Journal of the Science of Food and Agriculture *J Sci Food Agric* **85**:1143–1148 (2005)
DOI: 10.1002/jsfa.1990

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

M Carmen Gómez-Guillén,[1]* Óscar Martínez-Alvarez,[1] Argimiro Llamas[2] and Pilar Montero[1]

[1]*Instituto del Frío (CSIC), C/José Antonio Novais, No 10, 28040 Madrid, Spain*
[2]*Dpto de Ingeniería Química, Química Física y Química Orgánica, Facultad de Ciencias Experimentales, Campus de El Carmen, 21071 Huelva, Spain*

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

Keywords: melanosis; sulfites; 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 lower quality, and therefore a decrease in commercial value.[1] Blackspot or melanosis in crustaceans is a natural post-mortem phenomenon that involves the action of an enzymatic complex, polyphenoloxidase (PPO) which, in the presence of oxygen forms compounds which can polymerise into insoluble pigments.[2] Sulfite derivatives are widely used as antioxidants and/or preservatives (antimicrobial agents) in foods. The addition of sulfites, mainly metabisulfite, to avoid melanosis in raw prawns and shrimps has been a world-wide practice for many years.[3] Bisulfite appears to inhibit melanosis by two mechanisms: (1) reacting with intermediate quinones in the melanosis reaction, forming sulfoquinones, and (2) irreversibly reacting with PPO, causing complete inactivation.[4]

A common practice is to use commercial products containing another substance(s), in addition to sodium metabisulfite, which contribute to retard melanosis. Some of these are reducing agents, which act by causing 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 (ethylenediaminetretracetic acid) (EDTA). Nearly all the commercial antimelanotic products contain citric acid, ascorbic acid and/or EDTA.[5]

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

It is well known that sulfites produce certain adverse reactions in some groups of people, mainly asthmatics.[8,9] In clinical practice, metabisulfite is considered a precipitating cause of an asthmatic attack.[8] Because of this, the use of sulfites in food is limited. The regulatory authorities of many countries have indicated a maximum concentration of sulfites and derivatives in various foods. In Europe, the amount of sulfites in the edible part of fresh *Penaideae*

* Correspondence to: M Carmen Gómez-Guillén, Dpto de Ciencia y Tecnología de Carne y Pescado, Instituto del Frío (CSIC), C/José Antonio Nováis, 10, 28040 Madrid, Spain
E-mail: cgomez@if.csic.es
Contract/grant sponsor: Consejería de Agricultura y Pesca, Junta de Andalucía [Department of Agriculture and Fisheries of the Autonomous Government of Andalusia]; contract/grant number: CSIC-2000-664
(*Received 16 December 2003; revised version received 9 June 2004; accepted 28 June 2004*)
Published online 1 February 2005

crustacean family is restricted to 0.15–0.3 g SO_2 kg^{-1} according to the size of the crustacean.[10]

However, residual sulfite levels may depend not only on the size but also on harvest, treatment conditions, handling and processing of the products. In this sense, the information available on the level of residues generated as a consequence of different sulfite-based treatments is scarce for this and other species. There is one study where a number of commercial products containing sodium metabisulfite, applied with different times of immersion and concentrations, were analysed.[11]

The aim of the present work was to determine the effectiveness of different sulfite-based treatments, ie concentration of sulfites, method and time of application, synergy with other compounds, to avoid melanosis in fresh deepwater pink shrimp, and to determine the total content of sulfite 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 the spring. The temperature at the time of capture was around 20 °C. On board they were separated from the by-catch, washed with seawater and placed in perforated polystyrene boxes (approx 2 kg per box). All of these processes, before antimelanotic treatment (immersion or dust), took between 1 h and 1 h 45 min. For immersion treatments, sodium metabisulfite (Panreac Química, SA, Spain) at different concentrations ranging from 1.6 to 50 g kg^{-1} shrimps was used. Citric acid, ethylenediaminetetracetic acid (EDTA) and di-sodium dihydrogen pyrophosphate (PPi) were of reagent grade. The dip solutions were prepared with a seawater/shrimp ratio of 2/1 (w/w). The antimelanotic blend was dissolved in seawater and then the shrimps were introduced and covered with ice. At the end of 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 also performed on board, using three different commercial products (CP) which were spread in the form of dry powder on the surface of shrimp, followed by 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 SA (Spain), maximum content of SO_2 = 140 g kg^{-1}) added at a concentration of around 60 g kg^{-1} shrimps, which is the level normally used by the fishermen. Commercial product 2 (CP2) was Freskor (Hasenosa SA (Spain), maximum content of SO_2 = 600 g kg^{-1}), and commercial product 3 (CP3) was Melaplus (Turco SA (Spain), maximum content of SO_2 = 300 g kg^{-1}). CP2 and CP3 were added at a concentration of 40 g kg^{-1} shrimps, 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 2 °C. After 1 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 (ie double treatment).

Melanosis index

During storage, 14 shrimps per lot were evaluated every 2 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.[12]) The scale was: 1 = absent; 2 = very slight to moderate (up to 30% of shrimp surface affected in less than 50% of individuals); 3 = severe (30–70% of shrimp surface affected in less than 50% of individuals); 4 = extremely heavy (70–100% shrimp surface affected in most individuals). Results were average values of the scores delivered by the different assessors for a given lot during the storage period, considering the number of individual shrimps affected by each level of melanosis according to the 1 to 4 visual scale.

Sulfite determination

The amount of sulfites in shrimp was determined according to the Monier-Williams method.[13] Three muscle homogenates per lot, prepared from five to six shrimps per homogenate, were used for each determination.

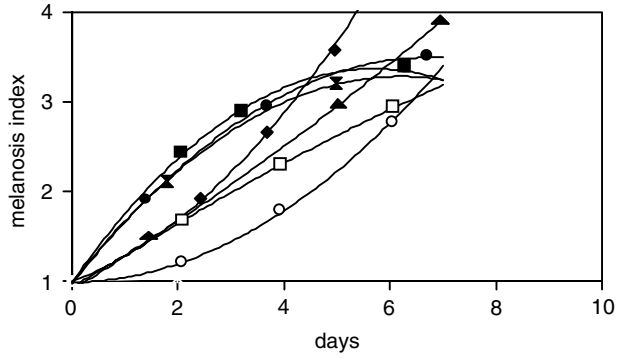
Statistical analysis

Regression analyses between average melanosis index values and days of storage, and between residual quantities of SO_2 and concentration in the treatment solution were performed. The significance of the differences between pair mean values was evaluated using one-way and two-way ANOVA. The 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, USA) was used for statistical processing.

RESULTS AND DISCUSSION

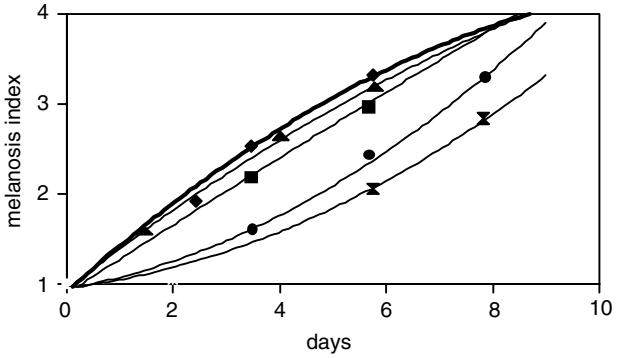
Immersion treatments

Melanosis development in shrimps treated with different concentrations of sodium metabisulfite for various immersion periods is shown in Fig 1. After 2 days of chilled storage only the shrimps that had been treated with 12.5 g kg^{-1} sulfite concentration for 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. However, treatments based on 0.5 h of 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



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

Figure 1. Melanosis index during storage in shrimps treated by immersion using different concentrations of sodium metabisulfite for several periods. (○) 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 of the table 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.



	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. Melanosis index during storage in shrimps treated by immersion using different concentrations of sulfites for 1 h. (⧗) 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 of the table 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.

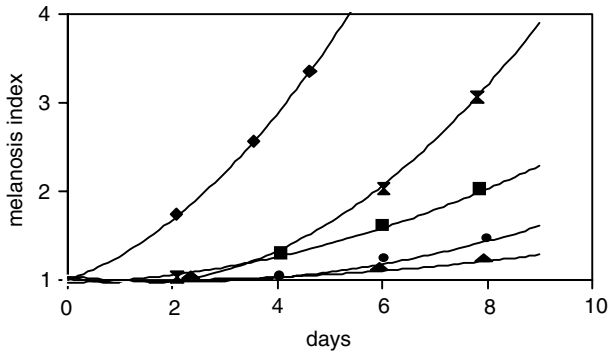
commercial products, which indicate 1 or 2 min of immersion treatment. Such indications were found completely ineffective to prevent melanosis for periods longer than 2 days of chilled storage (preliminary results not shown). Moreover, very short treatments are difficult to apply on board, given that usually the process is done largely by hand and is labour-intensive.

McEvily *et al*[2] found, for 1 min of dip treatment in a 12.5 g kg^{-1} sodium metabisulfite solution, an allowable residual sulfite level of 0.1 g kg^{-1}[14] on the shrimp. Treatments are not specified in the European Directive,[10] only the quantity of SO_2 remaining in the 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} sulfite (even after 2 h of immersion) was not effective for melanosis prevention beyond 2–3 days after capture. McEvily *et al*,[2] working with other species (*Penaeus aztecus* and *Penaeus duorarum*), found a dip of 1 min in 12.5 g kg^{-1} of sodium metabisulfite was effective; however after 7 days most of the shrimps showed noticeable melanosis.

However, perhaps 2 h of immersion is sometimes too much to be profitable and realistic on board; for this reason we propose the use of immersion for 1 h. As shown in Fig 2, higher concentrations of metabisulfite were tested in order to find out how much was effective, in terms of melanosis prevention, to increase shelf life to around 6–7 days. After 4 days of storage, shrimps treated with 6.3 or 12.5 g kg^{-1} sodium metabisulfite showed moderate melanosis, whereas those treated with 25 or 50 g kg^{-1} exhibited melanosis around 2 days later. These results indicated that concentrations of sulfite as high as 50 g kg^{-1} did not prevent melanosis for periods longer than 5–6 days.

Successful melanosis prevention has been reported in studies conducted with sulfite treatments applied on most occasions under controlled conditions. This was the case for McEvily *et al*[2] who dipped 1 lb (0.45 kg) of shrimp into a 12.5 g kg^{-1} sodium metabisulfite solution for 2 min, or Yu *et al*[14] who used 2 kg of shrimp dipped at 2 g kg^{-1} concentration of sodium bisulfite for 20 s. However, when the experiment is carried out on board under habitual conditions of weather, handling and processing, which was the case of the present work, there was a need for more additive and a longer time of application to inhibit the drastic increase in melanosis. In accordance with our findings, Arthur and Casedi[15] reported that immersion times from 1 to 15 min did not a prevent melanosis 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 (Henkel-Ecolab SA, Barcelona, Spain) (400 g kg^{-1} sodium metabisulfite 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 melanosis development during storage of shrimps treated with different combinations of 50 g kg^{-1} sodium metabisulfite with citric acid (20 g kg^{-1}) and/or chelants (0.45 g kg^{-1} EDTA + 30 g kg^{-1} PPi). Melanosis was efficiently retarded when, in addition to sulfites, chelating agents or citric acid were added, especially the latter. Thus, when



	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. Melanosis index during storage in shrimps treated by immersion (1 h) with 50 g kg^{-1} sulfites (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 of the table 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.

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 metabisulfite is shown in Fig 4. Citric acid alone did not inhibit the melanosis process. However, when added in combination with sodium metabisulfite, its action was favourable. This effect was more evident as the period of storage increased.

Dust treatments

A common practice is to add the antimelanotic product on board as a dust. This method normally involves 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 duration of application, since the dry powder is not removed. On many occasions throughout the distribution channel, when a single dose is not effective, another one is applied. This will increase the total content of sulfites in the edible part, frequently exceeding limits established by legislative authorities.

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

Residual sulfite levels

The total content of sulfites in the edible part (muscle) of shrimps treated by immersion (1 h) with metabisulfite (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 Fig 6a. In shrimps treated with 25 g kg^{-1}, the initial levels of sulfites 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

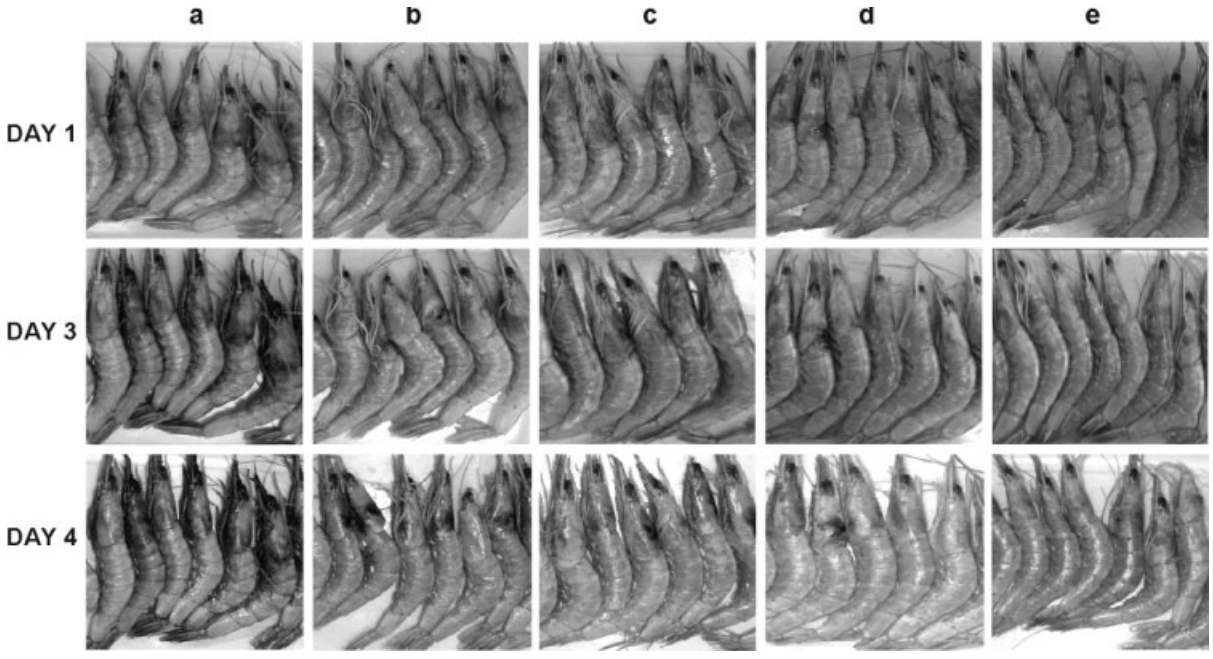
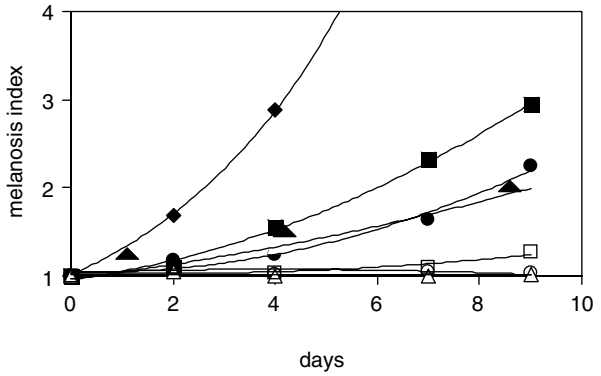


Figure 4. Photographs during storage of shrimps treated with (a) 20 g kg^{-1} citric acid alone, and 20 g kg^{-1} citric in combination with different concentrations of sulfites: (b) 12.5 g kg^{-1}, (c) 25 g kg^{-1}, (d) 37.5 g kg^{-1}, (e) 50 g kg^{-1}.



	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. Melanosis index during storage in shrimps 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 ($R^2 = 0.9963$); (○) 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 of the table 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.

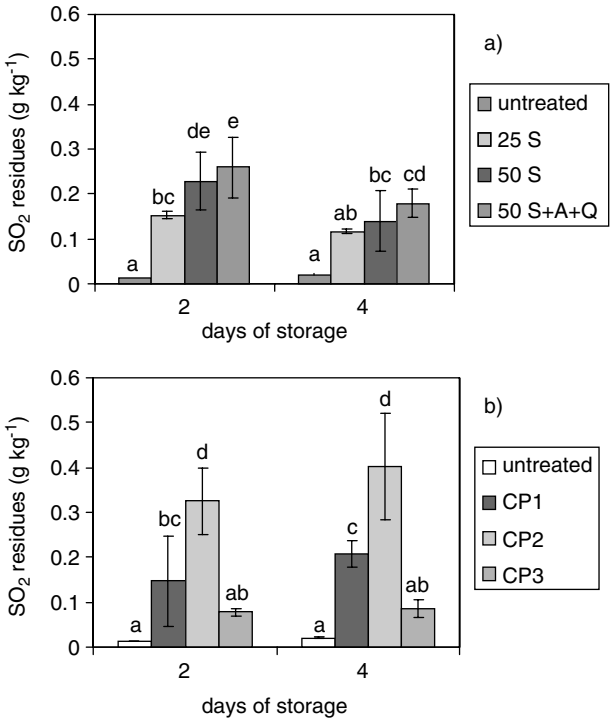


Figure 6. Residues of SO_2 present in the edible part of shrimps treated by (a) immersion for 1 h in a solution with sulfites at 25 g kg^{-1} and 50 g kg^{-1} concentration, and at 50 g kg^{-1} in combination with citric acid and chelants, and (b) dust with commercial products. A = citric acid; Q = chelants; CP1 = Melacide; CP2 = Freskor; CP3 = Melaplus. Error bars represent standard deviation. Different letters (a, b, c...) indicate significant differences ($p \leq 0.05$).

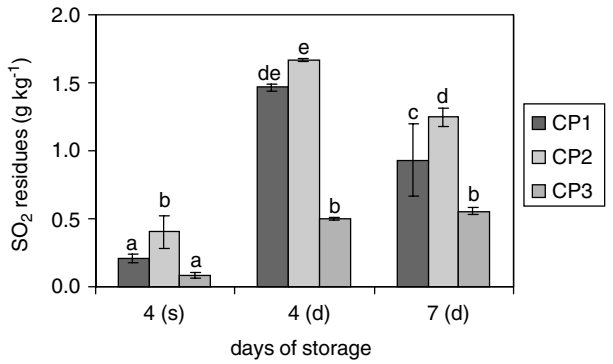


Figure 7. Residues of SO_2 present in the edible part of shrimps treated by dusting with commercial products in single dose (s) (residues determined after 4 days of storage) or double dose (d) (residues determined after 4 and 7 days of storage). CP1 = Melacide; CP2 = Freskor; CP3 = Melaplus. Error bars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

the residues, and probably induces softening of the carapace. However, differences were not significant ($p \leq 0.05$) in the results among analysed samples, given the wide dispersion of results. The wide dispersion causes some individuals to occasionally exceed the limit of 0.3 g kg^{-1} SO_2 in the edible part. After 4 days the general tendency is for the sulfite content in the edible part to decrease, probably because of drip with melting ice during storage.

In contrast, when the treatments were applied as dust (commercial products), the changes of residues with storage time were the opposite, ie they tended to increase (Fig 6b). In this case it is possible that the ice covering the shrimps, when melting, favoured penetration into the muscle of the sulfites present in the carapace. However, the dispersion of the results was again wide, as a consequence of the heterogeneity in the spread of dust. Thus, some shrimps could accumulate a large amount of additive, whereas others remained practically free. The amount of residual sulfite 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. In contrast, CP2, which inhibited blackspot formation more efficiently, produced a residual level exceeding the upper limit of 0.3 g kg^{-1} in the edible part (EU Directive[10]).

As expected, when a subsequent dose was applied the residual content of SO_2 increased drastically (Fig 7). The three commercial products, including CP3, exceeded the limits permitted in the EU Directive,[10] in some cases reaching values close to 1.6 g kg^{-1}, which represents more than five times the permitted 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 sulfite decreased in the case of CP1 and CP2; however residues remained still very high.

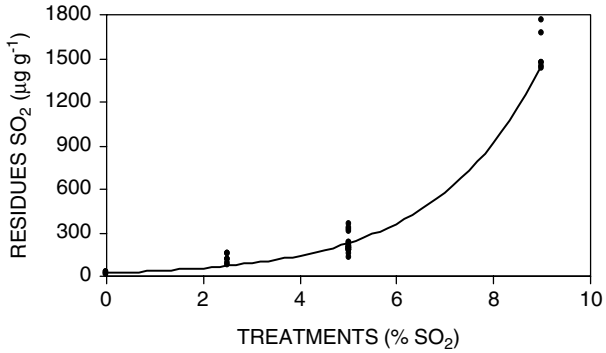


Figure 8. Regression analysis between residues of SO_2 in the edible part of shrimps and concentration of sulfites in the treatment solution ($R^2 = 0.9293$).

Regression analysis was performed between sulfite-based treatments having different concentrations of SO_2 with the corresponding residual contents in the muscle (Fig 8). It can be seen that the residual levels increased exponentially with the concentration of sulfites applied.

Hardisson *et al*[11] studied the sulfite content in edible and non-edible parts of frozen prawns and shrimps. They showed that sulfite concentration in the edible part of frozen crustaceans 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 reached $0.55\,g\,kg^{-1}$, which greatly exceeds the limit in the EU Directive,[10] indicating uncontrolled addition. We suggest also that exceptionally high residual levels may appear when the traditional method of dusting is applied, in which the additive is spread heterogeneously. In contrast, regarding immersion treatments, Rotllant *et al*[16] did not find differences in residual SO_2 levels in any part of the body among treatments as a function of immersion time. In this connection, Arthur and Casedi[15] observed in *P indicus* and *M monoceros* that, after 5 min of immersion, the residual SO_2 content in the muscle was about 90% of the value obtained after 15 min of immersion, indicating very little difference.

CONCLUSIONS

In deepwater pink shrimp (*Parapenaeus longirostris*), caught in the spring, metabisulfite concentrations as high as $50\,g\,kg^{-1}$, led to moderate melanosis after 5–6 days of storage. Citric acid and EDTA and PPi, especially the former, were shown largely to assist sulfites in melanosis prevention. Dip treatment of 1 h with $50\,g\,kg^{-1}$ sulfite together with citric acid and chelants was effective for melanosis prevention during one week. With this treatment, the limit of $0.3\,g\,kg^{-1}$ SO_2 in the edible part was not exceeded in the majority of analysed samples. In general, immersion treatments were more homogeneous and the shrimps tended to lose residual sulfites during storage. In contrast, dust treatment spread the additive more heterogeneously and favoured penetration into the muscle during the initial days of iced storage.

Further studies are needed to define the effectiveness of sulfite-based formulations and associated residual levels in different seasons of 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.

REFERENCES

1 Ramírez EC, Whitaker JR and Virador V, Polyphenol oxidase, in *Handbook of food microbiology*, ed by Whitaker JR, Voragen AGJ and Wong DWS. Marcel Dekker, New York, USA, Chapter 39, pp 509–523 (2003).
2 McEvily A, Radha I and Otwell S, Sulfite alternative prevents shrimps melanosis. *Food Technol* **9**:80–86 (1991).
3 Williams DJ, Slaterry SL and Notingham SM, A comparison of selected methods for determining sulfite in prawns. *J Food Prot* **53**:875–877 (1990).
4 Ferrer OB, Koburguer JA, Otwell WS, Gleeson RA, Simpson BK and Marshall M, Phenoloxidase from the cuticule of Florida spiny lobster (Panulirus argus): mode of activation and characterization. *J Food Sci* **54**:63–67 (1989).
5 Iyengar R and McEvily AJ, Anti-browning agent: alternative to the use of sulfites in foods. *Trends Food Sci Technol* **31**:60–64 (1992).
6 Ogawa M, Perdigão NB, Santiago ME and Kozima TT, On physiological aspects of blackspot appearance in shrimp. *Nippon Suisan Gakkaishi* **50**:1763–1769 (1984).
7 Cobb BF, Biochemistry and physiology of shrimp: effect on use as food, in *Proc 1st Annu trop and subtrop fish tech conf Americas*, pp 145–159 (1977).
8 Collins-Williams C, Intolerance to aditives. *Analysis of Allergy* **51**:315–316 (1983).
9 Gunnison A and Jacobsen D, Sulfite hypersensivity. A critical review. *Crit Rev Toxicol* **17**:185–214 (1987).
10 European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. *Official Journal* No L61, 18.3: 1–56 (1995).
11 Hardisson A, Rubio C, Frías I, Rodríguez I and Reguera JI, Content of sulphite in frozen prawns and shimp. *Food Control* **13**:275–279 (2002).
12 Otwell WS and Marshall MR, Report of the Florida Sea Grant. Screening alternatives to sulphiting agents to control shrimp melanosis (black spot). Florida Sea Grant Technical Paper No 46: pp 1–10 (1986).
13 AOAC, *Official methods of analysis*, 14th edn. Association of Offical Analytical Chemist. Washington, DC, (Sections 20.123–20.125) (1984).
14 Yu G, Wnag Y, Lui S and Tian X, Effects of low molecular weight chitosan (LCM-1) on shrimp preservation. *Chin J Oceanol Limnol* **14**:189–192 (1996).
15 Arthur L and Casedi E, Chemical treatment of prawns in Mozambique. *FAO Fisheries Reports* **400**:296–300 (1989).
16 Rotllant G, Arnau F, García JA, García N, Rodriguez M and Sardá F, Effect of metabisulphite treatments and freezing on melanosis inhibition in rose shrimp Aristeus antennatus (Risso, 1816). *Food Sci Tech Int* **8**:243–247 (2002).