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Original Paper

Controlled atmosphere as coadjuvant to chilled storage for prevention of melanosis in shrimps (*Parapenaeus longirostris*)

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Abstract The effect of a controlled atmosphere with high CO_2 and low O_2 levels on melanosis in deepwater pink shrimps (*Parapenaeus longirostris*) treated with sulphites was studied in samples in chilled storage. The application of atmosphere in shrimps without antimelanotics did not inhibit melanosis. Shrimps treated with 4% sulphites in combination with a concentration of 53% CO_2 and 7% O_2 totally inhibited darkening during storage. Covering with crushed ice produced an undesirable shrinking of muscle shrimp after cooking. The application of lower concentrations of CO_2 (45%) reduced the effectiveness in preventing darkening, compared with 53% CO_2, except where the melanosis inhibitor was 4-hexylresorcinol (0.1%). The residual effect of the gas when the shrimps were kept in a controlled atmosphere for 24 h was insufficient to prevent melanosis during further cold storage.

Keywords Shrimp · Melanosis · Controlled atmospheres · Sulphites · 4-Hexylresorcinol

Introduction

The shelf life of crustaceans, and especially fresh shrimps, is very short, because of natural deterioration of the organism and because of melanosis, which devalues the product by making it look less appetising. Melanosis is a biochemistry process produced by the action of an enzymatic complex called polyphenol oxidase (PPO). The active PPO catalyses the hydroxylation to benzoquinones of *o*-dihydroxyphenols. Benzoquinones react nonenzymatically with a variety of compounds, like amines, amino acids, or O_2, forming melanins, responsible for black coloration during storage.

Shrimps are normally sold commercially as fresh in small 1–2-kg boxes, covered with crushed ice. Immediately upon capture, they are treated on board ship with a product containing sulphites to prevent enzymatic browning. They are then covered with ice, in which manner they are freighted and stored for commercialisation. The problem is that even so, their shelf life is very short and this leads to substantial financial loss.

In the shelf life of crustaceans, particularly shrimps, melanosis is a much more important limiting factor than microorganisms. A number of ways have been proposed to deal with this problem: addition of new melanosis inhibitors, increased dosages of sulphites, modification of the storage process or a combination of all of these.

There are a number of possible alternative melanosis inhibitors, such as ascorbic, benzoic, sorbic, kojic and phytic acids, protease inhibitors, and resorcinol derivatives, all of which are considered to be safe compounds [1, 2, 3, 4, 5, 6, 7, 8]. 4-Hexylresorcinol appears to be one of the most effective, although the proportion required to inhibit melanosis is not clear: 50 ppm in brown shrimp (*Penaeus aztecus*), pink shrimp (*Penaeus duodarum*) [2], and in pink shrimp (*Penaeus duodarum*) [5]. Guandalini et al. [9] cite 100 ppm in deepwater pink shrimp (*Parapenaeus longirostris*), and others cite considerably higher concentrations: as much as 0.5% in tiger shrimp (*Penaeus japonicus*) [10] and 0.1% in deepwater pink shrimp (*Parapenaeus longirostris*) [11]. These differences may be due to species differences, to differences in physiological susceptibility, or to the volume of shrimps and the mode of application.

It is not feasible to raise the dosage of sulphites, as this would exceed permitted concentrations (in Spain, following a European Directive, the limit is 150 to 300 mg SO_2/kg in the edible parts, depending on the size of the crustacean [12]). Moreover, sulphites are known to be injurious to asthmatics, and therefore the tendency should be to reduce concentrations, except that the shelf life of shrimps would then be too short—hence the interest in modifying the storage process. It

has been suggested that an atmosphere poor (as far as possible) in oxygen and rich in CO_2 could act as a coadjuvant in delaying melanosis and microorganism growth, and hence spoilage of crustaceans.

Another unknown is the residual effect of the atmosphere on melanosis once the shrimps are released from the atmosphere, whereas the preservative effect post-treatment on fish stored at high CO_2 concentration is known. When a fish pack is opened, the CO_2 is slowly released by the product and continues to exert a useful preservative effect [13].

There are articles on the use of modified atmospheres in retail packaging of shrimps. Most of these articles refer to cooked shrimps, whether chilled or frozen [14, 15, 16], and very few refer to fresh shrimps [17, 18]. Moreover, they discuss microorganism-induced spoilage rather than melanosis. And there is hardly any information on the way that the use of post-capture controlled atmospheres affects the evolution of melanosis in shrimps or other crustaceans during chilled storage. The only such example is the effect of vacuum on storage of king shrimps packaged without the head [18].

The purpose of the present work was to study the effect of a controlled atmosphere with varying levels of CO_2 and O_2 on melanosis prevention in deepwater pink shrimps during chilled storage, and to evaluate the influence of a number of factors, such as the presence of crushed ice, different antimelanosics (sulphites and 4-hexylresorcinol) and the residual CO_2 effect.

Material and methods

First experiment: treatment of shrimps

An initial experiment was performed with deepwater pink shrimps (*Parapenaeus longirostris*), caught off the south coast of Spain (Cádiz) in March. Average and standard deviation shrimp sizes and weights were approximately 9±1 cm and 6±1 g, respectively. On board they were separated from the by-catch, washed, placed in perforated polystyrene boxes (approx. 2 kg per box) and kept in ice. All individuals were from a single catch. The temperature at the time of capture was 20 °C. On landing, several lots were made up for different treatments. The lots were identified as follows:

1. Shrimps not treated with antimelanosic (controls): covered with ice (Ice); covered with ice and stored under a controlled atmosphere (Ice+CA); or stored under a controlled atmosphere without ice (CA).

2. Shrimps treated with commercial antimelanosic (sulphites): covered with ice (Ice+S); covered with ice and stored under a controlled atmosphere (Ice+S+CA); or stored without ice under a controlled atmosphere (S+CA). The commercial antimelanosic product, containing approximately 28% sodium metabisulphite, was dusted in the amount recommended by the maker (4%, grams product per 100 g shrimps) before the ice addition.

All the CA-treated groups were placed in airtight steel containers (Innaves, Vigo, Spain) of approx. 215 L capacity. The chosen proportion of gases was 52–54% CO_2, 7–8% oxygen, with N_2 making up the rest. Preliminary trials showed that CO_2 proportions of 60% gave an acidic flavour and very pale colour. The atmosphere contained a small amount of oxygen to prevent the growth of anaerobic spoilage bacteria. The sulphite-treated shrimps were placed in containers different from those for shrimps without sulphites. All samples were placed in chilled storage at 2 °C.

In order to determine a residual effect of CO_2, the groups stored in controlled atmospheres with added sulphites (CA+S) were removed from their containers after 14 days, then covered with ice and further stored at 2 °C for one more week. All analyses were carried out in duplicate.

Second experiment: treatment of shrimps

A subsequent experiment was conducted on deepwater pink shrimps caught in July, in the Gulf of Cadiz by trawler. Average and standard deviation shrimp sizes and weights were approximately 9.5±1.3 cm and 6.7±1.5 g, respectively. All specimens were from a single catch. The temperature at the time of capture was 30 °C. On board ship, immediately after capture, the shrimps were divided into various groups. These groups were dusted with antimelanotics supplied by the Instituto del Frío (Spain), consisting of 2 and 4% of the commercial compound (Freskor, Hasenosa, Spain) containing approximately 28% sulphites and 0.1% 4-hexylresorcinol (H6250, Sigma, St. Louis, USA) with sorbitol as excipient. The amount of antimelanotic was calculated as grams product per 100 g shrimps.

The shrimps were then covered with ice until treated with atmospheres on land. The boxes were placed in airtight containers (Innaves, Vigo, Spain) at 2 °C with a gas mix consisting of 44–46% CO_2 and 7–8 % O_2, the remainder being N_2. Also, the effect of a residual atmosphere after a short interval (equivalent, for example, to transportation time) was studied. To that end, after 24 h in atmospheres, part of each group was removed from the containers then kept in ice and air at 2 °C. All treatments were carried out in duplicate.

The different lots were identified as follows:

1. Shrimps stored without antimelanosic: with ice (Ice); under a controlled atmosphere (CA); or 24 h under a controlled atmosphere, then stored with ice (CA+RE).
2. Shrimps stored with 4% antimelanosic (sulphites): with ice (S4+Ice); with controlled atmosphere (S4+CA); or 24 h in controlled atmosphere, then stored with ice (S4+CA+RE).
3. Shrimps stored with 2% antimelanosic: with ice (S2+Ice); with controlled atmosphere (S2+CA); or 24 h in controlled atmosphere, then stored in ice (S2+CA+RE).
4. Shrimps stored with 0.1% 4-hexylresocinol: with ice (R+Ice); with controlled atmosphere (R+CA); or 24 h in controlled atmosphere, then stored in ice (R+CA+RE).

Melanosis index

During storage, approximately every 2 days, the development of melanosis was evaluated visually by panellists with more than a year of training experience. Melanosis (manifested by black spots, especially on the shell heads) was assessed for each shrimp individually (14 per lot) according to a visual scale (a modified version of one developed by Otwell and Marshall [19]). The scale used was as follows: 1=absent; 2=very slight to moderate (up to 30% of shrimp surface affected); 3=severe (30–70% of shrimp surface affected); 4=extremely heavy (70–100% shrimp surface affected). In all lots, an average of melanosis index was obtained during storage. The location of melanosis and bonding of the cephalothorax to the abdomen in shrimps was also taken into account.

Statistical analyses

The significance of differences between mean value pairs was evaluated using two-way ANOVA. The Tukey HSD test was used to identify significant differences among main effects. Statistical processing was by the Statgraphics plus 2.1 computer program (STSC Inc., Rockville, MD). The level of significance setting was $P \leq 0.05$. Trend lines were used to represent melanosis index during storage.

Results and discussion

First experiment: shrimps treated with 53% CO_2 and 7% O_2

Effect of the atmosphere on additive-free shrimps

The onset of melanosis was observed in the first days of storage (Fig. 1) in additive-free shrimps, especially in samples stored without ice ($P \leq 0.05$) (Table 1). The lot Ice+CA showed a 2-day delay

in the development of melanosis. Although only partial, the melanosis was visible in heads, tails and pleopods in the three lots. Furthermore, the heads had a greenish tinge indicating slight melanosis.

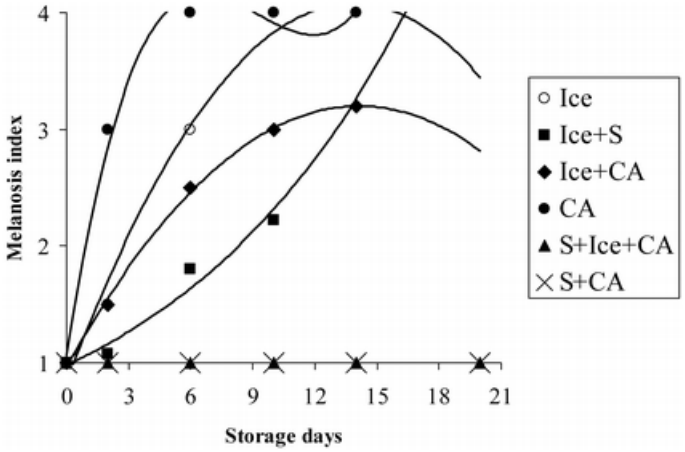

Fig. 1 Melanosis index versus storage time at 2 °C (trend lines). Ice: shrimps covered with ice. Ice+S: shrimps treated with 4% antimelanosic product and covered with ice. Ice+CA: shrimps covered with ice and stored under controlled atmosphere. CA: shrimps stored under controlled atmosphere. Ice+S+CA: shrimps treated with 4% antimelanosic product, covered with ice and stored under controlled atmosphere. S+CA: shrimps treated with 4% antimelanosic product and stored under controlled atmosphere.

[Table 1 will appear here. See end of document.]

After 6 days, melanosis was apparent in all lots (Ice, Ice+CA and CA, Figs. 1 and 2). It was most noticeable ($P \leq 0.05$) (Table 1) in the absence of washing by ice melt (Fig. 1). There were clear differences in the colouring of the shrimps: those stored in ice were pale, while the shrimps stored without ice presented a brighter and more intense pink colour. Also, the bond between abdomen and cephalothorax was weaker in the lots stored with ice.

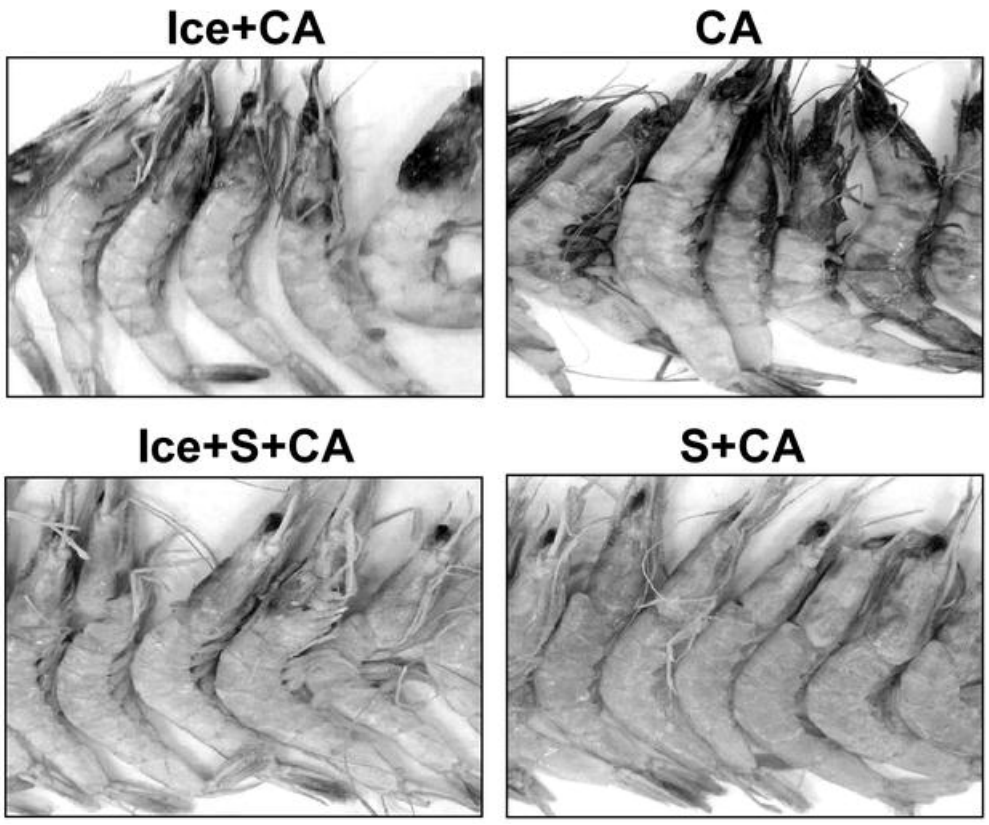

Fig. 2 Shrimps stored under controlled atmosphere (53%CO_2/7% O_2) at 2 °C for 6 days. Ice+CA: shrimps covered with ice and stored under controlled atmosphere. CA: shrimps stored under controlled atmosphere. Ice+S+CA: shrimps treated with 4% antimelanosic product, covered with ice and stored under controlled atmosphere. S+CA: shrimps treated with 4% antimelanosic product and stored under controlled atmosphere.

Effects of the atmosphere on shrimps with additives

The combination of an additive containing metabisulphite and the controlled atmosphere (Ice+S+CA and S+CA) inhibited melanosis ($P \leq 0.05$) for 14 days (Fig. 1), as compared to the browning symptoms detected after 6 days in the case of shrimps stored outside the containers in the conventional manner (Ice+S). From day 10 onwards, melanosis was noticeable in lot Ice+S.

From day 6 onwards, the lots with ice (Ice+CA and Ice+S+CA) presented a paler and more opaque aspect, resembling lightly cooked shrimp. In addition, all the shrimps stored in ice, with or without additives, presented softening and weakening of the abdomen–head bond.

During steam cooking (5 min at 90 °C) the muscle tended to shrink and detach itself from the shell (Fig. 3). This post-cooking effect was apparent in all specimens stored in ice with a controlled atmosphere, with and without sulphites (Ice+CA and Ice+S+CA). The effect of ice and a controlled atmosphere on muscle may be due to greater CO_2 solubility in the muscle; this would reduce the water holding capacity [17, 20], presumably thanks to a reduction of the pH. According to Henry's

law [21], CO_2 solubility will increase when the temperature falls [22] and the amount of water in the muscle increases, both of which will occur with the melting of the ice. The flavour loss is probably due to the leaching out of components such as free amino acids and organic phosphates [23].

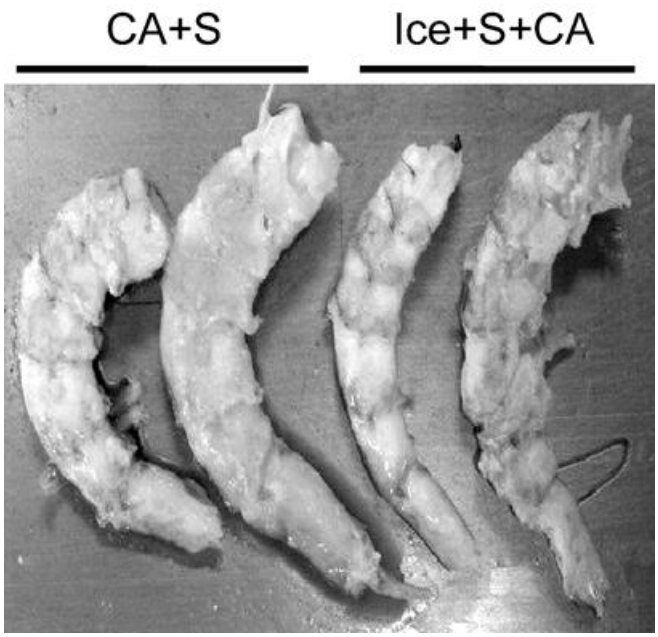

Fig. 3 Shrimps boiled after storage at 2 °C in controlled atmosphere, containing sulphites, with ice (Ice+S+CA) and without ice (S+CA). Ice+S+CA: shrimps treated with 4% antimelanosic product, covered with ice and stored under controlled atmosphere. S+CA: shrimps treated with 4% antimelanosic product and stored under controlled atmosphere.

The residual effect of the atmosphere after2 weeks' storage at 2 °C allowed samples to be kept outside the containers at the same temperature for one more week, with no sign of melanosis. However, the shrimps did not look fresh: the cephalothorax–abdomen bond was weak and the shrimps smelt of ammonia.

From these findings, the use of ice in combination with a controlled atmosphere at low temperatures is not recommended, since it did not delay the onset of melanosis, and produced weakening of the cephalothorax–abdomen bond, as well as the shrinkage of tail muscle and flavour loss after cooking. Furthermore, a controlled atmosphere composed of 53% CO_2/7% O_2 *per se* did not show beneficial effects on melanosis; however, in combination with 4% of commercial additive (sulphites), melanosis development was inhibited, although shrimps were characterised by a slightly pale appearance.

Second experiment: shrimps treated with 45% CO_2/7% O_2

A subsequent study was conducted in which the CO_2 was reduced from 52–54% to 44–46%, in order to avoid the pale colour of the shrimps. Given that the concentrations in the first experiment were highly effective, it could be assumed that there would be a possible loss of CA effectiveness, but a gain of a fresh pink colour. Since covering with ice had not proved to be beneficial in the aspect and flavour after cooking of shrimps stored under controlled atmosphere, no ice was used in this experiment, except for the controls stored without controlled atmosphere.

A slight presence of melanosis was evident in samples without antimelanosic by day 3 (Fig. 4a). There were no significant differences between lots stored with and without atmosphere (CA and Ice) ($P \leq 0.05$) (Table 2). Storage in atmospheres for 24 h followed by storage in ice (CA+RE) had no observable beneficial effect. The evolution of melanosis was much the same as in the first experiment, again highlighting the need for a melanosis inhibitor to palliate the problem.

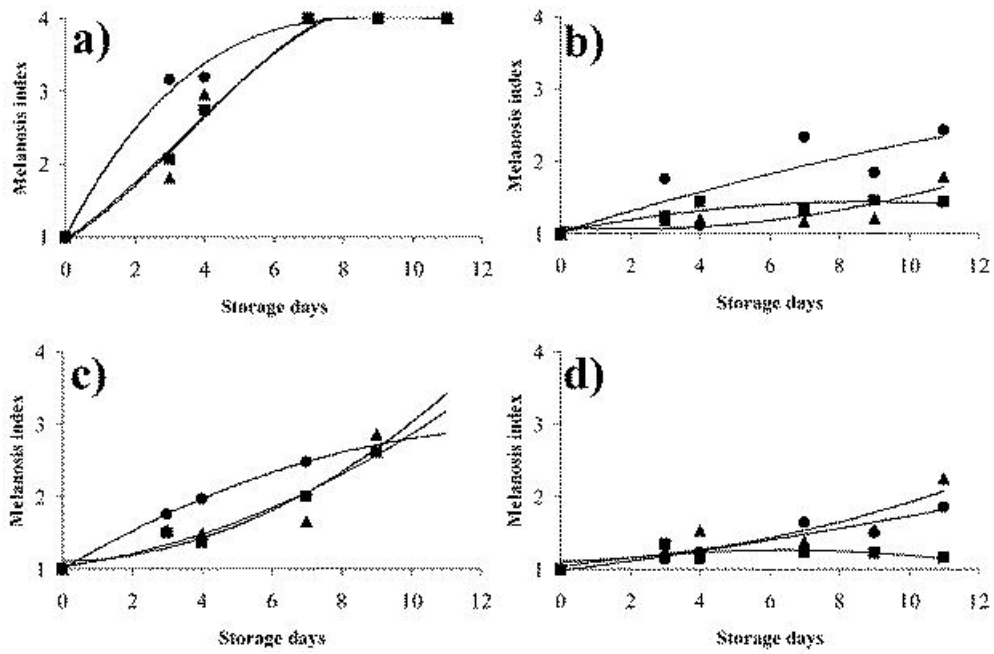

Fig. 4 Melanosis index versus storage time of shrimps at 2 °C. **a** Without antimelanosic. **b** With 4% antimelanosic (sulphites). **c** With 2% antimelanosic (sulphites). **d** With 0.1% 4-hexylresorcinol. Ice (▲); controlled atmosphere, CA (■); residual effect of controlled atmosphere, CA+RE (●).

[Table 2 will appear here. See end of document.]

In the first days of storage the evolution of melanosis in shrimps treated with 4% antimelanosic under controlled atmosphere (S4+CA) was similar to that in shrimps stored in air (S4+Ice) ($P \leq 0.05$); however, towards the end of the storage period melanosis stabilised more ($P \leq 0.05$) in the shrimps

stored in a controlled atmosphere (Fig. 4b, Table 2). The evolution of melanosis in lot control (S4+Ice) was similar to that of the first experiment (Fig. 1). This suggests that the higher melanosis shown in lot S4+CA in Fig. 4b is due to the lower CO_2 content in the controlled atmosphere with respect to the corresponding treatment (S+CA) shown in Fig. 1. When shrimps were kept for 24 h in a controlled atmosphere (S4+CA+RE), the residual effect of the gas was insufficient to slow down melanosis, and these samples presented higher ($P \leq 0.05$) melanosis values (Fig. 4b and Table 1). This may be due in part to the fact that the temperature in the chamber and the containers was 2 °C, while the shrimps without atmosphere (S+Ice) (kept at around 0 °C) were washed throughout by ice melt.

When smaller amounts of antimelanosic (2%) were added (Fig. 4c), the onset of melanosis was much faster than in samples with 4% and these samples were rejected on day 9. The evolution in this case was similar in lots with and without controlled atmospheres (S2+CA and S2+Ice) (Table 2), which would suggest that a higher quantity of sulphites is needed for the atmosphere to be an effective coadjuvant. There was no residual effect of the gas (S2+CA+RE), as was noted earlier in respect of shrimps treated with 4% melanosis inhibitor.

In order to observe the effectiveness of different compounds, 4-hexylresorcinol (0.1%) was also tested (Fig. 4d), and proved to be a potent melanosis inhibitor. There was no sign of melanosis in these lots for the first 11 days in a controlled atmosphere; as from day 7 post-capture, the results were significantly better than in a control sample stored in ice with normal air (Fig. 4d, Table 2). This concentration of 4-hexylresorcinol has been found to be effective in other studies [11]. Nevertheless, other authors have found that the effectiveness varies a lot depending on the physiological condition and season of capture of the specimen, the type of crustacean involved and the way in which the melanosis inhibitor is applied [2, 5, 6, 10].

In samples stored without atmospheres, by the end of storage melanosis indices were a little lower with 4-hexylresorcinol (R+Ice) than with 4% antimelanosic with sulphites (S4+Ice) ($P \leq 0.05$, Table 2). In samples stored in controlled atmospheres (S4+CA and R+CA), however, there were no significant differences, indicating that the coadjuvant effect of the atmospheres was similar in the presence of 0.1% of 4-hexylresorcinol or 4% of commercial antimelanosic (sulphites).

In shrimps treated with 0.1% 4-hexylresorcinol and kept in a controlled atmosphere for 24 h there was a residual CO_2 effect (R+CA+RE), as evidenced by the fact that at the end of storage they presented less melanosis ($P \leq 0.05$) than shrimps treated with 4-hexylresorcinol and kept in ice without CO_2 (R+Ice) (Fig. 4d and Table 2). Nevertheless, the effect was less intense than in the case of shrimps stored throughout in a controlled atmosphere.

Conclusions

The normal way of storing fresh shrimps is to apply a melanosis inhibitor and cover them with ice. The ice gives them a duller appearance and can wash microorganisms and other substances from the shell, thus slowing down spoilage to some extent. However, in storage with a controlled atmosphere containing 52–54% CO_2, covering with ice makes shrimps paler and softens the shell; moreover, on cooking there is considerable loss of flavour and shrinkage of the muscle. The atmosphere as a coadjuvant to chilled storage is useful in that it inhibits black spots; shrimps treated with sulphites presented no melanosis at any time during 20 days of storage in the atmosphere, but a slight decrease in the intensity of the typical pink colour was observed. Therefore, a controlled atmosphere with reduced CO_2 concentration (around 44–46%) has been also proved to be useful, especially when 0.1% 4-hexylresorcinol was added, rather than 4% of a sulphite-based commercial product. In these conditions, 2% of antimelanosic product was not effective either with or without a controlled atmosphere.

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Table 1 Analysis of variance (ANOVA) of melanosis index alongduring storage. Different letters (a, b, c...) in the same row indicate significant differences ($P \leq 0.05$) as a function of storage time; different letters (x, y, z...) in the same column indicate significant differences ($P \leq 0.05$) as a function of treatment.

	Days of storage				
	0	2	6	10	14
Ice	a/x	a/x	b/y	c/z	
Ice+S	a/x	a/x	b/x	c/x	
Ice+CA	a/x	b/y	c/y	c/y	c/y
CA	a/x	b/z	c/z	c/z	c/z
Ice+S+CA	a/x	a/x	a/v	a/v	a/x
S+CA	a/x	a/x	a/v	a/v	a/x

Table 2 Analysis of variance (ANOVA) of melanosis score alongduring storage. Different letters (a, b, c...) in the same row indicate significant differences ($P \leq 0.05$) as a function of storage time; different letters (x, y, z...) in the same column indicate significant differences ($P \leq 0.05$) as a function of treatment.

	Days of storage					
	0	3	4	7	9	11
Ice	a/x	b/x	c/xy	d/x	d/x	d/x
CA	a/x	b/y	c/x	d/x	d/x	d/x
CA+RE	a/x	b/z	b/y	c/x	c/x	c/x
S4+Ice	a/x	b/x	b/xy	b/x	b/x	c/x
S4+CA	a/x	b/x	b/x	b/x	b/x	b/y
S4+CA+RE	a/x	b/y	a/y	c/y	b/y	c/z
S2+Ice	a/x	b/x	b/x	c/x	d/x	d/x
S2+CA	a/x	b/x	b/y	c/y	d/y	e/x
S2+CA+RE	a/x	b/x	b/x	b/z	c/x	d/x
R+Ice	a/x	b/x	c/y	bc/y	c/x	d/z
R+CA	a/x	b/x	ab/x	b/y	b/y	ab/y
R+CA+RE	a/x	ab/x	a/x	c/x	c/x	d/x