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Effect of vacuum and modified atmosphere packaging on the microbiological, chemical and sensory properties of tropical red drum (*Sciaenops ocellatus*) fillets stored at 4 °C

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Abstract :

Aims

The effect of vacuum (VP – 4 °C) and CO2/N2–atmosphere (MAP – 4 °C) packaging on the quality of red drum fillets compared with whole gutted iced fish was investigated.

Methods and results

A metagenomic approach, bacterial enumeration and isolation, biochemical and sensory analyses were carried out. The organoleptic rejection of whole fish was observed at day 15 whereas VP and MAP fillets appeared unacceptable only after 29 days. At these dates, total mesophilic counts reached 107–108 CFU g− 1. According to Illumina MiSeq sequencing, *Arthrobacter, Chryseobacterium, Brevibacterium, Staphylococcus* and *Kocuria* were the main genera of the fresh red drum fillets. At the sensory rejection time, lactic acid bacteria (LAB), particularly *Carnobacterium sp*., dominated the microbiota of both types of packaging. The pH value of fresh samples was between 5.96 and 6.37 and did not vary greatly in all trials. Total volatile basic nitrogen (TVBN) and trimethylamine (TMA) concentrations were low and not represent reliable indicators of the spoilage, contrary to some biogenic amines (cadaverine, putrescine and tyramine).

Conclusion

Chilled packed fillets of red drum have an extended shelf-life compared to whole gutted iced fish. Overall, few differences in sensory and microbial quality were observed between the VP and MAP

samples.

Significance and impact of the study

Next-Generation Sequencing (NGS) provided data on the microbiota of a tropical fish.

Highlights

► A polyphasic approach to characterize the microbial ecosystem of red drum is used ► At day 0, less common genera (*Chryseobacterium*, *Brevibacterium*, etc.) dominated ► Chilled packed fillets had a longer shelf-life than whole gutted iced fish ► Packaging favored the dominance of the LAB (particularly *Carnobacterium* spp.) ► Cadaverine, putrescine and tyramine could be good indicators of the fillets spoilage

Keywords : Seafood, Biogenic amine, VP, MAP, NGS, 16S rRNA gene

1. Introduction

Red drum (*Sciaenops ocellatus*) culture began in the late 1970s and now represents a worldwide production of around 70,000 tons, mainly in China and then the USA (FAO, 2014). In France, this species is one of the main farmed marine fish with farms being mainly located in the overseas departments and territories. Currently, the annual production of Martinique (approximately 40 tons) remains lower than the potential maximum yield estimated at 200 tons. The usual form of commercialization is iced whole gutted and scaled red drums but farmers need to develop new products like fillets to gain the local markets (Falguière and Buchet, 2002).

In the study of Li et al. (2013b), the shelf-life of ice-stored red drum fillets was 8 days. This is shorter than that of whole fish, which was established as 13–15 days by Fauré (2009) and Régina et al. (2014). The use of vacuum and modified-atmosphere packaging in combination with chilled storage has been found to extend the shelf-life of meagre fillets (Genç et al., 2013; Sáez et al., 2015; Sáez et al., 2014), a fish belonging to the same family (*Sciaenidae*) as red drum, and also fillets of sea bream, sea bass and bogue (Kakouri et al., 1997; Kostaki et al., 2009; Mendes and Gonçalves, 2008), cod (Dalgaard et al., 1993), yellow grouper (Li et al., 2011), rainbow trout (Frangos et al., 2010; Rodrigues et al., 2016) and swordfish (Kykkidou et al., 2009). However, there are no data for packed fillets of red drum.

The proximate composition of fresh red drum flesh is 74–80% moisture, 0.6–2.7% fat, 19–24% protein and 1–1.3% ash and the muscle has a pH value of 6.3–6.8 (Leon et al., 2008; Li et al., 2013b). As for the majority of fish species, these intrinsic properties make the flesh an extremely perishable product due to both microbial development and biochemical reactions occurring during processing and storage

(Andrade et al., 2014). Bacterial growth is generally responsible for sensory deterioration (Dainty, 1996; Gram and Huss, 1996; Shewan, 1971).

The free amino acid content and bacterial composition of the fish influence the production of biogenic amines during storage and three of them (histamine, cadaverine and putrescine) are the most significant to monitor the fish safety and quality (Bulushi et al., 2009). In several studies, biogenic amines accumulation has been correlated with the sensory evaluation and used as chemical indicator (Jørgensen et al., 2000a, 2000b; Kim et al., 2009; Özogul et al., 2002; Veciana-Nogues et al., 1997). Rodríguez-Méndez et al. (2009) developed a multisensory system integrating the amount of biogenic amines to assess the fish freshness.

The main bacteria present in VP and MAP packed fish fillets are H_2S–producing bacteria (including *Shewanella putrefaciens* and *Photobacterium phosphoreum*), *Pseudomonas* sp., lactic acid bacteria (LAB) and *Enterobacteriaceae* (Dalgaard et al., 1993; Frangos et al., 2010; Kostaki et al., 2009; Kykkidou et al., 2009; Li et al., 2011), but also *Brochothrix thermosphacta* (Kakouri et al., 1997). All these microorganisms are often identified as the specific spoilage organism of various fishery products (Gram and Dalgaard, 2002; Gram et al., 2002; Gram, 2009).

In addition to the traditional enumeration on culture media, the microbiota composition can be analyzed by culture-independent methods such as denaturing gradient gel electrophoresis (DGGE) or temporal temperature gradient gel electrophoresis (TGGE). More recently, next-generation-sequencing (NGS), such as pyrosequencing 454 and Illumina MiSeq, has been successfully used to characterize the bacterial ecosystems of various seafoods such as cod and salmon fillets, cold-smoked salmon, cooked shrimp and yellowfin tuna raw steaks (Chaillou et al., 2014; Leroi et al., 2015; Silbande et al., 2016).

The first objective of this study was to investigate the effect of packaging (vacuum and CO_2/N_2–atmosphere) on the shelf-life and quality of red drum fillets from Martinique in comparison with whole gutted iced fish. The second was to monitor in detail the quantitative and qualitative evolution of the microbiota of VP and MAP fillets, using microbiological (culture-dependent and culture-independent techniques), chemical, biochemical and sensory analyses.

2. Materials and methods

2.1. Red drum sampling and storage conditions

2.1.1. First trial: comparison of whole fish and packed fillets

Red drum (*Sciaenops ocellatus*) provided from a fish farm located in the center of the Atlantic coast of Martinique (14°41'2.4''N; 60°54'7.8''W). Fish were caught with a dip-net, immediately placed under ice and prepared (scaling, gutting and filleting). Nine whole fish (approximate weight of 1 kg per fish) and fifteen fillets with skin (approximate weight of 250–300 g per fillet) were received at the PARM laboratory 6 h after harvesting.

The whole fish were stored in a cooler box by alternating a layer of fish placed on the belly with a layer of flake ice and kept in a cold room (4°C). To maintain these samples at 0°C, melting water was drained off and ice was replaced when necessary. Fillets were divided into 2 batches. For the first batch, fillets were vacuum-packed (VP) in 80-µm thick plastic bags (Garcia de Pou, Girona, Spain) made of polyamide/polypropylene with a gas-permeability of 2.78 cm^3/m^2/day for water vapor, 19.95 cm^3/m^2/day for O_2 and 164.87 cm^3/m^2/day for CO_2 using a packaging machine (Multivac, Lagny sur Marne, France). Fillets of the second batch were placed in the same type of plastic bags and packed under modified atmosphere

(MAP, 50% CO_2–50% N_2) using a Meca 500 machine (Mecapack, Pouzauges, France). VP and MAP samples were stored at 4°C. For each sampling date, 3 pieces (whole fish and fillets) were tested for sensory, chemical and bacteriological quality and a mean value of the triplicate results was used as a representative value of the sample. The sampling times were 0, 8 and 15 days.

2.1.2. Second trial: comparison of vacuum and modified atmosphere packed fillets

Twenty-seven red drum fillets were brought back to the laboratory in the same conditions as the first trial. Fillets were VP and MAP (50% CO_2–50% N_2). For this trial, the plastic bags of the MAP samples were replaced by a filmed plastic tray. The properties of the polyamide/polypropylene film (Pechiney, Paris, France) were a thickness of 90 µm and gas permeability (cm^3/m^2/day at 23°C, 50% RH) of 4, 30, 120 and 6 for water vapor, O_2, CO_2 and N_2, respectively. The packed fillets were stored at 4°C. Sensory, chemical, biochemical and microbiological (culture-dependent and culture-independent methods) analyses were carried out just before packaging (day 0) until the fillets were organoleptically unacceptable. A mean value of triplicate results (3 fillets) was used as a representative value of the sample for all the sampling dates (0, 8, 15, 22 and 29 days).

2.2. Sensory analyses

2.2.1. Organoleptic inspection (PARM laboratory)

The degree of freshness of the whole raw fish was assessed with the rating scale method developed by the Ifremer station in Martinique for farmed red drum (Fauré, 2009), based on visual properties. In this study, the gills could not be evaluated because they were removed during the evisceration step. In brief, 2 trained local judges had to score 8 criteria on a 6-point scale, with 0 representing good quality and

6 rotten fish (Table 1). When the mean of these scores (freshness index) was equal to or higher than 2.8, the fish was rejected.

For fillets, the appearance (color, texture, slime formation, etc.) and the odor were described in detail and an overall spoilage score was given to each fillet on a 10-point scale, with 0 representing fresh flesh and 10 rotten flesh.

2.2.2. Spoilage score and odor profiles (Ifremer laboratory)

At each sampling date, 50 g of flesh per fillet were diced. The triplicate were pooled in a single plastic bag (150 g), frozen at -80°C and shipped to the EM^{3}B laboratory (Ifremer, Nantes, France) under the same temperature condition. A sensory session was organized with 13 trained panelists to describe the odors in more detail. This session was carried out in individual testing booths according to the procedure NF V 09-105 (AFNOR, 1995), equipped with a computerized system (Fizz, Biosystèmes, Couternon, France). On the morning of the test, each packet (150 g diced) was thawed, divided into individual portions (20-25 g), placed in plastic bowls with lids and maintained in an oven at 18°C during the session. All products were coded with random 3-digit numbers and served to the panelists in a predefined order to avoid a bias due to the effect of the first group tested. The set of samples was scored by 2 different panelists with a minimum 20-min interval. This minimized the total quantity of red drum flesh for sensory analysis. The panelists had to score on a continuous scale from 0 to 10 (6 being the limit of acceptability) the following appropriate odor descriptors: fish, marine, plant, floor cloth, butter/caramel, rancid, sour/fermented, feet/cheese, red meat/blood. Data were processed by analysis of variance with 2 factors (product, panelist). Principal component analysis (PCA) was performed for the odor profile of samples. The statistical processes were carried out using Fizz 2.50 b 37 software (Biosystèmes, Couternan, France).

2.3. Bacterial counts

A 30-g portion of dorsal muscle without skin was collected from the whole fish and fillets with the most stringent hygienic precautions (12°C-room, disinfection of surfaces and equipment and use of a sterile scalpel). This portion was used to enumerate Total Mesophilic Viable Counts (TMVC), Total Psychrotrophic Viable Counts (TPVC), lactic acid bacteria (LAB), *Brochothrix* sp., *Enterobacteriaceae* and *Pseudomonas* sp., as described by Silbande et al. (2016).

2.4. Biochemical analyses

The pH was measured with a pH-meter (Inolab, Germany) in the mother solution (1/5 diluted flesh) prepared for microbial analysis.

Total volatile basic nitrogen (TVBN) and trimethylamine (TMA) were determined in 100 g of fish using the Conway micro-diffusion method (Conway and Byrne, 1933).

In the second trial, biogenic amines (putrescine, cadaverine, histamine, tyramine, spermidine and spermine) were quantified in 5 g of each sample by high pressure liquid chromatography (HPLC) using a Prominence 20A System (Shimadzu, Kyoto, Japan). The dansyl-chloride derivatization was realized in accordance with Duflos et al. (1999). Peaks were detected with a UV-detector (SPD-20A, Shimadzu) operating at 254 nm.

2.5. Isolation, purification and identification of bacterial isolates

In the second trial, twenty-two bacteria were isolated at day 0 (fresh fillet) and at the sensory rejection time (day 29) of VP and MAP red drum fillets. Isolates were selected by picking colonies with various morphologies from plates: 10 colonies from Plate Count Agar (PCA) or Long and Hammer agar (LH) and 3 colonies from Elliker agar (ELK), Streptomycin Sulfate Thallous Acetate Agar (STAA), Violet Red Bile

Glucose Agar (VRBGA) and CHROMagar *Pseudomonas*. The 66 resulting isolates were purified, characterized and identified as described by Silbande et al. (2016).

2.6. Next-generation sequencing (NGS)

2.6.1. Total bacterial DNA extraction from red drum flesh

At each sampling date of the second trial, the 3 independent mother solutions of the triplicate were pooled in equal proportions and the bacterial DNA was extracted and purified as described by Macé et al. (2012). The concentration and purity of DNA were assessed by the Quant-iT™ PicoGreen® dsDNA assay Kit (Invitrogen, Carlsbad, CA). DNA samples were stored at −20°C and sent to MATIS (Reykjavik, Iceland) for 16S rRNA gene amplification and sequencing.

2.6.2. Bacterial 16S rRNA gene amplification and barcoded sequencing

Bacterial DNA extracted from red drum at each sampling date was analyzed by Illumina MiSeq sequencing. The 16S Tag Sequencing workflow was performed according to the protocol provided by Illumina “16S Metagenomic Sequencing Library Preparation” (www.illumina.com). Modifications of this protocol are mentioned below. *Amplicon PCR:* The DNA samples received were diluted to 10 ng/µl. The variable V3-V4 region of the 16S rRNA gene was amplified with the primers S-D-Bact-0341-b-S-17 and S-D-Bact_0785-a-A-21 (single amplicon of approximately 460 bp). The standard PCR Master mix x1 contained 5 µl of template DNA, 0.5 µl of 10 mM dNTP, 1.25 µl of 10 µM forward primer, 1.25 µl of 10 µM reverse primer, 0.25 µl of Q5 high-fidelity polymerase, 5 µl of 5X Q5 high GC enhancer and 11.75 µl of molecular grade water. The thermocycler program consisted of a denaturation step of 30 s at 98°C, followed by 30 cycles of 10 s at 98°C, 30 s at 52°C and 30 s at 72°C, and a final elongation step of 2 min at 72°C.

PCR clean-up 1: HighPrep™ PCR beads (MAGBIO GENOMICS, Gaithersburg, USA) were used to purify the 16S V3-V4 amplicons from free primers and primer dimers. The PCR products were cleaned according to the MAGBIO protocol (www.magbiogenomics.com).

Index PCR: Dual indices and Illumina sequencing adapters were attached using the Nextera XT Index Kit. The index PCR Master mix x1 contained 23.5 µl of molecular grade water, 10 µl of buffer HF, 1 µl of dNTPs, 0.5 µl of HF Polymerase, 5 µl of Index 1, 5 µl of Index 2 and 5 µl of PCR product DNA. The thermocycler program consisted of a step of 30 s at 98°C, followed by 8 cycles of 10 s at 98°C, 30 s at 52°C and 30 s at 72°C, and a final step of 7 min at 72°C. The PCR products were cleaned as described above.

Library quantification and normalization: The DNA library was validated on a Bioanalyzer DNA 7500 chip (Agilent, Santa Clara, USA) to verify the size of the amplicons. Library quantitation was done by fluorometry using the Quant-iT PicoGreen dsDNA assay Kit (Invitrogen, Carlsbad, CA).

Library denaturing and MiSeq sample loading: Eight pM final loading concentration for the best cluster density and 20% PhiX control was spiked in.

Sequence processing, taxonomic assignment and analysis of diversity: Sequencing data were analyzed using the Qiime 1.9.1 pipeline (Caporaso et al., 2010a). Forward and reverse reads were joined using fastq-join (Aronesty, 2011) and quality filtered using default Qiime parameters (Bokulich et al., 2013). Chimera were detected using USEARCH (Edgar, 2010) and chimera-filtered reads were clustered into operational taxonomic units (OTUs) using UCLUST (Edgar, 2010) against the SILVA SSU database release 119 (Quast et al., 2013) at the 97% identify level, as well as *de novo*. A representative sequence was selected for each OTU and aligned against the

Silva core alignment using PYNAST (Caporaso et al., 2010b). Taxonomic assignment was performed with UCLUST (Edgar, 2010) using the SILVA taxonomy. Beta diversity was computed using the UniFrac method (Lozupone and Knight, 2005) and visualized in three-dimensional PCoA plots using EMPEROR (Vázquez-Baeza et al., 2013). A phylogenetic tree was built using FASTTREE (Price et al., 2010). Sequences have been deposited at the European Nucleotide Archive (ENA) under the project accession number PRJEB20568.

2.7. Statistical analysis

Chemical and cultural enumeration data were analyzed using the software R (version 2.14.0). Descriptive statistics of means, standard deviation, linear regression, two-way ANOVA and Tukey's HSD post-hoc test were applied. A significance level of 5% was used.

3. Results

3.1. Comparison of whole fish and packed fillets

3.1.1. Organoleptic inspection

At day 0, the whole red drums possessed all the characteristics of fresh fish (freshness index = 0, Table 1). After 8 days of iced-storage, the index reached a value of 1.8 due to a strong change in the shape (flat) and the color of the eyes (duller pupil and opalescent cornea), a softening of the flesh and a slight pinkish coloration of the muscle near the spinal column. The whole fish were rejected at day 15 with a freshness index equal to the acceptability limit of 2.8 (mean of the criteria underlined in gray in Table 1).

Fresh fish fillets (day 0) were mainly characterized by a firm texture with a uniform and normal color and a typical marine odor. After 8 days, all samples were of very

good quality and weak changes appeared at day 15. The MAP fillets were slightly discolored but still considered of better quality than VP fillets, which released a weak off-odor and were greenish in color. However, despite these impacts on the sensory quality, these fillets remained acceptable throughout the storage period (data not shown).

3.1.2. Chemical analyses

The pH value of fresh flesh was equal to 5.96 ± 0.01. The flesh of whole fish slightly alkalinized with a final pH of 6.10 ± 0.07 while the pH of VP products fell to a value of 5.87 ± 0.07 at day 15 (data not shown, significant difference with whole fish). The pH of MAP fillets remained more stable (very weak acidification) during the storage, with a final value of 5.92 ± 0.09.

Fig. 1 represents the TVBN and TMA results. At day 0, TVBN concentrations of whole fish and fillets (VP and MAP) were 22.0 ± 4.6 mg-N 100 g^{-1} and 24.0 ± 2.6 mg-N 100 g^{-1}, respectively. A decrease was observed in the whole fish (16.6 ± 1.9 mg-N 100 g^{-1} at day 15) whereas TVBN increased in the packed fillets, particularly in VP fillets where the value at the end of storage was equal to 28.4 ± 0.9 mg-N 100 g^{-1} ($p<0.05$). For all batches, the TMA concentrations dropped from approximately 6 mg-N 100 g^{-1} at day 0 to around 2 mg-N 100 g^{-1} at day 8 and remained stable until day 15.

3.1.3. Bacterial counts

As shown in Fig. 2, mesophilic bacteria (TMVC) were generally higher by 1 Log CFU g^{-1} than the psychrotrophic flora (TPVC) in all samples and throughout storage. At day 15, TMVC and TPVC ranged from 7.6 to 8.4 Log CFU g^{-1} and 6.3 to 7.3 Log CFU g^{-1}, respectively. LAB was the major group of bacteria enumerated in the 3 batches (5.6 to 6.9 Log CFU g^{-1}, depending on the storage), followed by *Brochothrix* sp. (4.8

to 6.2 Log CFU g^{-1}), *Enterobacteriaceae* (4.0 to 5.7 Log CFU g^{-1}) and *Pseudomonas* sp. (2.2 to 3.4 Log CFU g^{-1} at day 15). All bacterial groups/genera grew faster and reached higher counts in VP products than in whole fish and MAP fillets (significant difference, $p<0.05$). At the end of storage, counts of LAB, *Brochothrix* and *Enterobacteriaceae* were slightly higher than in iced whole fish.

3.2. Comparison of vacuum and modified atmosphere packed fillets

3.2.1. Sensory analyses

The results of the organoleptic evaluation performed at the PARM laboratory are presented in Table 2. Fresh red drum fillets (day 0) possessed a uniform bright appearance with a typical slight pink color and a marine/fresh fish odor. Until day 15, few changes were detected and the overall score remained lower than or equal to 4 for the 2 batches. After 22 days of storage, MAP products had a duller color and a softer texture than VP products but the off-odor levels were similar. VP and MAP fillets reached an overall spoilage score of 5.2 ± 0.3 and 5.5 ± 0.5, respectively (no significant difference). VP and MAP fillets were considered unacceptable at day 29 (overall score > 6). A strong discoloration of the flesh was observed. VP samples had a firmer texture than MAP products but released a stronger odor on opening the packaging. The off-odor profiles were obtained in more detail by the sensory panel from Ifremer. Fig. 3 shows the plane 1–3 (60.1% of the inertia) of the PCA performed with the mean scores of each odor descriptor. This plane was chosen because the third axis (15.1%), related to the plant characteristic, enabled a better discrimination of the VP and MAP fillets than the second one (19.1%). The first axis (45.0%) discriminated unspoiled samples (days 0 and 8) with fish and marine characteristics (left side of PCA) from more spoiled samples (days > 8) with slight off-odors such as sour/fermented and butter/caramel (right side). VP fillets were characterized by a

plant odor whereas MAP samples released a mixture of various slight odors such as butter/caramel, floor cloth and sour/fermented criteria.

3.2.2. Biochemical analyses

The initial pH (day 0) was equal to 6.37 ± 0.11. This value was fairly stable throughout the storage and no significant difference ($p > 0.05$) was observed between the 2 batches (data not shown). A very small production of TVBN was observed in samples with maximum concentrations of 18.5 ± 1.0 mg-N 100 g^{-1} and 16.6 ± 1.0 mg-N 100 g^{-1} for VP and MAP samples, respectively (data not shown). The TMA content was equal to 0.7 ± 0.2 mg-N 100 g^{-1} at the beginning and stabilized at around 2–3 mg-N 100 g^{-1} from the 15th day for VP products and from the 22nd day for MAP products (data not shown).

Fig. 4 shows the production of biogenic amines during storage. Putrescine and cadaverine were the major amines produced, particularly in VP samples where they respectively reached 95 ± 15 mg kg^{-1} and 93 ± 16 mg kg^{-1} after 29 days, versus 70 ± 12 mg kg^{-1} and 48 ± 8 in MAP samples. Tyramine, absent at day 0, increased from day 15 in VP and MAP products and reached 25 ± 5 mg kg^{-1} and 19 ± 6 mg kg^{-1}, respectively, at the end of storage. Among the six biogenic amines, only spermidine and spermine were present in the fresh fillets (day 0) at a respective level of 8 ± 1 mg kg^{-1} and 15 ± 2 mg kg^{-1} but no significant production was recorded (data not shown). Histamine was never detected in the red drum flesh.

3.2.4. Bacterial counts

The initial quality of fillets was poor with TMVC equal to 4.9 ± 0.1 Log CFU g^{-1} (Fig. 5). The TPVC was around 1 Log CFU g^{-1} lower ($p<0.05$) but increased more rapidly than TMVC with both counts reaching their maximum concentration of 7.5–8 Log CFU g^{-1} at day 15 in VP and MAP samples. The other enumerated bacteria were

present at a level of 3–4 Log CFU g^{-1} at the beginning of storage. The LAB count increased rapidly up to 7.5 Log CFU g^{-1} at day 15 and this group was predominant in both products (no significant difference), reaching around 8.0 Log CFU g^{-1} at the end of storage. *Brochothrix* sp. also developed very quickly in the VP and MAP fillets and reached 6.9 ± 0.2 Log CFU g^{-1} and 7.3 ± 0.1 Log CFU g^{-1}, respectively. The *Enterobacteriaceae* developed more slowly, except during the last week for VP storage when this family increased from 6.9 ± 0.4 Log CFU g^{-1} to 8.0 ± 1.0 Log CFU g^{-1}. The *Pseudomonas* sp. count remained below 5.8 Log CFU g^{-1} throughout the analysis period. Overall, slightly higher counts of total mesophilic and psychrotrophic bacteria, LAB and *Brochothrix* spp. were observed in MAP samples and conversely for *Pseudomonas* sp. and *Enterobacteriaceae*.

Sixty-six isolates (22 at day 0 and 22 at each sensory rejection time of VP and MAP products) were identified by the 16S rRNA sequencing gene (see Table 1 in Ref [Silbande and Leroi, 2017]). They were mainly *Pseudomonas* spp. (*azotoformans*, *plecoglossicida/monteilii*, *fluorescens*, *gessardii*, *poae/simiae/trivialis*, *psychrophila/fragi*) (23% of the isolates), *Enterobacteriaceae* (*Hafnia paralvei*, *Rahnella aquatilis*, *Serratia liquefaciens*-like and *S. myotis*) (20%), *B. thermosphacta* (20%) and LAB (*Carnobacterium divergens*, *C. maltaromaticum*, *Leuconostoc gelidum*) (12%). The others were found in smaller proportions: *Shewanella* spp. (*baltica*, *morhuae/glacialipiscicola*) (6%); *Acinetobacter soli* (3%); *Aeromonas salmonicida*, *Arthrobacter protophormiae*, *Paenibacillus glucanolyticus, Paracoccus yeeii*, *Psychrobacter fozii*, *Sphingobacterium multivorum* and *Stenotrophomonas rhizophila* (1.5% each).

3.2.5. Illumina sequencing analysis

MAP products at day 15 presented very few reads (240 reads) compared with all other samples (between 30,959 and 66,930 reads) and were excluded from the sequencing analysis. Following this quality check, sequencing of total DNA extracted from 8 samples (day 0, VP: days 8, 15, 22 and 29, MAP: days 8, 22 and 29) yielded a total of 214,735 bacterial 16S rRNA sequence-read counts and 2,972 OTUs. Table 3 summarizes the number of reads and OTUs and the top 15 (total abundance) bacterial genera for the different red drum samples. There were 887 OTUs at the beginning of the experiment and this varied between 771 and 1,084 and between 855 and 1,101 during the VP and MAP storage, respectively. At day 0, 128 different genera were identified and the most prevalent were *Arthrobacter* sp. (including *A. psychrochitiniphilus*) (11.9% of abundance), *Chryseobacterium* sp. (10.3%), *Brevibacterium* sp. (including *B. linens*) (8.5%), *Staphylococcus* sp. (6.5%) and *Kocuria* sp. (including *K. rhizophila* and *K. gwangalliensis*) (5.8%). The microbiota composition of the fillets changed with the packaging and, although the number of OTUs remained important, few genera dominated the ecosystem (43 for VP samples and 32 for MAP samples). At day 8, *Brochothrix* sp. were the main bacteria with an abundance of 50.0% and 51.8% of the VP and MAP products, respectively. Other genera such as *Carnobacterium* sp. (VP: 19.6%, MAP: 24.0%), *Pseudomonas* sp. (VP: 8.7%, MAP: 3.5%) and *Shewanella* sp. (VP: 6.3%, MAP: 2.1%) were also detected. The proportion of *Brochothrix*, essentially composed of *B. thermosphacta*, decreased over time but remained more abundant in MAP samples (18.1%) than in VP products (9.6%) at day 29. LAB became the major microorganisms at the end of storage with an abundance of 76.3% in VP samples and 72.1% in MAP samples. In the 2 batches, this bacterial group was mainly composed of the genera *Carnobacterium* (including *C. maltaromaticum*, *C. inhibens* and *C. gallinarum*) (VP:

35.5%, MAP: 54.5%), *Vagococcus* (including *V. teuberi* and *V. fluvialis*) (VP: 16.6%, MAP: 7.6%), *Lactococcus* (VP: 9.6%, MAP: 8.3%) and *Leuconostoc* (including *L. gelidum*) (VP: 13.3%, MAP: 0.7%) and to a lesser extent the genus *Enterococcus* (including *E. sulfureus*) (VP: 1.0%, MAP: 0.9%). In both conditions, *Pseudomonas* sp. (including *P. lundensis*) and *Shewanella* sp. (including *S. baltica* and *S. morhuae*) disappeared almost completely during storage. Conversely, some *Enterobacteriaceae*, particularly *Serratia* sp. and *Hafnia* sp., developed in VP and MAP samples, reaching a proportion of 3.8% and 1.3% at day 29, respectively. At the genus level, unclassified bacteria represented less than 10% of sample reads, except for fresh red drum fillets in which they were equal to 13.9%.

4. Discussion

In the present study, the quality of tropical farmed red drum (*S. ocellatus*) stored under different conditions was compared. The preliminary study confirmed the shelf-life of 15 days for the whole gutted fish stored under ice previously established by Fauré (2009) and Régina et al. (2014). These authors also showed variability in this sensory quality between local fish farms, particularly influenced by the composition of the food distributed to the livestock. A shelf-life of approximately 2 weeks is often observed for various lean to medium-fat white fish species. For example, in similar ice-storage whole fish conditions, *Dicentrarchus labrax* (European sea bass), *Otolithes ruber* (tiger tooth croaker), *Epinephelus merra* (wire-netting reef cod) and *Sparus aurata* (sea bream) were rejected after 15 to 18 days (Alasalvar et al., 2001; Jeyasekaran et al., 2005; Paleologos et al., 2004; Sharifian et al., 2011). At the rejection time of whole red drums, mesophilic and psychrotrophic counts reached 8 Log CFU g^{-1}, which is much higher than the 6 Log CFU g^{-1} obtained for spoiled

sciaenids in the study of Jeyasekaran and Sugumar (1997). The decrease in TVBN and the absence of TMA contents indicated that these indices are not reliable to detect the sensory spoilage of whole red drums. A similar result was found for European sea bass in which the change in volatile bases occurred after the rejection point (Castro et al., 2006). This phenomenon may be due to the washing effect of ice (Erkan, 2007; Ola and Oladipo, 2004). The initial pH of red drum (6.0/6.4) was lower than that of other Sciaenids, which have a pH close to neutrality (Genç et al., 2013; Li et al., 2012, 2013a, 2013b). However, the post-mortem pH often depends on various factors such as species, proximate composition of the flesh and constitution of the microbiota (Huss, 1999).

The preliminary study revealed a longer shelf-life of packed red drum fillets, which was studied in more detail in a second trial. The shelf-life of VP and MAP fillets was almost double that of whole fish. In the study of Li et al. (2013b), raw red drum fillets packed in air-proof polyethylene bags and stored at 4 ± 1°C were spoiled from day 8. In the majority of studies, the fillets stored in ice or at chilled temperature have a higher count of bacteria than whole fish and a similar or inferior shelf life (Chytiri et al., 2004a; Hernández et al., 2009; Paleologos et al., 2004; Poli et al., 2006; Taliadourou et al., 2003). However, when fillets are vacuum or modified atmosphere packed, a shelf-life extension of several days is often observed (Arashisar et al., 2004; Ayala et al., 2011; Dalgaard et al., 1993; Genç et al., 2013; Li et al., 2011; Mendes and Gonçalves, 2008; Sáez et al., 2014).

Just after packaging, the total bacterial count of red drum was 4–5 Log CFU g^{-1}, composed by diverse bacteria, mainly belonging to the phyla of actinobacteria (45.4% of abundance), proteobacteria (24.7%), bacteroidetes (12.3%) and firmicutes (11.1%). Gram-positive bacteria represented more than 40% of the identified OTUs

of the fresh red drum fillets. However, the genera frequently cited for fish, such as *Bacillus*, *Clostridium*, *Lactobacillus* and *Corynebacterium*, were absent or at very low abundance (Gram and Huss, 1996; Huss, 1999). Similarly, except for *Arthrobacter* sp., the gram-negative genera usually found in the fresh muscle of marine fish were detected at low levels (*Acinetobacter*, 0.8%; *Photobacterium*, 0.1%; *Pseudomonas*, 0.7%; *Psychrobacter*, 1.8%; *Shewanella*, 0.7%; *Aeromonas*, <0,1%; *Moraxella*, <0,1%) or absent or not identified (*Flavobacterium*; *Vibrio*). *Enterobacteriaceae* (*Serratia*, *Hafnia*, *Esherichia-Shigella*) represented only 2.0% of the microflora. Many strains of *Pseudomonas* (6 isolates, 239 reads) and *Brochothrix* (5 isolates, 598 reads) were isolated while their abundance were lower than *Arthrobacter* (4206 reads) which only one isolate was recovered, certainly due to the culture media and operator selectivity. Other strains (*Enterobacteriaceae*, *Shewanella*, *Psychrobacter*, etc.) were also obtained and will enable the spoilage potential of each strain to be analyzed.

Less common genera, such as *Chryseobacterium* for gram-negative bacteria and *Brevibacterium*, *Kocuria* and *Staphylococcus* for gram-positive bacteria, mainly composed the initial microbiota. However, these bacteria have not been isolated probably due to their difficulty to grow on the culture media used. In the study of Chaillou et al. (2014), which detailed the bacterial diversity of various meat and seafood products by pyrosequencing, *Chryseobacterium* was also part of the top 3 genera found in seafood samples. *Brevibacterium* spp. were isolated long ago from freshwater and sea fish and the Indian Ocean (Crombach, 1972; Johnson et al., 1968; Kazanas, 1966), while *Kocuria* spp. were found more recently in marine sediment (Kim et al., 2004). A high prevalence of *Staphylococcus* spp. (*S. epidermidis* and *S. warneri*) was also observed in Chaillou et al. (2014).

As frequently found in chilled packed seafood (Dalgaard et al., 2003; Franzetti et al., 2003; Leblanc et al., 1997; Leroi et al., 1998; Leroi, 2010; Lyhs, 2002; Paarup et al., 2002; Paludan-Müller et al., 1998), vacuum and modified atmosphere (without O_2) conditions favored the growth of LAB. The results of Illumina MiSeq and bacterial counts were correlated and highlighted the dominance of the LAB (8 log CFU g^{-1}) at the end of storage, particularly of *Carnobacterium* spp. (VP: 9723 reads, MAP: 12920 reads). The spoilage activity of these anaerobes shows interspecies and intraspecies variations and often appears less aggressive in pure culture than in combination with *Enterobacteriaceae* or *B. thermosphacta* (Gram et al., 2002; Joffraud et al., 2006; Leisner et al., 2007; Macé et al., 2013; Mejlholm et al., 2005; Paludan-Müller et al., 1998; Sivertsvik et al., 2002). The unpleasant odors identified on opening the packaging were probably due to a mixture of these species. These off-odors did not persist in the products and were not strongly detected by the sensory panel, probably due to a loss of the volatile compounds before the new packaging and storage at -80°C until the sensory test. The deteriorations in appearance and texture were important limiting factors for the shelf-life of red drum fillets.

Another well-known spoiler of CO_2-packed fish during chill storage is *P. phosphoreum*, responsible for a high level of TMA that contributes to the ammonia-like odors (Dalgaard, 1995a, 1995b; Dalgaard et al., 1997; Emborg et al., 2002; Gram and Dalgaard, 2002; Hansen et al., 2009). The low abundance of this species and others capable of reducing TMAO to TMA, such as some *Enterobacteriaceae*, *Vibrio and Aeromonas* sp., may explain the very weak formation of volatile bases in the VP and MAP fillets (Debevere and Boskou, 1996; Gram and Dalgaard, 2002).

The significant production of cadaverine, putrescine and, to a lesser extent, tyramine could be a good indicator of the spoilage of packed red drum fillets during storage. Similar biogenic amine formation has been reported in white fish flesh such as sea bass fillets (Paleologos et al., 2004), haddock fillets (Fernandes-Salguero and Mackie, 1987), rainbow trout fillets (Chytiri et al., 2004b), whole hake (Ruiz-Capillas and Moral, 2001) and whole sea bream (Koutsoumanis et al., 1999) during ice-storage. Furthermore, the tropical fish fillets (tambacu, hybrid *Colossoma macropomum* x *Piaractus mesopotamicus*) stored in the same conditions as our study (VP, 4°C) presented larger amounts of cadaverine and putrescine (around 2000-2500 mg kg^{-1}) after 6 days (Bottino et al., 2017). *Enterobacteriaceae*, particularly S. *liquefaciens*, are often responsible for cadaverine production while tyramine may be produced by *C. maltaromaticum*. Moreover, the level of putrescine was probably the result of a metabiosis between ornithine-forming LAB (precursor of putrescine) and putrescine-forming *Enterobacteriaceae* (Bover-Cid and Holzapfel, 1999; Dainty et al., 1986; Gram et al., 2002; Jørgensen et al., 2000b; Laursen et al., 2006; Lavizzari et al., 2010; Leisner et al., 1995). Other bacterial groups, identified in this study, possess the capacity to produce putrescine and cadaverine: *Pseudomonas* sp., particularly *P. fluorescens* and *P. putida*, and *Shewanella* sp., especially *S. putrefaciens* and *S. baltica* (Ge et al., 2017; López-Caballero et al., 2001; Özogul and Özogul, 2005; Rodriguez-Jerez et al., 1994). Conversely, *B. thermosphacta* not show a production of these two biogenic amines and even tends to attenuate the potential ability of others bacteria (Casaburi et al., 2014; Fall et al., 2012; Mejlholm et al., 2005; Nowak and Czyzowska, 2011). The absence or negligible production of histamine in MAP and VP samples, respectively, is due to either a low free-histidine content in the flesh (no data available) or the absence of

histamine-producing bacteria such as *Morganella morganii*, *Raoultella* spp. and *P. phosphoreum* (Drancourt et al., 2001; Kanki et al., 2002, 2004; Özoğul, 2004).

In conclusion, chilled packed fillets of red drum present a longer shelf-life than whole gutted iced fish. VP products retain a better appearance than MAP samples at the end of storage. The results of Illumina sequencing provide detailed data on the bacterial ecosystem evolution during the storage of packed red drum fillets and identify the bacterial species that do not grow on culture media. More research is needed to characterize the spoilage potential of the bacteria isolated from red drum and to develop rapid quality control methods for the local fish farming sector.

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List of figures

Table 1: Score and sensory criteria evaluated during the storage under ice (0°C) of whole red drums.

Criteria		Score						
		0	1	2	3	4	5	6
Eye	Color	shiny black pupil	shiny black pupil	duller pupil and transparent cornea	opalescent cornea	gray pupil and milky cornea	white pupil and milky cornea	white pupil and milky cornea
	Form	convex, bulging	convex, bulging	less bulging	flat	concave in the center	concave	very concave
Operculum	Color	silvery, slightly colored with red or brown	silvery, slightly colored with red or brown	silvery, slightly colored with red or brown	browning and blood seepage around eye	yellowish	yellow	excessive yellow
Peritoneum	Integrity	intact	adhesion	no adhesion	cracked	deteriorated	lysed	totally lysed
Belly cavity	Flesh	pre-rigor	firm	elastic	springy	soft	flaccid	very flaccid
	Wall	intact	intact	soft	fragile	perforated	perforated	perforated
Flesh near the spinal column	Adhesion	column breaks and flesh does not come off	column breaks and flesh does not come off	adhesion	less adhesion	no adhesion	flesh comes off easily	flesh comes off very easily
	Color	normal	normal	slight color	pink	red	brown	very brown

* Gray criteria indicate the unpleasant effects found at the rejection time (day 15).

Table 2: Spoilage score and sensory characteristics (appearance, texture, odor) of fresh red drum fillets and each storage condition (VP* and MAP**, 4°C) after 8, 15, 22 and 29 days.

	D0	D8		D15		D22		D29	
	Fresh fillet	VP	MAP	VP	MAP	VP	MAP	VP	MAP
Spoilage score	0.0 ± 0.0	1.5 ± 0.5	2.3 ± 0.3	3.2 ± 0.3	4.0 ± 0.0	5.2 ± 0.3	5.5 ± 0.5	8.0 ± 0.0	7.5 ± 0.0
Appearance	uniform color, slightly pink, bright	uniform color, slightly pink, bright, weak browning of the flesh near the spinal column	weak whitening/yellowing of the flesh, less bright, weak browning of the flesh near the spinal column	weak overall darkening of the flesh, slime formation, browning of the flesh near the spinal column	overall darkening of the flesh, dull, browning of the flesh near the spinal column	weak greening of the flesh, slime formation, strong browning of the flesh near the spinal column	strong darkening and weak greening of the flesh, very dull, strong browning of the flesh near the spinal column	strong discoloration with an overall greenish appearance, a lot of slime formation	strong discoloration with an overall greenish appearance, very dull
Texture	firm	firm	firm	firm	soft	firm	softer	slightly soft	disintegrated
Odor on opening the packaging	marine and fresh fish odors	very weak marine odor	very weak marine odor	cut grass and slightly pungent odors	moderate sweet, sour and amine odors	moderate cut grass odor	moderate sweet and meat odors	strong cut grass odor	moderate amine odor

*VP: Vacuum Packed fillets stored at 4°C;

**MAP: Modified Atmosphere (50% CO_2–50% N_2) Packed fillets stored at 4°C

Table 3: Sequencing information (reads, OTUs, genera) and sequence-read counts of bacterial genera identified by Illumina MiSeq sequencing for fresh fillets and each storage condition (VP* and MAP**, 4°C) after 8, 15, 22 and 29 days.

	Fresh fillet	**VP**				**MAP**		
	day 0	**day 8**	**day 15**	**day 22**	**day 29**	**day 8**	**day 22**	**day 29**
SEQUENCING INFORMATION								
Total number of reads	35265	20155	17628	22151	27398	31808	36661	23669
Total number of OTUs	887	801	771	960	1084	927	1101	855
Number of different identified genera	128	58	40	40	43	77	41	32
IDENTIFIED OTUs (genera-level)								
Carnobacterium	133	3960	5910	10320	9723	7623	14778	12910
Brochothrix	598	10075	6899	3344	2641	16478	11357	4295
Lactococcus	0	180	469	2275	2637	555	3170	1964
Vagococcus	8	375	904	1603	4555	491	1517	1789
Arthrobacter	4206	544	30	7	3	1447	100	16
Shewanella	249	1270	964	93	50	672	1297	280
Leuconostoc	0	6	22	78	3643	51	224	171
Pseudomonas	239	1757	324	86	71	1111	296	64
Chryseobacterium	3642	18	0	0	0	116	0	0
Brevibacterium	3006	12	3	1	0	109	1	0
Serratia	380	51	435	907	759	26	92	263
Staphylococcus	2283	1	0	1	0	38	0	0
Kocuria	2054	10	6	0	1	93	3	0
Deinococcus	1721	4	0	0	0	52	1	0
Enterococcus	18	56	72	206	277	79	433	210
Psychrobacter	623	68	13	5	0	480	23	2
Planomicrobium	6	185	216	228	171	95	197	111
Rhodovulum	1189	2	0	0	0	3	0	0
Geobacillus	2	139	207	220	207	50	111	95
Dermacoccus	969	5	3	0	1	37	1	0
Lactobacillus	11	1	1	786	25	3	47	17

Dietzia	634	1	0	0	0	4	0	0
Rhodococcus	547	7	0	0	0	75	6	0
Iodobacter	0	2	49	71	88	3	266	100
Streptococcus	469	2	6	19	34	2	10	9
Microbacterium	476	3	0	0	0	12	1	0
Propionibacterium	460	0	1	0	0	20	0	0
Acinetobacter	299	17	1	3	34	83	12	0
Halomonas	404	0	1	3	0	11	1	0
Paracoccus	409	1	0	0	0	6	0	0
Pelomonas	385	2	0	0	0	1	0	0
Bradyrhizobium	274	1	0	0	0	12	1	0
Zymomonas	274	0	0	0	0	0	0	0
Epilithonimonas	189	1	0	0	0	55	0	0
Hafnia	88	0	4	13	73	0	0	12
Rhodanobacter	180	0	0	0	0	4	0	0
Aeromonas	14	29	55	11	32	11	19	11
Myroides	0	4	12	5	46	56	45	11
Granulicatella	44	3	4	34	37	2	31	20
Stenotrophomonas	163	1	0	0	0	5	0	0
Brevundimonas	159	0	0	0	0	6	0	0
Proteiniclasticum	157	0	1	0	0	3	0	0
Enhydrobacter	158	0	0	0	0	1	0	0
Silanimonas	150	0	0	0	0	1	0	0
Rothia	143	1	0	0	0	1	0	0
Citricoccus	136	2	0	0	0	6	0	0
Niabella	137	1	0	0	0	4	0	0
Corynebacterium	116	2	0	0	0	7	0	0
Terrabacter	111	0	0	0	0	3	0	0
Luteimonas	108	0	0	0	0	0	0	0
Others (total abundance < 0.1%)	2339	36	48	93	173	155	58	34
Unassigned	4905	1320	968	1739	2117	1650	2563	1285

*VP: Vacuum Packed fillets stored at 4°C;

**MAP: Modified Atmosphere (50% CO_2–50% N_2) Packed fillets stored at 4°C

Fig. 1

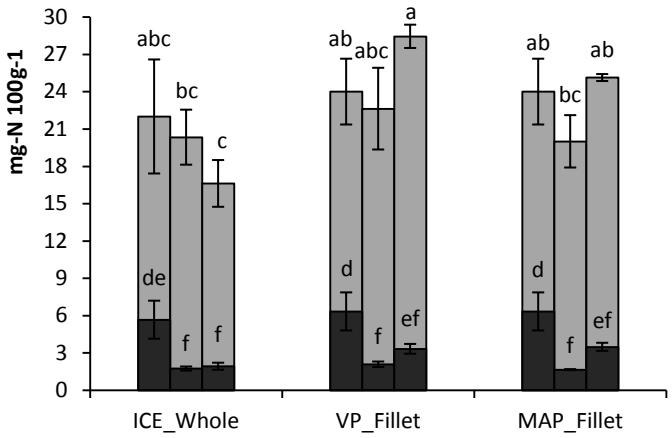

Fig. 2

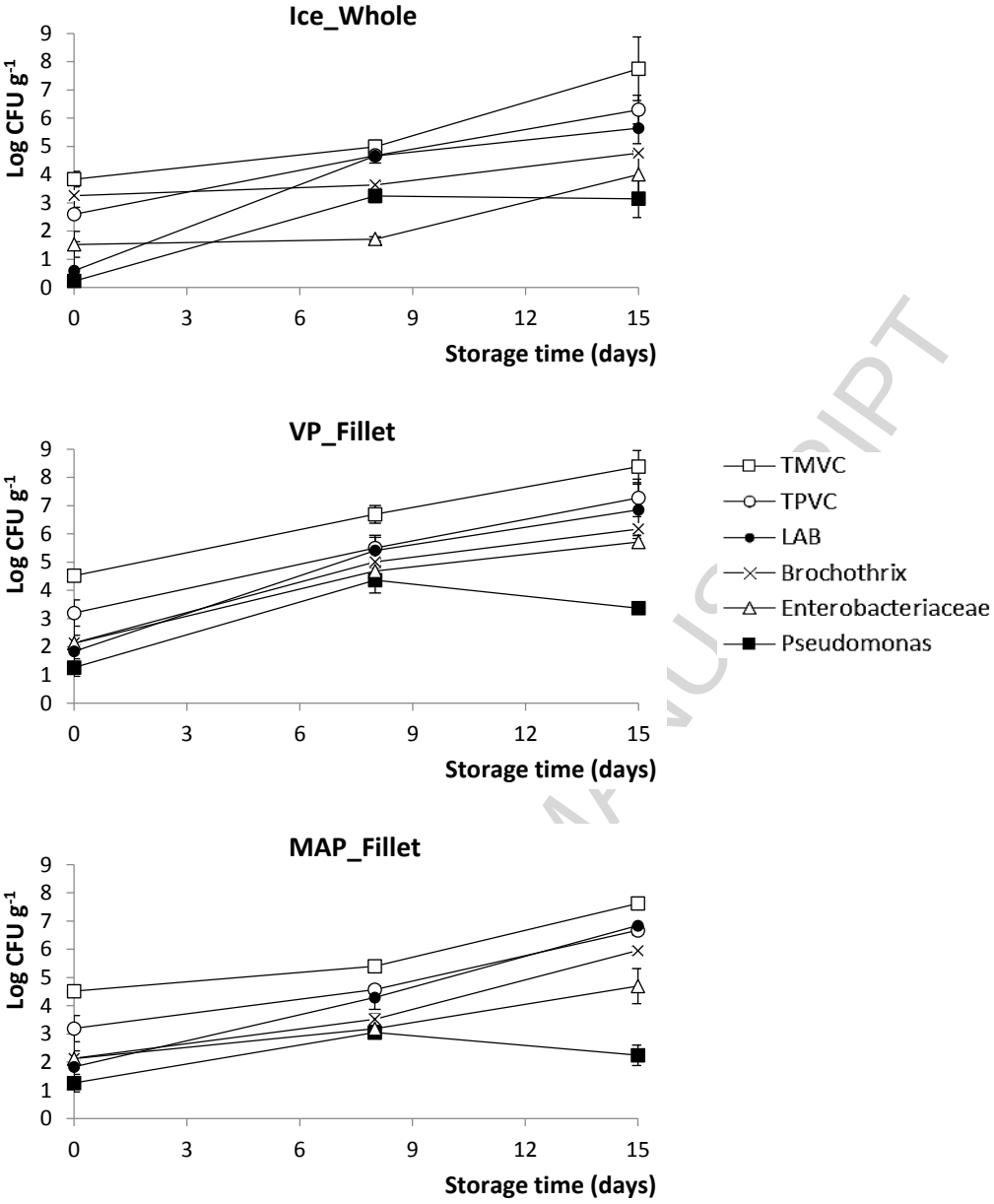

Fig. 3

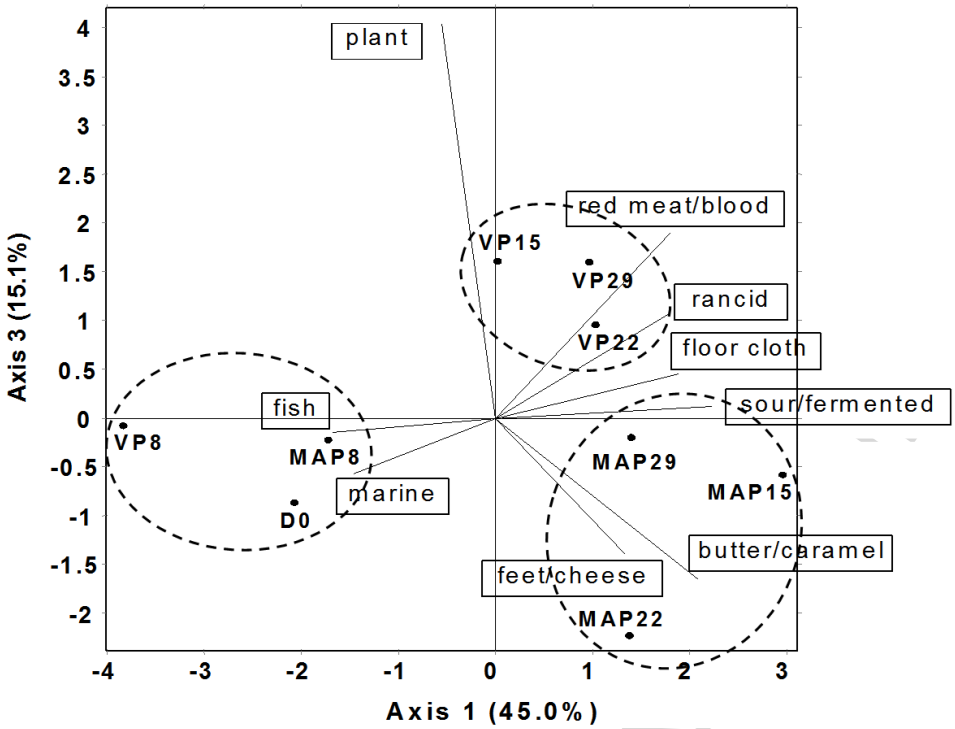

Fig. 4

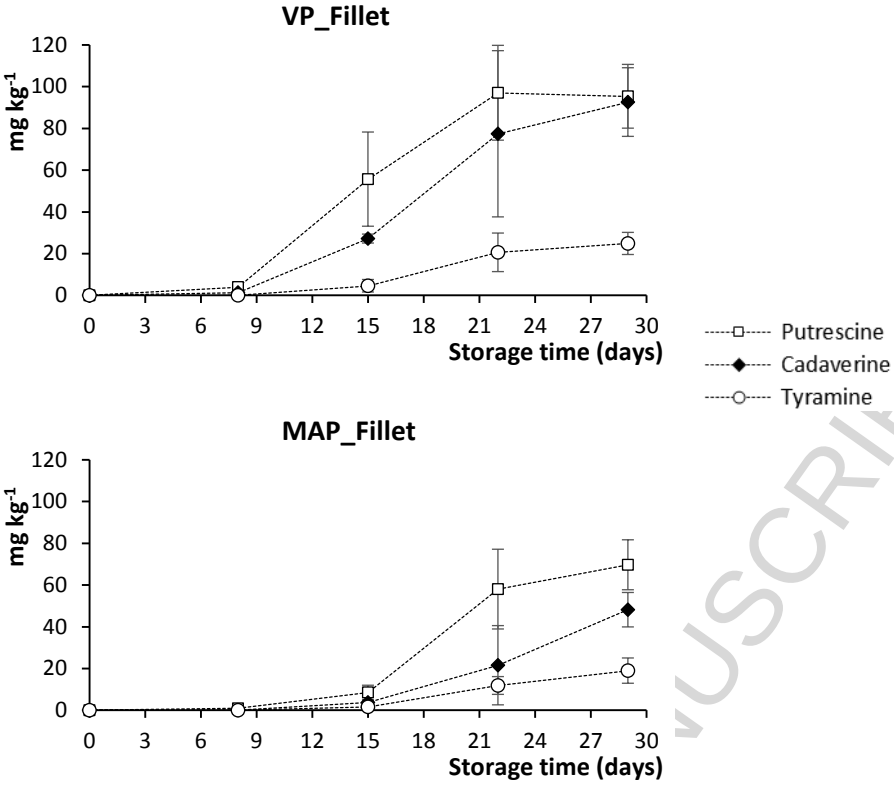

Fig. 5