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Improvement of the commercial quality of chilled Norway lobster (*Nephrops norvegicus*) stored in slurry ice: Effects of a preliminary treatment with an antimelanosic agent on enzymatic browning

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Abstract

The use of slurry ice is gaining increasing importance as an advanced method for the hygienic and efficient chilling and sub-zero storage of aquatic food products. In this work, this technology was applied as a novel technique for the chilling and storage of Norway lobster (*Nephrops norvegicus*) – a crustacean species of high-commercial value – under refrigeration conditions at −1.5 °C. In addition, the effects of a preliminary treatment with 0.5% Na HSO_3 on surface browning were evaluated and compared with the results obtained in control batches not subjected to such treatment. The processing of lobster in slurry ice significantly ($p < 0.05$) slowed down microbial spoilage, as determined by the counts of aerobes, psychrotrophs, proteolytic bacteria, and lactose-fermenting *Enterobacteriaceae*, and by the formation of volatile amines. Likewise, the autolytic breakdown mechanisms – as determined by the *K* value – were also significantly ($p < 0.05$) inhibited in the slurry ice batch. Remarkably, preliminary treatment with 0.5% sodium metabisulphite permitted better maintenance of the parameters involved in sensory quality – especially as regards the aspect of the carapace – as compared with non-treated batches, and allowed a shelf life of 9 days without surpassing the 150 mg/kg legal limit established for this food additive. On contrast, the non-treated batch stored in slurry ice exhibited a shelf life of 5 days. The combination of technological treatments proposed in this work – preliminary antimelanosic treatment and storage in slurry ice – may be successfully applied to other fresh and frozen shellfish species with a view to extending shelf life and to avoiding the legal and toxicological problems derived from current abuse of such antimelanosic agents to prevent shellfish browning.

Keywords: Refrigeration; Melanosis; Ice slurries; Norway lobster; Sensory quality; Enzymatic browning

1. Introduction

Marine species generate highly perishable food products whose quality and freshness rapidly decreases *post-mortem* due to a wide variety of microbial and biochemical degradation mechanisms that limit their commercial shelf life. Among aquatic food products, crustaceans are a type of shellfish of great economic importance in many countries across the world (FAO, 2004a). Crustacean species are often small, they are not gutted, and they have high contents of non-protein nitrogenous (NPN) compounds, polyunsaturated fatty acids (PUFAs), and melanin pigment (Cheftel & Cheftel, 1976; Piclet, 1987). The *post-mortem* modifications occurring in such compounds due to autolytic changes, microbial spoilage, and melanosis reactions

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have been shown to exert fast-acting negative effects on crustacean quality, also considerably limiting the shelf life of such shellfish (Ashie, Smith, & Simpson, 1996; Simpson, Marshall, & Otwell, 1987; Whittle, Hardy, & Hobbs, 1990). These undesirable events make the efficient refrigeration of crustaceans immediately after the catch mandatory.

The preservation of fresh aquatic food products has traditionally involved the use of flake ice (Mendes, Quinta, & Nunes, 2001), refrigerated sea water (Kraus, 1992), modified atmospheres (Ruíz-Capillas & Moral, 2004), brine solutions (Xiong, Xiong, Blanchard, Wang, & Tidwell, 2002) or the incorporation of chemical preservative agents (McEvily, Iyengar, & Otwell, 1991). Recently, slurry ice – also known as liquid ice or flow ice – has been reported to be a valuable technique for the chilling and refrigeration of fish species derived from both marine and aquaculture origin (for an updated review, see Piñeiro, Barros-Velázquez, & Aubourg, 2004). The main advantages of slurry ice are as follows: (i) the sub-zero storage of the fish material, (ii) its faster chilling rate as compared to other chilling techniques, due to its higher heat-exchange capacity, (iii) the reduced physical damage caused to the fish surface by its spherical microscopic particles, and (iv) the complete coverage of the fish surface, which reduces dehydration and oxidation events (Piñeiro et al., 2004).

While previous reports have described the advantages of the application of slurry ice systems to farmed sea bream (Huidobro, Mendes, & Nunes, 2001) and turbot (Rodrıguez, Barros-Velázquez, Piñeiro, Gallardo, & Aubourg, 2006) and to wild albacore (Price, Melvin, & Bell, 1991), hake (Losada, Piñeiro, Barros-Velázquez, & Aubourg, 2004a; Rodrıguez, Losada, Aubourg, & Barros-Velázquez, 2004), and sardine (Campos, Rodrıguez, Losada, Aubourg, & Barros-Velázquez, 2005; Losada, Barros-Velázquez, Gallardo, & Aubourg, 2004b), little information is currently available concerning the use of slurry ice for crustaceans (Chinivasagam, Bremner, Wood, & Nottingham, 1998; Huidobro, López-Caballero, & Mendes, 2002). In the case of Norway lobster (*Nephrops norvegicus*), an aquatic food product of remarkably high-commercial value in many European countries (FAO, 2004b), the use of slurry ice may offer advantages at microbial and biochemical level although, in contrast, it may accelerate the reactions involved in the enzymatic browning of shellfish carapaces. With a view to avoiding this defect and extending the shelf life of caught lobster, here we evaluated the incorporation of a preliminary treatment with an antimelanosic agent prior to storage in slurry ice. Our work also aimed at elucidating whether controlled addition of Na HSO_3 prior to storage in slurry ice might provide benefits without surpassing the legal limits established for this antimelanosic agent, thus avoiding the legal and toxicological events derived from the abuse of this type of additive. The evolution of quality was assessed by a number of microbial and biochemical parameters indicative of spoilage and the results were compared with sensory acceptability.

2. Materials and methods

2.1. Refrigeration systems

A slurry ice prototype (FLO-ICE™, Kinarca S.A.U., Vigo, Spain) was used. The composition of the liquid–ice binary mixture was 40% ice/60% water, prepared from filtered seawater with a 3.3% NaCl content. The temperature of the liquid–ice mixture was −1.5 °C. Flake ice was prepared from non-salted water by means of an Icematic F100 Compact device (CASTELMAC SPA, Castelfranco, Italy). The lobster specimens were surrounded by an equal weight of slurry or flake ice, and stored in a refrigerated room at +2 °C. The temperature of the lobster specimens stored in slurry ice along storage was in the −0.5/−1.5 °C range, while the temperature of the specimens stored in flake ice was in the +0.5 °C/+1.0 range. When required, the flake ice and the slurry ice mixture were renewed.

2.2. Lobster specimens, processing, and sampling

Norway lobster (*N. norvegicus*) specimens (carapace length 8–11 cm; weight range: 80–100 g) were obtained from a local aquaculture farm (REMAGRO, El Grove, Spain) in October 2005. Three specimens were separated for study as initial material on day 0. The remaining specimens were divided into four batches. Two of these batches were placed in an antimelanosic solution consisting of 0.5% sodium metabisulphite (Merck, Darmstadt, Germany) at room temperature (+15 °C) for 30 min. and then stored in flake ice (mFI batch) or slurry ice (mSI batch), respectively. The remaining two batches were not treated with the antimelanosic solution, the specimens being processed directly in flake ice (FI batch) or slurry ice (SI batch), respectively. All four batches were stored in a heat-insulated room at +2 °C for 9 days. Three specimens were taken from each batch on days 2, 5, 7, and 9 of chilled storage. Once the specimens had been subjected to sensory analyses, the edible flesh was separated and used to undertake both the microbiological and biochemical analyses.

2.3. Microbiological analyses

Samples of 10 g of muscle were dissected aseptically from chilled lobster specimens, mixed with 90 ml of 0.1% peptone water (Oxoid Ltd., London, UK), and homogenised in a stomacher (Seward Medical, London, UK). Serial dilutions from the microbial extracts were prepared in 0.1% peptone water. Total aerobes and psychrotrophic bacteria were investigated in Plate Count Agar (PCA, Oxoid) after incubation at 30 °C for 48 h or at 7–8 °C for 10 days, respectively, as previously described (Ben-Gigirey, Vieites Baptista de Sousa, Villa, & Barros-Velázquez, 1998, 1999). Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar, as previously described (Ben-Gigirey, Vieites-Baptista de Sousa, Villa, & Barros-Velázquez, 2000; Phaff, Starmer, Lachance, & Ganter,

1994). Lactose-fermenting *Enterobacteriaceae* (coliforms) were investigated in Violet Red Bile Agar (VRBA medium, Merck), following the manufacturer's instructions (Merck Microbiology Manual, 2002). All results were expressed as log CFU/g.

2.4. Chemical analyses

The evolution of pH in lobster muscle was determined by means of a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

Nucleotide extracts were prepared according to the method of Ryder (1985) and were stored at −30 °C until analysis. Analysis was carried out according to Aubourg, Piñeiro, Gallardo, and Barros-Velázquez (2005). The K value was calculated according to the following molar concentration ratio, in which the different molecules involved in the adenosine-triphosphate degradation pathway are considered: K value (%) = 100 × (hypoxanthine + inosine)/(adenosine-triphosphate + adenosine-diphosphate + adenosine-monophosphate + inosine-monophosphate + inosine + hypoxanthine).

The values concerning the formation of total volatile base-nitrogen (TVB-N) were determined by the Antonacopoulos (1960) method, with the modifications described elsewhere (Aubourg, Sotelo, & Gallardo, 1997), and the results were expressed as mg TVB-N/100 g of muscle. Trimethylamine-nitrogen (TMA-N) contents were measured by the picrate method, as previously described (Tozawa, Erokibara, & Amano, 1971), and the results were expressed as mg TMA-N/100 g of muscle.

The NaCl content in fish muscle was calculated from the amount of chlorine extracted by boiling in HNO_3 (The Sigma Chemical Co, St. Louis, MO) with an excess of 0.1 N $AgNO_3$ (Sigma), followed by titration with 0.1 N NH_4SCN (Sigma) (AOAC, 1990). The results were expressed as g NaCl/100 g of muscle.

The metabisulphite contents in lobster muscle were determined as follows. Briefly, a 5-g portion of lobster muscle was collected aseptically, minced, placed in a spherical flask, and distilled in the presence of the 20 ml of distilled water, glass beads, and 2 ml of concentrated HCl (Sigma). The resulting SO_2 from the reaction mixture was sent to a flask containing 0.5 ml of a 1% (w/v) starch solution (Sigma), 4.5 ml of distilled water, and one drop of an I_2 solution. The determination of SO_2 was performed with a 0.05 N I_2 solution (Sigma), as described elsewhere (De Vries, Hoon, Ebert, Magnuson, & Ogawa, 1986).

2.5. Sensory analyses

The sensory quality of the samples was evaluated by a taste panel consisting of five experienced judges, who based their evaluations on traditional guidelines for freshness assessment in crustacean species (Table 1) (DOCE, 1989). Carapace, eyes, gills, odour, and flesh were assessed, these allowing the classification of the lobster specimens into one of the following categories: E (extra), A (good), B (acceptable), and C (unacceptable).

2.6. Statistical analyses

The data from the different microbiological and biochemical analyses were subjected to one-way analysis of variance ($p < 0.05$). Comparison of means was accomplished using a least-significant difference (LSD) method (Statsoft, 1994). The SPSS software (SPSS Inc., Chicago, IL, USA) was also used to explore the statistical significance ($p < 0.05$) of the differences among batches, this including multivariate contrasts and multiple comparisons.

3. Results and discussion

3.1. Microbiological analyses

Microbial development in lobster muscle along refrigerated storage in the four batches is displayed in Figs. 1 and 2. As can be seen in Fig. 1, the use of slurry ice – the mSI and SI batches – led to lower counts for aerobic mesophiles, as compared to the mFI and FI batches. Statistical analyses revealed significant ($p < 0.05$) differences between the mSI/SI batches with respect to the mFI/FI batches. Remarkably, no significant difference deriving from the antimelanosic treatment was observed at this level. Thus, with respect to total aerobes, the average differences between the mSI/SI and mFI/FI batches were 0.94 log CFU/g and 1.15 log CFU/g on days 5 and 9 of storage, respectively (Fig. 1), the slurry ice batches exhibiting lower microbial counts than their counterpart flake-ice batches. Previous reports have also described significantly lower bacterial growth in shrimp stored in slurry ice, as compared to conventional flake ice (Huidobro et al., 2002). Likewise, storage in slurry ice has also been described to lead to a

Table 1
Scale employed for evaluating the sensory quality of chilled lobster

Attribute	Category			
	E	A	B	C
Carapace	Pink-orange and bright; without black spots	Pink-orange and pale; without black spots	Incipient formation of brown spots	Important formation of brown spots
Eyes	Black and bright	Black and lustreless	Grey	Grey
Gills	Pink	Incipiently grey	Dark grey	Green-grey
Odour	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and putrid	Sour and putrid

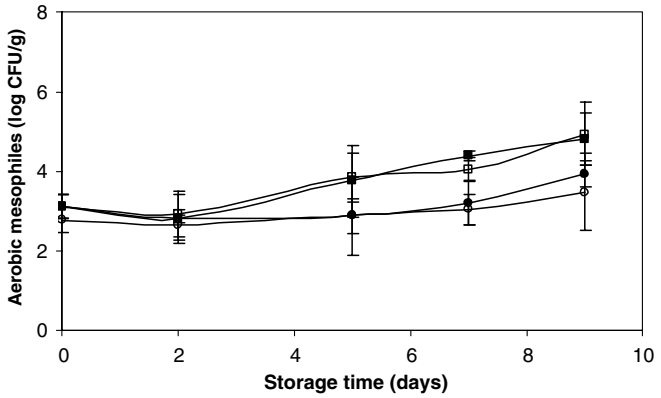

Fig. 1. Evolution of aerobic mesophiles during the refrigerated storage of lobster in the FI batch (■), mFI batch (□), SI batch (●), and mSI batch (○).

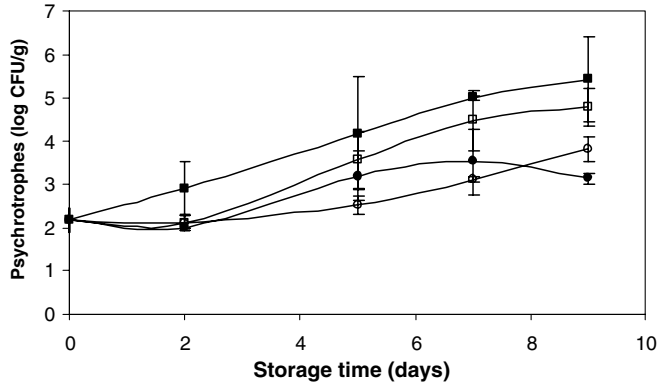

Fig. 2. Evolution of psychrotrophic microorganisms during the refrigerated storage of lobster in the FI batch (■), mFI batch (□), SI batch (●), and mSI batch (○).

significant slowing down of microbial activity in other aquatic food products, such as turbot (Rodríguez et al., 2006), horse mackerel (Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2005), hake (Rodríguez et al., 2004), and sardine (Campos et al., 2005). Although microbial growth was significantly slowed down in the slurry ice batches, aerobic bacteria did not reach counts higher than 10^5 CFU/g, suggesting that microbial spoilage might not represent the limiting factor to the sensory acceptability of lobster flesh.

With respect to psychrotrophic bacteria, the numbers of this group were also significantly ($p < 0.05$) lower in the mSI and SI batches as compared to the FI batch (Fig. 2). In contrast, notable but non-significant differences were observed between the mSI/SI batches and the mFI batch. As in the case of the aerobes, no significant effect deriving from the use of the antimelanosic agent on the psychrotroph numbers was observed. In global terms, the psychrotrophic bacteria in the mFI/FI batches reached average counts of 0.85 log CFU/g and 1.63 log CFU/g higher than in the mSI/SI batches after 5 and 9 days of storage, respectively (Fig. 2). These results provide additional support to previous studies performed on other aquatic food products such as sardine (Campos et al., 2005), hence underlining the benefits of the slurry ice-based storage systems to inhibit the growth of psychrotrophic bacteria.

The investigation of proteolytic bacteria in all four batches revealed significant ($p < 0.05$) differences only between the mSI batch and mFI/FI batches. However, the differences between such batches were in all cases below 1 log CFU/g and, in global terms, the counts were always below 10^4 CFU/g, indicating a very limited contribution of bacteria capable of producing extracellular proteases to the microbial breakdown of lobster muscle. In agreement with the above results, no significant effect of the addition of the antimelanosic agent on the growth of proteolytic bacteria was observed.

With respect to coliform counts, these were generally below 10^3 and 10^2 CFU/g in the mFI/FI and mSI/SI batches, respectively. Statistical analysis revealed that the use of slurry ice (mSI/SI batches) maintained coliforms at significantly ($p < 0.05$) lower numbers as compared to flake ice (mFI/FI batches). In agreement with the results obtained for other microbial groups, no significant effect of the preliminary antimelanosic treatment on coliform growth was determined. The very limited growth of coliforms in lobster muscle reported here is in agreement with previous reports on sardine (Campos et al., 2005), and jack mackerel (Figueroa, Galeno, Troncoso, & Aguilera, 1990).

3.2. Chemical analyses

The evolution of pH in lobster muscle along storage indicated significantly ($p < 0.05$) lower increases in the mSI/SI batches than in their mFI/FI counterparts. Thus, the initial 6.61 pH value rose to 7.94 and 7.30 in the FI and mFI batches, respectively, after 9 days of chilled storage. In contrast, final pH values of 7.21 were determined in lobster muscle in the SI and mSI batches. Previous reports on other non-crustacean aquatic food products stored in slurry ice have also described lower increases in the pH value as compared to flake icing (Campos et al., 2005; Rodríguez et al., 2004). The results presented here clearly

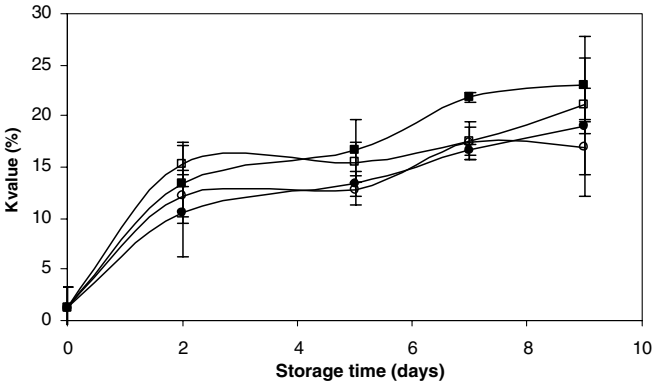

Fig. 3. Evolution of the autolytic breakdown mechanisms, as determined by the *K* value, during the refrigerated storage of lobster in the FI batch (■), mFI batch (□), SI batch (●), and mSI batch (○).

indicate that: (i) slurry ice allows a better control of endogenous and microbial alkalinising mechanisms involved in the spoilage of lobster muscle, and (ii) the preliminary antimelanosic treatment permits a better control of such alkalinising mechanisms when flake ice is used, but does not afford any significant advantage at this level when slurry ice is used.

The rates of autolytic degradation were evaluated in all four batches by means of the *K* value (Fig. 3). Both FI-based batches exhibited higher increases in the *K* value during storage, as compared to the SI-based batches. In our study, the FI batch showed significantly higher ($p < 0.05$) *K* values than both mSI/SI batches, this suggesting that slurry ice slows down the autolytic breakdown of lobster muscle. With respect to the effect of the preliminary antimelanosic treatment, this provided an additional slight advantage in the SI-based batch, while this effect was more pronounced in the FI-based batch, as also described above for the alkalinising mechanisms. However, in global terms the increases in the *K* value were generally low, thus confirming earlier works with other crustacean species (Mendes, Huidobro, & López-Caballero, 2002), and were remarkably lower than those reported for other non-crustacean aquatic food products (Aubourg et al., 2005; Losada et al., 2004a, 2004b).

With respect to the formation of TVB-N, slight increases were observed in all four batches along chilled storage. The initial concentration (37.2 ± 1 mg/100 g) was remarkably higher than those found in other non-crustacean aquatic food products (Campos et al., 2005; Rodríguez et al., 2004), which can be explained in terms of a higher NPN content in raw crustaceans as compared to fish species (Mendes et al., 2002; Piclet, 1987). A detailed comparison of the four batches afforded two main conclusions: (i) a better control of TVB-N formation in both SI batches was achieved, with concentrations in the range of 37.2–46.4 mg TVB-N/100 g muscle, as compared to the FI batches, which exhibited TVB-N concentrations in the 37.2–54.6 mg/100 g range, and (ii) in agreement with the results reported above, the preliminary treatment with metabisulphite significantly ($p < 0.05$) increased TVB-N formation in the mFI batch (final value: 53.3 mg/100 g) as compared to the FI batch (final value: 47.7 mg/100 g), this effect not being observed on comparing the mSI and SI batches. In this sense, the preliminary antimelanosic treatment did not provide any relevant advantage to the storage of lobster in slurry ice, but did prove to have detrimental effects in lobster batches stored in traditional flake ice.

The TMA-N content increased along the time of storage in lobster muscle in the FI batch and, to a lesser extent, in the mFI batch (Fig. 4). In contrast, the formation of such undesirable volatile amines in the SI and mSI batches was so low that the final TMA-N concentrations in these batches after 9 days of storage were not significantly different from the initial values. On the other hand, the preliminary antimelanosic treatment did not provide any relevant advantage in the SI-based batches, but did allow a remarkably better control of TMA-N formation in the batches subjected to flake icing.

The NaCl content in lobster muscle was higher in the mSI and SI batches than in the mFI and FI batches (Fig. 5). This result was expected, since the slurry ice considered in this work was prepared with seawater. However, the final NaCl concentrations determined in the mSI and SI batches were not above 0.80%. As expected, the preliminary antimelanosic treatment did not have any significant effect on the uptake of NaCl by the specimens stored in slurry ice.

The preliminary antimelanosic treatment tested in this work was aimed at achieving a better control of carapace browning, a defect frequently described as the limiting factor of acceptability of lobster and other crustaceans (Chen, Charest, Marshall, & Wei, 1997; McEvily et al., 1991). In this study, the antimelanosic treatment was applied as a preliminary step prior to chilled storage. The concentration of the sodium metabisulphite and the time of exposure to this agent should not lead to Na HSO_3 concentrations higher than 150 mg/kg in the edible parts of crustaceans, according to the current regulations (BOE, 2002). Fig. 6 shows the Na HSO_3 concentrations determined in the

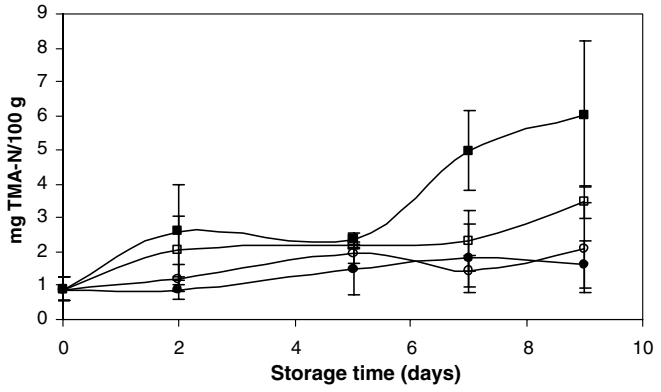

Fig. 4. Evolution of trimethylamine-nitrogen (TMA-N) content during the refrigerated storage of lobster in the FI batch (■), mFI batch (□), SI batch (●), and mSI batch (○).

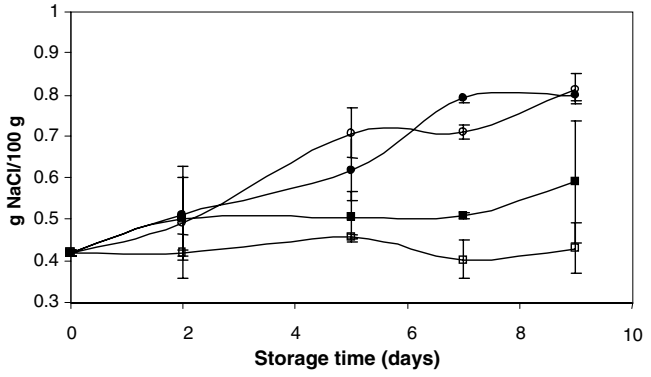

Fig. 5. Evolution of the NaCl content during the refrigerated storage of lobster in the FI batch (■), mFI batch (□), SI batch (●), and mSI batch (○).

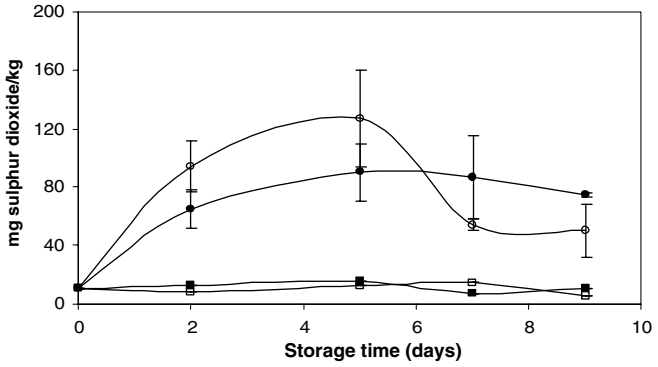

Fig. 6. Evolution of the Na HSO_3 content during the refrigerated storage of lobster in the FI batch (■), mFI batch (□), SI batch (●), and mSI batch (○).

edible part of the lobster muscle in all four batches. Thus, maximum Na HSO_3 concentrations of 126.7 and 90.1 mg/kg were determined in the mSI and mFI batches, respectively, after 5 days of refrigerated storage. Remarkably, the preliminary treatment applied here afforded lobster muscle with an acceptable residual content of the antimelanosic agent. Should this preliminary treatment prove to be effective in delaying lobster carapace browning, the residual presence of such antimelanosic would be below the limits established for this additive, thereby avoiding two main undesirable situations: (i) the uncontrolled addition of solid Na HSO_3 to crustaceans, mainly during their storage in flake ice, and (ii) the potential allergy and toxicological problems that may arise from such uncontrolled addition.

3.3. Sensory analyses

Table 2 shows the scores obtained at sensory analysis by all four lobster batches. Remarkably, the mSI batch exhibited an extended shelf life as compared to the other three batches. Thus, while the initial E quality decreased rapidly, especially in the FI batch, and to a lesser extent in the SI and mFI batches, their shelf lives being 2, 5, and 5 days, respectively, the mSI batch exhibited acceptable quality even after 9 days of storage. Fig. 7 shows the external appearance of the specimens from all four batches after 7 days of storage. Notably, the combination of the preliminary antimelanosic treatment and storage in slurry ice (mSI batch) allowed a better control of carapace browning, also allowing a reduction in the rate of other undesirable mechanisms directly affecting sensory quality.

Carapace browning is due to intense melanin oxidation. Melanin is a widely distributed polymeric compound that has been reported to undergo enzymatic oxidation during the processing of crustacean species, this leading to important quality losses in their sensory quality (Chen et al.,

Table 2
Effects of the antimelanosic preliminary treatment and storage system on the sensory acceptability of chilled lobster

Days of storage	Carapace				Eyes				Gills				Odour			
	FI	mFI	SI	mSI	FI	mFI	SI	mSI	FI	mFI	SI	mSI	FI	mFI	SI	mSI
0	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
2	B	A	A	E	A	A	A	A	A	A	A	A	B	E	A	E
5	B	A	B	A	A	B	A	A	B	B	B	A	B	A	A	A
7	C	B	C	A	B	B	B	A	C	B	B	A	C	B	B	B
9	C	C	C	B	C	C	C	B	C	C	C	B	C	C	C	B

Freshness categories as in Table 1; FI, flake icing; mFI, preliminary treatment with 0.5 N HSO_3Na + storage in flake ice; SI, slurry icing; mSI, preliminary treatment with 0.5 N HSO_3Na + storage in slurry ice.

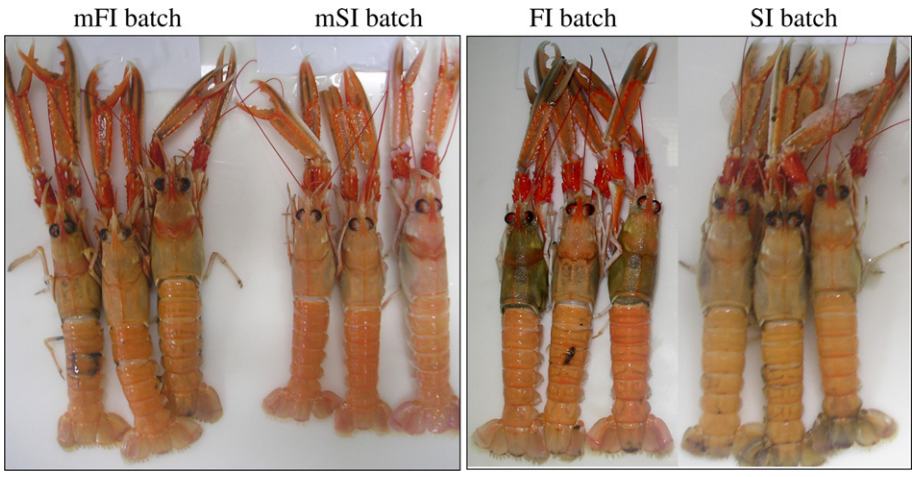

Fig. 7. Comparative development of melanosis in refrigerated lobster after 7 days of storage in the FI batch (■), mFI batch (□), SI batch (●), and mSI batch (○).

1997; McEvily et al., 1991). Preliminary research conducted at our laboratory has unveiled detrimental effects of slurry ice on lobster carapace due to an enhancement of the melanin oxidation pathway, in turn probably due to the presence of NaCl (Aubourg & Ugliano, 2002) in the slurry ice biphasic systems prepared from saline water. Nevertheless, the present study demonstrates that the preliminary antimelanosic treatment prevents such a drawback, leading to a better preservation of the sensory attributes of lobster and also extending its shelf life.

4. Conclusion

The combination of a preliminary antimelanosic treatment with 0.5% Na HSO_3 for 30 min, when followed by chilling and storage in slurry ice, afforded a better maintenance of sensory quality and an extended shelf life of lobster as compared to counterpart specimens not treated with the antimelanosic agent and/or stored in traditional flake ice. Such an effect was achieved without surpassing the 150 mg/kg legal limit established for Na HSO_3. Microbial and autolytic breakdown mechanisms were significantly slowed down in the slurry ice batches, this type of ice proving to be a better storage system for lobster than traditional flake ice. Our work also opens the way to the development of a combined system consisting of Na HSO_3 incorporated to the liquid phase of the ice slurries with the aim of extending the commercial shelf life of high-commercial value crustaceans.

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