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Spraying of 4-hexylresorcinol based formulations to prevent enzymatic browning in Norway lobsters (*Nephrops norvegicus*) during chilled storage

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Abstract

A comparison was made of the effects on melanosis development in Norway lobsters (*Nephrops norvegicus*) of treatment by dusting with a commercial sulphite-based product and of spraying with a formulation containing 4-hexylresorcinol (0.1% and 0.05%), in combination with organic acids and chelating agents. The following tests were performed during chilled storage: polyphenol oxidase (PPO) activity, melanosis score, colour parameters, tyrosine and tyramine content, as the main substrate of PPO. Differences among treatments were evaluated by means of statistical analyses (ANOVA, principal components and discriminant analyses). All formulations diminished PPO activity during storage successfully. The melanosis score was higher in sulphite-treated Norway lobsters, and a formulation with 0.05% 4-hexylresorcinol was enough to prevent the appearance of melanosis for 12 days. The tyrosine content decreased during storage, but the tyramine content was insignificant. Formulations with 4-hexylresorcinol improved the appearance of Norway lobsters, in comparison with the commercial sulphite-based product.

Keywords: Melanosis; Norway lobster; Polyphenoloxidase; Sulphites; 4-Hexylresorcinol; Spraying

1. Introduction

Norway lobster (*Nephrops norvegicus*) is one of the most economically important fishery resources, especially in the North-east Atlantic and Mediterranean areas (González-Gurriarán, Freire, Fariña, & Fernández, 1998). France, Britain, Denmark and Italy are the principal catchers. In the Spanish Mediterranean, an average of 20–30 mt of Norway lobsters is taken per port yearly (Maynou & Sardá, 2001). Norway lobster catches are not particularly high compared with those of other exploited demersal resources, such as fishes, but they account for around 15–20% of the total earnings of the local demersal fisheries (Aguzzi, Sardá, & Allué, 2004).

Norway lobsters rapidly develop black spots or melanosis during iced storage. Melanosis occurs in shellfish during storage as a result of the action of polyphenol oxidase (PPO) on tyrosine or its derivatives, such as tyramine, to form melanin (Rolle et al., 1991). Although the presence of black spots is not dangerous to human health, it reduces their marketability and makes necessary the use of antimelanotics. Sulphite-based formulations, mainly metabisulphite, are currently used to prevent or at least delay melanosis. However, the adverse reactions suffered especially by asthmatics (Collins-Williams, 1983; Gunnison & Jacobsen, 1987) caused by sulphites necessitates the use of alternative compounds to sulphite derivatives, and 4-hexylresorcinol appears to be a good alternative. The effectiveness of 4-hexylresorcinol as an antimelanotic has been demonstrated both in laboratory tests and on board (Guandalini, Ioppolo, Mantovani, Stacchini, & Giovannini, 1998; McEvily, Radha, & Otwell, 1991; Montero,

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Ávalos, & Pérez-Mateos, 2001; Montero, Martınez-Alvarez, & Gómez-Guillén, 2004; Otwell, Iyengar, & Mc Evily, 1992). Its use is permitted in the United States, Canada, Australia, and some Latin American countries, but not authorised in Europe. Presumably, its use will be approved as a preservative in the future (European Commission, 2003). Several studies on the effect of 4-hexylresorcinol based formulations on shrimp species exist, mainly by immersion (Guandalini et al., 1998; McEvily et al., 1991; Montero et al., 2001, 2004; Montero, Gómez-Guillén, Zamorano, & Martınez-Álvarez, 2003; Otwell et al., 1992). However, there is no information about the effect of this compound on Norway lobster when applied by spraying. Spraying could be the most useful application method, because dipping can cause mechanical damage in crustaceans (separation of the heads), and dusting presents a danger to fishermen (Montero et al., 2004). This method is very easy to apply by fishermen and was used by Montero et al. (2004) on shrimp, with good results regarding appearance. 4-Hexylresorcinol has also been found as very stable in sea water (Otwell et al., 1992). The presence of organic acids and chelating agents in 4-hexylresorcinol based formulations may improve the appearance of crustaceans, according to the studies of Montero et al. (2001, 2004) in prawn and shrimp.

The aim of this work was to determine the effected of 4-hexylresorcinol based formulations on melanosis of Norway lobsters (*Nephrops norvegicus*) during chilled storage, using spraying as the application method and to compare it with the traditional dusting sulphite-based treatment, in order to seek the best formulation to maintain a good appearance for as long a period of time as possible.

2. Materials and methods

2.1. General

The experiments were performed on Norway lobster (*Nephrops norvegicus*) caught by trawl off the south coast of Spain (Ayamonte, Huelva) in November 2004. Average and standard deviation sizes and weights were approximately 19.6 ± 1.7 cm (including the tail, carapace and clawed legs) and 46 ± 6 g respectively. Live crustaceans were placed on board in polystyrene boxes and covered with ice. The boxes were taken by refrigerated truck to the Instituto del Frío (Madrid), where most Norway lobsters arrived still living. Those dying Norway lobsters were separated into lots and treated with antimelanotics. Based on previous studies, two formulations were selected with 0.1% or 0.05% 4-hexylresocinol (H6250, Sigma Chemical Co., St Louis, Mo, USA), combined with citric acid (0.5%), ascorbic acid (0.5%), acetic acid (0.3%), EDTA (500 mg/kg) and disodium dihydrogen pyrophosphate (1.5%) (Montero et al., 2004). For simplicity, these formulations were designated as R-0.1 and R-0.05. The additives were dissolved in salted water (3.5%) and sprayed on the surface of crustaceans. A third lot was treated by dusting with a commercial sulphite-based product with sulphites (approximately 13%) and acids (citric and ascorbic) (Lot CS), at the concentration normally used by fishermen (around 4%). The treated Norway lobsters were placed in perforated polystyrene boxes, covered with ice and stored at 2 °C. A first control at day 0 of chilled storage was performed on Norway lobsters with no additives. Further analyses on the crustaceans with additives were carried out during chilled storage.

2.2. Preparation of crude enzyme

The crude enzyme was obtained from the cephalothorax. Each was separated from the abdomen on different days during chilled storage, and then frozen and stored at −80 °C until analysis. Crude extracts were obtained according to Wang, Taylor, and Yan (1992). Approx 30–40 g of cephalotorax were added to 1.5 parts of 0.1 M sodium phosphate buffer pH 6.4 and homogenized in an Omnimixer-Homogenizer (model 17106, OMNI International, Waterbury, USA) for 2 min. The homogenate was centrifuged at 50,000*g*, 30 min, 4 °C (Sorvall Combiplus, Dupont, Wilmington, DE, USA). The supernatant was used as the crude polyphenol-oxidase (PPO) preparation, and immediately frozen to −80 °C in order to prevent alterations prior to determination of enzymic activity.

2.3. Measurement of PPO activity

The enzyme activity was measured using the proline-catechol spectrophotometric assay (Rzepecki & Waite, 1989) under saturation conditions (calculated K_m and V_{max} were 3.3 mM and 2.33 Units/ml crude extract, respectively), according to Wang et al. (1992). The absorbance at 530 nm was monitored at 24 °C for 5 min in a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan) with a CPS-240 thermostatic controller. The results were expressed as Units/ml of crude enzyme, considering the unity as an increment of 0.01 absorbance/min.

2.4. Tyrosine and tyramine determination

The tyrosine content in the cephalotorax during chilled storage was determined by the method of multivaried calibration. Another aromatic aminoacid, tryptophan, was also determined in order to calculate the tyrosine content, because both aminoacids present similar absorbance spectra. The molar extinction coefficient for diluted tyrosine and tryptophan in a solution of 50% (w/v) trichloroacetic acid (TCA) was determined between 280 and 290 nm (ε^{Tyr} ($mM^{-1}\,cm^{-1}$): $\varepsilon_{280} = 1.4781$; $\varepsilon_{285} = 0.9785$; $\varepsilon_{288} = 0.3827$; $\varepsilon_{290} = 0.1601$; ε^{Trp} ($mM^{-1}\,cm^{-1}$): $\varepsilon_{280} = 7.7423$; $\varepsilon_{285} = 6.4302$; $\varepsilon_{288} = 6.2329$; $\varepsilon_{290} = 5.7361$). Approx 30–40 g of cephalotorax was added to 1.5 parts of 0.1 M sodium phosphate buffer pH 6.4 and homogenized in an Omnimixer-Homogenizer (model 17106, OMNI International, Waterbury, USA) for 2 min. The homogenate was centri-

fuged at 50,000*g*, 30 min, 4 °C (Sorvall Combiplus, Dupont, Wilmington, DE, USA). A volume of 450 μl of supernatant was mixed with 50 μl of cold 50% (w/v) trichloroacetic acid (TCA). The mixture was kept at 4 °C for 15 min to allow unhydrolyzed proteins to precipitate, followed by centrifugation at 5700*g* at 4 °C for 10 min (MicroSpin 24S, Sorvall Instruments, DuPont, Wilmington, Del., USA). The content of TCA-soluble tyrosine and tryptophan in the supernatant was determined by measuring the absorbance between 280 and 290 nm, according to Stoscheck (1990). The tyrosine content was expressed as mg of tyrosine per gram (mg/g) of wet sample.

Tyramine was determined in 10% aqueous trichloroacetic acid extract (relation acid:sample 2:1, v/w) by the post-column method described by Ritchie (1991). Detection was carried out by fluorescence measurement. Results of three replicates were expressed as mg of tyramine per kg (mg/kg) of wet sample.

2.5. Sensorial analyses

Throughout a period of 12 days after capture, the appearance of Norway lobster was evaluated by a group of trained panellists every two days (10 crustaceans per treatment per evaluation). Melanosis was scored separately in the cephalotorax, clawed legs, parapods, abdomen, telson, and pleopods, using a numeric scale from 1 to 4, where 1 = complete absence of black spots; 2 = a few small spots; 3 = considerable spotting; 4 = substantial spotting (Montero et al., 2004). The presence of a yellow-green colouration on the cephalotorax and marketability were also evaluated during storage.

2.6. Colour measurements

Lightness (L^*), yellowness (b^*), and redness (a^*) of well-pulverized cephalotorax carapace were measured with a Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia, USA), using a CIELab scale (Young & Whittle, 1985). With L^*, a^* and b^* values, whiteness (W) was calculated, according to Park (1994). Results are expressed as the mean of six measurements.

2.7. Statistical analyses

The significance of differences between mean value pairs of tyrosine content was evaluated using two-way ANOVA. The level of significance setting was $P \leqslant 0.05$. Furthermore, multivariate analysis was performed on all data values obtained from the different antimelanotic treatments for each control day, with the properties determined by the panellists together with colour measurements treated as a whole set. Correlation among properties was also calculated. Factorial analysis by principal components analysis (PCA) was done, and the results were subjected to stepwise discriminant analysis. All descriptive statistic and statistical tests were performed using the SPSS 12.0 computer program (Chicago, IL, USA).

3. Results and discussion

3.1. Determination of PPO activity

The activity of Norway lobster polyphenol-oxidase, extracted periodically from the cephalotorax carapace during chilled storage, is represented in Fig. 1. PPO activity measured at day 0 in the additive-free sample was higher than in the treated samples at any stage of storage. The presence of an active PPO in live lobsters has been attributed to rough handling on board (Ogawa, 1987) and also to an activation process of pro-PPO (Ali, Marshall, Wei, & Gleeson, 1994). Wang et al. (1992, 1994) and Zotos and Taylor (1996) reported the endogenous activation of PPO in crude extracts of Norway lobsters by the action of two thiol proteases and one alkaline zinc-serine protease, involving also one or more unidentified factors. Active PPO has also been found in extracts of late premoult cuticles from lobster and Norway lobster (Ali et al., 1994; Bartolo & Birk, 1998; Ferrer, Koburger, Simpson, Gleeson, & Marshall, 1989). Yan, Taylor, and Hanson (1990) reported a low activity latent form of PPO in Norway lobster, which can increase its affinity to substrate during the premoulting stage. In accordance with these authors, it is possible that Norway lobsters could have been ready to moult when they were caught (November). González-Gurriarán et al. (1998) also reported that moulting frequency increases in late autumn off the Atlantic coast of Spain and Portugal. Actually, in Norway lobster moulting occurs throughout the year, with peak periods in late autumn and early spring. However, the prevalence of these peaks depends on various physiological and environmental factors, such as temperature (Bartolo & Birk, 1998). According to these authors, the warm temperature of the south coast of Spain could even increase moulting.

Regarding treated samples, PPO had minor activity. Obviously, this is attributed to the effect of the chemicals added. However, a different trend was observed between the mode of action of the 4-hexylresorcinol and sulphite-based formulations. Sulphites efficiently inhibited PPO activity at the onset of storage; nevertheless, the recorded activity increased considerably with storage time. At the beginning of storage, sulphites were able irreversibly to inhibit PPO (Ferrer et al., 1989; Lambrecht, 1995; Ricquebourg, Robert-Da Silva, Rouch, & Cadet, 1996); they could interact with the formed quinones and reduce the coloured orthoquinones back to the colourless and less reactive diphenols (Lambrecht, 1995). During the rest of the storage, sulphites were gradually consumed and the quinones accumulated, forming melanin polymers (Lambrecht, 1995; Ricquebourg et al., 1996), implying that all PPO had not been completely inhibited by sulphites. On the contrary, formulas with 4-hexylresorcinol were less effective in the first days of storage, but produced a tendency to

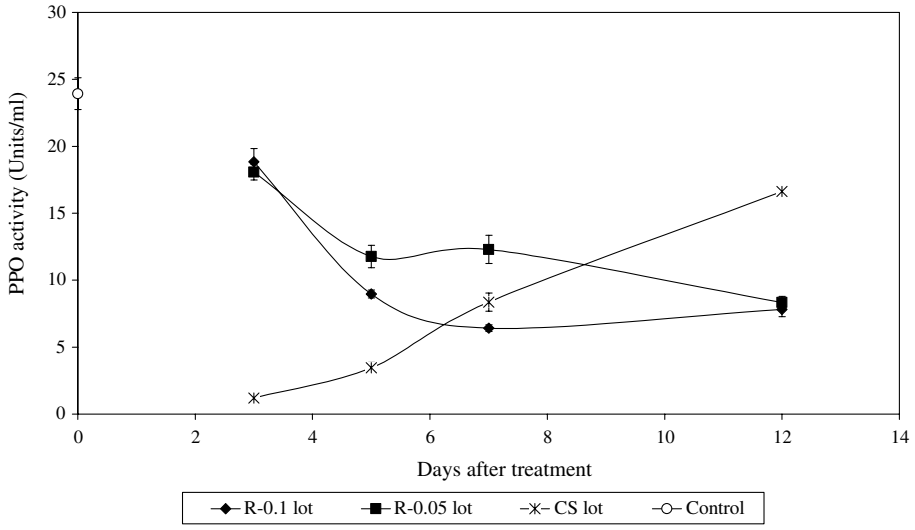

Fig. 1. PPO activity (Units/ml extract) in treated samples during chilled storage. Control: samples without inhibitors. R-0.1 lot: samples treated with 0.1% 4-hexylresorcinol-based formulation. R-0.05 lot: samples treated with 0.05% 4-hexylresorcinol-based formulation. CS lot: samples treated with commercial sulphite-based formulation.

decrease PPO activity during conservation. Nevertheless, the slight decline could be produced by a natural PPO inactivation process during the oxidation of substrate to product (Ramírez, Whitaker, & Virador, 2003, chap. 39). Differences in 4-hexylresorcinol concentration led to significant differences in PPO activity only at intermediate stages of storage, the R-0.1 formula showing slightly lower values than with the R-0.05, as could be expected. However, no differences were found at the end of storage. 4-Hexylresorcinol is a competitive inhibitor (Jiménez & García-Carmona, 1997), and it has been widely described as a good alternative to the use of sulphites by dipping (McEvily et al., 1991; Frankos et al., 1991; Iyengar & McEvily, 1992; Otwell et al., 1992; Guandalini et al., 1998). There are few studies of crustaceans treated by spraying; however, Montero et al. (2004) described comparable effectiveness of similar formulations with 4-hexylresorcinol on melanosis in pink shrimp (*Parapenaeus longirostris*).

3.2. Tyrosine and tyramine content in cephalotorax

Black spot development is the result of controlled oxidation of substrates, mainly tyrosine and tyramine, by PPO during storage. PPO activity on L-tyrosine has been detected in the hemolymph and gill of crayfish, in the hemolymph of tiger shrimp, and in fiddler crab blood (Rolle et al., 1991). In Norway lobster, an enzymatic controlled oxidation of tyrosine or its derivatives has also been reported by Yan, Taylor, and Hanson (1989). Free tyrosine, produced by the action of proteases, chiefly trypsin, chymotrypsin and carboxypeptidases (Celis-Guerrero, García-Carreño, & Navarrete del Toro, 2004), was present in all samples from the first day of storage (Table 1). After three days of chilled storage, the tyrosine content decreased in samples treated with 4-hexylresorcinol, unlike sulphite-treated samples, with significantly higher content of tyrosine. The existence of chelating agents in these formulations may reduce proteolytic activity, and consequently decrease the amount of free tyrosine. The tyrosine content declined during chilled storage in all lots, but especially in the CS lot after seven days of chilled storage, perhaps because of the use of tyrosine by PPO to form melanins after antimelanotics were used up, or even due to different reactions to form derivatives.

Tyramine is generated by decarboxylation of free L-tyrosine by both endogenous and microbial enzymes (López-Caballero, Álvarez, Sánchez-Fernández, & Moral, 2002). Therefore, the presence of microorganisms able to decarboxylate aminoacids, as well as favourable conditions for the growth of these microorganisms, may be important factors to generate PPO substrates during storage. However, the tyramine content was under the thresh-

Table 1
Tyrosine content in mg/g of wet sample (means ± standard deviations) during chilled storage in Norway lobsters treated with different formulations

Tyrosine content	Day 0	Day 3	Day 5	Day 7	Day 12
R-0.1 lot	$2.43 \pm 0.08^{ab/x}$	$2.18 \pm 0.11^{b/x}$	$1.73 \pm 0.14^{bc/xy}$	$1.47 \pm 0.06^{c/xy}$	$1.65 \pm 0.13^{c/x}$
R-0.05 lot	$2.43 \pm 0.08^{ab/x}$	$2.03 \pm 0.09^{bc/x}$	$1.25 \pm 0.10^{de/x}$	$1.71 \pm 0.07^{cd/x}$	$0.81 \pm 0.17^{e/y}$
CS lot	$2.43 \pm 0.08^{b/x}$	$3.13 \pm 0.30^{a/y}$	$2.07 \pm 0.02^{b/y}$	$1.17 \pm 0.15^{c/y}$	$0.72 \pm 0.03^{c/y}$

R-0.1 lot: samples treated with R-0.1 formulation. R-0.05 lot: samples treated with R-0.05 formulation. CS lot: samples treated previously with commercial sulphite formulation. Different letters (a, b, c, ...) in the same row indicate significant differences ($P \leqslant 0.05$) as a function of storage time; different letters (x, y, z, ...) in the same column indicate significant differences ($P \leqslant 0.05$) as a function of treatment.

old limit (<11.41 mg/kg) in all cases throughout the entire storage period. A possible explanation could be that the chemical compounds used to stop melanosis helped to hinder tyramine formation. However, it is also possible that the tyramine content was used by PPO during storage. Concerning this possibility, Rolle et al. (1991) reported that tyramine may become the primary substrate post-mortem for PPO-catalyzed melanosis, with a rate of oxidation 11-fold greater than tyrosine. Similar low tyramine levels were also detected by López-Caballero et al. (2002) in pink shrimp.

3.3. Sensorial analyses

The melanosis index was determined during chilled storage in various parts of the anatomy of Norway lobster: dorsal part of cephalotorax, ventral part of cephalotorax, clawed legs, cuticle of abdomen, telson and pleopods (Fig. 2).

It should be noted that melanosis was absent in any part of Norway lobster during the first seven days of storage, irrespective of treatment. The highest score was observed on the sides of cephalotorax after 12 days of chilled

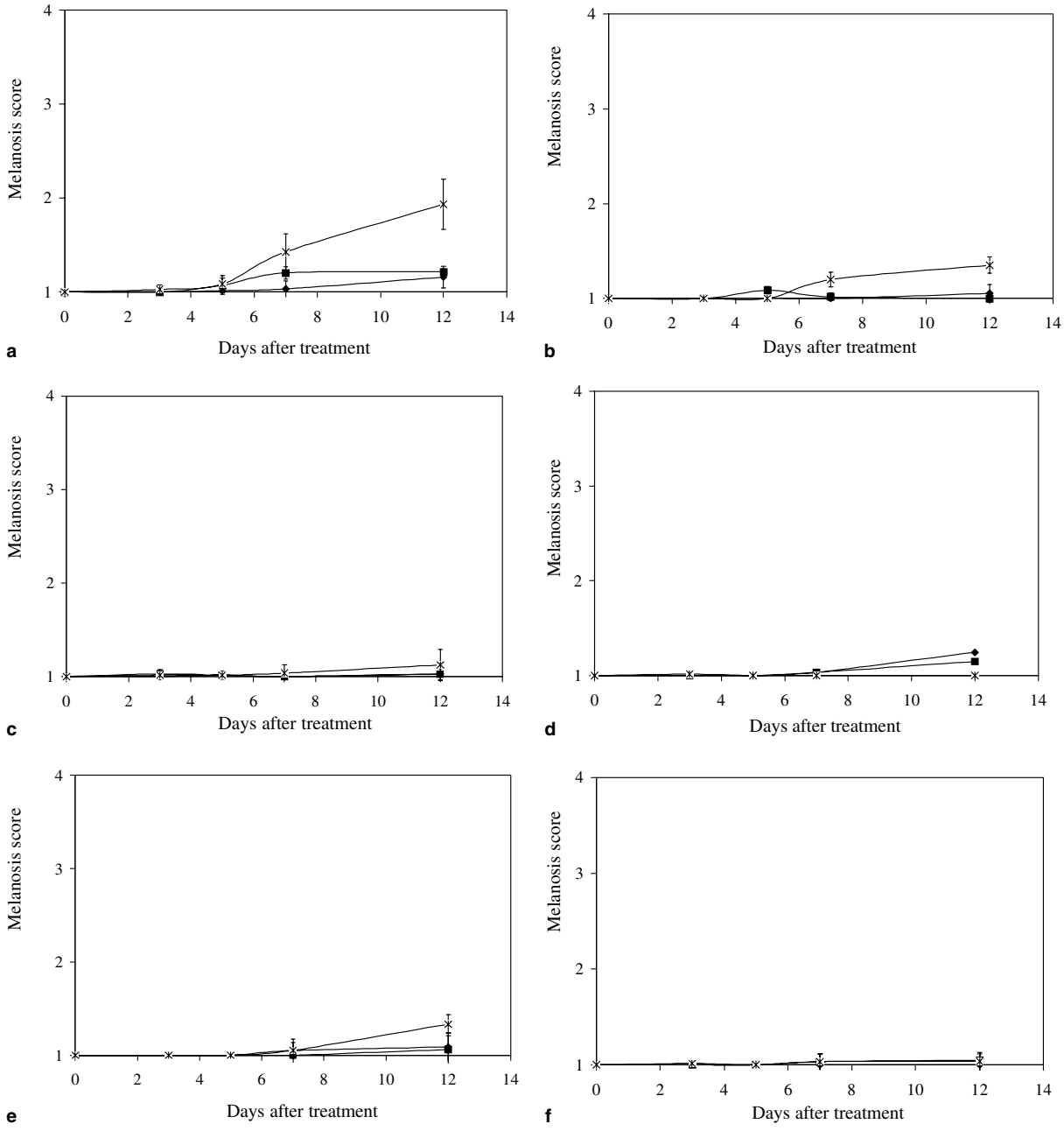

Fig. 2. Melanosis score of different parts of treated Norway lobsters during chilled storage: (a) dorsal part of cephalotorax; (b) ventral part of cephalotorax; (c) clawed legs; (d) tail carapace; (e) pleopods; (f) telson. R-0.1 lot (♦): samples treated with 0.1% 4-hexylresorcinol-based formulation. R-0.05 ■: samples treated with 0.05% 4-hexylresorcinol-based formulation. CS lot (*): samples treated with commercial sulphite-based formulation.

storage, especially in CS lot (Fig. 2(a)). A slight melanosis had also appeared previously on ventral part of cephalothorax of these samples. A small presence of black spot was also detected on pleopods (Fig. 2(e)), but not on clawed legs, telson, and tail carapace. R-0.1 and R-0.05 lots scarcely presented darkening during storage. Only a slight presence of melanosis was observed on the sides of cephalothorax and on tail carapace at the end of storage. Montero et al. (2004) obtained similar results with R-0.1-formulation applied to pink shrimp. Regarding the quantity of 4-hexylresorcinol presented in the formulation-treated samples, there were no appreciable differences involved in melanosis development.

Table 2
Greenish colouration and marketability (%) of Norway lobsters treated with different formulations during chilled storage

	Day 0	Day 3	Day 5	Day 7	Day 12
Greenish colouration					
R-0.1 lot	0	0	0	1	33
R-0.05 lot	0	0	0	33	34
CS lot	0	0	5	33	67
Marketability					
R-0.1 lot	100	100	100	100	25
R-0.05 lot	100	100	100	100	58
CS lot	100	100	100	72	8

R-0.1 lot: samples treated with R-0.1 formulation. R-0.05 lot: samples treated with R-0.05 formulation. CS lot: samples treated with commercial sulphite based formulation.

Besides melanosis, the colour of viscera is also a decisive factor by which to evaluate sensorial quality of crustaceans. In all lots the appearance of yellow-green colouration during chilled storage was observed, and it had influence on the final acceptability, in spite of the slight intensity of this colouration (Table 2). Yellow-green coloration appeared after one week of chilled storage in R-0.05 and CS-lot, unlike R-0.1 lot, where discolouration was detected later. The development of yellow to greenish colourations could be considered as an early stage of melanosis. It is probably related to some extent to the degree of endogenous proteolysis, and could be highly influenced also by the type of antimelanotic used. In deepwater pink shrimp (*Parapenaeus longirostris*) treated with formulations including 4-hexylresorcinol, the yellow-green colouration is the main factor used to reject the product, in spite of the absence of incidence of melanosis (Montero et al., 2004).

The panellists determined that Norway lobsters treated with a sulphite based commercial product were 100% marketable for five days of chilled storage, whereas the use of

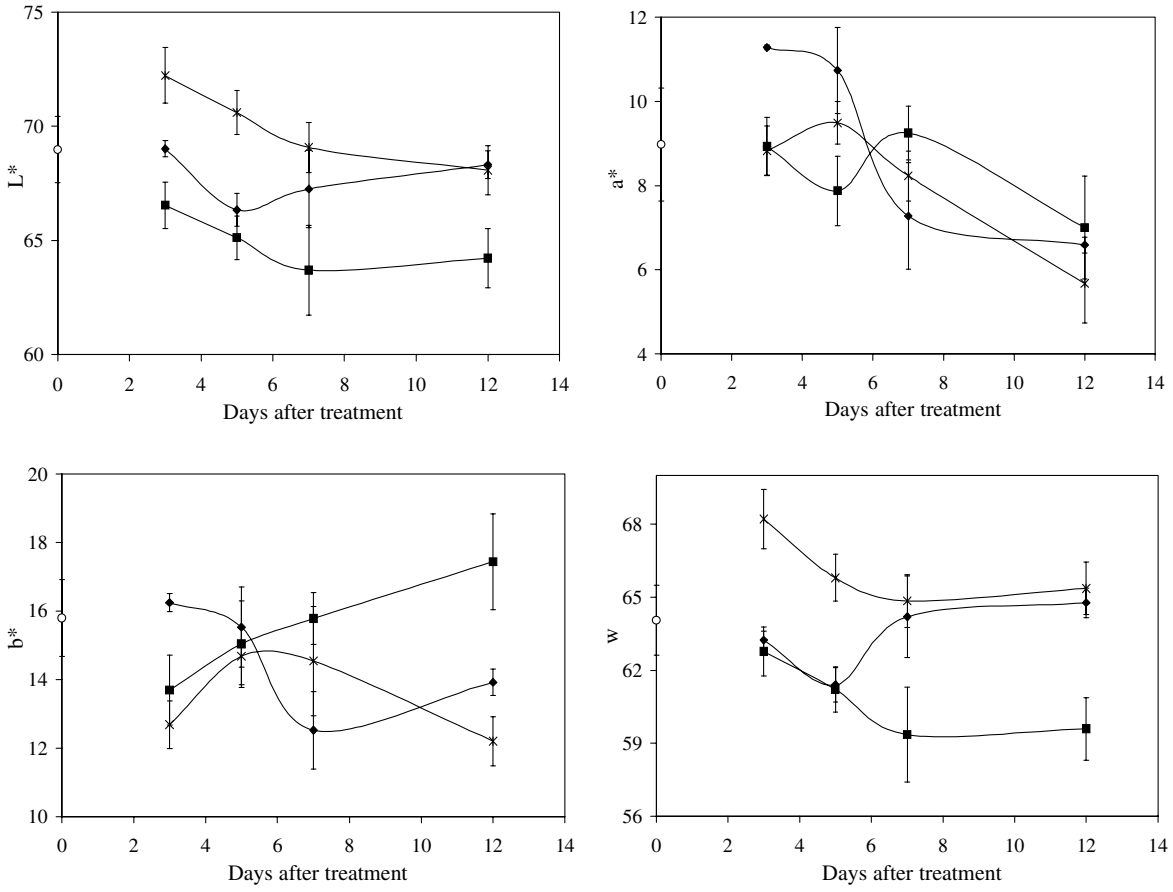

Fig. 3. Colour measurement during chilled storage: L^*, lightness; a^*, redness; b^*, yellowness; W, whiteness. Control lot (○): samples without inhibitors. R-0.1 lot (♦): samples treated with 0.1% 4-hexylresorcinol-based formulation. R-0.05 lot ■: samples treated with 0.05% 4-hexylresorcinol-based formulation. CS lot (*): samples treated with commercial sulphite-based formulation.

4-hexylresorcinol-based formulations allowed two more days of marketability.

3.4. Colour changes (CIE L*a*b*)

Changes in colour parameters L^* (lightness), a^* (redness), b^* (*yellowness*) and W (Whiteness) of head carapace of Norway lobsters during chilled storage are indicated in Fig. 3. A decrease in L^* value may be considered as indicative of browning.

Norway lobsters treated conventionally with a commercial sulphite-based product presented significantly higher lightness than control samples after treatment. This is due to the fact that sulphites are potent reducing agents and are able to bleach the samples (McEvily et al., 1991; Rotllant et al., 2002). Their lightness was also the highest during the first 7 days of chilled storage, despite the tendency to decrease, in contrast to the R-0.1 lot, where lightness was slightly affected, with values very close to those of the fresh untreated Norway lobster at day 0 of storage (Fig. 3). Thus, the presence of reducing agents, such as ascorbic acid, together with 4-hexylresorcinol was not enough to bleach the samples. The absence of bleaching capacity of 4-hexylresorcinol has also been reported by Iyengar and McEvily (1992).

In all cases redness (a^*) tended to decrease during storage, meaning that samples changed colour to greenish. It is important to emphasise that R-0.1 lot showed significantly the highest values after 3 days of storage. Yellowness (b^*) did not show a definite tendency during storage in any case. It was slightly higher at day 3 of storage when formulation with 0.1% 4-hexylresorcinol was used. With lower concentration of 4-hexylresorcinol yellowness increased at the end of storage.

A discriminant analysis was performed to determine differences among the treatments. In the graphic representation of discrimination analysis (Fig. 4) the canonical variables are a linear combination of the variables that best define the characteristic of the groups formed at all points on the X and Y axes. Three groups of treatments were differentiated with homogeneous distribution of elements. Group 1 contained all cases from treatment with 0.1% 4-hexylresorcinol and 25% of cases with 0.05% of 4-hexylresorcinol. Group 2 contained 75% of cases from treatment with 0.05% of 4-hexylresorcinol. Group 3 contained all cases from commercial sulphites treatment. The first canonical discriminant function (94.3% explained variance) showed differences among groups depending on the antimelanotic applied (4-hexylresorcinol or sulphites), while the second function (5.7% explained variance) showed differences depending on the concentration of 4-hexylresorcinol. Taken together, this suggests that the type of antimelanotic applied gives rise to significant different effects on Norway lobsters, the concentration of 4-hexylresorcinol in the formulations being less important.

In order to achieve a better understanding of the nature of the differences caused by the treatments, factorial analyses were performed, obtaining the correlation matrix and principal components represented in Tables 3 and 4, respectively. The data matrices were reduced to two principal components (PC) accounting for 89.6%, 95.7% and 99.6% of the total explained variance, respectively, for R-0.1, R-0.05 and CS lots. Yellow-green colouring showed positive correlation with melanosis score, as all data matrices indicated in the first component. Although yellow-greenish colour might be related to a^* and b^* parameters, no significant correlations were found in samples treated with 4-hexylresorcinol. In contrast, the sulphite-treated lot showed a high inverse correlation between redness (a^*) and yellow-greenish colour determined by sensorial analysis (Table 3(c) and Table 4(c), first component, 83.2% variance). This reflects the fact that in sulphite-treated individuals the heads turned green during storage; given that negative value of the redness parameter reflects a greenish tendency.

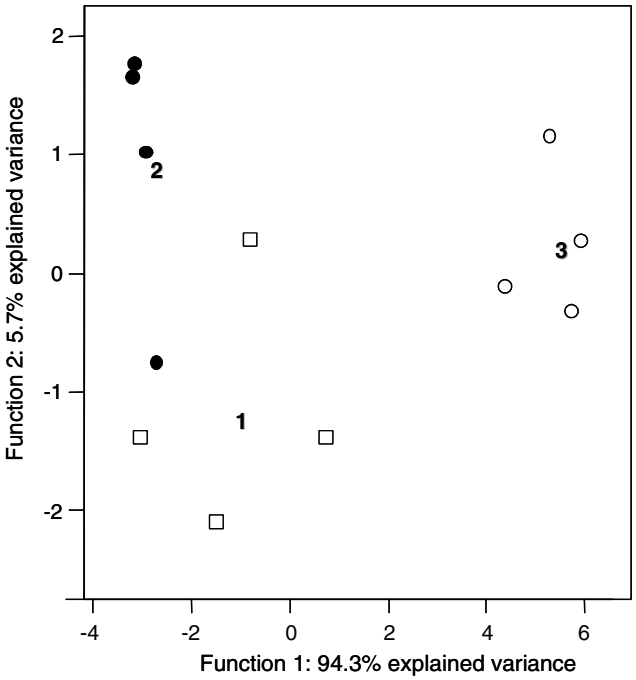

Fig. 4. Graphic representation of discrimination analysis of the different treatments used as antimelanotics on Norway lobsters: R-0.1 lot (1): samples treated with 0.1% 4-hexylresorcinol-based formulation. R-l0.05 lot (2): samples treated with 0.05% 4-hexylresorcinol-based formulation. CS lot (3): samples treated with commercial sulphite-based formulation.

Table 3(a) showed no significant ($P \leqslant 0.05$) correlation between melanosis score and $L^*a^*b^*$ parameters in R-0.1 LOT, probably because these samples hardly developed only slight melanosis during storage. However, melanosis score was positively correlated ($P \leqslant 0.05$) with yellowness and inversely with lightness (Table 3(b), 73.5% variance) in the lots treated with a smaller concentration of 4-hexylresorcinol (R-0.05). In CS lot the melanosis score was also inversely correlated with lightness ($P \leqslant 0.01$) (Table 3(c), 83.2% variance), showing also a significant ($P \leqslant 0.05$) negative correlation with redness. Taken together, these results indicate that lightness diminution was related to melanosis

Table 3
Correlation matrix of independent variables

	Melanosis score	Greenish	Marketability	Redness (a^*)	Lightness (L^*)	Yellowness (b^*)
(a) R-0.1 lot						
Melanosis score	1.000					
Greenish	0.984**	1.000				
Marketability	−0.986**	−0.999**	1.000			
Redness (a^*)	−0.780	−0.654	0.667	1.000		
Lightness (L^*)	0.249	0.301	−0.330	−0.011	1.000	
Yellowness (b^*)	−0.410	−0.240	0.254	0.889	0.236	1.000
(b) R-0.05 lot						
Melanosis score	1.000					
Greenish	0.961*	1.000				
Marketability	−0.617	−0.599	1.000			
Redness (a^*)	−0.334	−0.181	0.821	1.000		
Lightness (L^*)	−0.946*	−0.863	0.362	0.179	1.000	
Yellowness (b^*)	0.925*	0.842	−0.833	−0.665	−0.810	1.000
(c) CS lot						
Melanosis score	1.000					
Greenish	0.989**	1.000				
Marketability	−0.990**	−0.980**	1.000			
Redness (a^*)	−0.962*	−0.972*	0.986**	1.000		
Lightness (L^*)	−0.916*	−0.877	0.855	0.771	1.000	
Yellowness (b^*)	−0.456	−0.510	0.566	0.681	0.060	1.000

(a) R-0.1 lot: samples treated with R-0.1 formulation. (b) R-0.05 lot: samples treated with R-0.05 formulation. (c) CS lot: samples treated previously with commercial sulphite formulation.
* Indicate significant differences ($P \leqslant 0.05$) between samples.
** Indicate significant differences ($P \leqslant 0.01$) between samples.

and that the panellists could have confused preliminary step to melanosis, changing later to black.

Marketability was inversely correlated ($P \leqslant 0.01$) with both yellow-green colouring and melanosis score in R-0.1 and CS lots, according to the correlation matrices (Table 3) and the first principal component of the data matrices, which represent 64.5% and 83.2% of variance, respectively. It is possible that the presence of black and/or green colouring may have diminished the visual appraisal of crustaceans by the panellists. In CS lot, marketability was found positively correlated ($P \leqslant 0.01$) with redness (a^*), suggesting that the red tonality could be a highly appreciated value, intensified by sulphites. In contrast, marketability was not significantly correlated with L^*, a^* or b^* parameters in R-0.05 lot.

Table 4
Factor loadings for independent variables on the unrotated components

	Components	
	PC 1 (64.5% variance)	PC 2 (25.1% variance)
(a) R-01 lot		
Melanosis score	−0.984	0.116
Greenish	−0.938	0.270
Marketability	0.945	−0.274
Redness (a^*)	0.873	0.445
Lightness (L^*)	−0.250	0.746
Yellowness (b^*)	0.552	0.769
(b) R-0.05 lot		
Melanosis score	0.960	0.279
Greenish	0.902	0.357
Marketability	−0.803	0.533
Redness (a^*)	−0.573	0.789
Lightness (L^*)	−0.846	−0.463
Yellowness (b^*)	0.992	−0.102
(c) CS lot		
Melanosis score	−0.992	0.124
Greenish	−0.992	0.057
Marketability	0.997	0.005
Redness (a^*)	0.989	0.151
Lightness (L^*)	0.858	−0.512
Yellowness (b^*)	0.563	0.826

(a) R-0.1 lot: samples treated with R-0.1 formulation. (b) R-0.05 lot: samples treated with R-0.05 formulation. (c) CS lot: samples treated previously with commercial sulphite formulation.

4. Conclusions

In summary, the use of spraying of a sea water-dissolved formulation including organic acids, chelating agents and at least 0.05% 4-hexylresorcinol resulted in a good appearance of Norway lobsters (*Nephrops norvegicus*) during 12 days of chilled storage. 4-hexylresorcinol based formulations also decreased the availability of substrates for PPO from the first days of storage, compared with sulphites. Sulphites have only the advantage to bleach the samples, but it does not improve the appearance. The use of this formulation by spraying may therefore be a good alternative to commercial sulphite-based products in order to prevent melanosis.

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References

Aguzzi, J., Sardá, F., & Allué, R. (2004). Seasonal dynamics in *Nephrops norvegicus* (Decapoda: Nephropidae) catches off the Catalan coasts (Western Mediterranean). *Fisheries Research, 69*, 293–300.

Ali, M. T., Marshall, M. R., Wei, C. I., & Gleeson, R. A. (1994). Monophenol oxidase activity from the cuticle of Florida Spiny lobster (*Panulirus argus*). *Journal of Agricultural and Food Chemistry, 42*(1), 53–58.

Bartolo, I., & Birk, E. O. (1998). Some factors affecting Norway lobster (*Nephrops norvegicus*) cuticle polyphenol oxidase activity and black-spot development. *International Journal of Food Science and Technology, 33*, 329–336.

Celis-Guerrero, L. E., García-Carreño, F. L., & Navarrete del Toro, M. A. (2004). Characterization of proteases in the digestive system of spiny lobster (*Panulirus interruptus*). *Marine Biotechnology, 6*(3), 262–269.

Collins-Williams, C. (1983). Intolerance to additives. *Analysis of Allergy, 51*, 315–316.

European Commission (2003). Opinion of the Scientific Committee on Food on 4-Hexylresorcinol. Health and Consumer Protection Directorate – General, 5 March 2003.

Ferrer, O. J., Koburger, J. A., Simpson, B. K., Gleeson, R. A., & Marshall, M. R. (1989). Phenoloxidase levels in Florida Spiny lobster (*Panulirus argus*): relationship to season and molting stage. *Comparative Biochemistry and Physiology, 93B*(3), 595–599.

Frankos, V. H., Schmidtt, D. F., Haws, L. C., McEvily, A. J., Iyengar, R., Miller, S. A., et al. (1991). Generally Recognised As Safe (GRAS) evaluation of 4-hexylresorcinol for use as a processing aid for prevention of melanosis in shrimp. *Regulatory Toxicology and Pharmacology, 14*, 202–212.

González-Gurriarán, E., Freire, J., Fariña, A. C., & Fernández, A. (1998). Growth at moult and intermoult period in the Norway lobster *Nephrops norvegicus* from Galician waters. *ICES Journal of Marine Science, 55*, 924–940.

Guandalini, E., Ioppolo, A., Mantovani, A., Stacchini, P., & Giovannini, C. (1998). 4-hexylresorcino as inhibitor of shrimp melanosis: efficacy and residues studies; evaluation of possible toxic effect in a human intestinal in vitro model (Caco-2); preliminary safety assessment. *Food Additives and Contaminants, 15*(2), 171–180.

Gunnison, A., & Jacobsen, D. (1987). Sulphite hypersensivity. A critical review. *Critical Review in Toxicology, 17*, 185–214.

Iyengar, R., & McEvily, A. J. (1992). Anti-browning agents: alternatives to the use of sulfites in foods. *Trends in Food Science and Technology, 3*, 60–64.

Jiménez, M., & García-Carmona, F. (1997). 4-substituted resorcinols (sulfite alternatives) as slow-binding inhibitors of tyrosinase catecholase activity. *Journal of Agricultural and Food Chemistry, 45*(6), 2061–2065.

Lambrecht, H. S. (1995). Sulfite substitutes for the prevention of enzymatic browning in foods. In C. Y. Lee & J. R. Whitaker (Eds.), *Enzymatic browning and its prevention* (pp. 313–323). Washington: American Chemical Society.

López-Caballero, M. E., Álvarez, M. D., Sánchez-Fernández, J. A., & Moral, A. (2002). *Photobacterium phosphoreum* isolated as a luminescent colony from spoiled fish, cultured in model system under controlled atmospheres. *European Food Research and Technology, 215*, 390–395.

Maynou, F. X., & Sardá, F. (2001). Influence of environmental factors on commercial trawl catches of *Nephrops norvegicus* (L.). *ICES Journal of Marine Science, 58*, 1318–1325.

McEvily, A., Radha, I., & Otwell, S. (1991). Sulfite alternative prevents shrimps melanosis. *Food Technology, 9*, 80–86.

Montero, P., Ávalos, A., & Pérez-Mateos, M. (2001). Characterization of polyphenoloxidase of prawns (*Penaeus japonicus*). Alternatives to inhibition: additives and high-pressure treatment. *Food Chemistry, 75*, 317–324.

Montero, P., Gómez-Guillén, M. C., Zamorano, P., & Martínez-Álvarez, O. (2003). Estudio de inhibidores de melanosis en gamba blanca. In *Estudios de los agentes conservantes e inhibidores de la melanosis en crustáceos*. Spain: Junta de Andalucia. Consejería de Agricultura y Pesca.

Montero, P., Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2004). Effectiveness of on-board application of 4-hexylresorcinol in inhibiting melanosis in shrimp (*Parapenaeus longirostris*). *Journal of Food Science, 69*(8), 643–647.

Ogawa, M. (1987). Black spot occurrence in lobsters and shrimp. *Infofish Marketing Digest, 1*, 43.

Otwell, W. S., Iyengar, R., & Mc Evily, A. J. (1992). Inhibition of shrimp melanosis by 4-hexylresorcinol. *Journal of Aquatic Food Product Technology, 1*(1), 53–65.

Park, J. W. (1994). Functional properties additives in surimi gels. *Journal of Food Science, 59*(3), 525–527.

Ramírez, E. C., Whitaker, J. R., & Virador, V. (2003). Polyphenol oxidase. In J. R. Whitaker, A. G. J. Voragen, & D. W. S. Wong (Eds.), *Handbook of food microbiology* (pp. 509–523). New York: Marcel Dekker, Inc..

Ricquebourg, S. L, Robert-Da Silva, C. M. F., Rouch, C. C., & Cadet, F. R. (1996). Theoretical support for a conformational change of polyphenol oxidase induced by metabisulfite. *Journal of Agricultural and Food Chemistry, 44*, 3457–3460.

Ritchie, A. H. (1991). The Torry Station method for biogenic amines by HPLC. Technical Report Torry Research Station, Aberdeen, Scotland.

Rolle, R. S., Guizani, N., Chen, J. S., Marshall, M. R, Yan, J. S., & Wei, C. I. (1991). Purification and characterization of phenoloxidase isoforms from Taiwanese black tiger shrimp (*Penaeus monodon*). *Journal of Food Biochemistry, 15*, 17–32.

Rotllant, G., Arnau, F., García, J. A., García, N., Rodriguez, M., & Sardá, F. (2002). Effect of metabisulphite treatments and freezing on melanosis inhibition in rose shrimp *Aristeus antennatus* (Risso, 1816). *Food Science and Technology International, 8*(4), 243–247.

Rzepecki, L. M., & Waite, J. H. (1989). A chromogenic assay for catecholoxidases based on the addition of L-proline to quinones. *Analytical Biochemistry, 179*, 375–381.

Stoscheck, C. M. (1990). Quantitation of protein. *Methods in Enzymology, 182*, 50–69.

Wang, Z. K. D., Taylor, A., & Yan, X. (1992). Studies on the protease activities in Norway lobster (*Nephrops norvegicus*) and their role in the phenolase activation process. *Food Chemistry, 45*, 111–116.

Wang, Z. K. D., Taylor, A., & Yan, X. (1994). Further studies on the roles of proteases in the activation of phenolase from Norway lobster (*Nephrops norvegicus*). *Food Chemistry, 51*, 99–103.

Yan, X., Taylor, K. D. A., & Hanson, S. W. (1989). Sutdies on the mechanism of black spot development in Norway lobster (*Nephrops norvegicus*). *Food Chemistry, 34*(4), 273–283.

Yan, X., Taylor, K. D. A., & Hanson, S. W. (1990). Phenolase in Norway lobster (*Nephrops norvegicus*): activation and purification. *Food Chemistry, 36*, 19–33.

Young, K. W., & Whittle, J. (1985). Colour measurement of fish minces using Hunter *L*, *a*, *b* values. *Journal of Science of Food and Agriculture, 36*, 383–392.

Zotos, A., & Taylor, K. D. A. (1996). Partial purification and characterization of proteases from Norway lobster (*Nephrops norvegicus*) and their role in the phenolase activation process. *Food Chemistry, 56*(1), 61–68.