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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 CO_2/N_2–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 10^7–10^8 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.

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,

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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^3B 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).