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Shelf life extension of whole Norway lobster *Nephrops norvegicus* using modified atmosphere packaging

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ABSTRACT

Once a nuisance by-catch, today the Norway lobster (*Nephrops norvegicus*) is a valuable UK fisheries commodity. Unfortunately, the species is very susceptible to quality deterioration post harvest as it quickly develops black spots and also spoils rapidly due to bacterial growth. Treatment with chemicals can stop the blackening and carefully monitored cold storage can result in a sensory shelf life of up to 6.5 days. The high susceptibility to spoilage greatly restricts the extent to which *N. norvegicus* can be distributed to retailers and displayed for sale. The application of modified atmosphere (MA) could be extremely beneficial, allowing the chilled product to stay fresh for a long period of time, thus ensuring higher sales. In the present study, we identified a gas mix for the MA packaging (MAP) of whole *N. norvegicus* lobster into 200 g retail packs. Our results show that a shelf life extension to 13 days can be achieved when retail packs are stored in MAP at 1 °C. Effectiveness of the MAP was evaluated by using a newly developed QIM for MA-packaged whole *N. norvegicus* and also by analyzing bacterial plate counts. Changes in the microflora and effects of different storage temperatures on the quality of the MA packs are also presented. The main specific spoilage organism (SSO) of modified atmosphere packaged Norway lobster is *Photobacterium phosphoreum*.

1. Introduction

Once considered a nuisance by-catch, currently the Norway lobster (*Nephrops norvegicus*) represents one of the most valuable fisheries in the UK (Neil, 2012) and whole chilled animals are highly priced. Unfortunately however, the species is very susceptible to spoilage and exhibits rapid quality deterioration post-harvest (Gornik et al., 2011; Neil, 2012), especially when icing and/or cooling are delayed (Albalat et al., 2011). Furthermore, when left at elevated temperatures *N. norvegicus* also develops brown–black spots and lesions Neil, 2012 (Martínez-Álvarez et al., 2007) quickly, due to the accumulation of melanin triggered by the enzyme polyphenol oxidase (PPO) (Giménez et al., 2010). This browning (melanosis) is further accelerated after trawl capture and rough handling (Bartolo and Birk, 2002). Therefore, to inhibit melanosis the *N. norvegicus* processing industry routinely uses different anti-melanotic formulations (i.e. metabisulfite, 4-hexylresorcinol based products).

One processing method that is commonly used to extend the shelf life of seafood is modified atmosphere packaging (MAP) and, based on data from fish and other shellfish, a general shelf life increase of 30–60% is common (Sivertsvik and Jeksrud, 2002). Unfortunately, studies investigating the effects of MAP in crustaceans are sparse with shrimps and prawns being the most studied products. To date only two gas mix conditions have been tested for MAP application in *N. norvegicus* (Ruiz-Capillas et al., 2003). Unfortunately, that study only assessed these two gas mixes for bulk storage of lobster at 1 °C prior to sale, but not for MAP in retail packs.

Freshness and changes in sensory attributes are critical parameters for the quality assessment of seafood and form the basis of many quality index methods (QIMs) (Bremner, 1985; Hyldig et al., 2007), and as such they are also important for the assessment of *N. norvegicus* (Albalat et al., 2011; Neil, 2012). Numerous QIM schemes for various types of fish and crustaceans exist (e.g. Huidobro et al., 2000; Barbosa and Vaz-Pires, 2004; Cardenas Bonilla et al., 2007). Gómez-Guillén et al. (2007) and Martínez-Álvarez et al. (2008) have published QIMs for whole *N. norvegicus* treated with an anti-melanotic. However, these QIMs were only tested and optimized for air storage at 2 °C and 4 °C, respectively. Dependency on storage temperature and suitability for MA packs have not been tested for these QIMs.

The aim of the present study was to provide an optimal MAP gas mix and QIM for MA-packaged whole *N. norvegicus* lobster after treatment with an anti-melanotic stored within retail packs. Firstly, a QIM for whole *N. norvegicus* was tested and calibrated. The QIM scheme was then modified to work with animals that had been treated with an anti-melanotic. Then, a suitable gas mix for MAP of *N. norvegicus* was

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identified using a triangular, non-biased approach. At the same time the whole animal QIM was adapted for MA packs. The quality of the product stored in the MA packs was further evaluated using previously established microbiological methods. Changes in the microflora and effects of different storage temperatures on the quality of the MA packs were also assessed.

2. Material and methods

2.1. Trawl capture and post catch storage of whole N. norvegicus

N. norvegicus were caught in the Clyde Sea area, Scotland, UK (55°36.65′N and 04°52.99′W) as described by Gornik et al. (2011). Once caught, the lobsters were thoroughly washed for 5 min in a fishing basket using seawater derived from an on-board high-pressure hose, following common practice in the industry. Within 30 min (upon arrival at the pier) approximately 20 kg of lobsters (or around 500 animals) was placed in a fish box and 10 kg of ice was placed on top of the content. The box was then stored in a cold room at 5 °C until further processing, which was approximately 24 h later to simulate commercial practices.

2.2. QIM development and assessment procedure

The QIM for whole *N. norvegicus* lobsters consists of five different parameters and four demerit scores (from 0 to 3) per parameter. The detailed descriptions of the parameters and the demerit quality scores can be found in Supplementary Table S1. The QIM was firstly calibrated for iced storage. During calibration, per data point, three assessors sampled the product (10 animals per condition) independently. In all cases, prior to assessment, the samples were equilibrated to room temperature for 15 min. To avoid bias, sample identity was not disclosed until after evaluation.

2.3. Anti-melanotic treatment

All treatments were carried out at 5 °C. After overnight storage (approx. 12 h) whole animals were dipped into a 2% solution of Melacide® SC20 (Norkem, Cheshire, UK) in seawater for 15 min following manufacturer's instructions. The animal-to-solution ratio was 1:2 (w/v). After dipping, the animals were placed in a standard fish basket to drain off any remaining liquid.

2.4. MA-packaging of whole N. norvegicus

Following anti-melanotic treatment approximately 200 g of *N. norvegicus* (5–6 animals) was placed into Clearfresh® MA packs (R15–45, 260 × 177 × 45 mm, LINPAC, Yorkshire, UK) and packed using a PA1200 MA-packaging machine (Packaging Automation Ltd, Cheshire, UK). Gas mixes were delivered to the packaging machine through a WITT 3800 gas mixer (WITT, Cheshire, UK) fed by gas cylinders containing food-grade N_2, CO_2 and O_2 (BOC, Scotland, UK). The CO_2 was preheated to ambient temperature (5 °C) using a heated valve unit (BOC). The sealing film was anti-fog Esterpeel PS_2+ AF with a transmission rate of 8 cm^3 O_2 per m^2 day^{-1}. After packaging the headspaces of several packs were analyzed to verify the gas mixes using a WITT OXYBABY hand-held gas analyzer. Prior to sampling the gas content of the packs was measured to ensure that packs did not have leaks during storage. Leaky packs were always discarded and not analyzed.

2.5. Sample storage and sampling regime

During preliminary trials and during the identification of a suitable gas mix, a 24-h delay was incorporated into the packaging procedure, mimicking commercial fishing practice. All the animals were freshly caught, stored on ice for 24 h and packed on-site following anti-melanotic treatment. After packaging the samples were stored at 2 °C for 5 days. Then, after 5 days, the packs were transferred at 6 °C (mimicking consumer storage behaviors) for QIM sampling on day 8. See also Supplementary Fig. S1 for a schematic overview of the sampling regime.

During the evaluation of the OGM-MA-packs (see below for description), a 1-step and a 3-step temperature storage regime were employed to refine the analysis (Fig. S1). In the 1-step regime (intended to serve as a 'best practice' positive control) the MA packs were held at a constant temperature of 1 °C. In the 3-step regime (mimicking the current commercial retail chain from processor to consumer) the samples were stored at 1 °C for 3 days ("processor"), transferred at 4 °C for 2 days ("market") and finally stored at 6 °C for the remainder of the time ("consumer"). Temperature profiles were recorded using StowAway TidbiT® V2 (Onset, Massachusetts, USA) temperature loggers accompanying the stored packs. An excerpt of a typical temperature recording can be seen in Supplementary Fig. S2.

2.6. Microbiological analysis

2.6.1. Sample extraction and homogenization

Tail meat was sampled aseptically as described by Gornik et al. (2011). Briefly, the packs (3 packs per condition/sampling time) were opened and immediately 0.5–1.0 g of tail muscle was carefully removed per tail. Up to 5 samples were taken per pack. The extracted samples were transferred into a 'stomacher' bag. Per 1 g of meat, 9 ml of sterile seawater containing 0.1% bacteriological peptone (Difco/BD, Oxford, UK) was added. The samples were then homogenized and transferred into sterile plastic universals for further usage.

2.6.2. Compact Dry® plates

To perform high-throughput total bacterial counts (TBCs) during the developmental stage of the MAP process, Compact Dry® plates (HyServe, Uffing, Germany) were used following manufacturer's instructions. If not otherwise stated, 3 packs were analyzed per time point using 5 Compact Dry® plates per pack (3 × 5 samples).

2.6.3. MIA, IA and CFC plates

To obtain more accurate total TBCs as well as the number of H_2S-producing and luminous bacteria marine iron agar (MIA) plates were used and prepared following the method of Gram et al. (1987) with minor modifications (Gram et al., 1987). Instead of iron agar (IA) a marine broth (Difco/BD, Oxford, UK) was used and supplemented with 0.04% (w/v) L-cysteine (Sigma C8755; Sigma-Aldrich, Gillingham, Dorset, UK) and 0.03% (w/v) sodium thiosulfate (Sigma S7026). *Pseudomonas* species agar base (Oxoid, Basingstoke, Hampshire, UK) mixed with Cetrimide–Fucidin–Cephalosporin (CFC) supplement (Oxoid) was used according to the manufacturer's instructions to determine *Pseudomonas* counts in the samples. A series of 10-fold dilutions of the muscle homogenate (up to 10^{-6}) were prepared and 100 µl of each diluent was spread onto the various culture plates and incubated at 20 °C for 48 h, when colony-forming units were counted. Luminous bacterial numbers were determined by counting plates in the dark. If not otherwise stated, per sample point 3 MA packs were analyzed using 5 plates per pack (3 × 5 samples).

2.7. Statistical analysis

The software package SPSS v.15.0 was used for statistical analysis. A Tukey's range test was used to determine statistical differences between sample means and p-values <0.05 were considered statistically significantly different.