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Combined effects of ultrasound and antioxidants on the quality maintenance of bay scallop (*Argopecten irradians*) adductor muscles during cold storage

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ABSTRACT

The combined effects of ultrasound and the antioxidants of bamboo leaves (AOB) on the quality maintenance of the adductor muscle of scallops (AMSs) during cold storage was investigated. Ultrasound power at 350 W coupled with AOB solution (2% w/v) (UAOB-350) was applied to treat the AMSs according to Taylor diagram analysis. The microstructure, oxidative changes (lipid and protein oxidation), total numbers of colonies, total volatile basic nitrogen, and texture of the AMSs during 6 days of cold storage were analysed. The results indicated that UAOB-350 treatment could effectively retard protein and lipid oxidation and bacterial growth and maintain better microstructure and texture characteristics than AOB solution treatment alone, prolonging the shelf life of the AMSs by 2 days during storage at 4 °C. These results indicate that the UAOB-350 combination method has promising potential to maintain the quality and extend the shelf life of AMSs during cold storage.

1. Introduction

Scallops and their products are popular due to their unique taste and enjoyable flavour; they also contain a variety of nutrients that are easily absorbed by the human body, such as certain essential and delicious amino acids, polyunsaturated fatty acids, polysaccharides, taurine, iron and other trace elements [1]. However, scallops are highly perishable with a limited shelf life after capture, which is mainly associated due to oxidative changes (lipid and protein oxidation) [1], active endogenous enzymes [2], and microbial deterioration [3]. To suppress the above-mentioned chemical reactions, cold storage is the main short-term storage method of scallops, as it can temporarily maintain their quality and extend their shelf life. However, the use of only refrigeration as the fresh-keeping method is often not very effective. Additional safe ways to keep fresh scallops should be explored.

Numerous studies have shown that bacteria and oxidation are important factors that lead to the spoilage of aquatic muscle food products [4]. To effectively control microbial spoilage and oxidation reactions during the aquatic product storage, a variety of compound preservatives have been studied [5]. For example, Wu *et al.* showed that the antioxidants from bamboo leaf chitosan coating treatment can effectively inhibit bacterial growth and the lipid oxidation of scallops to effectively extend their shelf life. Yan *et al.* found that the combination treatment of weakly acidic electrolyzed water and ascorbic acid on shrimp (*Macrobrachium rosenbergii*) can effectively inhibit the growth of bacteria during the entire cold storage process, thereby slowing down the spoilage of shrimp meat and effectively extending its shelf life. In addition, endogenous proteases are an important factor leading to the deterioration of the quality of aquatic foods [2,6]. In view of this, it is particularly important to find a fresh-keeping method that can fully inhibit the oxidation reactions, microbial growth and endogenous enzymatic hydrolysis of aquatic foods during storage.

In recent years, ultrasound has been developed and become an emerging technology to minimize processing, maximize quality and ensure food safety [7,8]. Its applications on biological effects, such as the inactivation of microorganisms, and its effects on enzymatic

Abbreviations: AOB, The antioxidants of bamboo leaves; AMSs, The adductor muscle of scallops; UAOB-350, Ultrasound power at 350 W coupled with AOB solution; POV, Peroxide value; TBARS, Thiobarbituric acid-reactive substances; TVB-N, Total volatile basic nitrogen; TCA, Trichloroacetic acid; DSC, Differential scanning calorimetry; TPA, Texture profile analysis; SEM, Scanning electron microscopy; PCA, Principal component analysis.

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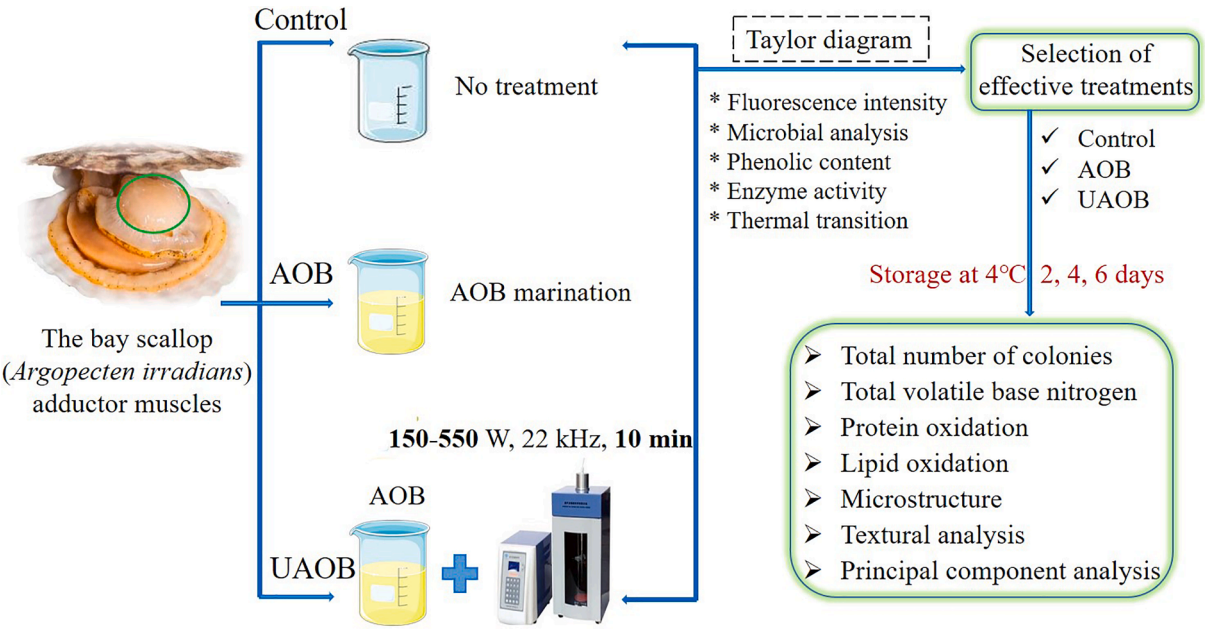

Fig. 1. Procedure flow illustration for the ultrasonic and AOB handling of the adductor muscles of scallops.

activities, has attracted the attention of researchers [7,9]. Ultrasonic technology has the advantages of a low operating cost, high safety, environmental friendliness and high efficiency during operation [8,10]. Some studies have also shown that ultrasound can inhibit the growth of enzymes and microorganisms in aquatic products [7,10–12].

Thus, the aim of this study was to investigate the combined effects of ultrasonic treatment and the antioxidants of bamboo leaves (AOB) on the quality maintenance of bay scallop (*Argopecten irradians*) adductor muscles (AMSs) during cold storage. To fulfil this goal, first, assays were performed to select the most effective ultrasound treatment conditions, including fluorescence intensity, microbial analysis, phenolic content, thermal transition, and enzymatic activity. Then, the AMSs were treated with ultrasound coupled with AOB under the best treatment conditions and stored at 4 °C for 6 days. The microstructure, carbonyl and sulfhydryl contents, peroxide value (POV), thiobarbituric acid-reactive substances (TBARS), total number of colonies, total volatile basic nitrogen (TVB-N), and texture of the samples after various lengths of storage were determined. The observations made in this study provide a promising method to preserve the quality of scallops during cold storage.

2. Materials and methods

2.1. Materials and chemicals

Fresh scallops were purchased from Qianhe Aquatic Market (Dalian, Liaoning, China), stored in a portable cooler containing ice and immediately transported to the laboratory. The AMSs were manually removed from the shell as soon as possible and stored at 4 °C refrigerator until treatment.

AOB (food-grade) were purchased from Zhejiang Shengshi Biotechnology Co., Ltd. (Zhejiang, China). Protein carbonyl and sulfhydryl assay kits and lipase activity assay kits were provided by Nanjing Jiancheng Technology Co., Ltd. (Nanjing, China). All other chemicals were of analytical grade and purchased from Dalian Bono Co., Ltd. (Dalian, China).

2.2. Application of ultrasound and antioxidants

Figure 1 summarizes the process design of all of the experiments. Ultrasound was performed using a TL-615HTD ultrasonic reactor chamber (Jiangsu Tenlin Instrument Co., Ltd.). The chosen concentration of AOB was 2% (w/v), according to previous results [1]. The experimental groups were set as follows. Unless otherwise indicated, the solid–liquid ratio of AMS to soaking solution in all groups was 1:5 (w/v), and the following treatments were performed in an ice water bath (water, ice and NaCl (1: 5: 1, w/w/w)) to keep the temperature of 4.0 ± 1.0 °C.

(1) Control group: the untreated AMSs were served as controls. The control AMSs were stored at 4 °C refrigerator, which was consistent with the ice water bath temperature during AOB and ultrasound treatments. In all the tests in this article, the use of control samples was taken from the refrigerator and tested directly; (2) AOB group: the AMSs were marinated in 0.2% AOB solution (w/v) for 1.5 h; (3–7) Ultrasound + AOB (UAOB) group: the AMSs were marinated in 0.2% AOB solution (w/v) and treated with ultrasound at different ultrasonic power levels for 10 min and then continued to marinate until the time of 1.5 h total was reached. The ultrasonic frequency remained unchanged (22 kHz), and the ultrasonic power was set to 150 W (UAOB-150), 250 W (UAOB-250), 350 W (UAOB-350), 450 W (UAOB-450), or 550 W (UAOB-550). The ultrasound was set to intermittent mode with a 4 s on/2 s off cycle.

After treatment, the fluorescence intensities of the treated AMSs were immediately determined. The remaining samples were frozen in liquid nitrogen and then kept in a −30 °C freezer for further use within 1 week. Each treatment was performed in three replicates.

2.2.1. Distribution of AOB in the adductor muscle

The distribution of AOB in AMSs was determined according to our previous study using a multi-functional in vivo imaging system [1]. All samples were prepared as described in Section 2.2. The AMSs were marinated in the corresponding solution (1:2, w/v) at 4 °C for 0.5 h (1), 1.0 h (2), or 1.5 h (3). The longitudinal section of each AMS was cut to a thickness of 0.5 cm with a knife from the middle position of the AMS. The fluorescence images of the AMSs were collected with a 470 nm bandpass excitation filter and a 525 nm longpass emission filter (exposure time of 200 ms).

2.2.2. Total phenolic content analysis

The total phenolic contents of the AMSs after different treatments were determined by the Folin method as described in our previous study [1]. Briefly, 1 g of sample powder was homogenized with 6 mL of water

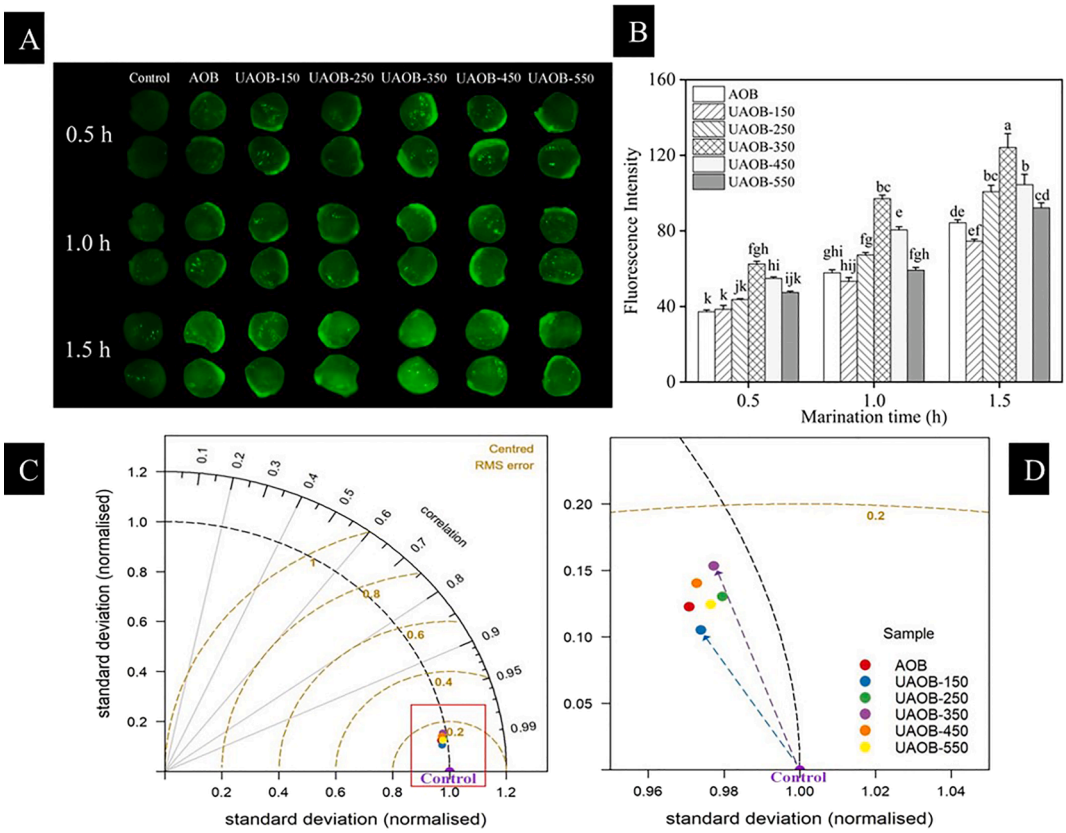

Fig. 2. Fluorescence images (A) and intensities (B) of the AOB in the adductor muscle longitudinal sections during processing. And Taylor diagram (C) and its partial enlarged view (D) for the adductor muscle of scallops after different treatments. The groups of AOB, and UAOB 150-550 represent the AMSs treated with AOB, and the combination of AOB and ultrasound at 150, 250, 350 450 and 550 W, respectively.

and 3 mL of chloroform. After centrifugation, 500 μL of Folin reagent and 1 mL of 10% sodium carbonate aqueous solution were added to 1 mL of the aqueous solution and vortexed. Then, the samples were placed in the dark for 2 h and detected with a microplate reader at 700 nm. The calculation of total phenolic contents used the sample from the control group as a blank. The results are expressed as μg/g of dry adductor muscle.

2.2.3. Microbial assay

The total number of AMS colonies were determined in plate count agar by the spread plate method [13]. Samples (25 g) with 225 mL of 0.1% sterile peptone water were added to stomacher bags and homogenized for 2 min using a T25 homogenizer (Ultra Turrax IKA, Taufen, Germany). The samples were diluted ten-fold with 0.1% protein in electropure (ept) water, and an aliquot (0.1 mL) was plated on an agar plate. Then, the above samples were incubated at 35 °C 48 times for mesophilic counting. All results are expressed as log CFU/g.

2.2.4. Enzyme activity assays

The lipase and cathepsin activities of the AMSs were measured according to Xie *et al.* [1]. The lipase activity of the AMSs after different treatments was measured using an assay kit from Zhejiang Shengshi Biotechnology Co., Ltd. (Zhejiang, China) strictly following the kit instructions. The activity of cathepsin was measured as follows. Briefly, minced samples were homogenized with extraction solution (50 mmol/L sodium acetate buffer (pH 5.0) containing 0.2% Triton X-100, 50 mmol/L cysteine and 1 mmol/L EDTA) at 4 °C at a ratio of 1:5 (w/v). After standing for 90 min, the homogenate was centrifuged at 12,000 g for 15 min. Then, the supernatant was collected, and cathepsin protease activity was determined using azocasein as a substrate. Substrate (0.5 mL) was mixed with 0.5 mL of crude protease sample and placed in a constant temperature shaking water bath at 40 °C for 1 h. After removal, 3 mL of 10% trichloroacetic acid (TCA) solution was added to stop the reaction. The mixture was allowed to stand at room temperature for 15 min and was then centrifuged (10,000 g for 5 min at 20 °C), 1 mL of the supernatant was removed and 1 mL of 1 mol/L NaOH solution was added; this solution was mixed well and the absorbance at 450 nm was measured with a UV spectrophotometer. In the control, deionized water was used to replace the protease sample, and the rest of the protocol was the same. One unit of enzyme activity (U) was defined as the amount of activity that released 0.001 absorbance units per min under the assay conditions.

2.2.5. Differential scanning calorimetry (DSC)

The thermal behaviour of the AMSs after different treatments was analysed according to our previous study [2] using a lDSC III differential scanning calorimeter (Setaram Instrumentation, Caluire, France).

2.3. Storage and physicochemical analysis

After determining the best ultrasound conditions, the effects of different treatments on the physicochemical quality characteristics of the AMSs during cold storage, including the microstructure, carbonyl and sulfhydryl contents, POV, TBARS, TVB-N, and texture profile analysis (TPA), were evaluated. Cold-stored AMSs were sampled on days 0, 2, 4, and 6, and scanning electron microscopy (SEM) and TPA were performed immediately. The remaining samples were stored at −30 °C for further analysis within 2 weeks.

2.3.1. Determination of TVB-N

The TVB-N contents in the AMSs were determined using the Conway micro-diffusion method according to Chinese standard GB 5009.228

Table 1
Changes in phenolics, total number of colonies, enzyme activity and the maximum transition temperature (Tmax) and denaturation enthalpy (ΔH) of myosin (peak 1) and actin (peak 2) of the scallop adductor muscles after different treatments.

	Phenolics μg/g dry basis	Total number of colonies (CFU/g)	Enzyme activity (U/g dry basis)		The maximum transition temperature (Tmax, °C)) and denaturation enthalpy (ΔH, J/g)			
			Lipoxygenase activity	Cathepsin activity	$Tmax_1$	$Tmax_2$	ΔH_1	ΔH_2
Control	0.11 ± 0.00	3.36 ± 0.21^{ab}	0.84 ± 0.04^{b}	12.44 ± 0.30^{ab}	48.35 ± 0.01^{a}	71.14 ± 0.00^{d}	0.78 ± 0.04^{abc}	0.42 ± 0.01^{ab}
AOB	9.81 ± 0.64^{bc}	3.49 ± 0.18^{a}	0.54 ± 0.03^{c}	12.79 ± 0.07^{a}	48.29 ± 0.06^{a}	71.28 ± 0.11^{d}	0.55 ± 0.03^{c}	0.28 ± 0.02^{c}
UAOB-150	8.56 ± 0.46^{d}	3.26 ± 0.15^{ab}	1.29 ± 0.07^{a}	12.47 ± 0.21^{ab}	48.21 ± 0.16^{a}	71.51 ± 0.05^{abc}	0.83 ± 0.03^{a}	0.45 ± 0.01^{a}
UAOB-250	10.02 ± 1.01^{bc}	2.90 ± 0.13^{bc}	0.44 ± 0.04^{d}	11.50 ± 0.14^{c}	48.33 ± 0.01^{a}	71.90 ± 0.06^{ab}	0.58 ± 0.03^{abc}	0.31 ± 0.02^{bc}
UAOB-350	11.78 ± 0.92^{a}	2.37 ± 0.11^{cd}	0.32 ± 0.02^{e}	11.77 ± 0.11^{bc}	48.56 ± 0.12^{a}	71.97 ± 0.03^{a}	0.61 ± 0.14^{abc}	0.33 ± 0.06^{abc}
UAOB-450	10.57 ± 0.94^{b}	2.26 ± 0.10^{d}	0.36 ± 0.04^{e}	10.93 ± 0.49^{c}	48.31 ± 0.24^{a}	71.21 ± 0.27^{d}	0.61 ± 0.02^{abc}	0.36 ± 0.04^{abc}
UAOB-550	9.48 ± 0.88^{cd}	2.36 ± 0.16^{cd}	0.28 ± 0.05^{e}	11.60 ± 0.06^{bc}	48.24 ± 0.03^{a}	71.41 ± 0.17^{cd}	0.80 ± 0.04^{ab}	0.44 ± 0.05^{ab}

Data are expressed as mean ± standard deviation, mean values in a column with different letters (a–e) are significantly different.

(2016) [14]. The TVB content was calculated and is expressed as mg N/100 g scallop meat.

2.3.2. Carbonyl and sulfhydryl content analysis

The protein carbonyl and sulfhydryl contents of the AMSs after different treatments were determined by a Protein Carbonyl and Sulfhydryl Assay kit from Zhejiang Shengshi Biotechnology Co., Ltd. (Zhejiang, China), and all operating steps strictly followed the instructions of the kit. The protein carbonyl and sulfhydryl contents are expressed as nmol/mg protein.

2.3.3. POV assays

The POV of the AMSs after different treatments was measured according to the first method of Chinese standard GB 5009.227 [15]. Briefly, 100 mg of extracted lipids from the AMSs and 0.002 N sodium thiosulfate were mixed for reaction, and the results are expressed as meq/kg lipid.

2.3.4. TBARS assay

The TBARS of the AMSs after different treatments were measured as described in a previous study with a slight modification [1]. In short, a mixture of 500 mg of sample powder, distilled water (2 mL) and trichloroacetic acid solution (10% (w/v), 2 mL) was vortexed for 2 min. The mixture was centrifuged at 8,000 g for 5 min. Then, the supernatant (1 mL) and 2-thiobarbituric acid solution (0.01 M, 1 mL) were reacted in a boiling water bath for 25 min. The absorbance of the mixture was measured at 532 nm. The TBARS value is expressed as mg malondialdehyde (MDA)/g dry adductor muscle.

2.3.5. SEM

SEM observations of the AMS tissues after different treatments were accomplished according to our previous study [2] using a JSM-7800F scanning electron microscope (Japan Electron Optics Laboratory, Tokyo, Japan) at a voltage of 3–10 kV.

2.3.6. Instrumental texture analysis

The TPA of the samples was measured using a TA.TX2 texture analyser according to our previous study [2]. The texture parameters, including hardness, springiness and chewiness, were obtained. Samples for each group were prepared and measured ten times.

2.4. Statistical analysis

The experiments were carried out in triplicate, and the results were compared by Duncan's multiple range tests and one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered to be significant. The Taylor diagram was analysed using R Version 3.6.2 software (Bell Laboratories, Auckland, New Zealand). Principal component analysis (PCA) was performed using SPSS analytical software version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Selection of an effective ultrasound treatment

3.1.1. Effects of ultrasound on the distribution of AOB and phenolic contents

The distribution of AOB in the AMSs after different treatments upon marination is shown in Fig. 2. The AOB show significant green fluorescence at an excitation of 470 nm and an emission of 525 nm (Fig. 2A). This might be attributed to the phenolic substances in AOB exhibit green fluorescence under aforementioned emission light. Similar phenomenon was reported in previous study [1]. During the marinating process, the AOB penetrated into the adductor muscle in all groups and showed time-dependent accumulation (Fig. 2A from top to bottom). Xie *et al.* [1] also found that the AOB gradually entered the scallop (*Argopecten irradians*) adductor muscle with the extension of the immersion time. In contrast, after marinating for 1.5 h, the fluorescence intensity of the AMSs in the UAOB-350 group reached a maximum value of 124.1 ± 7.3, which was 32% higher than that of the AOB treatment group (Fig. 2B). The phenolic contents in all groups after 1.5 h of marination are shown in Table 1. The phenolic contents of the samples treated with AOB were significantly higher than that of the control group. Compared with the control group, the phenolic contents for the samples in the AOB, UAOB-150, UAOB-250, UAOB-350, UAOB-450, and UAOB-550 groups increased by 88.2-, 76.8-, 90.1-, 106.1-, 95.1-, and 85.2-fold, respectively. Notably, the samples in the UAOB-350 group showed the highest phenolic contents, which is consistent with the fluorescence image results. The above results showed that 350 W of ultrasonic power can promote the penetration and diffusion of phenolic substances into AMSs, which may be attributed to the vibration and cavitation from ultrasonic treatment, which facilitates the penetration of phenolics into the scallop cells [7,10,16]. Based on this same principle, Zou *et al.* also reported that low-frequency ultrasound can accelerate the speed of marination in meat products, and a faster pickling process can effectively control the bloating and structural damage of pickled foods [8,17].

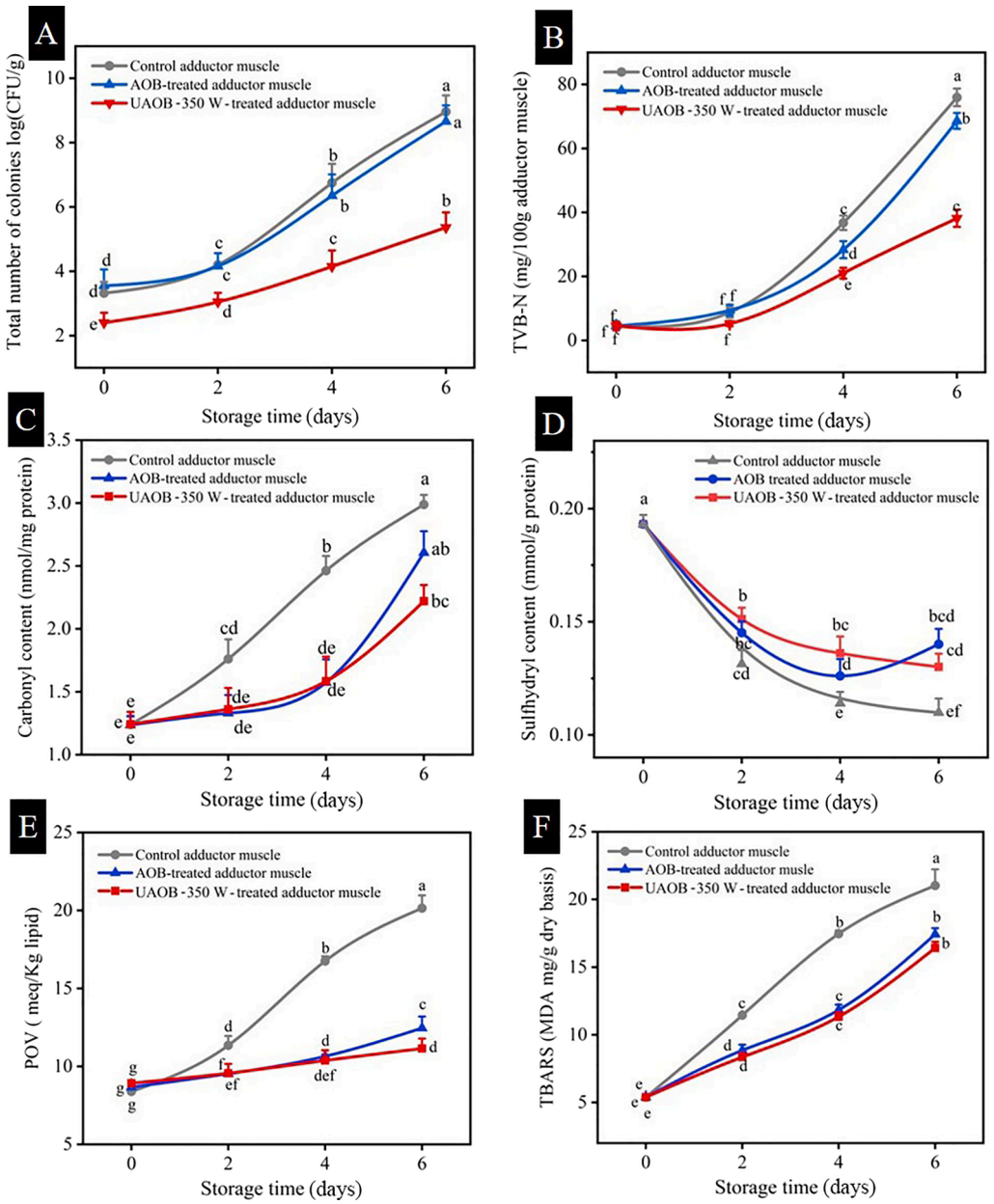

Fig. 3. Changes in total number of colonies (A), TVB-N (B), protein carbonyl content (C), protein sulfhydryl content (D), POV (E), TBARS (F) of the control adductor muscle, AOB-treated adductor muscle and UAOB-350-treated adductor muscle during cold storage (n = 3). Different letters (a-g) indicate significant differences among the samples ($p < 0.05$).

3.1.2. *Effects of ultrasound on the total number of colonies*

The total number of colonies of the control AMSs and AMSs after different treatments are shown in Table 1. The control AMSs showed initial values of 3.36 ± 0.21 log CFU/g for the total number of colonies. After ultrasonic treatment, the total number of colonies in the UAOB-150, UAOB-250, UAOB-350, UAOB-450, and UAOB-550 samples decreased by 3.0, 13.7, 29.5, 32.7 and 29.8%, respectively. Clearly, the AMS samples treated with UAOB from 350 to 550 W showed significantly fewer colonies than the control adductor muscle ($p < 0.05$). Numerous similar studies have shown that appropriate ultrasonic treatment can inhibit the growth of microbes in foods from aquatic muscles [11]. It has generally been speculated that due to the rapid formation and destruction of cavitation bubbles, ultrasonic treatment generates extremely high pressures, thereby inactivating bacteria and decomposing bacterial clusters or flocs [7,8,18]. The above results showed that appropriate ultrasonic treatment was able to inhibit microbial growth in AMSs.

3.1.3. *Effects of ultrasound on the enzyme activity*

Changes in the activities of lipase and cathepsin of the AMSs after different treatments are shown in Table 1. The lipase activity in the control sample was 0.84 ± 0.04 U/g meat. Compared with the control group, the lipase activity for the samples in the AOB, UAOB-250, UAOB-350, UAOB-450, and UAOB-550 groups decreased by 35.7, 47.6, 61.9, 57.1, and 66.7%, respectively. Obviously, UAOB-550 and UAOB-350 were the most effective treatment conditions to significantly inhibit ($p < 0.05$) the lipase activity of AMSs. The cathepsin activity in the control sample was 12.44 ± 0.30 U/g meat. Compared with the control group, the cathepsin activity for the samples in the UAOB-250, UAOB-350, UAOB-450, and UAOB-550 groups decreased by 7.6, 5.4, 12.1, and 6.8%, respectively. Notably, UAOB-450 were the most effective treatment conditions to significantly inhibit ($p < 0.05$) the cathepsin activity

of AMSs. Meanwhile, AOB treatment alone had no significant effect on cathepsin activity ($p < 0.05$). The above results showed that appropriate ultrasonic treatment was able to inhibit the activities of these two enzymes in AMSs. It is speculated that ultrasound uses vibrational energy to lyse cells and inactivate enzymes [19]. Moreover, the mechanical and chemical effects of ultrasonic cavitation make ultrasound particularly useful for enzyme inactivation [7].

3.1.4. Effects of ultrasound on the thermal behaviour of structural proteins

The thermal behaviour of the AMSs after different treatments is shown in Table 1. The transition temperature (T_{max}) and enthalpy (ΔH) involved in the denaturation of the AMS proteins were analysed using DSC. As shown in Table 1, peak 1 and peak 2 generally corresponded to the denaturation of myosin and actin, respectively [20]. After AOB and ultrasound treatment, both the T_{max} and ΔH of the two aforementioned peaks did not change significantly ($p < 0.05$). These observations indicate no changes in the thermal stability of the samples after AOB and ultrasound treatment. The changes in the thermal properties of the proteins caused by different treatments can be used to express the changes in protein structure [21,22]. Thus, our results indicated that ultrasound and AOB treatment had no significant effect on the protein structure of the AMSs.

3.1.5. Taylor diagram analysis

Taylor diagram can provide a concise statistical summary of how well groups match to each other in terms of their correlation and their centred root-mean-square (RMS) difference [2,23]. The above results showed that the phenol content of AMSs in the AOB and UAOB-(150–550) groups was significantly higher than that of the control group after AOB and ultrasonic treatment. Meanwhile, proper ultrasonic treatment can significantly inhibit the increase of enzymes and microorganisms activities in AMSs from the control group and had no significant effect on the protein structure of the AMSs. Therefore, in this study, a Taylor diagram was used to analyse all the detected indicators, including fluorescence intensity, microbial analysis, phenolic content, thermal transition, and enzyme activity, to quickly and clearly decide on the best treatment conditions. The control group was taken as a reference in Fig. 2C, D. Then, we quantified how difference of each group resembles the control group by comparing their correlation and centred RMS difference.

As shown in Fig. 2C, the results showed that the correlation coefficients between the control and the other groups decreased in the following order: UAOB-150 > AOB ≥ UAOB-550 ≥ UAOB-250 > UAOB-450 > UAOB-350. Additionally, the centred RMS difference between the control group and other groups was proportional to their distance apart (Fig. 2D), and the values of the centred RMS differences between the control and the other groups increased in the following order: UAOB-150 < UAOB-550 ≤ UAOB-250 ≤ AOB < UAOB-450 < UAOB-350. The group showing the lowest correlation and the highest centred RMS difference with the control group has the best quality. Thus, the Taylor diagram revealed that the best treatment conditions were those of the UAOB-350 W group. All results showed that an appropriate ultrasonic power (UAOB-350) promoted the immersion of phenols and reduced the activity of the enzymes and the total number of colonies. Moreover, UAOB-350 had no significant effect on the thermal stability of the proteins.

3.2. Combined effects of ultrasound and AOB on the quality of the AMSs during cold storage

3.2.1. Changes in microbial colonies and TVB-N of the AMSs during cold storage

Aquatic product microorganisms are an important reason for the deterioration of the quality of aquatic products during storage [3,10,24]. Therefore, inhibiting the growth of bacterial communities is a key means to maintain the quality of aquatic products during storage. As shown in Fig. 3A, the total number of colonies in the AMSs from the control, AOB and UAOB-350 groups had initial values of 3.32 ± 0.15, 3.55 ± 0.21 and 2.40 ± 0.19 log (CFU/g), respectively, which increased to 8.96 ± 0.51, 8.66 ± 0.49 and 5.36 ± 0.47 log (CFU/g), respectively, after 6 days of cold storage, showing increases of 1.70-, 1.44- and 1.23-fold, respectively. Clearly, UAOB-350 treatment significantly ($p < 0.05$) inhibited the growth of the total number of colonies after 6 days of storage.

TVB-N analysis is commonly used as an evaluation method for monitoring quality in aquatic products during storage [3,25]. Changes in the TVB-N of the AMSs after different treatments are shown in Fig. 3B. The initial TVB-N values of the AMSs in the control, AOB and UAOB-350 groups were 4.56 ± 0.49, 4.52 ± 0.55 and 4.48 ± 0.79 mg/100 g, respectively, which increased to 75.95 ± 2.75, 68.6 ± 2.49 and 38.15 ± 2.67 mg/100 g, respectively, after 6 days of cold storage, showing increases of 15.66-, 14.18- and 7.52-fold, respectively. Notably, after 6 days of storage, the TVB-N values of the AOB and UAOB-350 scallop samples were lower than those of the control group. In contrast, the TVB-N value of the UAOB-350 group was the lowest, indicating that the combination of ultrasound and AOB inhibited the increase in the TVB-N value of scallops significantly more than that of the single AOB treatment group.

In recent years, some studies have shown that ultrasonic technology can be used to sterilize aquatic products, extend their shelf life and improve their safety as food [12]. The principle is mainly the use the cavitation effect of ultrasonic waves, which mainly manifests according to the following two aspects [8,9]: one is that the instantaneous high temperature and high pressure produced by ultrasonic cavitation can have a sterilization effect and the other is that ultrasonic cavitation produces a relatively high temperature and high pressure, and the large shock waves make the microbes on the surface of the aquatic products fall off. Therefore, the results of this study showed that treatment of scallops with 350 W of ultrasound combined with AOB had the best preservation effect by inhibiting the growth of microbes during cold storage.

3.2.2. Changes in protein oxidation of the AMSs during cold storage

In this study, the changes in the degree of protein oxidation during scallop storage are expressed by the contents of protein carbonyls and sulfhydryls [26]. As shown in Fig. 3C, the AMSs from the control, AOB and UAOB-350 groups had initial protein carbonyl contents of 1.24 ± 0.07, 1.23 ± 0.06 and 1.24 ± 0.10 nmol/mg tissue, respectively, which showed increases of 1.41-, 1.11- and 0.79-fold, respectively, after 6 days of cold storage. Additionally, the sulfhydryl contents of the three aforementioned batches before storage were 0.19 ± 0.04, 0.18 ± 0.04 and 0.19 ± 0.03 nmol/mg tissue, respectively, which showed decreases of 43.1, 22.2 and 31.6%, respectively, after 6 days of cold storage (Fig. 3D). Clearly, UAOB-350 was able to effectively inhibit ($p < 0.05$) the increase in the protein carbonyl content of the AMSs. Moreover, the combined AOB and UAOB-350 treatment had a significant effect ($p < 0.05$) on the changes observed in the protein carbonyl and sulfhydryl contents of the AMSs during cold storage. In contrast, the combined treatment of AOB and ultrasound (UAOB-350) showed better inhibitory effects on protein oxidation of the AMSs caused by cold storage.

3.2.3. Changes in lipid oxidation of the AMSs during cold storage

POV and TBARS are two important indicators of lipid peroxidation [26,27]. Among them, the POV usually reflects the degree of lipid oxidation at the primary level; that is, the amount of hydroperoxide, the first-order oxidation product of the reaction. The TBARS value reflects the extent to which hydroperoxide is destroyed into the secondary oxidation product MDA [27,28].

In this paper, the POV (Fig. 3E) and TBARS value (Fig. 3F) continuously increased throughout the 6 days of cold storage, indicating that lipid oxidation occurred during storage. After 6 days of cold storage, the AMSs from the control, AOB and UAOB-350 groups showed a 1.40-,

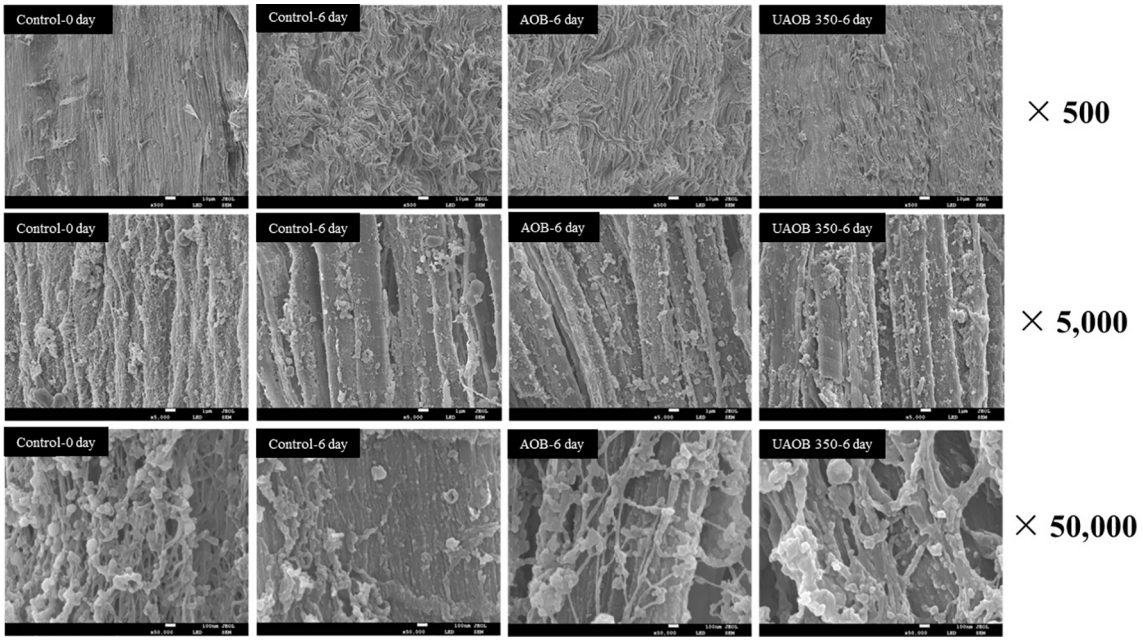

Fig. 4. Scanning electron micrographs of the fresh adductor muscle of scallop (Control-0 day) and cold-stored AMSs (after 6 days of storage) after different treatments. The groups of AOB and UAOB-350 represent the AMSs treated with AOB, and the combination of AOB and ultrasound at 350 W, respectively.

0.44- and 0.25-fold increase in POV content, respectively, and a 2.91-, 2.23- and 2.05-fold increase in TBARS content. Clearly, the AOB and UAOB-350 treatments effectively prevented lipid oxidation ($p < 0.05$). In contrast, the AOB- and UAOB-350-treated AMSs showed a significantly lower ($p < 0.05$) POV and TBARS value than the control AMSs during the entire storage process. Maqsood et al also reviewed the adverse reactions from lipid oxidation during the storage of aquatic products and revealed that phenolic compounds and phenolic substances extracted from plant sources as natural antioxidants can effectively inhibit the oxidation of oils in these products during storage, thereby significantly delaying the deterioration of aquatic products [27].

In summary, both AOB and UAOB-350 effectively inhibited lipid oxidation in AMSs ($p < 0.05$), and UAOB-350 had a better effect, confirming the synergistic antioxidant effects of ultrasound combined with AOB.

3.2.4. *Change in the microstructure of the AMSs during cold storage*

Changes in muscle structure can explain changes in the quality of muscle foods [6,29]. Thus, SEM micrographs of fresh AMSs (control-day 0) and 6 day-cold stored AMSs after different treatments were measured in this study (Fig. 4). Under a low-power microscope (×500), the muscle fibres of the fresh AMSs were tightly arranged. Under a high-power microscope (×5,000, ×50,000), the honeycomb arrangement of the connective tissue was tightly wound around the muscle fibres, and the muscle fibre bundles were densely arranged without the slightest gap. Similar phenomenon was also reported in previous studies [2,30]. After 6 days of refrigeration, under low magnification (×500), the scallop muscle fibres in the control group were clearly arranged in a disorderly manner. Under high magnification (×5,000, ×50,000), there were obvious gaps between the scallop muscle fibres in the control group, and the honeycomb arrangement of the connective tissue almost disappeared. These changes in the microstructure may be due to the self-degradation of the connective tissue and myofibril protein caused by endogenous proteases and oxidation [6,31]. Compared with the control samples, the AOB and UAOB-350 treatments significantly inhibited the degradation of the myofibrillar proteins and connective tissue proteins. In contrast, UAOB-350 has a better effect because, under high magnification, the arrangement of the muscle fibre bundles remained dense, and the connective tissue network structure was still surrounded by muscle fibres.

Table 2
Instrumental textural properties of the scallop adductor muscles after different treatments during cold storage.

Samples	Storage time (d)	Textural properties		
		Hardness (g)	Springiness	Chewiness
Control	0	1425 ± 39^{ab}	0.68 ± 0.03^{a}	459 ± 20^{a}
	2	1202 ± 23^{de}	0.60 ± 0.03^{abc}	359 ± 18^{cd}
	4	1051 ± 51^{f}	0.52 ± 0.01^{bcde}	285 ± 22^{f}
	6	773 ± 28^{g}	0.40 ± 0.03^{f}	206 ± 26^{g}
AOB	0	1413 ± 31^{abc}	0.67 ± 0.03^{a}	442 ± 14^{ab}
	2	1298 ± 18^{bcd}	0.56 ± 0.04^{bcd}	342 ± 10^{de}
	4	1096 ± 44^{ef}	0.51 ± 0.04^{cde}	303 ± 16^{ef}
	6	857 ± 25^{g}	0.43 ± 0.01^{ef}	269 ± 18^{f}
UAOB-350	0	1431 ± 16^{a}	0.67 ± 0.01^{a}	432 ± 13^{ab}
	2	1289 ± 42^{cd}	0.61 ± 0.01^{ab}	403 ± 20^{bc}
	4	1199 ± 33^{de}	0.56 ± 0.01^{bcd}	365 ± 15^{cd}
	6	1080 ± 20^{ef}	0.50 ± 0.02^{def}	315 ± 23^{def}

Data are expressed as mean ± standard deviation, mean values in a column with different letters (a–g) are significantly different.

3.2.5. *Changes in the texture of the AMSs during cold storage*

Texture is an important indicator that characterizes the freshness of aquatic muscle foods [2,29]. Textural changes in AMSs after different treatment and 6 days of cold storage are shown in Table 2. The hardness, springiness and chewiness of the AMSs in the control group remarkably decreased after 6 days of cold storage. Other publications have also reported that long-term storage of AMSs often results in a soft texture, which might be attributed to the proteolysis process that occurs in AMSs during 6 days of cold storage [2,32]. The AOB and UAOB-350 treatments retarded the decreases in hardness, springiness and chewiness of the AMSs; for example, the hardness values were 11 and 40% higher than that of the control AMS after 6 days of storage. In contrast, UAOB-350 significantly inhibited the deterioration of the AMS texture caused by cold storage compared with AOB treatment alone.

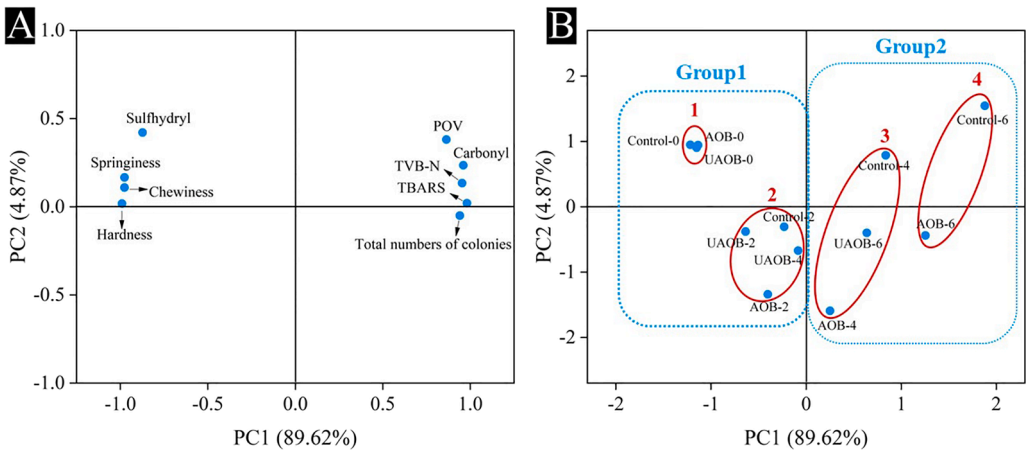

Fig. 5. Demonstrations of (A) interrelation among the analysed parameters and (B) positioning of analysed samples compared to each other. The AOB, and UAOB-350 samples represent the AMSs treated with AOB, AOB and ultrasound power at 350 W, respectively. Numbers represent storage time in days.

3.2.6. PCA

The above results shows that the AOB and UAOB-350 treatments have an important effect on muscle quality and the physical properties of the AMSs during cold storage, but there was no objective method to systematic measure their influence. It is difficult to accurately evaluate the degree of quality deterioration between samples overall according to the individual indicators. Therefore, PCA was used to process all of the tested indicators to visually reflect the degree of quality deterioration between the samples after different treatments. In addition, a correlation between these treatments and their muscle microstructure and physicochemical and textural characteristics of the cold-stored AMSs was provided.

As shown in Fig. 5, the first two principal components (PCs) explained 94.49% of the total variation in the data (PC1 and PC2 accounted for 89.62% and 4.87%, respectively), indicating that there is a strong interaction between the treatment conditions and the quality of the AMSs. The loading plot (Fig. 5A) shows that the hardness, springiness, chewiness, and sulfhydryl content parameters were positively correlated with each other and negatively correlated with POV, TBARS, TVB-N, carbonyl content, and total number of colonies. The parameters POV, TBARS, TVB-N, carbonyl content, and total number of colonies were positively correlated with each other. Additionally, it was speculated that the quality of the AMSs from different groups located in the first, second, third, and fourth quadrants were related to the variables in the corresponding quadrants.

The score plots (Fig. 5B) show that there are significant differences between the AMSs processed under different conditions and stored for different times. With the centre X axis as the boundary, all samples can be divided into two groups. The first group included the control-0 and control-2 day; AOB-0 and AOB-2 day; and UAOB-0, UAOB-2, and UAOB-4 day samples. These samples had the highest hardness, springiness, chewiness, and sulfhydryl content values. The second group was characterized by control-4 day and control-6 day; AOB-4 and AOB-6 day; and UAOB-6 day samples, which had the highest values of POV, TBARS, TVB-N, carbonyl content, and total number of colonies. According to the degree of quality deterioration, the samples can be subdivided into four parts (Fig. 5B, red): part 1 (control-0, AOB-0, UAOB-0) < part 2 (control-2, AOB-2, UAOB-2 and UAOB-4) < part 3 (control-4, AOB-4, UAOB-6) < part 4 (control-6 and AOB-6). These results indicated that quality deterioration occurred in samples from the control, AOB, and UAOB groups during cold storage between 2 and 6 days, 2–6 days, and 4–6 days, respectively. Therefore, it can be inferred that the shelf lives of the control, AOB and UAOB-350 samples are 2, 2 and 4 days, respectively. Clearly, compared with AOB treatment alone, the combination treatment of AOB with ultrasound at 350 W showed better inhibitory effects on the quality deterioration of AMSs caused by cold storage. UAOB-350 treatment prolonged the shelf life of the AMSs by 2 days during storage at 4 °C. This may be attributed to UAOB-350 treatment effectively retarding protein and lipid oxidation and bacterial growth and maintaining a better microstructure than treatment with AOB solution alone.

4. Conclusions

In this study, assays were performed to select the most effective ultrasound treatment conditions, including fluorescence intensity, microbial analysis, phenolic content, thermal transition, and enzymatic activity. Then, the combined effects of ultrasound and AOB on the quality maintenance of the AMSs during cold storage was investigated. The main results are summarized as follows:

(1) Taylor diagram clearly revealed that the best treatment conditions were those of the UAOB-350 W group. Because UAOB-350 promoted the immersion of phenols and reduced the activity of the enzymes and the total number of colonies, and UAOB-350 had no significant effect on the thermal stability of the proteins of the AMSs.

(2) Quality deterioration occurs in the AMSs (*A. irradians*) during 6 days of cold storage, and this process was retarded in groups of UAOB-350. The results indicated that UAOB-350 treatment could effectively retard protein and lipid oxidation and bacterial growth and maintain better microstructure and texture characteristics of AMSs than treatment with AOB solution alone.

(3) UAOB-350 treatment prolonged the shelf life of the AMSs by 2 days during storage at 4 °C. The results indicate that this combination (UAOB-350) has potential as a promising method to maintain the quality and extend the shelf life of AMSs during cold storage.

CRediT authorship contribution statement

Bing Liu: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **De-yang Li:** Resources, Investigation. **Zi-xuan Wu:** Software, Methodology. **Wen-jian Yang:** Methodology, Formal analysis. **Da-yong Zhou:** Conceptualization, Methodology, Supervision, Validation. **Bei-wei Zhu:** Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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