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Toward shrimp without chemical additives: A combined freezing-MAP approach

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ABSTRACT

The combined effects of freezing and modified atmosphere packaging (MAP) containing two different gas mixtures (100% N_2 or 50% N_2–50% CO_2) on the chemical properties and melanosis of deep-water rose shrimp (*Parapenaeus longirostris*) was investigated and compared to both a traditional sulfiting treatment and vacuum packaging (VP). Changes in pH, total volatile basic nitrogen (TVB-N) levels, thiobarbituric acid (TBA) levels and melanosis were measured over a one-year storage period.

Treatment with sodium sulfite solution before storage raised the pH and TVB-N levels rapidly, whereas both the MAP and VP samples exhibited minor increases in pH and TVB-N levels during the first six months of storage. Additionally, shrimp packed in MAP conditions, especially those packaged in 100% N_2, showed completely inhibited lipid oxidation (TBA levels remained very close to those of the sulfited samples) during the storage period. The melanosis scores revealed that the samples packed in 100% N2 maintained natural color for up to six months of storage.

These data suggest that the use of modified atmosphere packaging in combination with freezing during shrimp processing could be a safe and effective alternative to artificial additives.

1. Introduction

The deep-water rose shrimp (*Parapenaeus longirostris)* is the most valuable demersal species caught by Italian trawl fisheries and is widely appreciated in other Mediterranean countries, such as Spain, France, Algeria, Tunisia, Greece and Turkey. Similar to what has been reported by Otwell and Marshall (1986) for the East Coast of the USA, interest in Penaeidae emerged in this part of the Mediterranean Sea when the economy prospered after the Second World War. The construction of more powerful boats and the availability of chemical additives (sulfite compounds) to address problems related to melanosis (Kim, Marshall, & Wei, 2000) contributed to the rapid development of the shrimp fishery industry. Until the 1980s, most accumulated wealth was invested in increasing fishing capacity instead of encouraging the full valorization of the catch.

This inappropriate and unsustainable fishing requires the adoption of resource conservation measures that will best serve long-term environmental, social and economic interests (Defeo, MacClanahan, & Castilla, 2007; Lleonart, 2005). In other words, it is necessary to reverse the exploitation pattern by reducing the fishing effort (Hilborn, Orensanz, & Parma, 2005) and by maximizing the commercial value of shrimp through the introduction of new processing and packaging techniques that protect the natural quality and safety of the product. The traditional on-board freezing techniques, almost indispensable for offshore fisheries, may be combined with value-added on-board processing techniques that use little or no sulfite derivatives. Despite the demonstrated efficacy of other low-dosage retardants, such as 4-hexylresorcinol (Montero, Martinez-Alvarez, & Gomez-Guillen, 2004), sulfites are still extensively used, and residual levels of sulfites in flesh tissue are often above the limits established by law (Hardisson, Rubio, Fras, Rodriguez, & Reguera, 2002). Sulfite derivates have potentially pathological effects (Gunnison & Jacobsen, 1987); therefore, there is a need to develop alternative techniques that minimize the risk to public health (Nirmal & Benjakul, 2011) but that are easy to introduce into the fishing supply chain.

Significant results have been obtained in improving the chemical, microbial and sensory qualities of refrigerated deep-water rose shrimp and white shrimp by combining different concentrations of CO_2, O_2, N_2 and additives (Gonçalves, Lopez-Caballero, & Nunes, 2003; Lopez-Caballero, Gonçalves, & Nunes, 2002; Martínez-Alvarez, Montero, & Gomez-Guillen, 2005; Thepnuan, Benjakul, & Visessanguan, 2008). However, few advances have been made in the offshore fishing industry, where the catch must be frozen. Bak, Andersen, Andersen, and Bertelsen (1999) suggested pre-cooking cold-water shrimp (*Pandalus borealis*) before freezing, a system

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that is not practiced by the supply chain partners of the Mediterranean shrimp fishery industry. Montero, Avalos, and Perez-Mateos (2001) and Adachi and Hirata. (2011) reported that decreasing the oxygen in the packs led to a significant reduction of the enzymatic activity responsible for dark discoloration.

Therefore, in this study, the potential effects of two different oxygen-free gas-based mixtures combined with freezing (Rosnes, Sivertsvik, & Skara, 2003) and of freezing combined with vacuum packaging were compared with the effects of the standard sulfite-based preservation method on the preservation of deep-water rose shrimp. The aim of this study was to verify whether these novel shrimp-packaging techniques could be proposed as valid substitutes to common sulfite preservatives, thus eliminating the need for chemical additives and extending the storage life of shrimp. The results were assessed over 12 months of frozen storage.

2. Materials and methods

2.1. On-board shrimp handling and packaging

Deep-water rose shrimp were caught in the Strait of Sicily (Central Mediterranean) during the spring season. A new bottom trawler equipped with a semi-automatic modified atmosphere packaging (MAP) system (Mondini, Brescia, Italy) was used to collect 50 kg of shrimp in one haul with an average carapace length of 22 ± 5 mm. The shrimp were washed in flowing seawater and pre-chilled (1 ± 0.5 °C) within an hour of capture by dipping them in a 1:1 mixture of seawater and ice. After approximately 15 min, when the core temperature of the shrimp was equal to the temperature of the ice-seawater mixture, the shrimp were dripped, randomly divided into sub-lots of approximately 800 g and rapidly subjected to the following treatment and packaging protocols:

- One sub-lot was frozen in a blast freezer room at −35 °C and, after 8 h, when the water at the thermal center of the shrimp had been converted into ice, was packed in N_2 (100%).
- A second sub-lot of shrimp was frozen in a blast freezer room at −35 °C for 8 h and then packed in a vacuum.
- A third sub-lot was packed in a gas mixture of N_2 and CO_2 (50%–50%) and subsequently frozen in a blast freezer room at −35 °C for 8 h. This was necessary to allow the full dissolution of CO_2 into the shrimp tissue.
- A fourth sub-lot was dipped in a seawater solution (4% w/v) of commercial anhydrous sodium sulfite (the shrimp-to-dipping solution ratio was 1:4) according to the preservation techniques and materials usually employed by Mediterranean fleet crews. After 20 min of dipping, the shrimp were dried and frozen in a blast freezer room at −35 °C.
- Finally, a fifth sub-lot was frozen in a blast freezer room at −35 °C without any treatment; this sub-lot was used for comparative purposes.

All the samples were stored at −18 °C, which, according to Blond and Le Meste (2004), is the optimum storage temperature when considering both the financial costs of freezing and the shelf-life of frozen foods.

2.2. Packaging materials

All samples, including the control, were packed in semi-rigid A.PET/EVOH/PE barrier trays (volume: 1800 cc; laminate density: 1.39 g cm^{-3}; thickness: 500 μm) with an oxygen permeability of 1.8 cm^3 m^{-2} day^{-1} atm^{-1} and water vapor permeability of 4 g m^{-2} day^{-1} (Arcoplastica srl, Andenzeno, Italy). The trays were heat-sealed with a multiflex OPP/EVOH/PE film (weight: 72 g m^{-2}; thickness: 75 μm) with an O_2 permeability of 3 cm^3 m^{-2} day^{-1} atm^{-1}, a CO_2 permeability of 10 cm^3 m^{-2} day^{-1} atm^{-1} and a water vapor permeability of 3 g m^{-2} day^{-1} (Cryovac, Sealed Air Corp.). To avoid shrimp horn damage to the multiflex OPP/EVOH/PE film, an additional anti-pinhole lamina (weight: 340 g m^{-2}; thickness: 250 μm) was inserted into the headspace of the trays.

2.3. Gas composition

The gas blends were prepared on-board using a gas mixer (MAP Mix 9000, PBI-Dansensor A/S, Denmark). The gas compositions of the samples were analyzed both upon arrival in the laboratory and after one year of storage using a Food Package Analyzer series 1400 (Servomex Ltd, Crowborough, England). On board, the gas compositions of the samples packed in modified atmospheres were monitored using an O_2/CO_2 checkpoint (PBI-Dansensor A/S, Denmark).

2.4. pH measurement

Shrimp muscle tissue was homogenized with distilled water (1:5 w/v), and the pH of the homogenate was measured using a digital pH meter in a Level 1 Lab (WTW GmbH & Co. KG, Weilheim, Germany).

2.5. Determination of the chemical parameters

The total volatile basic nitrogen (TVB-N) levels in the edible flesh were determined every two months throughout the one-year storage period using the method proposed by Billon, Ollieuz, and Tao (1979). Thiobarbituric acid (TBA) levels were measured every two months throughout the one-year storage period using the method proposed by Tarladgis, Watts, and Younathan (1960).

2.6. Melanosis assessment

The melanosis, or dark discoloration, of deep-water rose shrimp was evaluated by a trained panel of four experienced judges. After a general inspection of the packaging (wrappings and glazes), shrimp were removed from each pack, thawed at ambient temperature and immediately presented to the panelists. Melanosis was evaluated on a five-point scale, where 0 was natural or the absence of discoloration; 1 was yellowing and 2, 3, and 4 referred to slight, moderate and intense black spotting, respectively.

2.7. Statistical analyses

All experiments were conducted in triplicate. Statistical analyses were performed using SPSS software (version 7.1). The pH, TVB-N levels and TBA levels were analyzed using ANCOVA (to help control for the effect of storage time). This analysis was followed by a post-hoc comparison of means based on Fisher's least significant difference (LSD), using data from the entire study period (from month 0 to month 12) and from the second half of the storage time (from month 6 to month 12). A 5% significance level was used for all statistical analyses.

3. Results and discussion

3.1. Gas measurements

Three days after packaging, the samples packed in an atmosphere of N_2 and CO_2 showed a reduction in CO_2 concentration from 50% to 23.4%. Previous studies observed larger reductions in CO_2 both in a different Penaeidae species (Dalgaard & Jørgensen,

2000; Layrisse & Matches, 1984) and in the same species (Gonçalves et al., 2003), which was attributed to the dissolution of CO_2 in the water phase and the fat phase of the shrimp (Devlieghere, Debevere, & Van Impe, 1998). However, after 12 months of frozen storage, the percentage of CO_2 increased significantly to 33.1% ($P < 0.05$). This increase was detected at the same proportion in samples packed in N_2 (+8.5% after 12 months) and may have been due to enzymatic decarboxylation of free amino acids (Zhang, Li, Luo, & Chen, 2010). Other variations (Table 1) included a small amount of oxygen found at the end of the test period that could have been due to a non-optimal barrier effect of the packaging material over prolonged storage time.

3.2. pH changes

The initial pH for freshly caught shrimp was 7.20 (results not shown on the graph). This value is in accordance with pH values reported for the same species caught outside the Strait of Gibraltar (Gonçalves et al., 2003; López-Caballero, Martinez Alvarez, Gomez Guillen, & Montero, 2007). Furthermore, this slight alkalinity of fresh shrimp is associated with the high non-protein, nitrogenous content typical of decapod shellfish (Shahidi, 1994).

At the beginning of storage (the third day after being caught), the pH value of sulfited samples quickly increased to 7.46, reaching 7.90 after two months. It was significantly higher than the pH of the other samples ($P < 0.05$), for which the values remained constant for six months (Fig. 1). These data indicates that the high alkalinity (pH 8.5) of the sodium sulfite (Na_2SO_3) solution used for the sulfiting treatment has a considerable effect on the pH of shrimp meat and on the production of volatile basic nitrogen, as noted later. Similar effects have been reported in the same species treated with analogous sulfiting agents by Mendes, Gonçalves, Pestana, and Pestana (2005).

This pattern changed between the sixth and eighth months of storage, when the pH increased in all the samples. Across the entire storage period time, the pH of the sulfited samples was significantly higher than all other samples ($P < 0.05$), whereas in the groups not treated with sulfites, the best performance was obtained with vacuum packaging, followed by the control and N_2 (100%) MAP samples.

According to Ashie, Smith, and Simpson (1996), the general increase in pH observed in the second half of storage may be attributed to the production of dimethylamine (DMA), which is considered an important compound of TVB-N, especially in frozen seafood (Botta, 1995; Hebard, Flick, & Martin, 1982).

3.3. TVB-N changes

Changes in the TVB-N levels are presented in Fig. 2. Three days after the catch (time 0 on the graph), the TVB-N values of the three samples packed in a vacuum or in a modified atmosphere ranged between 33.5 and 36.5 mg, whereas the TVB-N value of the sulfited sample was 42.0 mg. Although higher than the limit of 30 mg/100 g suggested by Angel, Basker, Kanner, and Juven (1981), our TVB-N results are comparable with those obtained from chilled samples

Table 1
Gas composition changes [mean value ± S.D. ($n = 3$)] of deep-water rose shrimp packed in either N_2 or N_2/CO_2.

Month	Shrimp frozen under N_2			Shrimp frozen under N_2/CO_2		
	N_2 (%)	CO_2 (%)	O_2 (%)	N_2 (%)	CO_2 (%)	O_2 (%)
1st	95.6 ± 1.8	2.9 ± 1.4	1.8 ± 1.0	77.0 ± 7.3	23.4 ± 4.8	0.5 ± 0.1
12th	88.7 ± 5.0	11.4 ± 4.4	0.4 ± 0.1	66.7 ± 6.2	33.1 ± 3.0	0.4 ± 0.1

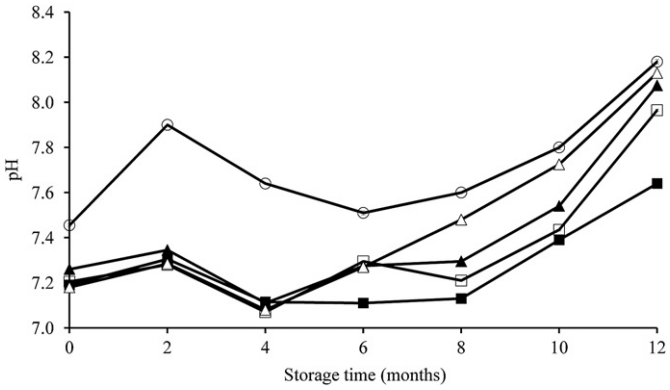

Fig. 1. Temporal changes in the pH value of frozen deep-water rose shrimp packed according to different methods. Error bars ($n = 3$) are smaller than symbols. □: Control; ■: Vacuum; ▲: N_2–CO_2; △: N_2; ○: Sulfited.

of the same species caught along the southern coast of Portugal (Lopez-Caballero et al., 2002; Mendes et al., 2005). Furthermore, according to Martínez-Alvarez, López-Caballero, Gómez-Guillén, and Montero (2009), the TVB-N pattern in frozen shrimp is consistent with the suggestion that ammonia production by tissue enzymes far exceeds that produced by bacteria. During the first four months of storage, the higher levels of TVB-N already observed in sulfited samples increased continuously up to 59 mg, whereas the TVB-N in the other samples was generally lower than 40 mg. This early increase in TVB-N, which was not reported in previously published works, could be due to the formation of DMA from trimethylamine oxide (TMAO) by non-enzymatic reactions that rapidly accelerate when sulfite treatments are used (Spinelli & Koury, 1979). Regarding the formation of TVB-N in sulfite-treated foods, Harada (1975) postulated that high pH might play a significant role in the enzymatic production of DMA and formaldehyde (FA) from TMAO. To the best of our knowledge, this mechanism constitutes the only convincing explanation for these elevated TVB-N levels, which, in addition to the results of the current study, were also measured in *Aristaemorpha foliacea* and *Nephrops norvegicus* (unpublished data). DMA in frozen seafood can be readily nitrosated to N-nitrosodimethylamine (Fay et al., 1997); thus, if our hypothesis is plausible, the adverse effects of sulfites on human health have been underestimated. After the sixth month, the TVB-N levels remained almost constant until the end of storage. This plateau may be associated with a decrease in TMAO.

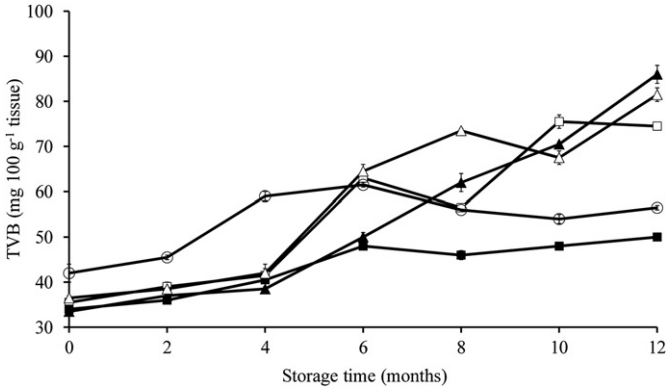

Fig. 2. Temporal changes in the TVB levels of frozen deep-water rose shrimp packed according to different methods. Bars represent the standard deviation ($n = 3$). □: Control; ■: Vacuum; ▲: N_2–CO_2; △: N_2; ○: Sulfited.

In contrast, no significant changes in TVB-N levels were observed in the vacuum-packed samples, which consistently showed the smallest changes in pH. There are no references on the potential effects of vacuum packing on TVB-N production in frozen shrimp or in any other seafood. The vacuum technique is generally used when anoxic conditions are required; however, in our study, the alternative anoxic technique (MAP) showed an increase in TVB-N levels during the second half of the storage period. Therefore, the causes of this phenomenon are more likely physical than chemical. One possibility is that a closer relationship exists between the low pressure that characterizes the headspace of these samples and the high volatility of nitrogen compounds.

For the first six months, the TVB-N levels of the control samples and of the samples packed in N_2 and in N_2/CO_2 remained constant. From the eighth month onward, those levels increased to 86 mg (+53 mg/100 g) for the sample packed in N_2/CO_2. Total exclusion of oxygen from the headspace alone was not enough to control the production of TVB-N after the sixth month of storage. Considering that TMA production was not expected during frozen storage and that non-enzymatic breakdown of TMAO was not assumed to be the cause for TVB-N increases in sulfited samples, other factors, such as free amino acid deamination and the degradation of nucleotides, may be involved. This potential mechanism was corroborated with the increase in CO_2 in the headspace observed during the final period of storage.

Overall, it appears that during the first six months of storage, packaging techniques that do not use sulfites suitably control the factors associated with TVB-N production, especially vacuum packaging. However, after this time period, the levels of TVB-N begin to increase substantially in all samples toward a plateau that is not compatible with the standard index for seafood freshness quality.

3.4. TBA changes

While the shrimp were frozen, lipid oxidation (as measured by malonaldehyde content) remained well below the threshold level of 1–2 μmol per 1 g fat as proposed by Connell (1995), considering that the lipid fraction was equal to 0.7% of the total constituents.

As illustrated in Fig. 3, the samples packed in a modified atmosphere, the sulfited samples and the control samples all had initial malonaldehyde value of less than 0.2 mg kg^{-1}. Furthermore, no significant changes were observed during the first six months of frozen storage. Tsironi, Dermesonlouoglou, Giannakourou, and Taoukis (2009) reported a similar pattern of lipid oxidation during frozen storage (at −15 °C) of shrimp in atmospheric air. During the second half of the year, the control samples tended to deteriorate more quickly than the others, with values almost doubling from 0.25 to 0.52 mg kg^{-1}. The sulfited samples reacted best in terms of lipid oxidation, with malonaldehyde values that ranged from 0.15 to 0.23 mg over 12 months. It is worth noting that no significant changes in TBA were observed during the entire period of frozen storage in the samples packed in N_2 ($P > 0.05$). This confirms that the exclusion of oxygen from the headspace is decisive in controlling lipid oxidation in frozen shrimp (Bak et al., 1999). However, a significant difference in malonaldehyde content ($P < 0.05$) was observed during the second half of the storage time between the sulfited samples and the samples packaged in N_2/CO_2. The increase in TBA values during the final period of storage in samples packed in N_2/CO_2 may be due to a slight depressurization in the headspace caused by CO_2 dissolving in the fat phase (Devlieghere et al., 1998).

Although the TBA levels of samples packaged in a vacuum increased approximately the same amount as did the TBA levels in the control samples, these samples showed evident signs of lipid oxidation (0.31 mg kg^{-1}) that, increased to about 0.60 mg kg^{-1} after 12 months. The cause of this unexpected behavior is not clear, and these results conflict with the widely reported advantages of packaging in a vacuum (Kerry & Murphy, 2007; Sikorski, Kolakowaka, & Pan, 1990). Because the analyses had already revealed increased oxidation at the first measurement (time 0) and because all of the malonaldehyde values increased in a pattern similar to that of the control, it is possible to attribute such oxidation pathways to the physical stress that may have occurred during the vacuum packaging stage, which changed both the physical and chemical conditions of the muscle tissue. It was at this stage that the muscular tissue could have been damaged due to squashing, which, consequently, favored the oxidation of the lipid fraction. Another possibility is that there is an inverse relationship between pH and the degree of lipid oxidation (Van Laack, 1994). When both datasets were compared, particularly the data from the tenth and twelfth months, an increase in lipid oxidation (0.6 mg) was observed in the samples packaged in a vacuum, which showed the lowest pH and TVB-N levels (7.64 and 50 mg, respectively) of the five packaging methods examined.

3.5. Melanosis

The melanosis scores of deep-water rose shrimp are presented in Fig. 4. At the beginning of analysis (time 0 in the graph), which took place three days after the shrimp were caught and packed, the control and vacuum-packed samples showed significant blackening (score 2) that intensified over the first six months and then

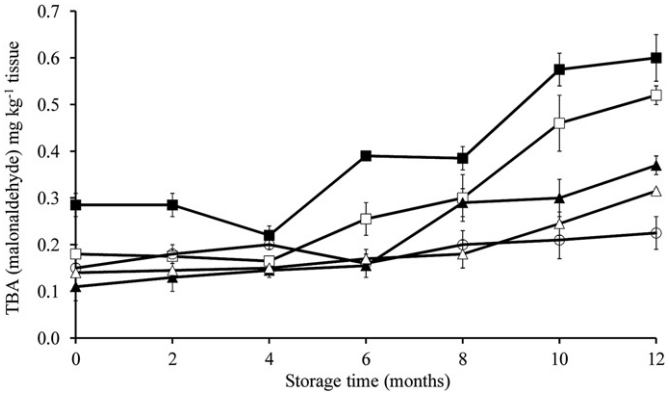

Fig. 3. Temporal changes in the TBA levels of frozen deep-water rose shrimp packed according to different methods. Bars represent the standard deviation ($n = 3$). □: Control; ■: Vacuum; ▲: N_2–CO_2; △: N_2; ○: Sulfited.

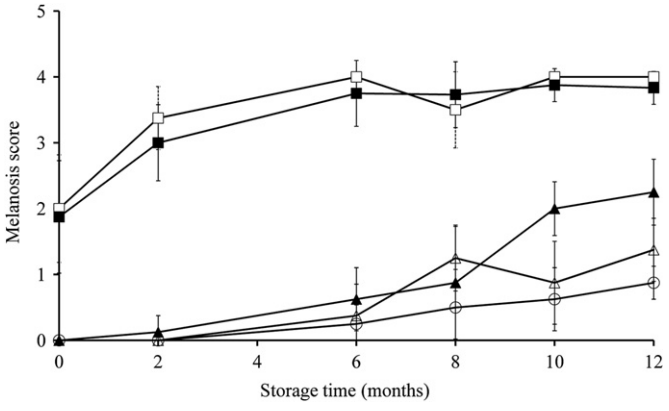

Fig. 4. Melanosis scores of deep-water rose shrimp packed according to different methods. It should be noted that the shrimp were captured and packed on-board three days before the onset of analysis. Bars represent the standard deviation ($n = 4$). □: Control; ■: Vacuum; ▲: N_2–CO_2; △: N_2; ○: Sulfited.

remained constant until the end of the study period. Consequently, the combination of vacuum packaging and frozen storage cannot be used to inhibit or delay blackening in shrimp. This result may be related to trace amounts of atmospheric air (i.e., oxygen) that remained in the spaces between shrimp because of the hardness of the exoskeleton, even though the vacuum was otherwise complete.

Conversely, until the sixth month of storage time, the samples packed in N_2 did not show any signs of the classic black spotting observed in the vacuum-packed samples. This result corroborates the observation that melanosis is induced by oxygen (Adachi & Hirata, 2011), and therefore, the N_2 (100%) packaging method could be used to delay the post-mortem blackening of shrimp. A slight loss of the natural color was, however, observed between the sixth and eighth months of storage. This discoloration, most likely associated with lipid oxidation rather than the progression of melanosis, involved a form of yellowing that was more obvious in the cephalothoracic area and that was more apparent in the samples packed in N_2/CO_2.

4. Conclusions

Because the high levels of TVB-N detected in the sulfited samples in the initial stage of storage should be positively correlated with DMA production and, hence, the suspected carcinogen N-nitrosodimethylamine, it appears that the use of sulfites as black spot inhibitors is not sustainable, especially because they are deleterious to human health. Other antimelanotic additives, such as a 4-hexylresorcinol-based formula, have been examined in recent papers, but they will always carry the burden of being categorized as chemical treatments.

Alternatively, the results of this study reveal that early application of modified atmosphere packaging techniques, especially packaging in N_2 (100%), in combination with freezing and frozen storage, should be considered for preventing melanosis and other chemical deteriorations induced by oxygen. Moreover, this packaging technique may promote the safety and quality of marine crustaceans as well as maximize their economic value.

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