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Quality Assessment of Frozen *Solenocera crassicornis* Treated with Sodium Metabisulphite by Soaking or Spraying

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Abstract The present work was carried out to evaluate the safety of shrimp (*Solenocera crassicornis*) treated with different concentrations of sodium metabisulfite (SMB) by soaking or spraying during frozen storage. Shrimps soaked in higher concentrations of SMB showed higher sensory scores, lower total color differences, and better anti-melanosis effects than shrimps in the control and other treatment groups throughout frozen storage (−18℃). Lower total volatile basic nitrogen and thiobarbituric acid reactive substances and higher salt soluble protein contents were detected in shrimp soaked with high doses of SMB compared with other samples. In addition, lower counts of total aerobic plates and psychrotrophic bacteria were observed in shrimp treated by soaking with higher doses of SMB than those in control shrimp and shrimp treated with other methods during frozen storage (−18℃). However, the SO_2 content of 5% SMB-soaked samples exceeded the maximum allowable limit of 100 mg kg^{-1}. Overall, the use of 1.5% SMB soaking to treat shrimp results in good antioxidant and antimicrobial effects and, thus, may be suggested to preserve *S. crassicornis* under frozen conditions. The results of this study present important guidance on the use of SMB to maintain the quality of marine-trawling shrimp from manufacturing to consumption.

Key words *S. crassicornis*; frozen storage; SMB; soaking; spraying; quality changes

1 Introduction

The marine-trawling red shrimp (*Solenocera crassicornis*) is an important commercial species mainly distributed in China, India, Malaysia, Indonesia, the Arafura Sea, and Japan. China is one of the world's top *S. crassicornis* exporters, and frozen peeled prawns, as the main processed products of this species, are exported to over 10 countries. *S. crassicornis* is a rich source of astaxanthin, and possesses a series of biological properties, including immunostimulation, anti-tumor, anti-oxidation, anti-hypertension, and anti-cardiovascular disease activities. Furthermore, as a delicious and nourishing aquatic product, *S. crassicornis* contains large amounts of protein, fatty acids, and mineral elements (*e.g.*, calcium, iron, phosphorus, iodine) (Wang, 2014; Liu, 2016). Thus, *S. crassicornis* is an ideal food resource with high nutritional value.

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Shrimp has a limited shelf-life and is highly perishable due to microbial spoilage and melanosis (Gokoglu and Yerlikaya, 2008; Farajzadeh *et al*., 2016). Melanosis is a biochemical reaction triggered by polyphenoloxidase (PPO). PPO oxidizes phenols into quinones, which subsequently undergo further oxidation or polymerization to form high-molecular weight black pigments (Benjakul *et al*., 2006; Sae-Leaw *et al*., 2017). Melanosis significantly reduces the market value of shrimp and results in considerable economic loss (Martínez-Álvarez *et al*., 2005). Freezing is usually employed as an effective method to extend the shelf-life of shrimp (Tsironi *et al*., 2009; Wachirasiri *et al*., 2016). However, even with freezing, melanosis may still occur in shrimp, albeit slowly, because PPO remains slightly active under freezing conditions (Nirmal and Benjakul, 2010). Thus, traditional on-board freezing techniques combined with value-added on-board processing techniques using small amounts of sulfites and its derivatives is indispensable for offshore fisheries to overcome or alleviate melanosis (Bono *et al*., 2012, 2016).

Sulfites and their derivatives are widely used to inhibit

melanosis in seafood (Montero *et al*., 2001; Martínez-Álvarez *et al*., 2008). These chemical substances prevent browning by combining irreversibly with quinones and interfering with their polymerization to melanins; they have also been demonstrated to possess antimicrobial activity and retard microbial spoilage (Nirmal and Benjakul, 2009). Sodium metabisulfite (SMB, $Na_2S_2O_5$), a known sulfite, is applied to harvest marine shrimp by soaking with ice. However, SMB reacts with the oxygen dissolved in water to release SO_2, which produces acidic substances and sodium acid sulfate, while these compounds are toxic chemical agents for human consumption (Rencüzo□ullari *et al*., 2001).

At present, direct dusting of SMB, due to its convenient operation, is widely used by many fishermen to preserve marine-trawling shrimp. However, SMB is often used in excess owing to the difficulty of spreading evenly, which frequently results in SO_2 residues exceeding established limits in shrimp. If the shrimp is reasonably treated by soaking or spraying with SMB solution, the problem of uneven spreading and excessive use can be avoided. Therefore, the aim of the present work is to study the quality changes of *S. crassicornis* soaked with 1.5%, 3.0%, or 5.0% SMB respectively, or sprayed with 5.0% SMB, and evaluate the safety of SMB applied at different concentrations and by using different treatment methods during frozen storage (−18℃). The results of this study will provide a theoretical basis for the specific use of SMB as a preservative for *S. crassicornis*.

2 Materials and Methods

2.1 Shrimp Collection and Preparation

Fresh red shrimp (*S. crassicornis*) treated without chemicals was provided by Dongqing Marine Fisheries Cooperative. The shrimps were immersed in ice-cold water after harvest and transported to the laboratory within 12 h. In the laboratory, the shrimps were soaked in 1.5%, 3.0%, or 5.0% SMB solution at 4℃ for 5 min. The shrimps were then taken out and drained. A batch of *S. crassicornis* was sprayed evenly with 5.0% SMB solution and allowed to sit for 5 min as another treatment group. *S. crassicornis* without any chemical treatment was considered the control. All procedures were performed at 4℃. Finally, all shrimp samples were frozen and stored at −18℃. The samples were frozen for 6 months. During this period, the qualities of shrimp in different groups were analyzed with an interval of 30 days. Sensory quality, color, melanosis, total volatile basic nitrogen (TVB-N), salt soluble protein (SSP), thiobarbituric acid (TBA), and microbial counts were selected as quality indices. At the beginning of the analysis, shrimps were manually deheaded and de-shelled. Then they were thawed at 4℃ and analyzed immediately when the core temperature was 0℃. A schematic of the experiment is shown in the supplementary material.

2.2 Determination of SO_2

Usage amount of SMB was calculated in terms of SO_2 residue. SO_2 was determined according to GB 5009.34-2016 (Chinese National Standard). A 5 g sample was placed in a distillation flask with 250 mL of distilled water and then installed in a condensing device. One end of the condenser was immersed in 25 mL of lead acetate absorbing solution in an iodometric bottle, capped immediately, and then heated to distill. The end of the condenser was taken out the liquid level when the distillate was about 200 mL, and distilled again for 1 min. The device in the lead acetate solution was rinsed with a small amount of distilled water. A blank test was also conducted. Exactly 10 mL of HCl and 1 mL of starch indicator solution were added to the iodine bottle. After shaking, the mixtures were titrated with iodine standard solution until the solution could maintain a blue coloration for 30 s. The volume of iodine standard solution used to achieve this endpoint was recorded, and SO_2 content was calculated as follows:

$$X = \frac{(V - V_0) \times 0.032 \times c \times 1000}{m},$$

where X represents the total SO_2 content ($g\,kg^{-1}$); V and V_0 represent the titration volumes of the iodine standard solution in the test sample (mL) and blank (mL), respectively; c represents the concentration of the iodine standard solution ($mol\,L^{-1}$); and m represents the weight of the shrimp sample (g).

2.3 Sensory Analysis

Sensory evaluation was performed as described by Farajzadeh *et al*. (2016) and Wu (2014). A panel of 10 trained panelists with basic sensory knowledge and test skills was used in this study to evaluate the sensory quality of the shrimp samples. Appearance, flavor, odor, texture, and overall acceptability in appropriate forms with descriptive terms were used to describe the organoleptic evolution of quality deterioration. Appearance, texture, and odor were evaluated in raw shrimp, whereas flavor was analyzed in cooked shrimp which was boiled for 5 min. Panelists were asked to score the samples independently *via* a 9-point descriptive hedonic scale (9=highest score, 1=lowest score) for each descriptor. The scores were calculated as mean values, and overall acceptability was defined as the average of these sensorial attribute values as weighted.

2.4 Instrumental Color Analysis

The color of shrimp was measured with a Hunter Lab colorimeter (SC-80C, Kangguang Optical Instrument Co., Ltd., Beijing, China) according to the method of Jin *et al*. (2018). The middle section of the shrimp meat was used for color analysis, and CIE Lab values were measured for color quantization. In the CIE Lab system, 'L' represents lightness from black and white, 'a' represents redness or greenness, and 'b' represents yellowness or blueness. Three different single shrimp specimens were used for each measurement, and average values were recorded. Color change was also evaluated by using the total color differ-

ence (TCD) during frozen storage (Dai *et al.*, 2016; Yuan *et al.*, 2016). TCD values (ΔE) were calculated as follows:

$$\Delta E = \left[(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2 \right]^{0.5},$$

where L_0, a_0, and b_0 respectively represent the values of L^*, a^*, and b^* color parameters at a storage time of zero.

2.5 Total Volatile Basic Nitrogen (TVB-N) Analysis

TVB-N was determined according to GB 5009. 228-2016 (Chinese National Standard). A 20 g sample was blended evenly with 100 mL of distilled water, stirred thoroughly, and sonicated for 1 min. After impregnated for 30 min, the homogenate was filtered through filter paper and added with boric acid (10 mL, 20 g L^{-1}) and mixture indicators (5 drops). The filtrate was slowly transferred into the reaction chamber, the beaker was washed, and the eluents were collected. After adding 5 mL of magnesia (10 g L^{-1}, MgO) to the reaction system, steam distillation was carried out for 5 min using a Kjeldahl distillation unit. The distillate was titrated with 0.01 mol L^{-1} HCl. Measurements were conducted in triplicate, and a blank assay was performed. TVB-N content was calculated as follows:

$$\text{TVB-N (mg (100 g)}^{-1}) = \frac{(V_1 - V_2) \times c \times 14}{m \times (10/100)} \times 100,$$

where V_1 and V_2 represent the titration volumes (mL) of the tested sample and blank, respectively; m represents the weight of the shrimp sample (g); and c represents the actual concentration of HCl (mol L^{-1}).

2.6 Salt Soluble Protein (SSP) Contents

SSP content was determined using the method of Al-Bulushi *et al*. (2013). Approximately 6.67 g of sample was blended with 100 mL of chilled NaCl (5%, w/v), adjusted to pH 7−7.5 using 0.02 mol L^{-1} $NaHCO_3$ (pH 7.2), and then homogenized for 2 min. The homogenate was centrifuged at 4000 r min^{-1} for 30 min at 4℃, and the supernatant was collected. Finally, 10 mL of chilled trichloroacetic acid (15%, TCA) was added to the supernatant, and non-protein nitrogen compounds were removed by centrifugation as described above. The biuret method (Gornall *et al*., 1949) with bovine serum albumin as the standard was used to determine SSP contents. All experiments were performed at less than 10℃.

2.7 Lipid Oxidation

TBA reactive substances (TBARS) were determined for lipid oxidation analysis of shrimp using the method of Dong *et al*. (2018) with slight modifications. Shrimp samples (5 g) were homogenized with 15 mL of distilled water for 2 min. Then, 2.5 mL of TBA solution containing 0.25 mol L^{-1} HCl, 15% TCA, and 0.375% TBA was added to 1 mL of the homogenates. The reaction was conducted in a boiling water bath for 35 min. The mixture was cooled to room temperature and then centrifuged at 4000 r min^{-1} for 20 min. The absorbance of the solution at 532 nm was determined with a digital spectrophotometer (Cary 50, Varian Australia Pty Ltd., Australia). Malondialdehyde (MDA) levels based on 1,1,3,3 tetraethoxypropane at gradient concentrations of 0−10 μg mL^{-1} were used for standard curve preparation. TBARS values were calculated and expressed as mg MDA per kg of sample.

2.8 Microbiological Analysis

Approximately 25 g of shrimp samples were homogenized with 225 mL of sterile phosphate-buffered saline (PBS) at 10 Hz for 2 min. Decimal dilutions were carried out using sterile PBS. Three suitable gradients of the diluted homogenates were spread on the surface of sterile Petri dishes, pour-plated with 15−20 mL of plate count agar, and then incubated at 30℃ for 3 d to determine total aerobic plate counts (TPCs) and at 7℃ for 10 d to determine psychrotrophic bacterial counts (PBC) (FDA, 2001). Two replicates of three appropriate dilutions were performed. Microbial counts were calculated as follows:

$$\text{Microbial count (CFU g}^{-1}) = \frac{\sum C}{(n_1 + 0.1 n_2) d},$$

where C represents the total microbial count in all enumerated plates; d represents the dilution factor of the first dilution gradient; n_1 ($30 \le n \le 300$) represents the numbers of the flat plate in the first dilution gradient; and n_2 ($30 \le n \le 300$) represents the numbers of the flat plate in the second dilution gradient.

2.9 Statistical Analysis

In this work, an apparent first-order equation was used to model TVB-N and SSP values and clarify protein changes, which could be considered failure/formation phenomena (Tsironi *et al*., 2009). The relevant equation may be written as follows:

$$\frac{A}{A_0} = e^{kt},$$

where k represents the kinetic rate; t represents the frozen storage time; and A_0 and A represent the TVB-N values at frozen storage times of zero and t, respectively.

Analysis of variance was performed, and Duncan's multiple range test was used to determine mean comparisons. Statistical analysis was conducted using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). A probability value of $P < 0.05$ was considered significant. All experiments were carried out thrice, and the results are reported as mean ± standard deviation.

3 Results and Discussion

3.1 SO_2 Contents Analysis

SO_2 contents in shrimps subjected to different treatments at −18℃ for 180 d of storage are presented in Fig.1. Ini-

tially, the 1.5% SMB-soaked shrimps had lower SO_2 contents than the 3.0% and 5.0% SMB-soaked shrimps ($P<0.05$). However, the SO_2 levels of 5.0% SMB-sprayed shrimp were between those of the 1.5% and 3.0% SMB-soaked shrimp ($P<0.05$). Thus, soaking allows better penetration of SMB into the shrimp than spraying. In general, decreased SO_2 contents were found in all samples as the storage time increased ($P<0.05$). On day 180, the SO_2 contents of the 1.5%, 3.0%, and 5.0% SMB-soaked and SMB-sprayed samples were 29.4, 44.9, 103.4 and 32.5 mg kg^{-1}, respectively. Among the samples, the 1.5% SMB-soaked sample possessed the lowest SO_2 at all storage times ($P<0.05$).

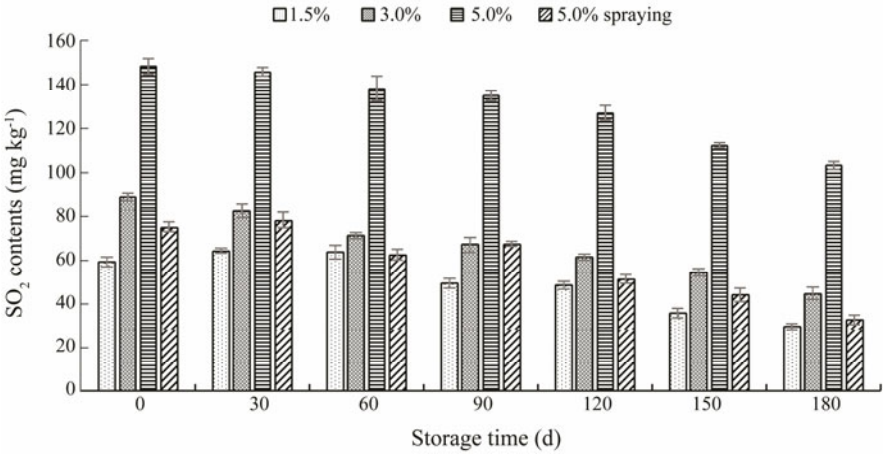

Fig.1 SO_2 content of *S. crassicornis* treated with SMB at different concentrations using different methods over 180 d of frozen storage. Bars represent the standard deviation ($n=3$) with three parallel samples. Soaking method: 1.5%, 3.0%, and 5.0% SMB; spraying method: 5.0% SMB.

SO_2 is widely used as an indicator of food safety, especially in seafoods. Food and Agriculture Organization of the United Nations (FAO) (1999), U.S. Food and Drug Administration (FDA) (Taylor *et al.*, 1986), and China (GB 2760−2014) have stipulated a threshold value of 100 mg kg^{-1} SO_2 in fresh shrimp meat. SO_2 contents in the 1.5% and 3.0% SMB-soaked and 5.0% SMB-sprayed samples were consistently lower than 100 mg kg^{-1} over 180 d of frozen storage ($P<0.05$), but the SO_2 contents of the 5% SMB-soaked sample were in the range of 103.4−148.3 mg kg^{-1}, which exceeds maximum limits ($P<0.05$). Thus, from a food safety perspective, soaking with 5.0% SMB is unsuitable for short-term freezing preservation of *S. crassicornis*.

3.2 Sensory Evaluation

Fig.2 shows the changes in the sensory quality of *S. crassicornis*. All indices declined with increasing storage time. In general, SMB treatment improved the gloss of shrimp, and, therefore, the appearance scores of shrimp in the treatment groups were higher than those in the control group ($P<0.05$). Moreover, the observed effects became more significant as the storage time increased ($P<0.05$). The appearance scores of 5.0% SMB-soaked shrimp were higher ($P<0.05$) than those of 5.0% SMB-sprayed shrimp and samples soaked with SMB at lower concentrations. Thus, treatment of shrimp by soaking with SMB, especially at higher doses, could effectively improve appearance scores during frozen storage. These results are consistent with previous studies (Gonçalves and Ribeiro, 2008; Wachirasiri *et al.*, 2016), showing that sulfites can improve appearance properties and increase consumers' preference for red shrimp.

No difference in odor was noted in all samples ($P>0.05$). Therefore, the use of SMB does not potentially affect the odor of shrimp. At the beginning of frozen storage, slightly lower texture scores were noted in all treated samples compared with the control ($P<0.05$), and no difference in texture was observed among the SMB-treated samples ($P>0.05$). Similarly, no difference in texture was noted among all samples as the storage time increased ($P>0.05$). SMB treatment may increase the initial moisture content of the shrimps and affect their texture. However, loss of moisture occurs during frozen storage, and, thus, the influence of SMB is weakened. Flavor was analyzed using cooked shrimp after tasting. Some panelists noted a slight sourness after tasting the treated samples, which also directly reflects the lower flavor scores of treated samples compared with the control at all storage times ($P<0.05$). Indeed, the 5.0% SMB-soaked shrimp received lower flavor scores than those soaked with SMB at lower concentrations or sprayed with 5.0% SMB at all storage times ($P<0.05$). This result is in agreement with the high SO_2 content of 5.0% SMB-soaked shrimp.

Overall acceptability was calculated by taking the average of all four parameters. The overall acceptability of the samples changed from the initial values of 8.62, 8.56, 8.65, 8.65, and 8.88 to 6.61, 6.57, 6.59, 6.54, and 6.45 in the 1.5%, 3.0%, and 5.0% SMB-soaked, 5.0% SMB-sprayed, and control samples, respectively, after 180 d of frozen storage. At the beginning without freezing, slightly higher scores of overall acceptability were noted in the control sample compared with those of all treated samples ($P<0.05$). The overall acceptability of all samples decreased as the storage time increased ($P<0.05$). Nonetheless, the rate of decrease varied depending on the SMB concentra-

tions and treatment methods. Compared with other samples, the 1.5% SMB-soaked shrimp possessed slightly higher overall acceptability at the end of storage ($P>0.05$). Thus, the results suggest that treatment by soaking with 1.5% SMB could ideally improve the sensory quality of red shrimp during frozen storage.

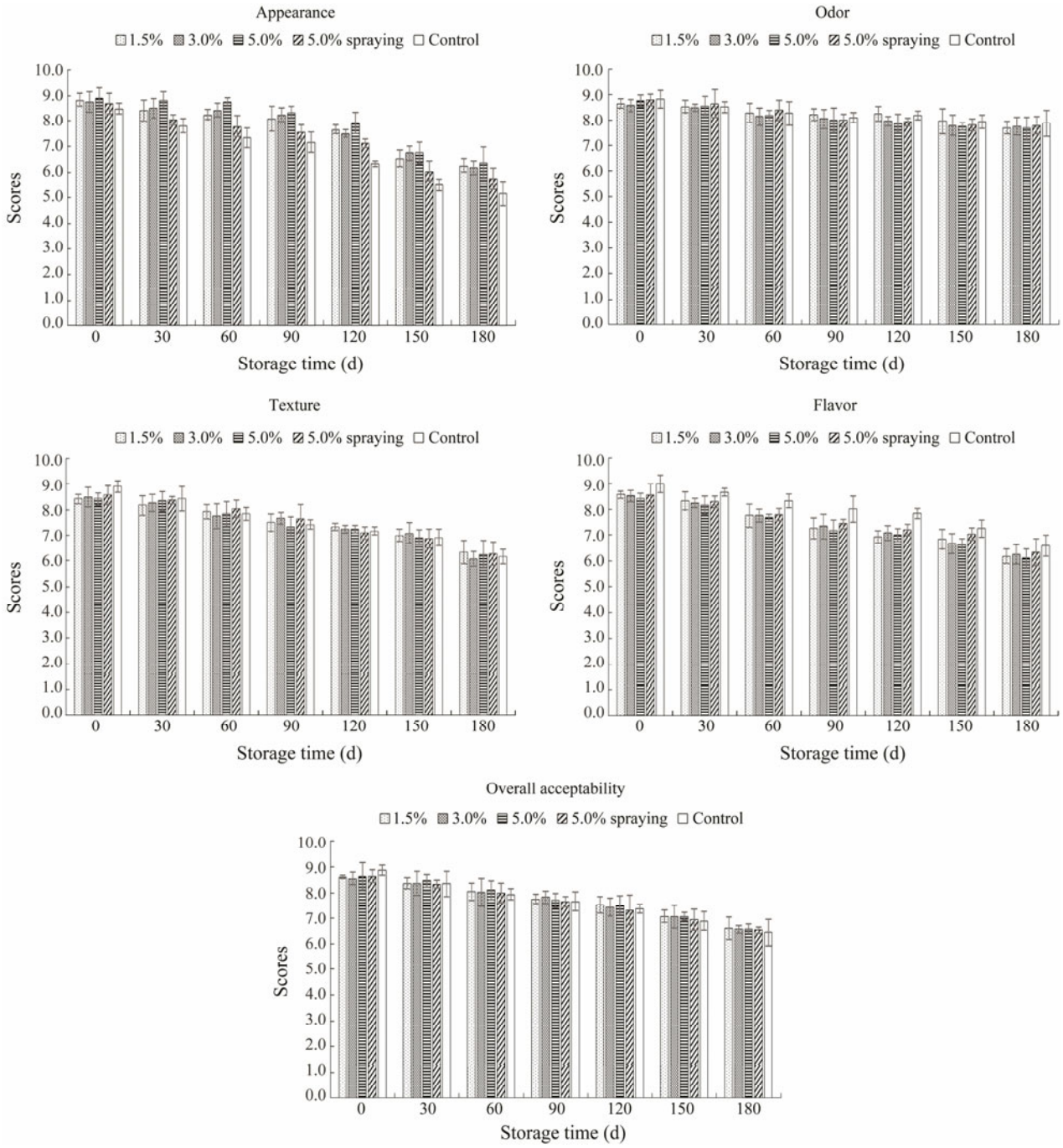

Fig.2 Appearance, odor, texture, flavor, and overall acceptability of *S. crassicornis* treated with SMB at different concentrations using different methods over 180 d of frozen storage. Bars represent the standard deviation ($n=3$). Soaking method: 1.5%, 3.0%, and 5.0% SMB; spraying method: 5.0% SMB; control: treated without chemicals.

3.3 Total Color Difference (TCD) Changes (ΔE) Evaluation

Color fading is an important quality change in shrimp during frozen storage (Solval *et al*., 2014). TCD changes (ΔE) in the control and treated shrimp during storage are shown in Fig.3. On day 30, no difference in ΔE was noted in all samples ($P>0.05$), and the corresponding values were determined as 3.38, 3.17, 3.40, 3.25 and 3.27 in the 1.5%, 3.0%, and 5.0% SMB-soaked, 5.0% SMB-sprayed, and control samples, respectively. The TCD values of all samples increased during storage. Interestingly, a faster increase in TCD was observed in the control ($P<0.05$) compared with the treated samples. However, shrimp treated with 1.5%, 3.0%, and 5.0% SMB soaking and 5.0% SMB spraying showed no difference in TCD until after 90 d of frozen storage ($P>0.05$). Shrimp treated with 5.0% SMB soaking had lower TCD values than shrimp treated with other

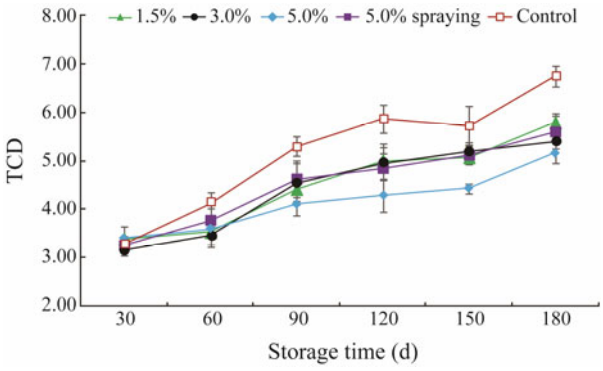

Fig.3 Total color difference (TCD) values (ΔE) changes in *S. crassicornis* treated with SMB at different concentrations using different methods over 180 d of frozen storage. Bars represent the standard deviation (n=3). Soaking method: 1.5%, 3.0%, and 5.0% SMB; spraying method: 5.0% SMB; control: treated without chemicals.

means after 90 days (P<0.05), and no difference was found among other treatment groups (P>0.05). At the end of storage, the 5.0% SMB-soaked shrimp showed the lowest TCD (5.15), followed by those treated with 3.0% SMB soaking (5.40), 5.0% SMB spraying (5.59), and 1.5% SMB soaking (5.78). By comparison, the control showed the highest TCD (6.75) on day 180. The low TCD of SMB-treated shrimp is likely related to the SO_2 content of shrimp meat (Fig.1). In the present study, treatment of *S. crassicornis* with SMB, especially by soaking at higher doses, could delay color changes effectively during frozen storage, which is in agreement with the work reported by Chantarasuwan *et al*. (2011).

Evaluation of melanosis was performed by visual inspection of postharvest images captured by a digital camera (Canon, Japan) at the end of frozen storage (Fig.4). Black spots on the heads of shrimp treated with 1.5% SMB soaking and 5.0% SMB spraying were visualized, than other treated shrimp (3.0% and 5.0% SMB soaking) that showed only slight melanin deposition visually. However, among the samples, the control shrimps showed the largest black centers, and the tails of these shrimps darkened slightly. The mechanism of melanosis development is related to the enzymatic action of PPO. Application of SMB at high doses by soaking may allow penetration of the sulfite into PPO active sites underneath the shrimp shell more effectively than through other means (Shiekh *et al*., 2019). Thus, SMB is capable of preventing melanosis in *S. crassicornis* kept under frozen conditions. The effect of soaking, especially at high concentrations, is especially significant.

Fig.4 Photographs of *S. crassicornis* treated with SMB at different concentrations using different methods on day 180. Soaking method: 1.5%, 3.0%, and 5.0% SMB; spraying method: 5.0% SMB; control: treated without chemicals.

3.4 Changes in Chemical Parameters

The TVB-N contents of all samples over 180 d of storage are depicted in Fig.5. Initially, no significant difference in TVB contents was observed in all samples (P>0.05), and TVB-N contents ranged from 11.7 to 12.2 mg(100 g shrimp)$^{-1}$ meat. During frozen storage, the TVB-N contents of all samples showed a gradual increase (P<0.05). However, TVB-N contents in the control (k=0.0027) increased more significantly throughout the storage (P<0.05) than those in the SMB treatments. Shrimp soaked with SMB at higher concentrations (5.0%) showed lower increase rate (k=0.0016) of TVB-N contents than those soaked with SMB at lower concentrations (1.5%, 3.0%) or sprayed with 5.0% SMB (P<0.05). No difference in TVB-N contents was noted among shrimp soaked with 1.5%, 3.0%, and 5.0% SMB (P>0.05; final values, 16.9, 16.4, and 15.6 mg(100 g)$^{-1}$, respectively) after 180 d of frozen storage. By comparison, higher TVB contents were found in the control and sprayed shrimp (P<0.05; final values, 19.2 and 17.7 mg(100 g)$^{-1}$, respectively) on day 180. Hence, soaking shrimp with SMB could retard the formation of TVB-N more effectively than spraying during frozen storage, and the degree of inhibition depends on the treatment dose. These results may be ascribed to high SMB contents decreasing the capacity of bacteria to induce oxidative deamination of non-protein nitrogen compounds. TVB-N, as a product of protein breakdown, is an important indicator of chemical spoilage, and increases in TVB-N value are related to bacterial spoilage and the activity of endogenous enzymes (Soncin

et al., 2009; Arancibia *et al*., 2015). In general, a TVB-N value of 30 mg TVB-N per 100 g in fresh shrimp is considered to reflect spoilage (GB 2733-2015). In the present study, none of the samples exceeded the spoilage limit of shrimp after 180 d of frozen storage. Nevertheless, among the samples, the 5.0% SMB-soaked shrimp revealed the lowest TVB content on day 180 ($P<0.05$). This result agrees with the high SO_2 content of 5.0% SMB-soaked shrimp.

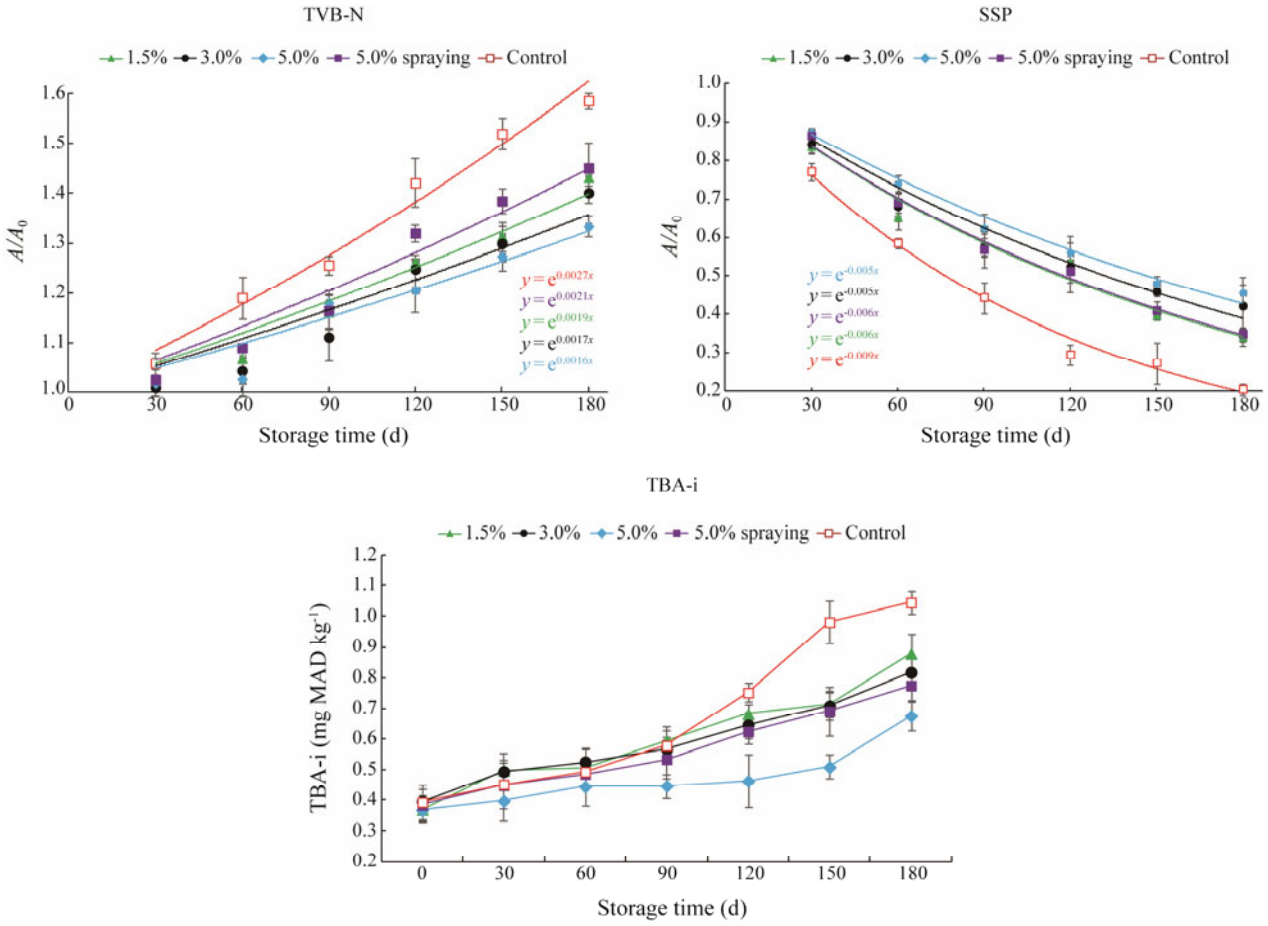

Fig.5 Total volatile basic nitrogen (TVB-N), salt soluble protein (SSP) and thiobarbituric acid reactive substances (TBARS) changes in *S. crassicornis* treated with SMB at different concentrations using different methods over 180 d of frozen storage. Bars represent the standard deviation ($n=3$). Soaking method: 1.5%, 3.0%, and 5.0% SMB; spraying method: 5.0% SMB; control: treated without chemicals.

SSP refers to the myofibrillar fraction of shrimp protein and influences the water holding capacity and texture of shrimp greatly during frozen storage. Loss of protein solubility can indicate myofibrillar protein denaturation (Remya *et al*., 2015). On day 0, no significant difference in SSP was found among all samples ($P>0.05$), which revealed initial SSP contents of 29.16, 30.02, 29.70, 29.97, and 30.60 μg kg^{-1} in the 1.5%, 3.0%, and 5.0% SMB-soaked, 5.0% SMB-sprayed, and control samples, respectively. Extractible SSP decreased with increasing storage time. However, SSP contents in the control showed a faster decrease ($k=-0.009$) than those in the treatment groups. Among the SMB treatments, the decrease rates of SSP ($k=-0.005$) in the 3.0% and 5.0% SMB-soaked shrimp were significantly lower ($P<0.05$) than those in the 1.5% SMB-soaked and 5.0% SMB-sprayed shrimp ($k=-0.006$). At the end of the storage, shrimp soaked at higher SMB doses revealed higher SSP contents ($P<0.05$). However, no significant difference was observed between 3.0% and 5.0% SMB-soaked shrimp ($P>0.05$; final SSP contents, 12.71 and 13.52 μg kg^{-1}, respectively). Furthermore, 1.5% SMB-soaked and 5.0% SMB-sprayed shrimp revealed SSP contents of 9.96 and 10.47 μg kg^{-1} on day 180, and no difference was noted between these two samples ($P>0.05$). The lowest SSP content (final value, 6.27 μg kg^{-1}) was observed in the control group. The results suggest that SMB treatment exerts beneficial effects during shrimp storage by retaining a high percentage of SSP content. Moreover, soaking with high SMB concentrations suppresses SSP denaturation more effectively than other methods. It is well known that decreases in myofibrillar content can affect the quality of shrimp. As previously reported by Remya *et al*. (2015) and Yoon and Lee (1990), decreases in myofibrillar protein content could be attributed to the denaturation and aggregation of proteins due to the formation of disulfide bonds followed by rearrangement into hydrophobic and hydrogen-bonded regions on an intra- and intermolecular basis (Buttkus, 1974). Considering the findings in the present study, high levels of SMB may be speculated to reduce the exposure of hydrophopic residues to

the protein surface and maintain the structural stability of myofibrillar proteins, ultimately slowing down the kinetics of protein aggregation (Herrera and Mackie, 2004; Al-Bulushi *et al*., 2013).

The TBARS content reflects the concentration of secondary lipid oxidation products in a sample. Changes in TBARS content in the samples are presented in Fig.5. Initially, no difference in TBARS contents was observed among all samples ($P>0.05$). Thereafter, TBARS contents increased up to day 180 ($P<0.05$). The lowest increase in TBARS content was observed in the 5.0% SMB-soaked shrimp ($P<0.05$), reaching 0.676 mg MDA kg^{-1} after 180 d of frozen storage. However, shrimp treated with SMB (soaking with 1.5% and 3.0% SMB and spraying with 5.0% SMB) and the control showed no difference in TBARS contents until day 120 of the storage ($P>0.05$). On day 120, a rapid increase in TBARS content was found in the control; indeed, the TBARS content of the control reached a maximum value of 1.046 mg MDA kg^{-1} at the end of the storage ($P<0.05$). Soaking with 5% SMB prevented lipid oxidation, as shown by the lower TBARS content observed in this sample on day 180 ($P<0.05$) compared with those of the 1.5% SMB-soaked, 3.0% SMB-soaked, and 5.0% SMB-sprayed shrimp (0.878, 0.818, and 0.775 mg MDA kg^{-1}, respectively). Since shrimp treated with SMB had lower TBARS contents than those without treatment, SMB may be speculated to function as an antioxidant that effectively retards lipid oxidation in shrimp during frozen storage. Higher antioxidant activity was found in shrimp soaked with higher levels of SMB. Previous studies (Cacciuttolo *et al*., 1993; Solval *et al*., 2014) reported that several reactive oxygen species, especially hydroxyl radicals (·OH), which have strong chemical activity, can easily react with biomolecules, such as DNA, proteins, amino acids, and fatty acids. SMB may react with ·OH to reduce concentrations of free ·OH and inhibit lipid oxidation.

3.5 Microbiological Analysis

Fig.6 shows the evolution of microbial counts in all investigated samples plotted as a function of storage time, and all values are reported as log(CFU g^{-1}) of fresh weight. The TPC values of all samples were in the range of 4.11−4.16 log(CFU g^{-1}) on day 0, and no significant difference in TPC was found ($P>0.05$). TPC showed an increasing trend during frozen storage, and the highest TPC was obtained in the control samples ($P<0.05$). TPC increased rapidly in the control shrimp over the first 30 d of frozen storage, decreased slightly, and then increased once more up to the end of storage. The TPC of 5.0% SMB-soaked shrimp was lower ($P<0.05$) than those of SMB-sprayed and low-concentration SMB-soaked shrimp ($P<0.05$). On day 180, the TPCs of the control, 1.5%, 3.0%, and 5.0% SMB-soaked, and 5.0% SMB-sprayed shrimp were 5.01, 4.64, 4.61, 4.53, and 4.68 log(CFU g^{-1}), respectively. Overall, the ability of SMB to inhibit increases in TPC was dependent on the treatment method and dose, which correlates well with the TVB-N levels of the corresponding samples.

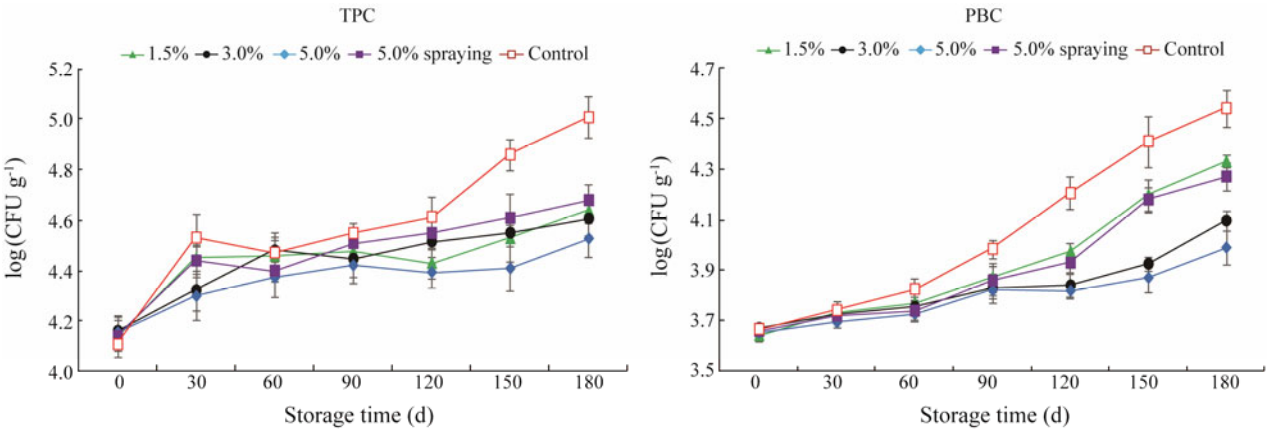

Fig.6 Total aerobic plate count (TPC) and psychrotrophic bacteria count (PBC) of *S. crassicornis* treated with SMB at different concentrations using different methods over 180 d of frozen storage. Bars represent the standard deviation ($n=3$). Soaking method: 1.5%, 3.0%, and 5.0% SMB; spraying method: 5.0% SMB; control: treated without chemicals.

PBC, as a major cause of spoilage in raw shrimp during frozen storage, remains active even in a frozen environment. Increased PBC was found in all samples as the storage time increased. On days 0 and 30, no obvious difference in PBC was found among all samples ($P>0.05$). Thereafter, a rapid increase in PBC was observed in the control shrimp ($P<0.05$), which revealed a higher PBC after 60−180 d of storage compared with treated shrimp ($P<0.05$). No significant difference in PBC was observed among SMB treatments over 0−90 d of storage ($P>0.05$). During 120−180 days, however, differences in PBC were noted, and significantly lower PBC values were found in 3.0% and 5.0% SMB-soaked shrimp ($P<0.05$). At the end of the storage, the control, 1.5%, 3.0%, and 5.0% SMB-soaked, and 5.0% SMB-sprayed shrimp revealed PBCs of 4.54, 4.33, 4.10, 3.99, and 4.27 log(CFU g^{-1}), respectively. Thus, treatment of shrimp with SMB could retard the growth of PB during frozen storage, and soaking with higher concentrations is a more effective treatment than spraying or soaking with lower SMB concentrations.

Interestingly, the TPC and PBC of all samples did not exceed the maximum permission limit of 7.0 log(CFU g^{-1})

recommended by the ICMSF (1986) over the entire observation period. Therefore, the use of 1.5% SMB soaking treatment demonstrates good antimicrobial effects in preserving *S. crassicornis* under frozen conditions.

4 Conclusions

During frozen storage, soaking shrimp with high dose of SMB can improve sensory scores, delay color changes, and prevent melanosis more effectively than soaking shrimp with lower doses of SMB or by spraying SMB. Moreover, shrimp soaked with SMB at higher concentrations revealed lower increases in TVB, TBARS content, and microbial growth and lower decreases in SSP over 180 d of frozen storage. However, the SO_2 contents of 5% SMB-soaked samples exceeded the limit of 100 mg kg^{-1} throughout the storage. In conclusion, soaking with 1.5% SMB revealed good antioxidant and antimicrobial effects and may be recommended in efforts to preserve *S. crassicornis* under frozen conditions.

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