Melanosis inhibition and SO_2 residual levels in shrimps (*Parapenaeus longirostris*) after different sulphite based treatments.

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ABSTRACT

The effectiveness of different sulphite based treatments to prevent melanosis in fresh deepwater pink shrimp (*Parapenaeus longirostris*) was evaluated. Increasing concentration of sulphites, different methods (immersion and dust) and synergy with other compounds, such as citric acid and chelants were investigated. A selection of the most effective treatments was chosen to determine the level of SO_2 residues in the muscle. One-hour dip treatment with 50 g kg^{-1} sulphite, together with citric acid and chelants, was effective for melanosis prevention during at least one week. With this treatment, restricted limit of 0.3 g kg^{-1} SO_2 in edible part was not exceeded by the majority of analysed samples.

Key words: Melanosis; sulphites; shrimps; iced storage; residues.

INTRODUCTION

Browning is one of the major problems in the food industry and can cause deleterious changes in the organoleptic properties of foods, resulting in shorter shelf life and quality, and therefore a decrease in commercial value (1). Black spots or melanosis in crustaceans is a natural postmortem mechanism that involve the action of an enzymatic complex, polyphenoloxidase (PPO), which in the presence of oxygen form compounds which can polymerise into insoluble pigments (2). Sulphite derivatives are widely used as antioxidant and/or preservatives (antimicrobial agent) in foods. The addition of sulphites, mainly metabisulphite, to avoid the melanosis in raw prawns and shrimps has been world-wide a practice for many years (3). Bisulphite appears to inhibit melanosis by two mechanisms: 1) by reacting with intermediate quinones in the melanosis reaction, forming sulfoquinones, and 2) by irreversibly reacting with PPO causing complete inactivation (4).

A common practice is to use commercial products containing another substances, in addition to sodium metabisulphite, which contribute to retard melanosis. Some of them are reducing agents, which act by causing the chemical reduction of the pigment precursors (ascorbic acid, ascorbyl derivatives), acidulants (citric acid, phosphoric acid) or chelating agents to reduce the level of copper available (ethylene-diaminetretraacetic) (EDTA). Almost the majority of the commercial antimelanotic products contain citric acid, ascorbic acid and/or EDTA (5).

In chilled crustacean the intensity of melanotic reaction, the point of beginning and the rate of spread differ among species, being deepwater pink shrimp (*Parapenaeus longirostris*) one of most susceptible. In addition, depending on the season, the melanosis is higher coinciding with moulting cycle (6; 7). For that reason, higher concentration of sulphites is often required for an effective prevention of melanosis. This will increase the total content of additive in edible part, exceeding the limits established by the legislative authorities.

It is well known that sulphites produce certain adverse reactions in some groups of population, mainly asthmatic (8; 9). In fact, metabisulphite is considered as a precipitating cause of an asthmatic attack (8). Because of this, their used in food is limited. The regulatory authorities of many countries have indicated a maximum concentration of sulphites and derivatives in different foods. In Europe, the limit of sulphites in edible part of fresh *Penaideae* crustacean family is restricted from 0.15 to 0.3 g $S0_2$ /kg according to the size of the crustacean (10).

However, the dependency of residual sulphite levels may not be only on the size but also on harvest, treatment conditions, handling and processing of the products. In this sense, only scarce information is available on the level of residues generated as a consequence of different sulphite based treatments in this and other species. There is one study where a number of commercial products containing sodium metabisulphite, applied by different times of immersion and concentrations, were analysed (11).

The aim of this work is to determine the effectiveness of different sulphite based treatments, i.e. concentration of sulphites, method and time of application, synergy with other compounds, to avoid melanosis in fresh deepwater pink shrimp, and to determine the total content of sulphite residues in the edible part.

MATERIAL AND METHODS

Harvesting and treatment of shrimps

Deepwater pink shrimp (*Parapenaeus longirostris)* were caught off the South coast of Spain (Cádiz) by trawl in spring. The temperature at the time of capture was around 20 ºC. On board they were separated from the by-catch, washed with seawater, placed in perforated polystyrene boxes (aprox. 2 kg per box). All of these processes, before antimelanotic treatment (immersion or dust), delayed between 1h and 1 h and 45 min. For immersion treatments, sodium metabisulphite (Panreac Química, S.A., Spain) at different concentrations ranging from 1.6 to 50 g kg^{-1}

(expressed as g compound per kg shrimps) was used. Citric acid, ethylenediaminetetraacetic acid (EDTA) and di-sodium-dihidrogene-pyrophosphate (PPi) were also of reagent grade. The dip solutions were prepared with a seawater/shrimp relation of 2/1. The antimelanotic blend was dissolved in seawater and afterwards the shrimps were introduced and covered with ice. Once finished the treatment time, they were taken away, placed in perforated polystyrene boxes of 2 kg of capacity, and covered with ice.

The dust treatments were performed also on board, using three different commercial products (CP) which were spread in the form of dry powder on the surface of shrimp, followed by a slight manual mixing. Then they were placed in perforated polystyrene boxes, and covered with ice. The commercial product 1 (CP1) was Melacide Fresh (Técnicas Químicas Industriales, S.A. (Spain), maximum content in SO_2 = 140 g kg^{-1}) added in a concentration of around 60 g kg^{-1} (60 g per kg shrimps, w/w), which is normally used by the fishermen. Commercial product 2 (CP2) was Freskor (Hasenosa, S. A. (Spain), maximum content in SO_2 = 600 g kg^{-1}), and commercial product 3 (CP3) was Melaplus (Turco, S.A. (Spain), maximum content in SO_2 = 300 g kg^{-1}). CP2 and CP3 were added in a concentration of 40 g kg^{-1} (40 g per kg shrimps, w/w), which was the one recommended by the manufacturers. All the treatments (immersion and dust) were carried out in duplicate at the same time, using different boxes.

Once the boat arrived at harbour, all the boxes were sent in isothermal transport to the Instituto del Frío in Madrid, where they were kept in iced storage at 2ºC.

After one day of storage at the Institute, an extra dose of additives, similar to the first one, was added to part of the dust-treated lots (double treatment).

Melanosis index

During storage, 14 shrimps per lot were evaluated every two days by a trained panel. Melanosis (manifested by black spots, especially on the shell heads) was assessed on a visual scale (a modified version of one developed by Otwell and Marshall 1986). The scale was: 1= absent; 2= very slight to moderate (up to 30 % of shrimp surface affected) in less than 50 % individuals; 3= severe (30-70 % of

shrimp surface affected) in less than 50 % individuals; 4= extremely heavy (70-100% shrimp surface affected) in most individuals. Results were average values of the scores emitted by the different assessors for a given lot along the storage period, considering the number of individual shrimp affected by each level of melanosis according to the 1 to 4 visual scale.

Sulphite determination

The amount of sulphites in shrimp was determined according to Monier-Williams method (13). Three muscle homogenates per lot, prepared from 5 to 6 shrimps per homogenate, were used for each determination.

Statistical analyses

Regression analyses between average melanosis index values and days of storage were performed. The same analysis was carried out between residual quantities of SO_2 and concentration in the treatment solution. The significance of the differences between pair mean values was evaluated using one-way and two-way ANOVA. Tukey HSD test was used to identify significant differences ($p \leq 0.05$) among residual levels. The Statgraphics plus 2.1 computer program (STSC Inc., Rockville, MD) was used for statistical processing.

RESULTS AND DISCUSSION

Immersion treatments

Melanosis development in shrimps treated with different concentrations of sodium metabisulphite for various immersion periods is shown in Figure 1. After 2 days of chilled storage only the shrimps that have been treated with 12.5 g kg^{-1} sulphite concentration during 2 h did not show melanosis at all. The degree of melanosis in this batch after 4 days was moderate, and spread along the whole shrimp after 7 days. On the other hand, treatments based on 0.5 h immersion, even at 12.5 g kg^{-1} concentration, led to noticeable melanosis at day 2 of storage. These results are in

disagreement with the specifications suggested for commercial products, which indicate 1 or 2 min immersion treatment. In fact, such indications were found completely ineffective to prevent melanosis for periods longer than 2 days of chilled storage (preliminary results not shown). Moreover it is difficult to apply on board, given that usually the process is done largely by hand, and require more time.

McEvily et al. (2) indicated, for 1 min dip treatment into a 12.5 g kg^{-1} sodium metabisulphite solution, an allowable residual sulphite of 0.1 g kg^{-1} on the shrimp (Federal USA register, 1985). Treatments are not specified in European Directive, only the quantity of $S0_2$ remaining in edible part, depending on the species and number of individuals per kg. In the case of deepwater pink shrimp, as observed above, 12.5 g kg^{-1} sulphite (even during 2h immersion) is not effective for melanosis prevention beyond 2-3 days after capture. McEvily et al. (2), working with another species (*Penaeus aztecus* and *Penaeus duorarum*), found effective 1 min dip in 12.5 g kg^{-1} of sodium metabisulphite, however after 7 days the shrimps showed noticeable melanosis on most of them.

On the other hand, perhaps two hours immersion is too much to be sometimes profitable and realistic on board, for this reason we propose to apply the immersion during 1 hour. As shown in Fig. 2, higher concentrations of metabisulphite were tested in order to know the effective amount, in terms of melanosis prevention, to increase shelf life to around 6-7 days. After 4 days of storage, shrimps treated with 6.3 g kg^{-1} or 12.5 g kg^{-1} sodium metabisulphite showed moderate melanosis, whereas those treated with 25 g kg^{-1} or 50 g kg^{-1} exhibited melanosis around two days later. These results indicated that concentrations of sulphite as high as 50 g kg^{-1} did not prevent melanosis for periods longer than 5-6 days.

Successful melanosis prevention has been reported by studies conducted with sulphite treatments applied on most occasions under controlled conditions. It is the case of Mc Evily et al. (2) who dipped 1 lb of shrimp into a 12.5 g kg^{-1} sodium metabisulphite solution for 2 min, or Yu et al. (14) who used 2 kg of shrimp dipped at 2 g kg^{-1} concentration of sodium bisulphite for 20 sec. However, when the

experiment is carried out on board under habitual conditions of weather, handling and processing, which is the case of the present work, the need of additive amount and time of application to inhibit melanosis increase drastically. In accordance with our findings, Arthur and Casedi (15) reported that immersion times from 1 to 15 min did not affect melanosis prevention in *P. Indicus* and *M. monoceros*. Rotllant et al. (16) observed that 25% of shrimps (*Arsisteus antennatus*) treated with 60 g kg^{-1} HQ-bacterol F, (400 g kg^{-1} sodium metabisulphite content), had small black spots on the tips of the appendages after 27 h, whereas with 20 or 40 g kg^{-1} all the shrimps (100%) were affected.

Figure 3 shows the melanosis development along storage of shrimps treated with different combinations of 50 g kg^{-1} sodium metabisulphite with citric acid (20 g kg^{-1} w/w) and/or chelants (0.45 g kg^{-1} EDTA + 30 g kg^{-1} PPi, w/w). Melanosis was efficiently retarded when, in addition to sulphites, chelating agents or citric acid were added, especially the latter. In fact, when citric acid was included melanosis was almost absent after 9 days.

The development of melanosis in shrimps treated with 20 g kg^{-1} citric acid and increasing concentrations of sodium metabisulphite is shown in Figure 4. The citric acid alone did not inhibit the melanosis process, however when added in combination with sodium metabisulphite, favoured the action of this. This effect is more evident along storage.

Dust treatments

A common practice to add the antimelanotic product on board is by dust. This method involves normally a heterogeneous spread of the additive in the shrimps, resulting in irregular appearance of melanosis in the different individuals. Moreover it is difficult to control the time of application, since the dry powder is not removed. In many occasions along the distribution channel, when a unique dose is not effective, another one is usually applied. Obviously, this will increase the total

content of sulphites in the edible part, frequently exceeding limits established by legislative authorities.

In Figure 5 is shown the melanosis index of samples treated by dust, in single or double dose, with three commercial additives containing sulphite. All treated samples exhibited less blackspot formation than in untreated shrimps. Slight differences could be observed depending on the commercial product used. In this case, CP3 led to notably more melanosis development along the storage period. This is largely attributed to differences in metabisulphite content. When a subsequent dose (double treatment) was applied, there was complete absence of melanosis in all batches along the entire storage period, i.e. around 9 days (Figure 5).

Residual sulphite levels

The total content of sulphites in the edible part (muscle) of shrimps treated by immersion (1 h) with metabisulphite (25 and 50 g kg^{-1}), alone or in combination with citric acid (20 g kg^{-1}) and chelants (EDTA 0.45 g kg^{-1} + PPi 30 g kg^{-1}), is shown in Figure 6a. In shrimps treated with 25 g kg^{-1}, the initial levels of sulphites were around 0.15 g kg^{-1}, whereas 50 g kg^{-1} led to more than 0.2 g kg^{-1} in the edible part. The presence of citric acid and chelants may increase the residues, probably inducing softening of the carapace. Anyhow, differences were not significant ($p \leq 0.05$) given the high degree of dispersion in the results among analysed samples. This will cause that some individuals may occasionally exceed restricted limit of 0.3 g kg^{-1} SO_2 in edible part. After 4 days the general tendency is to decrease the sulphite content in edible part, probably due to drip with melting ice along storage.

On the other hand, when the treatments were applied by dust (commercial products), the evolution of residues with storage time was the opposite, i.e. they tended to increase (Fig. 6b). In this case it is possible that the ice covering the shrimps, when melting, favoured the way inside the muscle of the sulphites present in the carapace. However, the results showed a high standard deviation as a

consequence of the heterogeneity in the spread of dust. Thus, some shrimps could accumulate a great amount of additive, whereas others remained practically free. The amount of residual sulphite varied considerably from one commercial product used to another, and was in consonance with the melanosis development. The treatment with CP3 led to a residual level lower than 100 ppm, but was less effective for melanosis prevention. On the contrary, CP2, which inhibited more efficiently blackspot formation, produced a residual level exceeding the upper limit of 0.3 g kg^{-1} in edible part (EU Directive).

As expected, when a subsequent dose was applied the residual content increased drastically (Fig. 7). The three commercial products, including CP3, exceeded the limits permitted in the EU Directive, reaching values in some cases close to 1.6 g kg^{-1}, which represents more than five times the mentioned limit. In terms of melanosis prevention, these treatments were the most effective, since all the shrimps were absolutely free of blackspot for more than one week. After 7 days of storage, the residual content of sulphite decreased in the case of CP1 and CP2, however residues remained still very high.

A regression analysis was performed between sulphite-based treatments having different concentrations of SO_2 with the corresponding residual contents in the muscle (Fig. 8). It can be observed that the residual levels increased exponentially with the concentration of sulphites applied.

Hardisson et al. (11) studied the sulphite content in edible and non-edible part of frozen prawns and shrimps. They showed that sulphite concentration in edible part of frozen crustacean was very variable with standard deviations around 0.15 g kg^{-1} in prawns and 0.12 g kg^{-1} in shrimp. In some cases, residual levels achieved up to 0.55 g kg^{-1} that highly exceeds the limit in EU Directive, indicating uncontrolled addition. We suggest also that exceptionally high residual levels may appear when the traditional method of dust is applied, in which the additive is spread heterogeneously. In contrast, regarding immersion treatments, Rotllant et al. (16)

did not found differences in residual SO_2 levels among treatments as a function of immersion time in any part of the body. In this connection, Arthur and Casedi (15) observed in *P. indicus* and *M. monoceros* that, after 5 min immersion, the residual SO_2 content in the muscle was about 90% of the value obtained after 15 min, indicating a very short difference.

Conclusions

In deepwater pink shrimp (*Parapenaeus longirostris*), caught in spring, metabisulphite concentrations as high as 50 g kg^{-1}, led to moderate melanosis after 5-6 days of storage. Citric acid and EDTA and PPi were shown largely to assist sulphites in melanosis prevention, especially the former. One-hour dip treatment with 50 g kg^{-1} sulphite together with citric acid and chelants was effective for melanosis prevention during one week. With this treatment, restricted limit of 0.3 g kg^{-1} SO_2 in edible part was not exceeded by the majority of analysed samples. In general, immersion treatments were more homogeneous and the shrimps tended to loose residual sulphites along storage. On the contrary, dust treatment spread the additive more heterogeneously, and favoured the penetration into the muscle during the first days of iced storage.

Further studies will be needed to define the effectiveness of sulphite-based formulations and associated residual levels in different seasons along the year, where melanosis may appear with more intensity than in springtime.

Acknowledgements

This work was funded by the Consejería de Agricultura y Pesca, Junta de Andalucía [Department of Agriculture and Fisheries of the Autonomous Government of Andalusia] (Project CSIC-2000-664). The authors wish to express their thanks to the Centro de Investigación de Cultivos de Especies Marinas (CICEM) [Marine Species Culture Research Centre] and particularly to its Director, José Naranjo, for cooperation in carrying out this study.

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LEGEND TO FIGURES

FIGURE 1: Melanosis index along storage in shrimps treated by immersion with different concentrations of sodium metabisulphite for several periods.

(0) 12.5 g kg^{-1} / 2 h (R^2= 0.9963); (□) 6.2 g kg^{-1} / 2 h (R^2= 0.8291); (▲) 3.1 g kg^{-1} / 2 h (R^2= 0.953); (●) 12.5 g kg^{-1} / 0.5 h (R^2 = 0.9865); (■) 6.2 g kg^{-1}/ 0.5 h (R^2= 0.997); (⧗) 1.6 g kg^{-1}/ 4 h (R^2= 0.9665); (◆) untreated (R^2= 0.9999).

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.

FIGURE 2: Melanosis index along storage in shrimp treated by immersion with different concentrations of sulphites for one hour.

(⧗) 50 g kg^{-1} (R^2= 0.9772); (●) 25 g kg^{-1} (R^2 = 0.9805); (■) 12.5 g kg^{-1} (R^2 = 0.9571); (▲) 6.2 g kg^{-1} (R^2 = 0.9857); (◆) untreated (R^2= 0.9745).

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.

FIGURE 3: Melanosis index along storage in shrimp treated by immersion (1 hour) with 50 g kg^{-1} sulphites (S) in combination with 20 g kg^{-1} citric acid (A) and/or chelants (Q) (0.45 g kg^{-1} EDTA and 30 g kg^{-1} PPi).

(⧗) S (R^2= 0.9999); 5 % (■) S+Q (R^2 = 0.9931); (●) S+A+Q (R^2 = 0.8889); (▲) S+A (R^2 = 0.9221); (◆) untreated (R^2= 0.9999).

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.

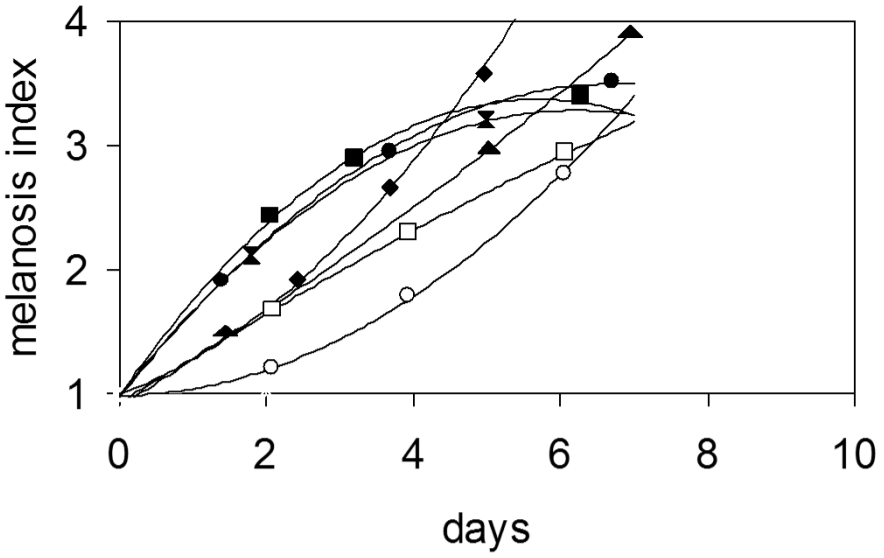
FIGURE 4: Photographs along storage of shrimp treated with (a) 20 g kg^{-1} citric acid alone, and 20 g kg^{-1} citric in combination with different concentrations of sulphites: (b) 12.5 g kg^{-1} , (c) 25 g kg^{-1}, (d) 37.5 g kg^{-1}, (e) 50 g kg^{-1}.

FIGURE 5: Melanosis index along storage in shrimp treated with different commercial products (CP1=Melacide; CP2=Freskor; CP3=Melaplus) in single dose (s) or double dose (d).
(●) CP1s (R^2= 0.9814); (▲) CP2s (R^2= 0.9351); (■) CP3s (R^2 = 0.9963); (o) CP1d (R^2= 0.9951); (△) CP2d (R^2= 0.8999); (□) CP3d (R^2= 0.7626); (◆) untreated (R^2= 0.9999).
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.

FIGURE 6: Residues of $S0_2$ present in the edible part of shrimps treated by (a) immersion for one hour in a solution with sulphites at 25 g kg^{-1} and 50 g kg^{-1} concentration, and at 50 g kg^{-1} in combination with citric acid and chelants, and (b) dust with commercial products.
A= citric acid; Q= chelants; CP1=Melacide; CP2=Freskor; CP3=Melaplus.
Error bars represent standard deviation.
Different letters (a, b, c...) indicate significant differences ($P \leq 0.05$).

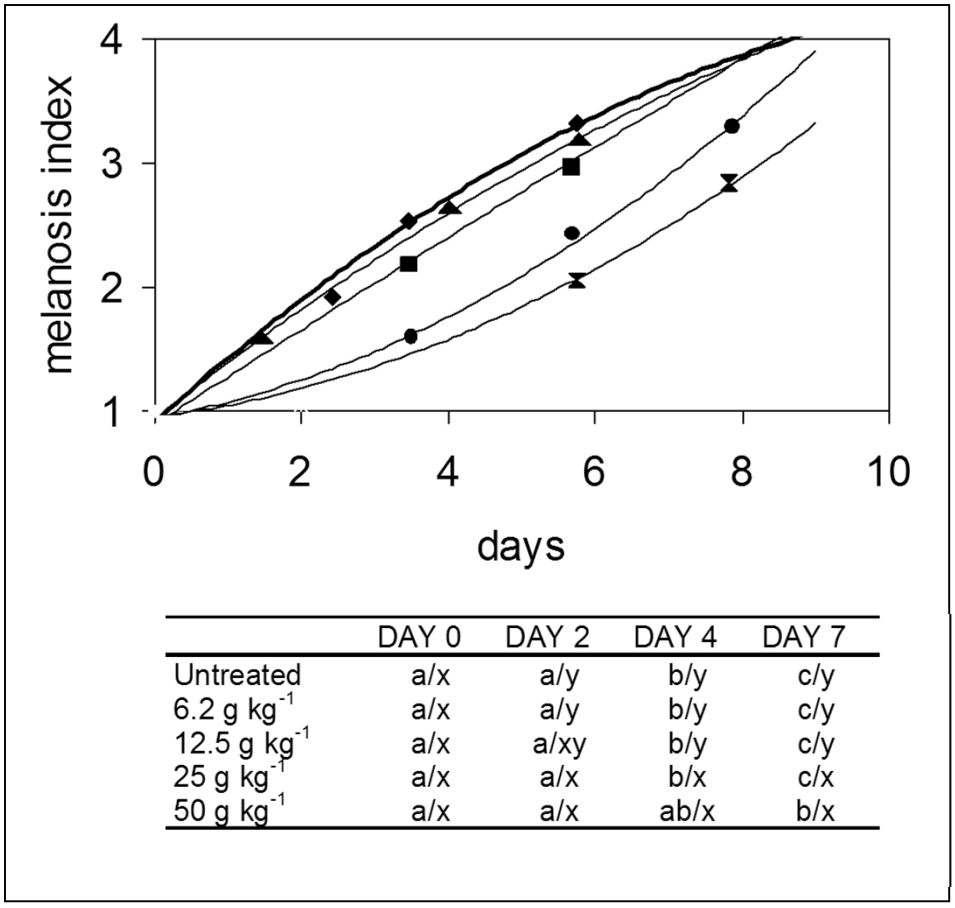
FIGURE 7: Residues of SO_2 present in the edible part of shrimps treated by dust with commercial products in single dose (s) (residues determined after 4 days of storage) or double dose (d) (residues determined after 4 and 7 days of storage).
CP1=Melacide; CP2=Freskor; CP3=Melaplus.
Error bars represent standard deviation.
Different letters (a, b, c...) indicate significant differences ($P \leq 0.05$).

FIGURE 8: Regression analysis between residues of SO2 in edible part of shrimp and concentration of sulphites in treatment solution (R^2=0.9293).



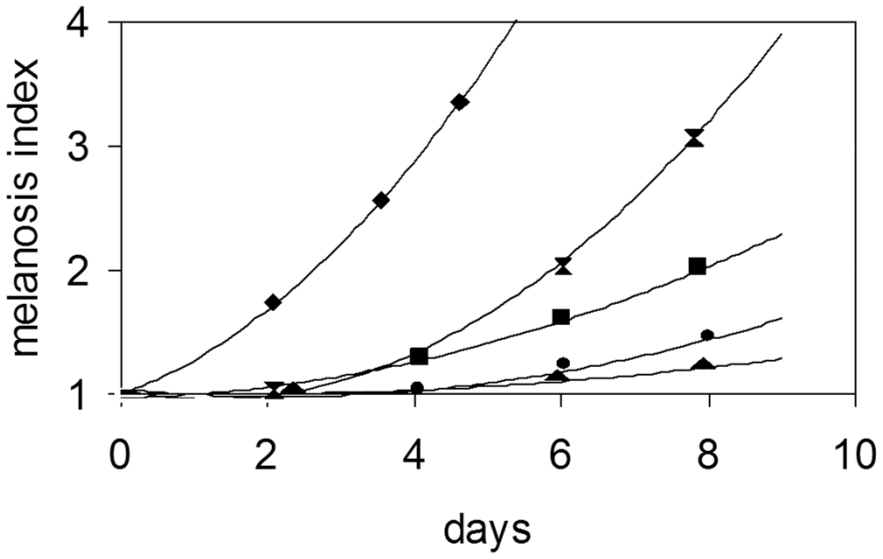
	DAY 0	DAY 2	DAY 4	DAY 7
Untreated	a/x	b/yz	b/x	c/x
1.6 g kg^{-1}/4h	a/x	b/z	b/x	c/x
3.1 g kg^{-1}/2h	a/x	a/xy	b/x	c/y
6.2 g kg^{-1}/0.5h	a/x	b/z	c/y	c/x
6.2 g kg^{-1}/2h	a/x	a/x	b/x	c/x
1.25 g kg^{-1}/0.5h	a/x	b/z	c/y	c/x
1.25 g kg^{-1}/2h	a/x	a/x	b/x	c/x

Figure 1



	DAY 0	DAY 2	DAY 4	DAY 7
Untreated	a/x	a/y	b/y	c/y
6.2 g kg^{-1}	a/x	a/y	b/y	c/y
12.5 g kg^{-1}	a/x	a/xy	b/y	c/y
25 g kg^{-1}	a/x	a/x	b/x	c/x
50 g kg^{-1}	a/x	a/x	ab/x	b/x

Figure 2



	DAY 0	DAY 2	DAY 4	DAY 7	DAY 9
Untreated	a/x	a/y	b/y	c/z	c/z
S	a/x	ab/x	b/x	c/y	d/z
S+A	a/x	a/x	a/x	a/w	a/w
S+Q	a/x	a/x	a/x	b/x	c/y
S+A+Q	a/x	a/x	a/x	a/w	b/x

Figure 3

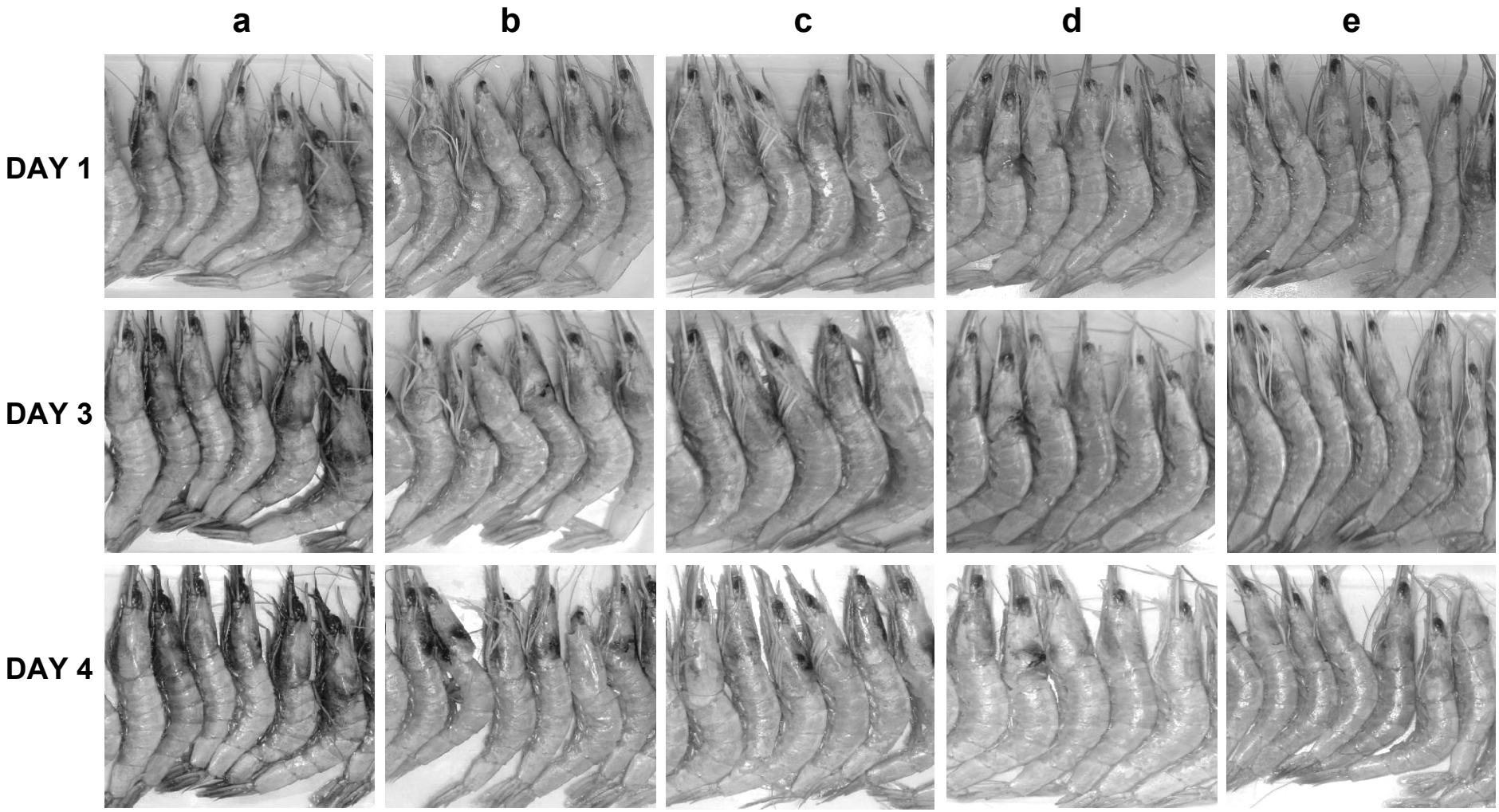
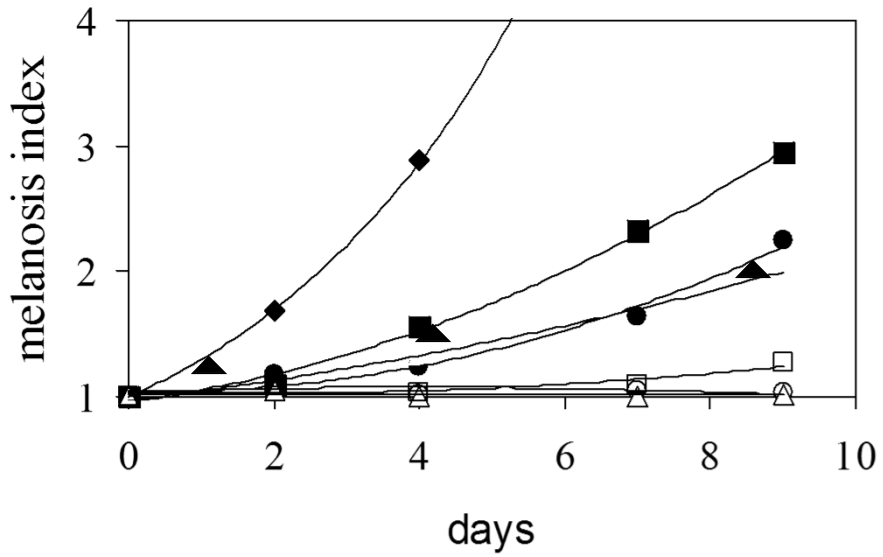


Figure 4



	DAY 0	DAY 2	DAY 4	DAY 7	DAY 9
Control	a/x	b/y	c/z		
CP1s	a/x	a/x	a/wx	b/y	c/z
CP2s	a/x	a/x	b/x	c/y	c/y
CP3s	a/x	a/x	b/y	c/z	d/w
CP1d		a/x	a/w	a/x	a/x
CP2d		a/x	a/w	a/x	a/x
CP3d		a/x	a/w	a/x	a/x

Figure 5

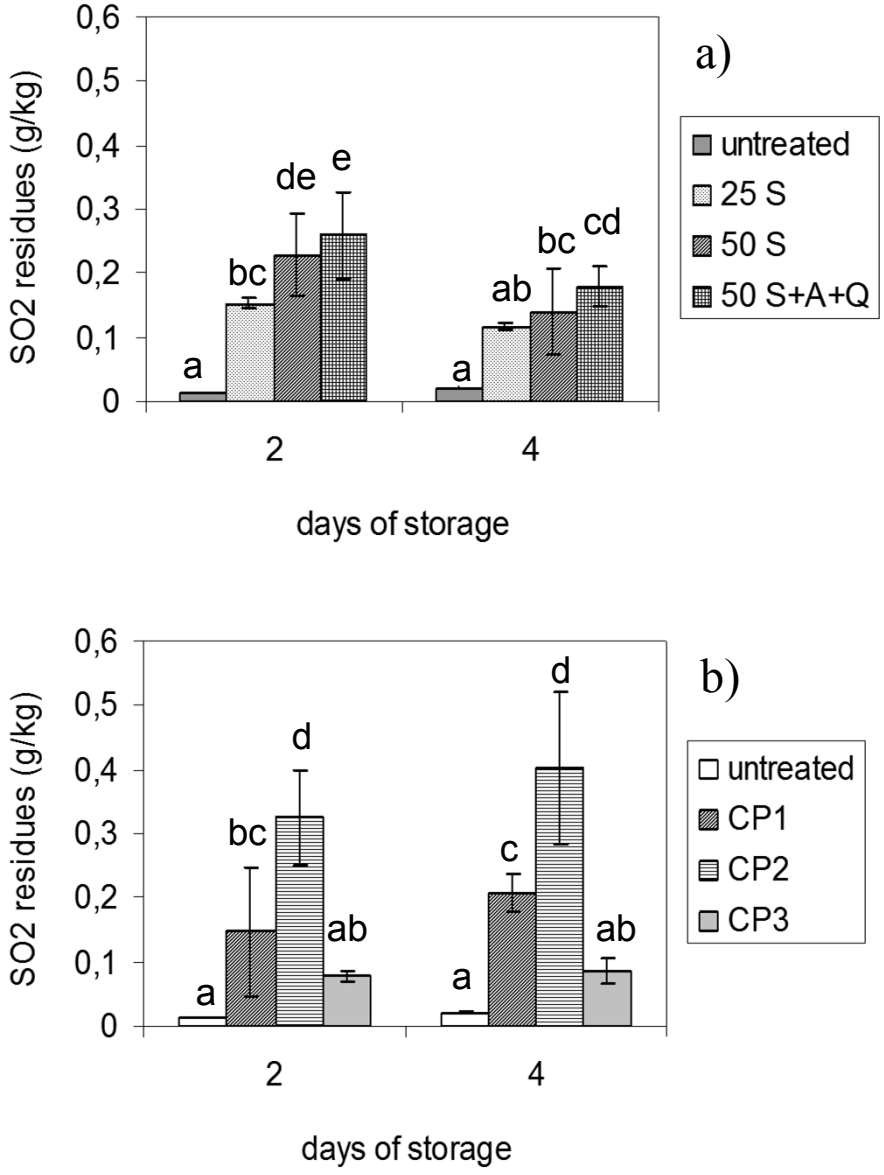

a)
SO2 residues (g/kg)
0,6
0,5
0,4
0,3
0,2
0,1
0
a
bc
de
e
a
ab
bc
cd
2
4
days of storage
untreated
25 S
50 S
50 S+A+Q
b)
SO2 residues (g/kg)
0,6
0,5
0,4
0,3
0,2
0,1
0
a
bc
d
ab
a
c
d
ab
2
4
days of storage
untreated
CP1
CP2
CP3

Figure 6

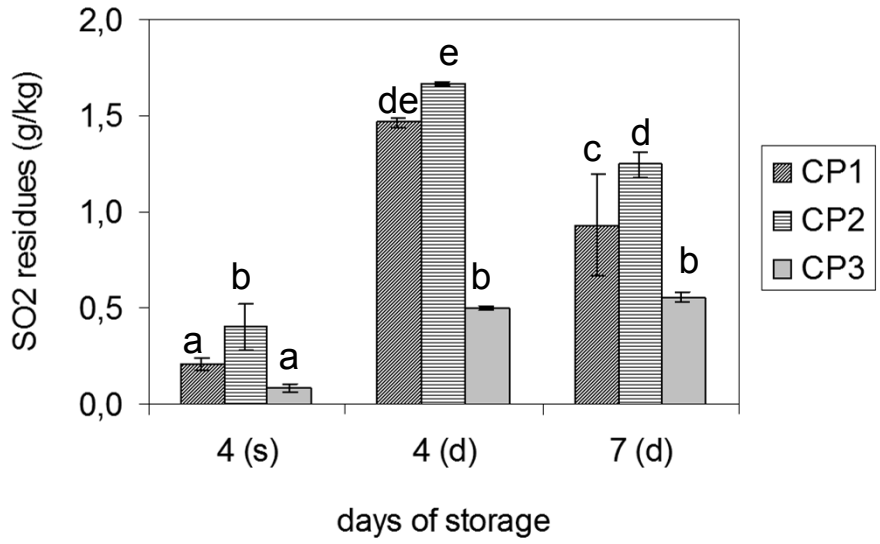

2,0
1,5
1,0
0,5
0,0
SO2 residues (g/kg)
a
b
a
de
e
b
c
d
b
4 (s)
4 (d)
7 (d)
days of storage
CP1
CP2
CP3

Figure 7

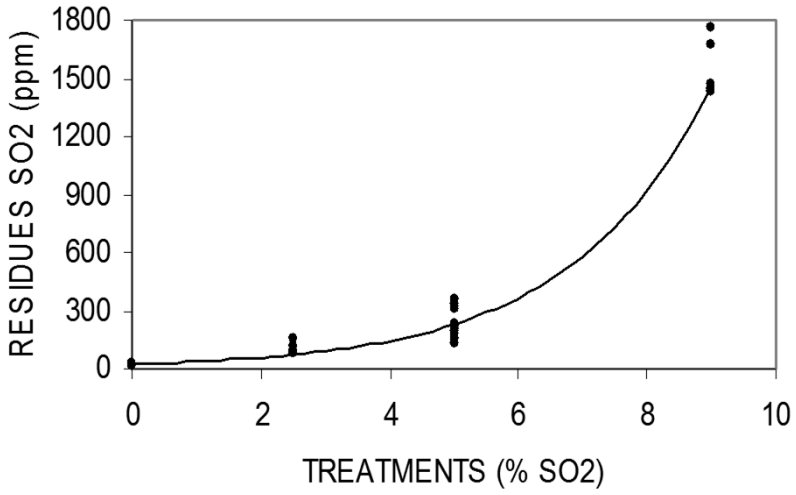

RESIDUES SO2 (ppm)
1800
1500
1200
900
600
300
0
0
2
4
6
8
10
TREATMENTS (% SO2)

Figure 8