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EFFECT OF SLURRY ICE COOLING DURING HARVESTING AND TRANSPORTATION OF EUROPEAN SEA BASS ON FLESH MICROBIAL QUALITY

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Introduction

Post-harvest fish deterioration process is accelerated by increased temperatures, physical damage, and contamination. Therefore, the key to fish preservation is the immediate chilling upon catch or harvest to a temperature slightly above the freezing point and maintaining this temperature throughout the cold chain. Slurry ice is a biphasic system consisting of small spherical ice particles surrounded by seawater at subzero temperature (Cakli et al., 2006). Its reported advantages over traditional fresh-water ice include its lower temperature, faster chilling due to rapid heat exchange, and lower rate of physical damage due to its spherical microscopic particles (Kauffeld et al., 2010). The objective of the study was the evaluation of the effect of slurry ice composition during harvesting and transportation of European sea bass (*Dicentrarchus labrax*) on fish flesh and skin microbiome using conventional and novel "omics" analytical tools that have the capacity to detect non-culturable or poorly characterized microorganisms and emerging bacteria relevant to the quality level and shelf life of fish and fish products (Tsironi et al., 2019).

Materials and methods

Whole European sea bass (*Dicentrarchus labrax*) was harvested from the sea cages in Philosofish S.A. farming facilities (Greece) in slurry ice prepared from seawater, and was transported to the laboratory in polystyrene boxes within 24 h after slaughtering. Three different combinations of slurry ice and conventional flake ice were tested and coded as C: slaughtered and transported in flake ice, SC: slaughtered in slurry ice and transported in flake ice, S: slaughtered and transported in slurry ice. The ratio of ice (slurry or flake) to fish (w/w) was 1:1 and the temperature of the slurry ice was -3.2°C. Upon receipt at the laboratory, all fish samples were stored isothermally at 0±0.2°C. Microbial growth in fish flesh (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria yeasts/molds and *Enterobacteriaceae* spp.) was monitored using conventional culture-based techniques and the experimental data were fitted to the Baranyi growth model. Fish skin microbiome was characterized, analyzing the 16S rRNA gene bacterial diversity from each individual sample, targeting the V3-V4 region. Samples were collected on slaughter day and four days post-slaughter, scraping the skin with different sterile scalpel for each sample. DNA extraction was performed using the PureLink™ Genomic DNA Mini Kit (Thermofisher) with minor modifications. Sequencing of samples was performed by BGI Genomic solution and the operational taxonomic units (OTUs) were filtered and classified using Ribosomal Database Project classifier. Relative abundances of these groups were compared to highlight the differences between sampling days.

Results

Microbial counts increased with storage time, in contrast to the counts of *Brochothrix thermosphacta*, yeasts/molds (<2.0 log CFU/g) and *Enterobacteriaceae* (<1.0 log CFU/g), which remained below the detection limit during the 33-day storage period. TVC, *Pseudomonas* spp. and H_2S-producing bacteria had a similar growth pattern, with the two latter being the dominant bacteria at the end of the storage period, responsible for quality deterioration of whole sea bass. Initial counts of the aforementioned microorganisms were low (i.e. 2.0±0.2, 2.0±0.1 and 1.0±0.1 log CFU/g for TVC, *Pseudomonas* spp. and H_2S-producing bacteria, respectively) and comparable with those reported in the literature for fresh fish stored aerobically (Tsironi et al., 2019).

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Metabarcoding analysis revealed 170 OTUs common between slaughter methods on harvest day, rolling down to 52 OTUs common in all three groups four days post-harvest. Microbial composition revealed *Proteobacteria* (74-96%), and *Bacteroidetes* (1-21%) as the most abundant phyla, in accordance with the current knowledge on the skin microbiome of European seabass (Rosado et al., 2019). On harvest day, *Pseudoalteromonas* and *Marinobacter* were the dominant genus in C and S samples, respectively. Only 19 common OTUs were identified between S4 and SC4 samples that followed different storage (S4: storage in slurry ice and SC4: storage in ice flakes), indicative of the effect of storage conditions on microbiome composition. Storage in slurry ice established *Pseudoalteromonas* as the dominant genus (65%), as opposed to *Psychobacter* (39%) following storage in ice flakes. *Pseudomonas* represented just around 2% of relative abundance on day 4 post-harvest. Significant differentiation in genus composition were observed among the sample groups between the two time points, which was the combined result of slaughter and storage method followed.

Discussion and conclusion

The use of slurry ice as an alternative slaughtering method for farmed European sea bass resulted in a significantly different microbiome composition at slaughter and during storage. The comparison with conventional slaughter in ice flakes indicated that ice flake microbiome may reflect on the start microbiome of the fish and storage can dictate different trajectories in microbiome composition. Microbiome characterization of fish may provide promising new markers for fresh fish quality assessment and optimizing the slaughter and storage methods.

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