Synergistic effect of combined treatment with allicin and antioxidants of bamboo leaves and preservation of bullfrog (Lithobates catesbeiana) during refrigeration storage

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Abstract

The effects of allicin (All) and antioxidant of bamboo leave (AOB) on the quality of bullfrog (Lithobates catesbeiana) during refrigerated storage were investigated. The results demonstrated that the treatment of All and AOB could reduce the decline of moisture loss and maintain the water-holding capacity (WHC). In addition, combination treatment inhibited the increase of total viable counts (TVC), exhibited good antibacterial activity, delayed the decrease of amino acid content and retarded the sensory deterioration. The reduction of thiobarbituric acid (TBA) and total volatile basic nitrogen (TVB-N) indicated that allicin combined with antioxidant of bamboo leave (AA) could restrain the degradation of protein and lipid oxidation. As a consequence, AA treatment prolonged the shelf-life of bullfrogs for another 6 days. Allicin combined with antioxidant of bamboo leave treatment could be an effective approach to extend the shelf-life and delay the biochemical reaction of refrigerated bullfrog.

1. Introduction

Bullfrog (Lithobates catesbeiana) is one of aquacultural species in China, which is considered as one of the most worth cultured frog species. The cooked frog meat is white in color, tender in texture and lightly sweet in taste, which is similar in mouthfeel to chicken (Nóbrega et al. 2007). Frog legs are popular among consumers due to its high nutritional value, specifically, rich in moisture and protein, and low in calories. They are considered as delicious food and popular in many countries around the world (Gratwicke et al. 2010). Frog meat is popular not only because of its delicious taste, but also it is rich in high-quality proteins, which are easily in digestion and abundant in many essential amino acids (Silva et al. 2015). Compared with frozen meat (especially after frozen-thawed cycles), fresh bullfrog meat is preferred by customers, because the frozen-thawed frog meat has diminished sensorial qualities of taste, compared with the fineness and juiciness of the fresh one after cooking. Therefore, local breeders usually take live bullfrogs to markets rather than sacrificing them. However, live frog transportation is also flawed. Because the frogs can be easily frightened, causing fatal crush. Moreover, cannibalism is more frequent in crowded space during transportation and subsequent processing (Chen et al. 2021). As a result, the development of post-slaughter processing based on refrigeration is urgent for the bullfrog industry.

Allicin (All) is an oxygenated sulfur compound, which was isolated from crushed garlic petals in 1944 and identified as a compound with antibacterial activity. As one of the important organic sulfur compounds, it is beneficial to human health but also accompanied by typical penetrating odor (Salehi et al. 2019; Marchese et al. 2016). The interaction of alliin (S-allyl-L-cysteine sulfoxide) with the catalytic action of alliinase (alliin lyase; EC 4.4.1.4) during the crushing of garlic leads to the production of allicin.

Antioxidants of bamboo leaves (AOB) extracted from bamboo leaves, which is a brown-yellow powder and mainly made up with lactones, flavonoids and phenolic acids (He et al. 2020). It is a natural additive and has been widely used in food industry (Nirmala et al. 2018). AOB has widely used in food
preservation, such as fish, meat and edible oils. Some research showed that AOB has good antioxidant activity on the preservation of aquatic products (Xie et al. 2019; Hu et al. 2022; Nirmala et al. 2018).

At present, fresh delivery is very popular in China. As one kind of fresh food, bullfrog quality is particularly critical in the process of cold chain delivery. Frozen bullfrog products have severe moisture loss and poor taste. The cost of live bullfrog transportation is expensive. Therefore, it is important to develop post-slaughter storage of bullfrogs with refrigeration as the mainstay. But most of them are thermal treatment, there are no researches on the effect of natural bio-preservative treatment on bullfrog. The objective of our study is to investigate the effects of All and AOB treatment on the quality of bullfrogs during refrigerated storage.

2. Materials And Methods

2.1. Materials and reagents

The allicins (purity: ≥95%) were purchased from the Aladdin Industrial Inc., Shanghai, China. AOB (≥ 40.0% of total phenols) was provided by Shaanxi Zhenghe Phar Biotechnology Co., Ltd. (xi’an, Shaanxi, China). All reagents used in the study were analytical grade.

2.2 Preparation of bullfrog samples and sample treatment

Live bullfrogs were purchased from aquatic market (Shanghai, China) and transported to the laboratory immediately. Subsequently, samples were sacrificed and removed the head, internal organs and then flushed with water. They were stochastically split into four groups and underwent different treatment: (1) samples treated with deionized water (CK); (2) samples treated by 0.5 g/L (w/v) allicin solution (All); (3) samples treated by 0.5 g/L (w/v) antioxidants of bamboo leaves (AOB); and (4) samples treated by 0.25 g/L allicin solution and 0.25 g/L AOB solution (AA). Each group was dipped in respective solutions for 10 min, drained and then stored in polythene bags at 4°C in refrigerator for subsequent analysis at different period. Microbiological, physicochemical, sensory evaluation and protein indexes were analyzed at 2-day intervals in triplicate.

2.3 Microbiological analysis

The microbial were carried by the method of Liu (Liu et al. 2020). TVC and PBC were performed on samples incubated at 30°C for 72 h and at 4°C for 10 days using plate counting agar (PCA). All counts were done in triplicate and expressed as log CFU/g of samples.

2.4 Physicochemical analysis

2.0 g bullfrog sample was homogenized with 18 mL of distilled water. The final solution measured by digital pH meter (Mettler Toledo, Zurich, Switzerland).

The determination of TVB-N values were followed by the method of Li (Li et al. 2012) with slightly modification. 2.0 g of minced sample after adding MgO was determined by FOSS Kjeldahl analyzer
(FOSS China Shanghai Co. Ltd). The TVB-N was denoted by mg N/100 g.

The measurement of TBA value was followed by the measurement of Sanchez (Sánchez-Alonso et al. 2008). TBA values were expressed as (MDA)/kg.

The WHC of bullfrog was detected by the method of Zhao (Zhao et al. 2021). The sample weights before and after centrifugation were marked as \( m_1 \) and \( m_2 \). The WHC value was expressed as following:

\[
WHC(\%) = \left( 1 - \frac{m_1 - m_2}{m_2} \right) \times 100
\]

TPA was measured by the Analyzer of Texture (Stable Micro Systems Ltd., England) and the parameters followed by the method of (Merlo et al. 2019).

Free amino acids (FAAs) determination: 2.0 g frog sample mixed with 15 mL cold trichloroacetic acid (5%). The mixture was centrifuged after 5 min of sonication. Kept static at 4°C for 2 h, homogenized and centrifuged at 4°C at 10 000 \( \times g \) for 10 min. Combined the supernatants, adjusted pH to 2.0 then diluted to 10 mL. The solution was filtered with 0.22 µm water phase filter then analyzed with analyzer of amino acid (model L-8800; Hitachi, Tokyo, Japan).

2.5 Protein characteristic

2.5.1 Fluorescence spectroscopy analysis

The intrinsic fluorescence emission spectra of 1 mg/mL protein solution were determined by F-7100 fluorescence spectrometer (Shanghai Smaio Analytical Instruments Co. Ltd).

2.5.2 Myofibril fragmentation index (MFI)

MFI was measured as described as Zhao(Zhao et al. 2021). And the MFI value was the absorbance value at 540 nm multiplied by 200.

2.6 Sensory evaluation

Sensory characteristics of bullfrogs with different treatments were assessed by ten experienced members with similar product evaluation experience. Sensory analysis was performed on each group according to color, texture, odor and overall acceptability scores of 40, 30, and 30 percentages were weighted based on a 10-point descriptive analysis. A score of 8–10 indicated “very good,” “good,” corresponded to 5.0–7.9, and 1.0–4.9 represented as spoiled.

2.7 Statistical analysis

The results are denoted as the mean ± standard deviation and all measurements repeated at least in replicate. The data were analyzed by SPSS software (vision 13.0) and Origin 2018. Statistical analysis
was performed with Duncan's multiple range tests and one-way ANOVA, and significant differences were set at $p < 0.05$.

3. Results And Discussion

3.1 Microbiological analysis

Different superscript lowercase letters represent significant differences within groups ($p < 0.05$).

From Fig. 1, the number of bacteria in treated groups was lower than that in CK group ($P < 0.05$). The initial value of TVC in CK group was $4.03\log$ CFU/g, and reached $9.02\log$ CFU/g at day 12 (Fig. 1a). The bacterial growth rate of CK group was higher than those of other groups. The above results demonstrated that All and AOB treatment could effectively inhibit the increase of microorganism. Psychrophilic is the principal microorganism contributing to the deterioration of fish products during storage. Initial PBC was $4.0\log$ CFU/g in CK group (Fig. 1b). The PBC in other treated groups were 2.95, 3.10 and 2.94 log CFU/g at day 0, which significantly lower than CK group, indicated that the preservative treatment could inhibit the PBC at the early storage. The number of psychrophilic bacteria was consistent with the trend of TVC.

3.2 Physicochemical analysis

3.2.1 Change in pH, TVB-N and TBA value

The accumulation of alkaline substances was mainly the activity of endogenous enzymes and microorganisms, which caused the rise of pH value. And volatile bases are converted from proteins and other nitrogenous substances (Liu et al. 2020). In Fig. 2a, the initial pH value of bullfrog was 6.44, which was a little bit lower than the results reported by Boga (Büyükdeveci et al. 2019). The increase of pH value in CK group had the largest range of changes; the pH value in CK group was varied from 6.44 to 7.27 throughout storage. While samples in All, AOB and AA groups had relatively lower pH value. The fluctuations in pH value might be caused by the dissolution of CO$_2$ into meat aqueous phase, further to form carbonic acid.

TVB-N is extensively used to assess the quality of aquatic products. TVB-N value also can reflect the quality of bullfrog, it is closely related with the activities of bacteria and relative enzymes. The TVB-N value (Fig. 2b) in CK group increased significantly, ranged from 6.65 to 43.81 mgN/100 g ($p < 0.05$). Lang (Lang 1979) reported that the acceptability limit of TVB-N is 30 mgN/100 g. However, Sohrab (Sohrab et al. 2009) documented that different seafood had different levels of acceptability. In the present study, the corresponding TVB-N value in the CK group was 25.26 mgN/100 g on the 8th day of sensory rejection. The TVB-N value in other groups exceeded 25.26 mgN/100 g on the 12th, 12th and 14th days.

TBA value is a vital criterion to evaluate the lipid oxidation. MDA was a kind of secondary lipid oxidation product. In the initial and intermediate stages, the level of thiobarbituric acid active substance increased. Some substances, such as amino acids, proteins, nucleosides and nucleic acids in bullfrog, led to the
increase of TBA value at the end of storage (Li et al. 2020). As illustrated in Fig. 2c, the TBA values rose from 0.06 mg MDA/kg sample at day 0 to 0.10, 0.10, 0.09, and 0.09 mg MDA/kg sample at day 10 for CK and day 14 for All, AOB and AA, respectively. The AOB and AA treatments were valid in retarding the lipid oxidation, which may be related to the antioxidant effect of AOB ($P < 0.05$).

3.2.2 Change in WHC and TPA

The WHC of samples was shown in Fig. 3a. There was a downward trend among groups, while the CK group exhibited lower WHC compared to All, AOB and AA group. The WHC in CK group reduced from 63–49%, and the All, AOB and AA group decreased to 52%, 54% and 54%, respectively. The results showed that the treatment reduced the water loss. The sharply decrease of WHC in CK group may be explained by the degeneration of actin and myosin impaired the water holding capacity (Liu et al. 2021). The WHC of AA group was the highest, suggesting that allicin combined with AOB treatment could effectively reduce the moisture loss. The results showed that the treatment protected muscle protein, reduced water loss and maintained the quality of bullfrog.

To a certain extent, the acceptability of consumers depends on texture, because textural properties were reflected gel properties and taste characteristics of bullfrog. Musculature is influenced by WHC and some inherent biological factors (Zhao et al. 2012). The effect of hardness and springiness of bullfrog with different treatments were showed in Fig. 3b. It could be observed that the hardness of sample showed a downward trend. Moreover, the AA group had higher hardness value than CK, All or AOB groups. Under the action of external force, the ability of sample was restored its initial condition, which called springiness, named elasticity as well. Springiness reflects the degree of recovery of flesh after external force (Liu et al. 2020). It was found that only CK group showed a significant decrease in springiness during the first 2 days of storage. After that, the springiness gradually decreased. The springiness of AOB and AA groups decreased significantly at day 6. It is noteworthy that the springiness of bullfrog was higher after the treatment with the compound solution than the other groups. The results of TPA were compatible with the variation trend of WHC. The texture-protective effect of the preservative could be ascribed to its ability to inhibit the proteolytic enzymes activity in muscle tissue, the enzyme that destroyed cells and caused structural changes rapidly (Xu et al. 2014). The results demonstrated that combination treatment had a protective effect on the texture of bullfrogs during refrigerated storage.

3.2.3 Free amino acids
Table 1
Changes in FAA of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Asp</th>
<th>Thr</th>
<th>Ser</th>
<th>Glu</th>
<th>Gly</th>
<th>Ala</th>
<th>Val</th>
<th>Met</th>
<th>Ile</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-0</td>
<td>1.09±0.16c</td>
<td>3.08±0.05a</td>
<td>2.63±0.00b</td>
<td>3.68±0.03b</td>
<td>2.24±0.01c</td>
<td>5.54±0.04c</td>
<td>3.54±0.40a</td>
<td>1.31±0.39b</td>
<td>1.47±0.02c</td>
</tr>
<tr>
<td>CK-6</td>
<td>2.82±0.02b</td>
<td>1.78±0.02b</td>
<td>6.10±0.03a</td>
<td>3.84±0.03b</td>
<td>7.39±0.05b</td>
<td>6.25±0.00b</td>
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<td>2.26±0.09b</td>
</tr>
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<td>CK-12</td>
<td>5.02±0.38a</td>
<td>1.29±0.06c</td>
<td>6.39±0.36a</td>
<td>4.43±0.08a</td>
<td>13.23±0.39a</td>
<td>8.50±0.13a</td>
<td>0.79±0.02b</td>
<td>3.65±0.16a</td>
<td>3.27±0.20a</td>
</tr>
<tr>
<td>All-0</td>
<td>2.17±0.03c</td>
<td>2.04±0.05b</td>
<td>1.51±0.03c</td>
<td>4.28±0.15c</td>
<td>1.64±0.01c</td>
<td>6.27±0.03b</td>
<td>3.67±0.57a</td>
<td>1.30±0.06b</td>
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<td>All-6</td>
<td>3.86±0.13a</td>
<td>1.89±0.04b</td>
<td>5.16±0.08a</td>
<td>2.47±0.02b</td>
<td>6.25±0.00b</td>
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<td>1.34±0.00b</td>
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<tr>
<td>All-12</td>
<td>1.33±0.02b</td>
<td>2.46±0.07a</td>
<td>1.74±0.05b</td>
<td>8.20±0.21a</td>
<td>6.30±0.04a</td>
<td>9.32±0.05a</td>
<td>3.80±0.03a</td>
<td>2.51±0.01a</td>
<td>2.20±0.08a</td>
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<tr>
<td>AOB-0</td>
<td>0.67±0.02b</td>
<td>0.67±0.02c</td>
<td>1.76±0.09b</td>
<td>2.78±0.33b</td>
<td>1.62±0.01b</td>
<td>3.95±0.05c</td>
<td>2.86±0.02b</td>
<td>1.41±0.01b</td>
<td>1.49±0.01b</td>
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<tr>
<td>AOB-6</td>
<td>0.96±0.08b</td>
<td>1.45±0.02b</td>
<td>1.74±0.01b</td>
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<td>2.19±0.06a</td>
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<td>7.43±0.12a</td>
<td>2.07±0.03a</td>
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<td>3.87±0.17a</td>
<td>2.20±0.10a</td>
<td>1.99±0.10a</td>
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<td>0.17±0.01c</td>
<td>1.62±0.24a</td>
<td>5.05±0.83a</td>
<td>1.97±0.27a</td>
<td>4.71±0.34b</td>
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<td>1.37±0.37a</td>
<td>1.31±0.03c</td>
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<tr>
<td>AA-6</td>
<td>0.22±0.01b</td>
<td>2.36±0.07a</td>
<td>1.61±0.06a</td>
<td>5.72±0.23a</td>
<td>1.84±0.00a</td>
<td>5.03±0.12a</td>
<td>3.25±0.16a</td>
<td>1.54±0.00b</td>
<td>1.51±0.00b</td>
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<td>1.98±0.00b</td>
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<td>6.52±0.02a</td>
<td>2.11±0.01a</td>
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<td>Phe</td>
<td>Lys</td>
<td>His</td>
<td>Arg</td>
<td>Pro</td>
<td>Total</td>
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<td>2.18 ± 0.02c</td>
<td>3.20 ± 0.09ab</td>
<td>7.99 ± 0.00a</td>
<td>8.38 ± 0.09b</td>
<td>1.86 ± 0.15c</td>
<td>2.47 ± 0.31a</td>
<td>54.01 ± 1.87</td>
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<td>4.40 ± 0.15b</td>
<td>1.75 ± 0.20b</td>
<td>5.97 ± 0.41b</td>
<td>8.85 ± 0.02b</td>
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<td>6.74 ± 0.22b</td>
<td>12.17 ± 0.71a</td>
<td>0.50 ± 0.03a</td>
<td>3.72 ± 0.67a</td>
<td>86.03 ± 4.67</td>
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<tr>
<td>All-0</td>
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<td>2.20 ± 0.18c</td>
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<td>4.45 ± 0.09a</td>
<td>6.96 ± 0.05b</td>
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<td>6.32 ± 0.14a</td>
<td>6.89 ± 0.24ab</td>
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<td>3.09 ± 0.05a</td>
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<td>53.87 ± 2.10</td>
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</tr>
</tbody>
</table>

Different superscript lowercase letters represent significant differences within groups (p < 0.05).

FAAs can be the precursors of volatile organic compounds for aquatic products and they are closely related to the flavor. In Table 1, sixteen FAAs were detected. Among all kinds of FAAs, the contents of alanine (Ala), glutamic acid (Glu) and glycine (Gly) were the top three amino acids. From 0 to 12 day, the total FAA content increased in each group, although the increments vary in size. Then, the final value of FAA contents reached 86.03 mg/100g in the CK group, 81.38 mg/100g in All group, 72.56 mg/100g in AOB and 53.87 mg/100g in AA group.

The TAV value was calculated based on different FAA thresholds and reflected the effect of different treatments on the flavor property of bullfrog. In Fig. 4, the value of umami-TAV decreased during storage. Nevertheless, at day 12, the flavor amino acids content increased in each treated group, which enhanced the flavor value of bullfrog. Bitterness-TAV showed an upward trend among groups. AA treatment could retard the increase of histidine content by controlling microbial metabolism and water loss. Histidine
content ranged from 8.38 mg/100g to 12.17 mg/100g in CK group, but the corresponding contents in AA were only 5.89 mg/100g at day 12, which led to bitterness reduction for bullfrog meat throughout the storage.

3.3 Protein characteristics

3.3.1 Fluorescence spectroscopy analysis

Figure 5 showed that the variation of intrinsic fluorescence intensity (IFI) of bullfrog. The highest fluorescence intensity was observed in the fresh sample at 335 nm, signifying that the structure of protein was complete, because residues were enclosed inside the MP. The IFI in myofibrillar protein (MP) reflecting the protein conformational changes (Zhao et al. 2021). It could be seen that the IFI in CK group dropped sharply throughout the storage, which mainly caused by the unfolding of oxidized MP. It can be seen that the AA group had a higher IFI compared with the other three groups at the later stage of storage, which showed that the combination treatment could delay the denaturation, aggregation or destruction of tertiary structure in MP.

3.3.2 Myofibril fragmentation index (MFI)

The MFI value reflects the tenderness of meat and represents the myofibril integrity (Silva-Buzanello et al. 2018). High value of MFI indicated the severe disruption of myofibril structure, which was associated with the improvement of tenderness. In Fig. 6, the MFI value significantly increased from 9.90 to 32.65 in the CK group \(P < 0.05\). On day 0, the MFI values in treated group were lower than that in CK group, which indicated that the addition of preservative inhibited the increase of MFI. All and AA group showed better effect than CK and AOB group during storage, it may be related to the bacteriostasis of allicin, which inhibited the growth of bacteria and restrained the protein degradation. Therefore All and AA group had lower MFI value, and the MFI results were consistent with the change of microbiological index.

3.4 Sensory evaluation

Sensory is a visible index of freshness, containing texture, odor, color and overall acceptability (Xuan et al. 2016). The overall acceptability for human consumption was inedible when point below 5. The original sensory scores of all parameters were close to 9, indicating that bullfrog meat was very fresh. Originally, as the progress of storage time, the sensory scores in Fig. 7 showed a downward tendency. However, the sensory scores in All and AA groups were upper than those of others. The All and AOB samples were acceptable till 12th and 14th day, while the over acceptability in AA sample still above 5 at the 14th day, by the contrast, the samples of CK group was acceptable until 8th day of storage. According to the results, sensory analysis was considered as a good indicator because its values significantly correlate with TVB-N and other indexes. Besides, the results illustrated that combination solution treatment could keep better quality and prolong the shelf-life of bullfrog for another 6 days.

3.7 Correlation analysis
Correlation analysis between quality indexes of bullfrog during refrigerated storage was exhibited in Fig. 8. There was a positive correlation among TVB-N, TBA, TVC, PBC and MFI. While these indicators showed a negative correlation with WHC, hardness, springiness and sensory analysis. Not surprisingly, with the extension of storage time, the increase of TVC, PBC, TVB-N and TBA values which reflected the degradation of protein and lipid oxidation, resulted in reduction of water-holding capacity, deterioration of texture and decline of sensory analysis. There was a significant correlation between sensory analysis and TBA, TVB-N, TVC, PBC, WHC, hardness, springiness.

### 3.8 Diagram of antibacterial mechanism of allicin and AOB

Allicin can rapidly pass through cell membranes with ease owing to its hydrophobic nature, reaching cellular compartments where reacts rapidly with free thiol groups. Acetyl-CoA synthetases blockage and glutathione oxidation initiation, which result in a shift on cellular redox-potential, membrane disruption leading to cellular content leakages (Salehi et al. 2019). AOB play a major role in scavenging free radicals (Fig. 9). Based on these variations, microbial growth is effectively inhibited. Therefore, retard the increase of TVC, TVB-N and TBA and extend the shelf-life of bullfrog.

### 4. Conclusions

The results of present study suggested that allicin, antioxidants of bamboo leaves and combination treatment could retard the growth of microorganisms, slow down the augment of TBA, TVB-N and texture changes in bullfrog during refrigerated storage. The combined treatment was effective in sensory evaluation of bullfrogs, and the sensory quality was well maintained, at the same time, the shelf-life of bullfrog during refrigerated storage could prolong for another 6 days. Therefore, the excellent antioxidant and antimicrobial activity of allicin and AOB, as revealed in the present study, provide a potential approach for increasing the shelf-life of bullfrogs and keeping its quality better during storage. However, the mechanism of allicin and AOB on improving the quality and inhibiting the specific spoilage microorganisms of bullfrog needs to be further investigated.

### Declarations

#### Data Availability

The data supporting the results of this study are available from the corresponding author, Weiqing LAN (wqlan@shou.edu.cn).

#### Acknowledgments

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#### Declaration of competing interest
We confirm that the manuscript has no interest issues.

Author contribution

Jintao DU designed the experiment, finished the study, collected test data, and drafted the original manuscript. Weiqing Lan and Xiao XU reviewed the data interpretation and edited the manuscript. Shengyun ZHU assisted in finishing the experiment. Jing Xie was responsible for project administration.

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3. Gratwicke, Evans, MJ, Jenkins, PT, Kusrini, MD, Moore, RD & ENVIRON SJFE (2010) Is the international frog legs trade a potential vector for deadly amphibian pathogens?


Figures

Figure 1

Changes in Total viable counts (a), *Psychrophilic bacteria counts* (b) of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage.
Figure 2

Changes in pH (a), TVB-N (b) and TBA (c) of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage.
Figure 3

Changes in WHC (a), Hardness (b) and Springiness(c) of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage
## Figure 4

The thresholds of different amino acids taste (a) and changes in TAV-umami (b) and TAV-bitter (c) of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage.

<table>
<thead>
<tr>
<th>FAA</th>
<th>Taste</th>
<th>Threshold (mg/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>Umami</td>
<td>100</td>
</tr>
<tr>
<td>Glu</td>
<td>Umami</td>
<td>30</td>
</tr>
<tr>
<td>Ser</td>
<td>Sweet</td>
<td>150</td>
</tr>
<tr>
<td>His</td>
<td>Bitter</td>
<td>20</td>
</tr>
<tr>
<td>Gly</td>
<td>Umami/Sweet</td>
<td>130</td>
</tr>
<tr>
<td>Thr</td>
<td>Sweet</td>
<td>260</td>
</tr>
<tr>
<td>Arg</td>
<td>Sweet/Bitter</td>
<td>50</td>
</tr>
<tr>
<td>Ala</td>
<td>Umami/Sweet</td>
<td>60</td>
</tr>
<tr>
<td>Val</td>
<td>Sweet/Bitter</td>
<td>40</td>
</tr>
<tr>
<td>Met</td>
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<td>Phe</td>
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</tr>
<tr>
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<tr>
<td>Leu</td>
<td>Bitter</td>
<td>190</td>
</tr>
<tr>
<td>Lys</td>
<td>Sweet/Bitter</td>
<td>50</td>
</tr>
<tr>
<td>Pro</td>
<td>Sweet/Bitter</td>
<td>300</td>
</tr>
</tbody>
</table>
Figure 5

Changes in the protein tertiary structure of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage. 0d (a), 4d(b), 8d(c) and 12d(d)
Figure 6

Changes in the MFI of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage
Figure 7

Changes in the color(a), odor(b), texture(c) and overall acceptability(d) of bullfrog (*Lithobates catesbeiana*) with different treatments during refrigerated storage
Figure 8

Correlation analysis between quality indexes in bullfrog (*Lithobates catesbeiana*) during refrigerated storage
Figure 9

Diagram of antibacterial mechanism of allicin and AOB