Food Microbiology 35 (2013) 99–107

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

# Biodiversity and dynamics of the bacterial community of packaged king scallop (*Pecten maximus*) meat during cold storage

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ARTICLE INFO

*Article history:*
Received 4 October 2012
Received in revised form
22 January 2013
Accepted 14 February 2013
Available online 1 March 2013

*Keywords:*
King scallop
Microbial biodiversity
Modified atmosphere packaging (MAP)
TTGE

ABSTRACT

The microbial biodiversity and dynamics of king scallops meat and coral during cold storage (cold chain rupture: 1/3 storage time at 4 °C followed by 2/3 at 8 °C), was assessed by combining culture-dependant and -independent methods. Products were packaged as follows: aerobic, vacuum packed and 3 different $CO_2/N_2$ modified atmospheres and the impact of these conditions on the microbial communities was assessed. Results indicated that under air (current packaging condition), the dominant species corresponded to *Brochothrix thermosphacta*, *Pseudomonas* spp. and *Shewanella* spp. These species have regularly been associated in the literature with food (especially seafood), and product spoilage. *Moellerella wisconsensis* was the only species detected on VRBG medium, however, its impact on the food product is unclear. Packaging conditions influenced the ecosystem equilibrium and biodiversity. Except for day 8, the lowest counts for all studied flora were observed for modified atmosphere packaging (MAP) containing >80% $CO_2$. Moreover, in these conditions, higher biodiversity by Temporal Temperature Gradient Gel Electrophoresis (TTGE) and the non-detection of specific flora (i.e. *Pseudoalteromonas haloplanktis*) were observed. At day 8, scallops packaged using these conditions were still acceptable from a sensorial point of view (odour), although the initial load of the king scallop was high (total psychrotrophic flora reached 5.5 log CFU/g).

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## 1. Introduction

The king scallop *Pecten maximus* is a valuable bivalve mollusc as it is a highly appreciated food product, especially in France ("coquilles Saint-Jacques"). France produces around 25,000 tons of king scallops per year, a significant portion originate from the Normandy region. A large quantity of scallops is also imported to France and mainly originates from Scotland and North America (*P. maximus* and *Placopecten magellanicus*, respectively). In the context of new food consumption habits, in particular, ready-to-eat foods, a larger proportion of this bivalve mollusc production is sold shelled. King scallop meat is sold either frozen or fresh. As for other seafood products, shelf-life during cold storage is limited; thus, the study of alternative packaging or storage conditions is important.

Like other seafood products, scallop quality during packaging and storage is associated with biochemical and sensorial changes that are mainly affected by storage temperature (Ehira and Uchiyama, 1987; Kawashima and Yamanaka, 1992) and the development of microbial flora that can lead to spoilage (Ocaño-Higuera et al., 2006). Several methods have been used to evaluate the freshness of fish and shellfish. These methods are based on measuring chemical, physical and microbiological changes (Luong et al., 1991; Ohashi et al., 1991; Olafsdottir et al., 1997). The main indices used in relation to fish spoilage (Botta, 1995; Hebbard et al., 1982) correspond to trimethylamine nitrogen (TMA) and total volatile basic nitrogen (TVBN), as well as sensory changes; however, there is very limited data based on the changes observed during mollusc's spoilage and especially king scallops. Indeed, studies have rather been carried out on alterations of closely related species including sea scallops (*P. magellanicus*) (Hilts and Dyer, 1970), queen scallops (*Chlamys opercularis*) (Thomson et al., 1974), yesso scallops (*Patinopecten yessoensis*) (Kawashima and Yamanaka, 1992) and lion-paw scallops (*Nodipecten subnodosus*) (Ocaño-Higuera et al., 2006) or have been based on the characterization of bacterial

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http://dx.doi.org/10.1016/j.fm.2013.02.011

communities during the early life stages of scallops that may have an impact on the survival of bivalve larvae (Lane et al., 1985; Nicolas et al., 1996; Sandaa et al., 2003; Torkildsen et al., 2005).

Studies based on the impact of packaging conditions on bivalve mollusc spoilage during cold storage are rare. Ruiz-Capillas et al. (2001) compared the biochemical and sensory changes of frozen king scallops that were thawed and stored at 4 °C in melting ice to samples wrapped in aluminium foil and cling film. Only few differences were observed between aluminium foil and cling film while the levels TMA and TVBN were low due to the leaching effect of the ice meltwater. Kimura et al. (2000) showed that storage at 5 °C of scallop adductor muscle packaged under air or various $O_2$ and $CO_2$ atmospheres ranging from 20 to 40% had an impact on the bacteria found in the samples (inhibition from 20% $CO_2$) and that 100% $O_2$ allowed to prolong the shelf life by nearly two days in comparison to aerobic conditions.

Finally, the microflora associated with king scallops and their respective spoilage potential as well as the impact of packaging on the microbial diversity is not well documented. Thomson et al. (1974) indicated that the spoilage flora of the queen scallop (*C. opercularis*) was similar to spoilage flora found in fish. While Llanos et al. (2002) showed that in the Peruvian scallop (*Argopecten purpuratus*), the initial flora was associated with culture conditions, farming localization and the feed used. Moreover, this flora would have a direct impact on spoilage. Bremner and Statham (1983) showed that *Vibrio* spp. were the dominant flora and persisted in vacuum packed *Pecten alba* scallops.

This study aimed at evaluating the microbial diversity of French king scallops meat and coral during cold storage and the impact of various packaging conditions on the bacterial ecosystem. The different modified atmosphere conditions were selected to test for their potential inhibiting properties towards spoilage bacteria at temperatures encountered by the consumer during food storage. Indeed, practical industrial studies tend to suggest that TVBN content is lower in $CO_2$ rich environments for some seafood products. Both microbiological and molecular tools were used to follow and identify the bacterial communities during cold storage. To our knowledge, this is the first study on the bacterial community of king scallops using microbial and molecular tools.

## 2. Materials and methods

### 2.1. Sample preparation and bacterial numerations

King scallops (*P. maximus*) harvested in the Basse-Normandie region were obtained just after shelling (meat and coral) on ice. They were then placed into trays (6 King scallop meats with coral per tray) and packaged under different conditions using a Multivac Galaxy TS 355 semi-automatic tray sealer (Multivac, France) with 150 μm thick polyethylene film. A total of 5 different modified atmosphere packaging conditions were tested for each sampling date (day D1, D4, D6 and D8) and corresponded to air conditions, vacuum packed and three different modified atmospheres (50% $CO_2$/50% $N_2$, 80% $CO_2$/20% $N_2$, 100% $CO_2$). A 3:1 ratio of modified atmosphere to scallops was obtained in the sealed trays. The different modified atmosphere conditions were selected to test for their potential inhibiting properties towards spoilage bacteria at temperatures encountered by the consumer during food storage.

A cold chain rupture, 1/3 storage time at 4 °C and 2/3 at 8 °C, was applied to each sample to mimic conditions encountered by the consumer during cold storage according to the guidelines given for best before date determinations (Norme NF V01-003). Microbial sampling was performed in triplicate. For each sampling date and packaging condition, the 6 King scallop meats per tray were separated into 3 samples (2 meats and coral) to obtain 3 sample repetitions. Then, for each repetition, 2.5 g of each meat and 2.5 g of each coral, corresponding to 10 g in total per repetition, were placed in a sterile stomacher bag and homogenized for 2 min in 90 ml tryptone salt buffer using a stomacher (AES laboratories, France). In order to numerate total aerobic flora, homogenates were serially diluted and plated on modified Long and Hammer's medium (van Spreekens, 1974), then, incubated for 5 days at 15 °C. For enterobacteria, serial dilutions were plated onto Violet Red Bile Glucose (VRBG) agar (AES, France) and incubated for 48 h at 30 °C. *Pseudomonas* spp., were plated and enumerated on Cephalosporine Fucidine Cetrimide (CFC) medium (AES, France), lactic acid bacteria (LAB) on De Man Rogosa Sharpe (MRS, pH 6.4) (AES, France), *Brochothrix thermosphacta* colonies were enumerated on Streptomycin Thallous Acetate Actidione (STAA) agar (Oxoid, France) and $H_2S$ producing bacteria on Iron Agar (IA) (tryptone 20 g/L, NaCl 5 g/L, beef extract 3 g/L, yeast extract 3 g/L, ferric citrate 0.3 g/L, sodium thiosulfate 0.3 g/L and agar 12 g/L). CFC, MRS, STAA and IA plates were incubated at 25 °C for 48 h, 5 days, 48 h and 72 h, respectively. For each Petri dish containing $n < 300$ and at each sampling date, the square root of the total number of colonies were selected according to morphological, microscopic and biochemical aspects (sampling was performed according to morphotypes, Gram-staining, catalase and oxidase testing to ensure proper species representation). Overall, 311 isolates were selected for further study using molecular tools. In parallel, the king scallop homogenates used for microbiological analyses were filtered on Minisart 5 μm filters (Sartorius, France) and kept frozen at −20 °C for latter use in Temporal Temperature Gel Electrophoresis (TTGE) analyses. Indeed, previous studies performed by our laboratory have shown that there is no impact on TTGE results after freezing samples at −20 °C (data not shown).

### 2.2. Culture conditions

All representative strains were cultivated in tryptic soy broth (TSB, AES) supplemented with 2.5 g/L yeast extract (TSBYE) except LAB which were cultivated in MRS broth (AES, France). All isolates, except LAB, were incubated at 25 °C for 24 h under agitation. LAB were incubated at 30 °C for 24–48 h. Strains were finally conserved in cryotubes with 30% v/v glycerol at −80 °C.

### 2.3. Isolate M13-PCR grouping and identification

#### 2.3.1. Preparation of template DNA

DNA was extracted from bacterial cultures grown to stationary phase using 1 ml of culture with the NucleoSpin Tissue Kit (Macherey Nagel, France) according to the manufacturer's instructions for bacteria. Purified DNA samples were stored at −20 °C.

#### 2.3.2. PCR amplification

For strain grouping, genetic profiles were generated for each isolate as described by Guinebretière and Nguyen-The (2003). Briefly, 1 μl purified DNA (~50 ng) was used for each reaction in the presence of 2.0 μM M13 primer (5′-GAGGGTGGCGGCTCT-3′), 400 μM dNTP in the presence of 1.25U *Taq* polymerase (5 PRIME, Germany). Amplification conditions were as follows: 95 °C 5 min, 45 cycles of 95 °C 1 min, 36 °C 1 min, 72 °C 4 min.

For strain identification, amplification and sequencing of a 1533 bp 16S rRNA gene fragment was carried out using the universal primers BSF8 and BSR1541 (Edwards et al., 1989) in the presence of ~50 ng DNA, 0.2 μM each primer, 1.5 mM $MgCl_2$, 200 μM dNTPs and 1U *Taq* polymerase (Invitrogen, France). Amplification conditions were 94 °C for 5 min, 30 cycles of 94 °C for 45 s, 59.5 °C for 45 s, 72 °C for 2 min with a final extension at 72 °C for 5 min. All PCR were performed in a Master Gradient thermocycler (Eppendorf, France).

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