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

O. Martínez-Alvarez, M.E. López-Caballero *, P. Montero, M.C. Gómez-Guillén

Instituto del Frío (CSIC), José Antonio Novais, No. 10, 28040 Madrid, Spain

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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,

* Corresponding author. Tel.: +34 91 549 23 00; fax: +34 91 549 36 27.
E-mail address: mlopez@if.csic.es (M.E. López-Caballero).

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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-