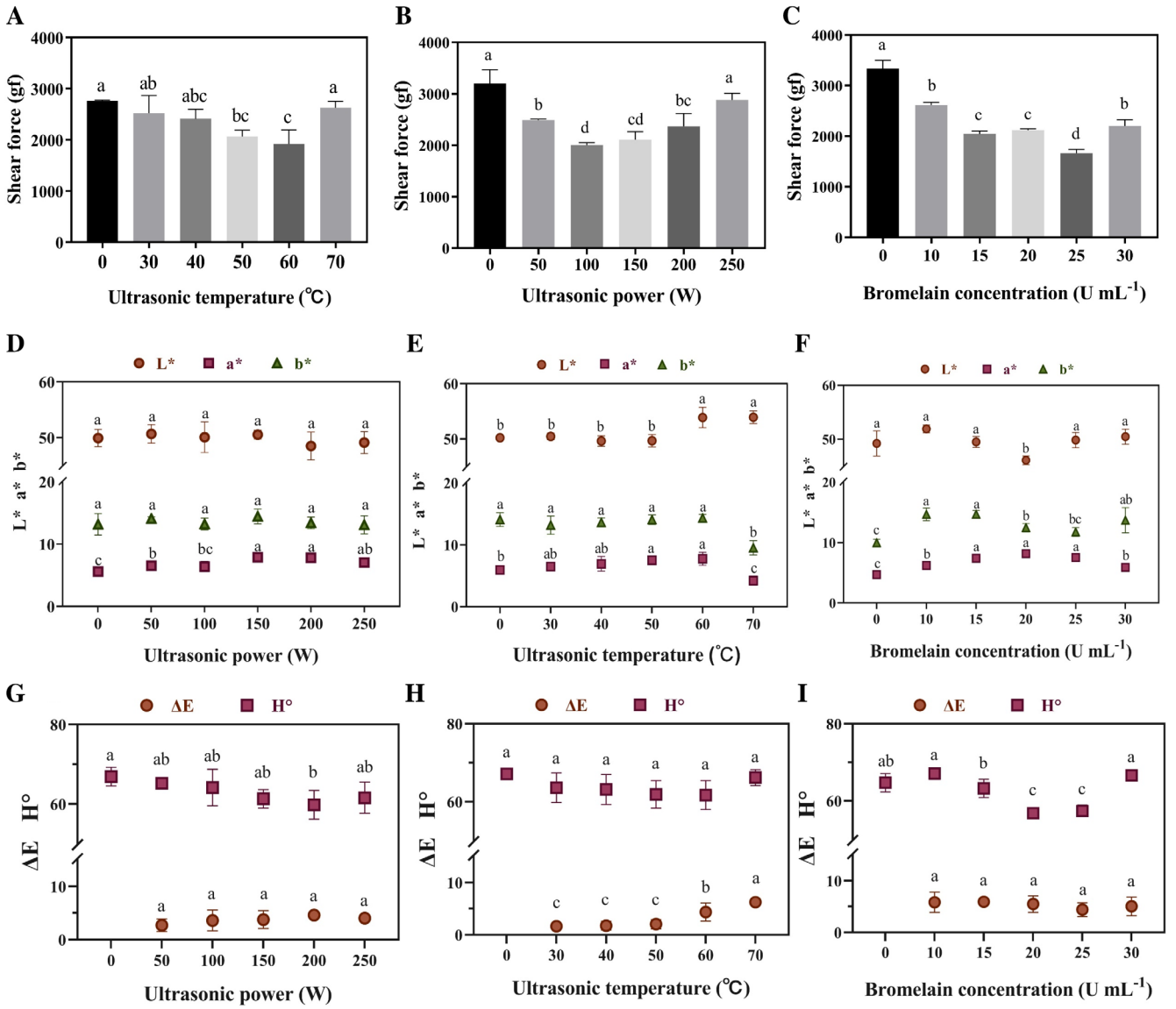

Figure 1. Single-factor analysis for the shear force and color measurement. Effect of ultrasonic power on the shear force (A) and color (D,G). Effect of ultrasonic temperature on shear force (B) and color (E,H). Effect of bromelain concentration on the shear force (C) and color (F,I). Data represent a mean value from five replicate experiments, and error bars represent standard deviation.

Color measurement

The redness value (a^*) is notably influenced by ultrasound power, temperature, and enzyme concentration ($P < 0.05$). The change in a^* value was mainly related to the content of free astaxanthin, with astaxanthin degradation and lipid oxidation being the primary factors influencing the color changes observed in shrimp.[42,52] Notably, ultrasound power does not exert any significant impact on the L^* (brightness) and b^* (yellowness) values (Fig. 1(D)). Comparable results have been reported in studies where ultrasound treatment led to an increase in the a^* value of hake.[53] As the ultrasonic temperature increases, the b^* value demonstrates a significant increase ($P < 0.05$). However, in the 70 °C treatment group, both the L^* value and the a^* and b^* values experience concurrent increases and decreases, respectively (Fig. 1(E)). This phenomenon is attributed to decreased pigment activity and water migration within and outside the cells. These processes result in reduced light reflection intensity and a subsequent decrease in lightness, somewhat similar to the characteristics observed in pale, soft, exudative (PSE) meat.[54,55] Additionally, the a^* and b^* values of dried shrimp treated with bromelain exhibit significant increases compared to the control group ($P < 0.05$) (Fig. 1(F)). These shifts in the a^* and b^* values are associated with alterations in methemoglobin content and enzyme reactions within the meat, leading to reduced methemoglobin activity. This can be attributed to the lower pH observed in the bromelain-treated samples, which exerts an impact on the color stability of the muscle.

The ΔE was used to indicate the overall color difference of dried shrimp (Fig. 1(G–I)). There were no significant differences observed between samples when changing the ultrasonic power and enzyme concentration ($P > 0.05$). However, the difference in ΔE values increased significantly when the temperature exceeded 60 °C, which could be explained by the higher L^* values of the shrimp samples. Characterizing the overall color with $H°$ values provides a more complete measure of color variation, where 0° represents red, 90°

represents yellow, 180° represents green, and 270° represents blue.[34] The results showed that most of the shrimps were orange-yellow in color and the $H°$ values of the shrimp samples decreased significantly ($P < 0.05$) after ultrasonication and bromelain treatment (Fig. 1(G–I)). Therefore, pre-tenderization of shrimp before drying may improve the overall color of dried shrimp.

Therefore, based on the above conclusions, the following groups were used to analyze the tenderization mechanism: control group: no treatment; UB 1 group: subjected to 100 W of ultrasonic power, 50 °C of ultrasonic temperature, and 20 U mL^{-1} bromelain; UB 2 group: subjected to 150 W of ultrasonic power, 60 °C of ultrasonic temperature, and 20 U mL^{-1} bromelain; UB 3 group: subjected to 150 W of ultrasonic power, 50 °C of ultrasonic temperature, and 25 U mL^{-1} bromelain.

Analysis of the tenderization mechanism

MFI value

The MFI reflected the degree of MP degradation and positively correlated with meat tenderness.[56,57] As shown in Fig. 2(A), the MFI value for the control group was 178.53 ± 3.06 – significantly lower than that of both the UB1 and UB3 groups, yet notably higher than that of the UB2 group ($P < 0.05$). These results suggest that bromelain exhibited its highest activity at 50 °C, leading to the disruption of MP structures. Zhao *et al.*[58] demonstrated that the enzyme could disrupt the Z-line of the muscle structure, causing a reduction in the length of MPs and a decrease in the number of muscle segments, ultimately resulting in an increased MFI value. The increase in shrimp tenderness is also attributed to ultrasound treatment, which destroyed the bonds between protein molecules, resulting in the raw material structure being loosened, promoting water absorption and MP hydrolysis.[57] However, it is possible that high temperatures led to protein denaturation and muscle contraction, contributing to the decreased MFI values observed in the UB 2 group.

SDS-PAGE results of tenderized shrimp

The SDS-PAGE profiles of MP extracted from tenderized shrimp of each group are shown in Fig. 2(B). The protein pattern of the

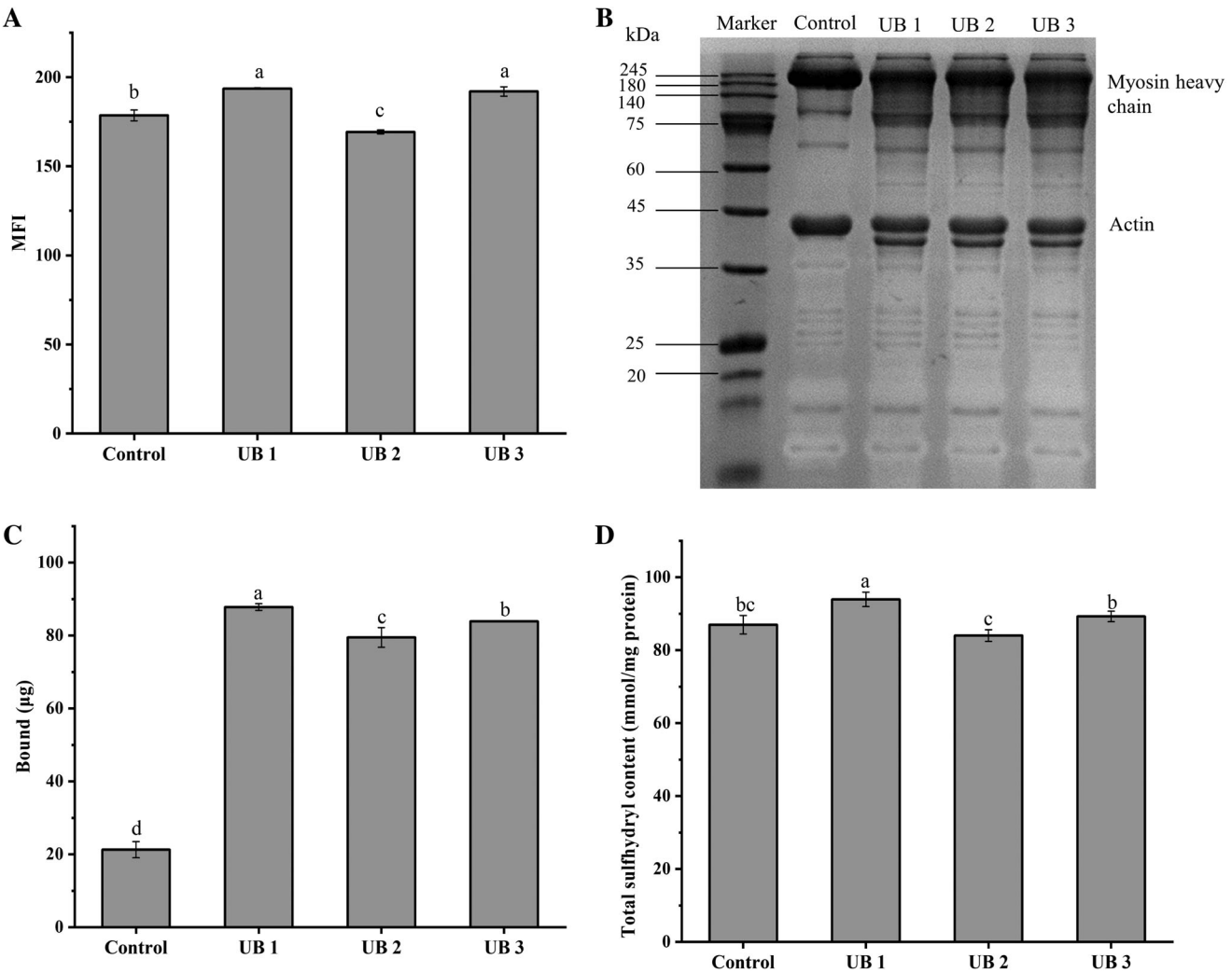

Figure 2. Myofibrillar fragmentation index (MFI) (A) of tenderized shrimp meats and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (B), surface hydrophobicity (C), and total sulfhydryl content (D) of myofibrillar protein from tenderized meat. Control: no treatment; UB 1: subjected to 100 W of ultrasonic power, 50 °C of ultrasonic temperature, and 20 U mL^{-1} of bromelain; UB 2: subjected to 150 W of ultrasonic power, 60 °C of ultrasonic temperature, and 20 U mL^{-1} of bromelain; UB 3: subjected to 150 W of ultrasonic power, 50 °C of ultrasonic temperature, and 25 U mL^{-1} of bromelain. Bars represent the standard deviation from triplicate determination. Different letters on the bars between different treatment methods indicate their significant differences ($P < 0.05$).

tenderized groups was significantly different compared to the control group. In the control group, the predominant proteins were myosin heavy chain (MHC, 220 kDa) and actin (43 kDa). However, in the tenderized groups, clear bands in the range of 100–230 kDa indicated the degradation of MHC during the tenderization process. This degradation is likely a result of enzymatic action on MPs.[17] Additionally, it is worth noting that ultrasound treatment not only facilitates enzyme penetration but also has a disruptive effect on cell membranes and basic structures.[57] In terms of tenderized groups, the UB 1 group exhibited the most significant degradation, which aligns with the hardness and shear force results (Table 1). Chaurasiya *et al*.[59] reported similar findings, with major bands such as MHC and actin being degraded in bromelain-treated samples. Mehrabani *et al*. supported the conclusion that the combination of enzymes with ultrasound technology can disrupt muscle fibers and alter MP structure, ultimately improving meat tenderness.[48] Zou *et al*. also demonstrated that ultrasound treatment, when used in conjunction with exogenous additives, can accelerate molecular diffusion to achieve a tenderizing effect in beef.[43]

Surface hydrophobicity and total sulfhydryl content analysis of tenderized shrimp

Bromophenol blue (BPB) is a commonly employed tool to monitor conformational alterations in MPs and to assess the exposure of hydrophobic sites.[32] In general, partial swelling of animal proteins leads to the exposure of hydrophobic amino acid residues that are typically concealed within their structures, thereby leading to an

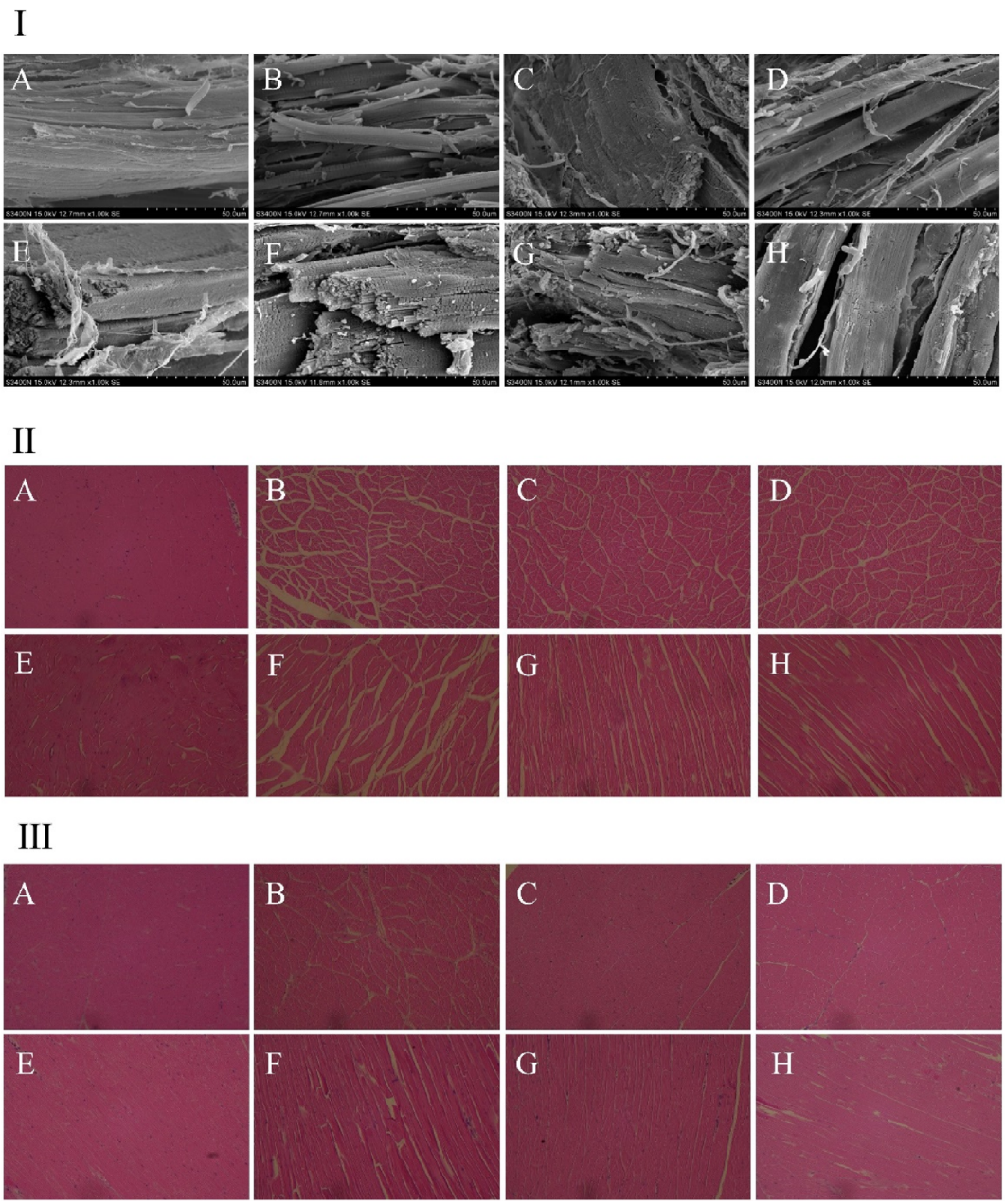

Figure 3. I: Scanning electron microscopy images of longitudinal section of tenderized (A–D) and dried (E–H) shrimp meat. II: Histology of different treatments on tenderized shrimp meat. III: Histology of different treatments on dried shrimp meat. From left to right are the control, UB 1, UB 2, and UB 3 groups. (A–D) Transverse section of myofibril; (E–H) longitudinal section of myofibril. Scale bar = 200 μm.

increase in surface hydrophobicity.[60] Figure 2(C) illustrates a significant increase ($P < 0.05$) in the surface hydrophobicity of the treatment group. This phenomenon can be attributed to the partial denaturation of protein structures induced by enzymes and ultrasound. Consequently, this leads to the unfolding of the protein structure and the exposure of non-polar and hydrophobic groups that are normally concealed within the myofibril structure.[39]

Sulfhydryl groups play an important role in the functional properties of protein. In comparison to the control group, the tenderized groups exhibited an increase in the total sulfhydryl content following ultrasound and enzyme treatment (Fig. 2(D)). The change in the sulfhydryl group may be explained by the unfolding of the protein structure, leading to the exposure of sulfhydryl groups within the protein molecule.[32] Similar results were reported by Amiri *et al.* on bulls after different ultrasounds.[61] Myosin is composed of two long intertwined tails as well as a circular head. The mechanical impact of ultrasound could cause these myosin filaments to dissociate, and the protein structure to unfold, resulting in the release of sulfhydryl groups.[62] This is contrary to the results for UB 2, which showed a decrease in sulfhydryl groups under the treatment condition of 60 °C. This difference may be due to the oxidation of the applied treatments. Consequently, a higher sulfhydryl content is associated with greater surface hydrophobicity and a higher degree of protein denaturation.[12] The notably elevated total sulfhydryl content in the UB 1 group suggests a more significant degradation of MHC (Fig. 2(B)).

Scanning electron microscopy

The SEM results of tenderized shrimp and dried shrimp muscle subjected to the combination of ultrasound and bromelain treatments are shown in Fig. 3 I. In terms of tenderized shrimp, the results showed that the muscle structure of shrimp meat in the control group was more compact, while the structure of the tenderized shrimp meat was significantly broken. This structural transformation can primarily be attributed to the cavitation effect induced by ultrasound and the enzymatic reaction.[63,64] Peña-Gonzalez *et al.* further corroborated these findings by confirming reductions in fascicle size of muscle fibers and endomysium after 14 days of ultrasound treatment.[50] Moreover, ultrasound-facilitated enzyme penetration emerged as one of the key drivers behind the substantial structural changes observed in the muscle. Barekat and Soltanizadeh suggested that the muscle treated with 0.1% (w/v) of papain solution and 100 W of ultrasound treatment for 20 min showed the most pronounced microstructural alterations compared to other treatments.[21] In contrast, the smooth and dense microstructure of shrimp muscle fibers after treatment

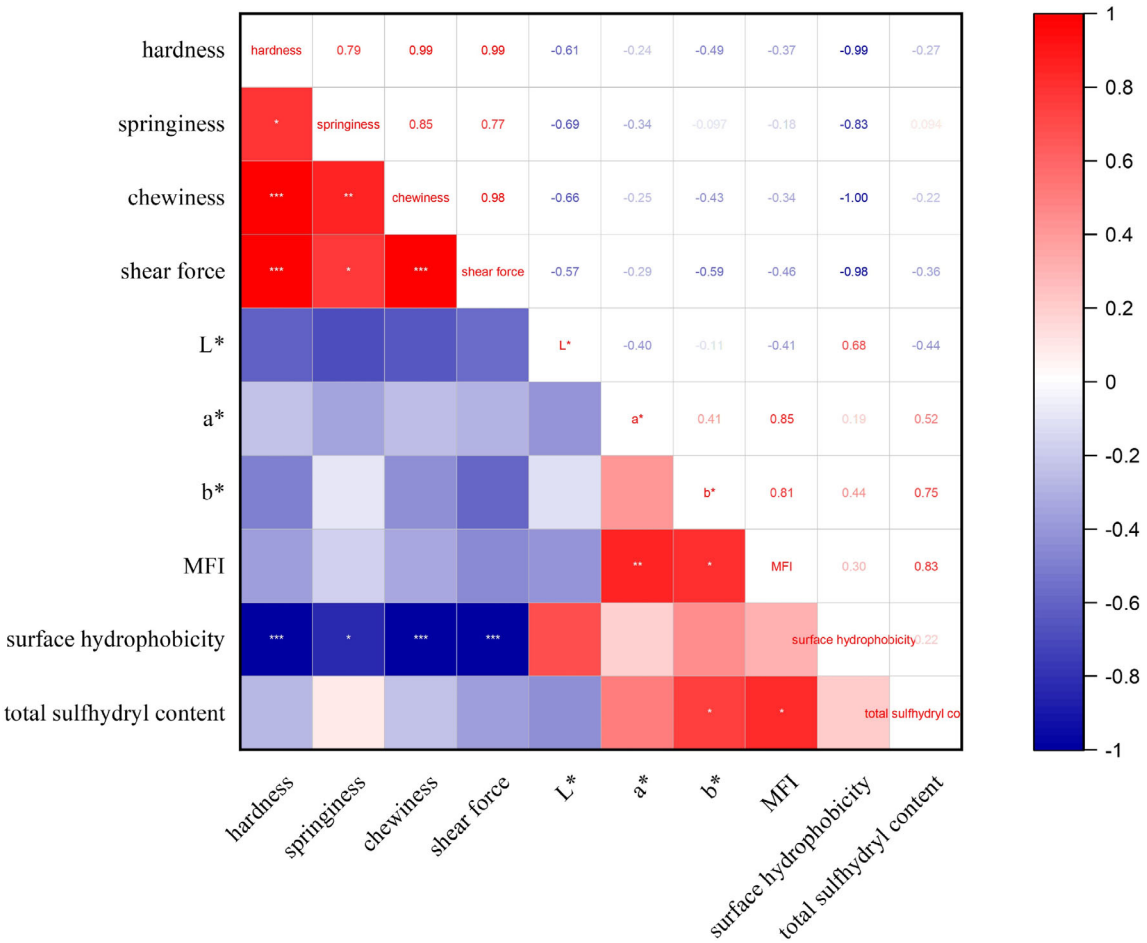

Figure 4. Correlation analysis between physical properties (hardness, springiness, chewiness, cohesiveness, gumminess, and shear force), color attributes (L^*, a^*, b^*), and biochemical parameters (myofibril fragmentation index (MFI), surface hydrophobicity, total sulfhydryl content). Significance levels are denoted as follows: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

at 60 °C (UB 2) may be due to the gelation of muscle proteins caused by high temperature. Similar outcomes were reported by Yang *et al.*[36] However, for dried shrimp, muscle contraction occurred in all groups after drying. This muscle contraction results from the denaturation of muscle proteins during the heating process, leading to muscle fiber contraction and aggregation.[65] The degree of muscle breakage was more significant in dried shrimp after tenderization, indicating that the tenderization treatment effectively enhances the tenderness of dried shrimp. These findings are in line with the analysis of shear force values and TPA results.

Histological observations

The microstructure of both cross-sections and longitudinal sections of tenderized shrimp and dried shrimp is shown in Fig. 3 II. In the control group, the muscle fibers of shrimp meat were closely aligned, exhibiting uniform size and narrow spacing. In contrast, the tenderized group displayed varying degrees of fiber breakage. Notably, the UB 1 group exhibited larger interfibrillar gaps compared to the other groups. This observation might be attributed to water absorption within the intracellular gaps of muscle fibers.[31] Previous studies by Zou *et al.* have shown that the application of ultrasound combined with adenosine monophosphate resulted in histiocyte damage, leading to the separation of beef fibers with larger intracellular spaces.[43] During the drying process, the pineapple protease solution formed a barrier on the meat's surface, preventing tissue contraction and effectively blocking the outward diffusion of water. This mechanism contributed to improved meat tenderness. In addition, microstructural changes in muscle might also lead to the release of cellular contents, which could enhance the proteolytic activity of enzymes in the tissue.[47] Meanwhile, the enhanced proteolytic activity of bromelain might trigger the enzymatic disassembly of muscle fiber structure.[66] Overall, the UB 1 group had more significant muscle gaps with better tenderness, thereby corroborating the conclusions drawn from MFI values and shear forces.

Correlation analysis

It has been observed that alterations in protein structure have a significant impact on meat tenderness.[67] Consequently, a correlation analysis was conducted to determine the relationship between physical properties (hardness, springiness, chewiness, cohesiveness, gumminess and shear force), color attributes (L^*, a^*, b^*), and biochemical parameters (MFI, surface hydrophobicity, total sulfhydryl content). As illustrated in Fig. 4, there were negative correlations between the physical properties of dried shrimp and the color attributes, particularly the L^* value, as well as the biochemical parameters. This suggests that more tender dried shrimp exhibited more appealing color characteristics. The structural modifications in muscle fibers resulting from the combined influence of ultrasound and bromelain led to an improvement in the tenderness of dried shrimp while exposing non-polar amino acids within the protein structure, thus enhancing surface hydrophobicity.

CONCLUSIONS

In conclusion, the hardness and shear values of the samples treated with ultrasound combined with pineapple protease were significantly reduced; both springiness and cohesiveness were improved, and the color of the dried shrimp was redder and yellower. The optimal treatment conditions were determined as follows: power of 100 W, temperature of 50 °C, and bromelain concentration 20 U mL^{-1}. In addition, surface hydrophobicity and total sulfhydryl content were significantly increased in shrimp MPs after ultrasonic cooperation with pineapple protease treatment. Due to protein degradation and destruction, fractures and wide gaps appeared within the muscle. The results of this study provide a solid theoretical basis and technical support for the selection of optimal pre-tenderization conditions for shrimp meat. In the future, we will focus on the development of new types of tenderized dried shrimp products with the addition of various nutrients, as well as research related to the digestive properties and nutritional functions of shrimp products.

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AUTHOR CONTRIBUTIONS

Xinyi Zhou: Conceptualization, methodology, formal analysis, investigation, data curation, writing original draft, visualization. **Jian Wang:** Methodology, validation, formal analysis, investigation. **Jingxu Zhao:** methodology, investigation. **Chunhong Yuan, Xiaojun Zhang** and **Tao Huang:** investigation. **Wenge Yang:** Methodology, investigation, supervision, funding acquisition. **Huamao Wei:** Conceptualization, writing – review and editing, supervision, project administration. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. The raw/derived data supporting the findings of this study are available from the corresponding authors upon request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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