International Journal of Food Microbiology 438 (2025) 111233

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Multi-omics signature profiles of cold-smoked salmon from different processing plants: Insights into spoilage dynamics

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ARTICLE INFO

Keywords:
Seafood
Microbiota
Multi-omics
bacteria
Volatile compounds
Sensory analysis

ABSTRACT

Cold-smoked salmon (CSS) is highly susceptible to spoilage due to its processing and storage requirements. This study leverages a multi-omics approach to unravel the complex interactions between microbiota, biochemical changes, and sensory characteristics during the storage of CSS produced in three distinct processing plants. By integrating high-throughput metabarcoding, volatile organic compound (VOC) profiling, biochemical assays, and sensory evaluations, plant-specific spoilage trajectories and molecular signatures that influence product quality were identified. Initial storage phases revealed a shared unspoiled profile across all samples marked by high levels of phenolic VOCs. However, as storage progressed, spoilage pathways diverged depending on the processing plant, driven by variations in microbiota composition and metabolic activity. Distinct bacterial communities, including genera such as *Photobacterium*, *Aliivibrio*, *Carnobacterium*, and *Brochothrix*, shaped the production of spoilage-related VOCs. Statistical analyses using the DIABLO framework uncovered strong correlations between bacterial taxa, volatile organic compounds (VOCs), and sensory attributes, emphasizing the distinct spoilage signatures associated with each processing plant. This study provides new insights into the spoilage mechanisms of cold-smoked salmon by integrating multi-omics data to identify plant-specific microbiota and their metabolic contributions. Beyond identifying distinct spoilage signatures, this study highlights the potential of multi-omics approaches to develop targeted interventions for maintaining product quality.

1. Introduction

The fisheries and aquaculture sector significantly expanded since 1990 and total world fisheries and aquaculture production reached a record 214 million tonnes in 2020 (FAO, 2022). Unfortunately, the FAO estimate that 35 % of this global harvest is either lost or wasted (FAO, 2020) and reducing this phenomenon is urgent to meet the Sustainable Development Goals of the United Nations. Seafood are very perishable products and during their storage the development of microorganisms leads to spoilage. The microbiota of a specific product is influenced by abiotic parameters like the process undergone, the type of packaging (MAP, vacuum, aerobic, etc.) and storage temperature (Sivertsvik et al., 2002). Biotic parameter like endogenous microbiome composition and bacterial interaction occurring during storage also play an important role in off-odours production (Joffraud et al., 2001, 2006; Mace et al., 2014; Macé et al., 2013; Silbande et al., 2016).

Cold-Smoked Salmon (CSS) is a lightly preserved seafood product with a high commercial value. In 2020, its production reached 158,000 t in EU. Poland, France and Lithuania are the main producer of smoked salmon covering respectively 45 %, 11 and 10 % of the EU production. In 2021, French consumer purchase >16,000 tons representing around 564 million euros (France Agrimer, 2022).

In the last decade, omics approaches have revolutionized food microbiology and help scientists to characterize food microbiota and decipher molecular mechanisms linked with bacterial interaction (Borges et al., 2022; Junker et al., 2024; Kergourlay et al., 2015; Zagdoun et al., 2020). High-throughput amplicons sequencing targeting 16S rDNA region have been used to describe and monitor many seafood microbiota during storage for different product like salmon (fresh, smoked and gravlax), shrimps, cod, hake, red drum, yellow fin tuna, mussel, blue crab and cuttlefish (Antunes-Rohling et al., 2019; Chaillou et al., 2015; Maillet et al., 2021; Odeyemi et al., 2019; Parlapani et al.,

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https://doi.org/10.1016/j.ijfoodmicro.2025.111233
Received 7 February 2025; Received in revised form 28 March 2025; Accepted 30 April 2025
Available online 2 May 2025

2018, 2019; Silbande et al., 2016, 2018; Wiernasz et al., 2020, 2021).

CSS core microbiome is composed of cultivable bacteria with an important taxonomic and phenotypic diversity that evolves during storage (Chaillou et al., 2015). It results of an association of bacteria originating from fish and its marine environment but also from *hygienic conditions in the smokehouses*. Recently, specific bacterial signature related to plant processing environment were highlighted by comparing 16S rRNA-based CSS microbiota during the process (Maillet et al., 2021).

A good knowledge of the CSS microbiota and its evolution during storage is necessary to develop strategies to reduce the development of pathogens and delay sensory degradation to limit food waste. Gentle food preservation, like biopreservation, consists of preventing the development of undesirable microorganisms (pathogens or spoilers) by introducing into the product harmless microorganisms selected for their antimicrobial properties. Based on bacterial competition, the efficiency of this technology is thus, closely linked with the food matrix microbiota and the occurring interaction (Macé et al., 2024).

This current work focuses on the characterisation of the CSS signature microbiota of three different processing plants and its impact on the organoleptic and biochemical changes occurring during storage. To achieve this goal, an integrated approach combining multiple techniques like metabarcoding, volatilome analysis, biochemistry assays, classic microbiology and sensory analysis was used on each sample. Sensorial perceptions of seafood expert panellists have also recently been correlated to volatile compounds directly extracted from salmon matrix (Cardinal et al., 2020; Wiernasz et al., 2020). In this study, a statistical approach was performed to draw links between all the different groups of data and highlight the existence of specific multi-omics signatures and their influence on spoilage.

2. Materials and methods

2.1. Cold-smoked salmon sampling

Three batches of cold-smoked salmon slices (*Salmo salar*), processed from three different production plants (France, confidential) named L1, L2 and L3, were purchased. In the three different processing plants, all the samples were beech wood smoked and salted with dry salt. For all batches, the samples were individually packaged under vacuum (two slices per pack) on the production date (day 0) with their expiration date set at day 28. Immediately after production (D0), samples were stored for 8 days at 4 °C and transferred to 8 °C for 21 days of storage, according to the shelf-life evaluation protocol monitored by industrial plants. Sensory evaluation, biochemical and microbiological analyses, including culture and NGS methods, were carried out at 8, 15, 22 and 29 days in triplicate. The composition of each product including dry matter, % fat content as well as the salt and phenol concentration was determined at each sampling point, as described by Leroi et al. (2015). At each time point, the samples designated for sensory evaluation were stored at −80 °C for no longer than two months until the evaluation session, ensuring that samples could be presented in the same session. Previous tests have demonstrated that this freezing process preserves the product's initial characteristics (Alterobio, final report, French regional project, 2022).

2.2. Sensory analysis

The sensory evaluation was performed by an internal trained panel of 11 judges experienced in seafood, especially in CSS (Cardinal et al., 2004; Wiernasz et al., 2017, 2021). A conventional sensory profiling test was conducted on smoked salmon slice, according to ISO 13299 (2016). After the assessment of global spoilage based on off-odour perception, panellists characterized the main properties of the product using a list of sensory descriptors: for odour, smoked, butter, pungent/acid, sour, amine, sulphur and cheese, for appearance, orange colour and fatty surface, for texture, firmness, pasty and fat, and for flavour, smoked, salty, acid, and amine. The global spoilage and all the descriptors were scored depending on their intensity on a continuous scale from 0 (low intensity) up to 10 (high intensity). Three sessions were organised, one for each processing plant. At each session, four samples of smoked salmon, corresponding to each sampling date, 8, 15, 22 and 29 days were presented to the panellists.

The day before the sensory evaluation, samples were thawed overnight at 4 °C. Sessions were performed in individual partitioned booths, as described in procedure NF V-09-105 (ISO 8589, 2007) and equipped with a computerized system (Fizz, Biosystèmes, Couternon, France). Each panellist was given a 1/2 slice of smoked salmon, cut into three strips and rolled up to make it easier to assess the texture. Samples were presented in covered plastic container. Samples were assigned with three-digit numbers and randomized for the order presentation within panellists.

2.3. Chemical analysis

Total volatile basic nitrogen (TVBN) and trimethylamine (TMA) were measured in duplicate by the Conway micro diffusion method (Conway and Byrne, 1933) in 100 g of raw salmon flesh was homogenised in a Waring Blender (New Hartford, CO, USA). The pH value was measured with a pH meter (Mettler Delta, AES, Combourg, France) in the five-fold diluted flesh prepared as described below for microbiological analysis.

Biogenic amines concentrations were determined during storage. Four milliliter aliquots from stomached solution obtained were mixed with 2 mL of a 9 % trichloroacetic acid solution (Panreac, Darmstadt, Germany) was added. Samples were kept frozen at 20 °C until analysis. Putrescine, cadaverine, tryptamine, spermidine, tyramine and histamine were quantified by high pressure liquid chromatography (HPLC) according to the method described by Wiernasz et al. (2017).

2.4. Volatile compounds analysis

The protocol used to determine the volatilome was adapted from Wiernasz et al. (2020) using Headspace Solid-Phase MicroExtraction (HS-SPME) for the extraction of volatile organic compounds (VOCs), and Gas Chromatography (GC) coupled with Mass Spectrometry (MS) for their identification and quantification. Smoked salmon was stored at −80 °C and placed at 4 °C during 24 h before analysis. Sixty grams of smoked salmon were mixed with 40 mL NaCl solution (30 % *w/v*) in a blender (Multi Moulinette, Moulinex, France) during 20 s. Five grams of sample were placed in 20 mL glass vials closed with screw caps equipped with septa. Equilibration between sample and headspace lasted 30 min at 50 °C. Extraction of the volatile compounds was performed with a PDMS/DVB-coated 65 µm fiber (Supelco Inc., Bellefonte, PA) during 30 min at 50 °C. A gas chromatography device equipped with a Flame Ionization Detector (Agilent 7890 A, Wilmington, DE, USA) and coupled to a mass spectrometer (electronic impact source, Agilent 5975CNetwork, Wilmington, DE, USA) was used to analyse VOCs. The inlet temperature was 260 °C, the FID detector temperature 300 °C and the MS detector temperature 280 °C. The carrier gas was helium and the pressure was 150 kPa. The splitless mode was used for the injection, and the desorption time was 10 min. The capillary column was a HP-5 MS (30 m, 0.25 mm, 0.25 µm, Agilent J&W Scientific, Folsom, CA). The program used was 40 °C for 2 min, ramped up to 300 °C at 10 °C/min then equilibrium at 300 °C for 5 min. Effluent from the end of the GC was split 1/1 between the MS and FID. Peaks were integrated with MSD Chemstation software (Agilent Technologies). Mass spectra were recorded in electron impact mode (70 eV) between 33 and 300 *m/z* mass range at a scan rate of 2.7 scan·s^{-1}. The volatile compounds were identified according to 2 criteria: comparison of their Kovats retention index with literature values and comparison of their mass spectra with those of the Wiley 6 library. The semi-quantified results were obtained from the FID

chromatogram and expressed as peak areas. Analyses were performed in triplicate.

2.5. Enumeration of bacterial groups

For each CSS sample, a 30-g portion from a slice was stomached for 2 min with 120 mL of sterile physiological saline solution with 1 % of Tween 80 to obtain a 5-fold dilution. From this CSS solution, several appropriate 10-fold dilutions were carried out in sterile physiological saline solution and 0.1 mL of each was spread-plated. Different bacterial populations were enumerated with appropriate media. Total Psychrotrophic Viable Counts (TPVC) were determined using Long and Hammer agar (LH) supplemented with 1 % NaCl (Van Spreekens, 1974) and incubated at 15 °C for 7 days. Among the TPVC on LH, bioluminescent bacteria were enumerated in a dark room by counting bioluminous colonies. In CSS, these bioluminescent bacteria are mainly *Photobacterium* colonies. Total lactic acid bacteria (LAB) were numbered on Elliker agar (Biokar Diagnostic, Beauvais, France) at 20 °C for 3 days under anaerobic conditions (Anaerocult A; Merck, Darmstadt, Germany), *Brochothrix* spp. on Streptomycin sulphate Thallous Acetate Agar (STAA, Oxoid, Basingstoke England) after 3 days at 20 °C and Enterobacteriaceae in pour plate of Violet Red Bile Glucose Agar (VRBGA, Biokar) incubated for 2 days at 30 °C. Bacterial concentrations were expressed as the decimal logarithm (Log $_{10}$) of colony forming units (CFU) per gram (Log CFU g-1). Each type of samples was enumerated in triplicate and the mean of (Log CFU g-1) was calculated for the triplicate.

2.6. Salmon microbiota metabarcoding analysis

2.6.1. Direct bacterial DNA extraction from the salmon matrix

To separate bacterial cells from salmon matrix, 2 mL of the homogenised sample suspension were first centrifuged 5 min at 400*g* at room temperature. Pellet was discarded and the supernatant, transferred into a new tube, was centrifuged for 10 min at 13,000*g*. The supernatant was removed and the bacterial cell pellet was used for DNA extraction following the DNeasy PowerFood Microbial kit procedure (Qiagen, S.A.) with slight modification of the standard procedure described by (Jérôme et al., 2022).

2.6.2. 16S metabarcoding analysis

Extracted DNA samples were sent to Microsynth company (Balgach, Switzerland) for 16S rDNA V3-V4 region amplicon sequencing. Library preparation was performed by the company targeting the V3-V4 region of the 16S rRNA gene using the primers 341F-5'-CCTACGGGNGGCWGCAG-3′ and 785R-5′- GACTACHVGGGTATCTAATCC-3′ (Klindworth et al., 2013) following the recommended method for 16S rRNA amplicon sequencing on the Illumina platform (Illumina, 2021). The resulting sequences were analysed using SAMBA (Standardized and Automated MetaBarcoding Analyses workflow) (https://gitlab.ifremer.fr/bioinfo/workflows/samba;v4.0.1). SAMBA is a FAIR scalable workflow integrating, into a unique tool, verification of the integrity of raw reads and metadata, and bioinformatics processing using QIIME 2 (Bolyen et al., 2019) and DADA2 (Callahan et al., 2016); it also adds new steps relying on dbOTU3 (Olesen et al., 2017) and microDecon to build high quality ASV count tables (McKnight et al., 2019). The SILVA 138.1 SSU Ref NR 99 database (Quast et al., 2012) was used to assign taxonomy to the amplicon sequence variants (ASVs) using a naive-bayesian classification. Diversity analyses were performed based on CSS-normalized phyloseq object generated by the SAMBA workflow. The species richness and Shannon diversity were used to investigated alpha diversity. Beta diversity analyses were carried out using Non-metric Multi-Dimensional Scaling (NMDS) with Bray-Curtis dissimilarity matrices (Bray and Curtis, 1957; Lozupone et al., 2007) Sequenced data have been deposit on Bioproject accession number on ENA: PRJEB84917.

2.7. Multi-omics integration analysis

Supervised multiblock partial least square-discriminant analysis (sPLS-DA) was conducted to integrate metabarcoding, volatile compounds, microbiology and sensory data using DIABLO framework from the R package "mixOmics" (Rohart et al., 2017). This approach allowed to identify discriminant features in both datasets that drive differences between batches. Prior to the analysis and in order to reduce the background noise, a selection of the most discriminating volatile compounds between plants and affected by time was performed using the VIP approach (Wold et al., 1998) (VIP score > 1). The analyses were carried out only from the most abundant bacterial genera in fish alteration. The "*plotVar*" function was used to visualize the correlation between the key features from each matrix. Correlation between the metabarcoding component and others components was calculated using the "*cim*" function and visualized by a correlation heatmap built using the *ComplexHeatmap* R package. Multi-omics molecular signature expression for each plant was highlighted using the "*cimDiablo*" function of *mixOmics*. The data and script used for this analysis, as well as the raw figures obtained, are available in the following Git repository: https://gitlab.ifremer.fr/bioinfo/bioanalysis/public/salmonics

3. Results

3.1. Chemical analysis

Chemical composition of the samples was close for the three different batches with an average water content of 63.2 ± 2.5 % for L1 61.5 ± 1.3 % for L2 and 65 ± 1.1 % L3 and an average fat content of 10 ± 1.9 % for L1, 11.4 ± 1.9 % for L2 and slightly inferior for L3 with 7.6 % ±1.3 %. The three batches present a similar salt content with respectively 3 ± 0.4 %, 2.9 ± 0.5 % and 3.2 ± 0.4 % for L1, L2 and L3. There is a significant difference in phenol concentration between the three processing plants: L2 samples present the lowest phenol concentration with 0.5 ± 0.2 mg/100 g, followed by L3 with 0.7 ± 0.1 mg/100 g and 0.9 ± 0.1 mg/100 g for L1.

Until the end of the storage where the products have reached the expiration date, TMA and TVBN never exceeded 2.6 mg/100 g and 9.2 mg/100 g in L1 and L3 samples, whereas, their concentration increased during storage and reached up to respectively 7.5 and 29.5 mg/100 g for L2 samples.

Biogenic amine analyses included tryptamine, putrescine, cadaverine, tyramine, histamine, spermidine quantification. For L1 samples, most of the biogenic amine concentration stayed under 27 mg/kg during the storage except for the cadaverine that reached 64 mg/kg at D29 (expiration date reached). For L2 samples, cadaverine and tyramine concentration increased the most during storage and reached respectively 162 and 62 mg/kg. The other biogenic amines stayed above 25 mg/kg. In L3, three biogenic amines increased during storage and reached a concentration of 313 mg/kg for cadaverine, 95 mg/kg for tyramine and 165 mg/kg for histamine while tryptamine, putrescine, and spermidine concentration stayed under 10 mg/kg.

3.2. Sensory characteristics of smoked salmon

The results of the analysis of variance carried out on scores of each product and each sensory attribute given by the 11 panellists showed that the products are significantly different on eleven descriptors ($p < 0.10$): seven on odour description, one on texture and three on flavour. Comparison of F values for the product effect showed that spoilage odour had the highest score followed, in decreasing order, by amine flavour, pasty texture, acid flavour, smoked flavour, sour odour, smoked odour, sulphur odour, amine odour, pungent odour and cheese odour (data not shown).

The first plane of the standardized Principal Component Analysis (PCA) accounted for 82.6 % of the total information (Fig. 1). The first

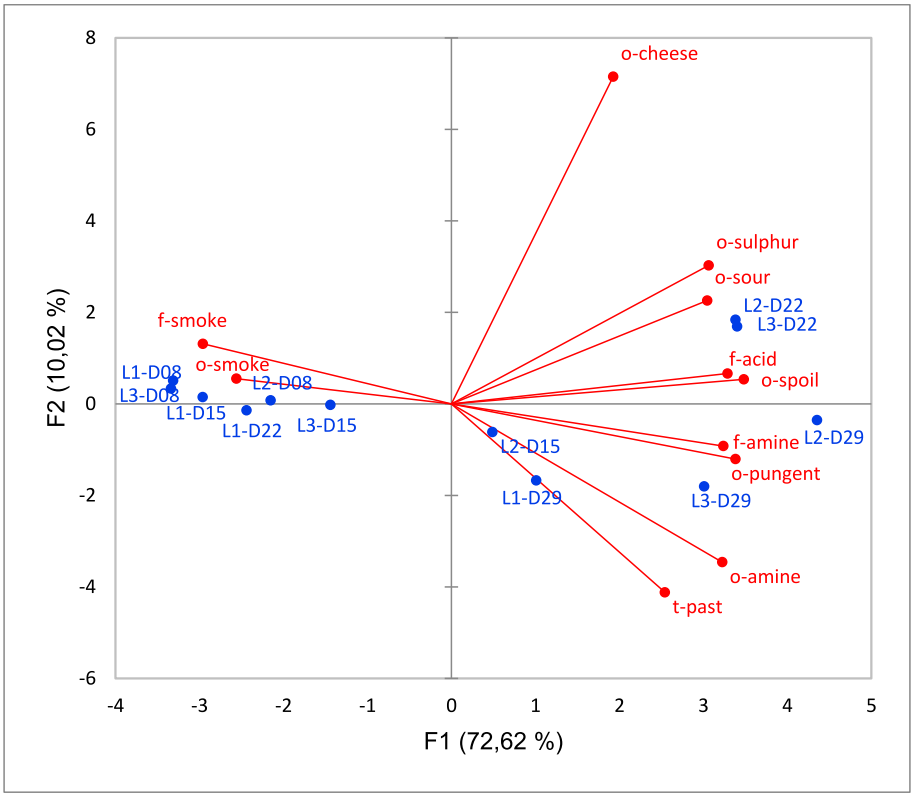

Fig. 1. Representation of sensory descriptors and samples on the first two dimensions of PCA. Labels: o = odour; f = flavour; t = texture.

axis (72.6 % of total inertia) was mainly created by the criteria smoked odour and flavour on the negative part of the axis, amine odour and flavour, acid flavour, sour, sulphur and pungent odour on the positive part of the axis. The first axis was clearly an axis of spoilage. Pasty texture and cheese odour were mostly involved in the creation of the second component (10.0 % of the inertia). A clear discrimination between samples appeared on this plane. Along axis 1, two groups of products were separated, according to the smoked odour and flavour odour on one part as well as the global intensity of spoilage and specific characteristics of spoilage (amine note, sour odour, pungent odour, acid flavour) on the other part. Almost all the samples at the beginning of storage (D08, D15), were gathered on the left part of the figure. These products still presented characteristics of fresh smoked salmon. Only the L2 product, after 15 days of refrigerated storage, differs from this group. This sample was closer to the products with spoilage characteristics. This group of spoiled products mainly included D22 and D29 samples from plants L2 and L3. Only the products from plant L1 seems to stand out, with a much less marked deterioration in quality than the other two samples.

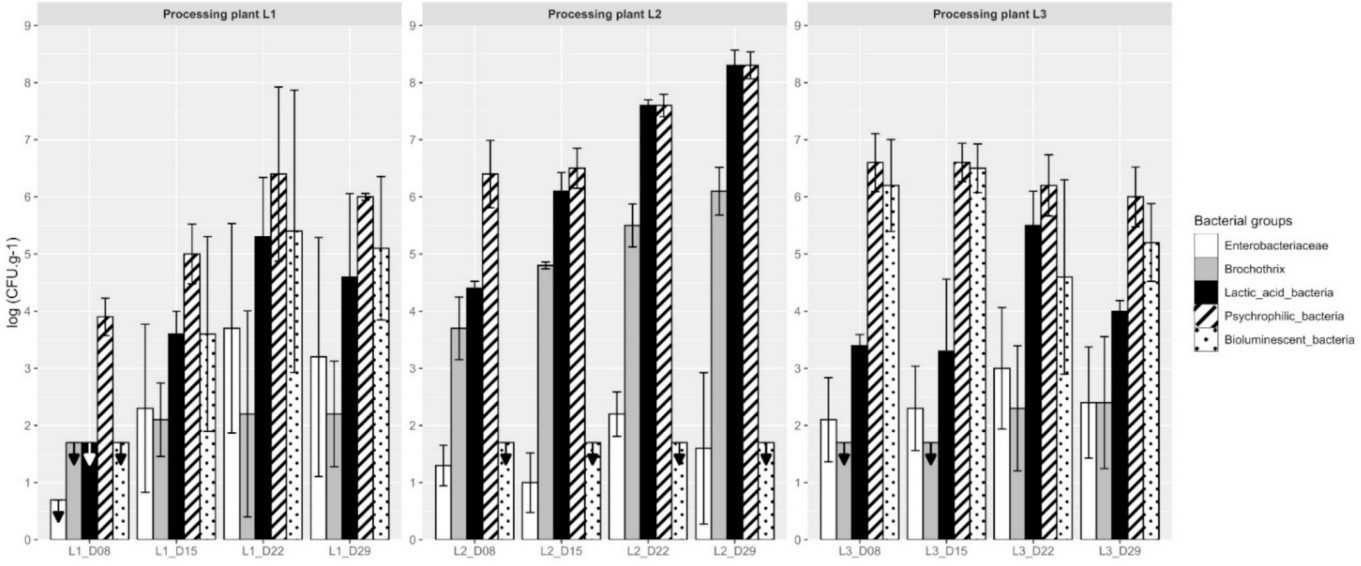

Fig. 2. Bacterial groups concentration of the different processing plants samples during storage. Black/white arrow symbol represents the results under the limit of plate counting quantification. Results are expressed as means of (Log CFU g-1) and standard deviation of 3 biological replicates.

3.3. Enumeration of the different bacterial group

Concentration of the different bacterial group during the storage are presented in Fig. 2. For processing plant L1, the TPVC after 8 days of storage did not exceed 4 Log (CFU.g^{-1}) and reached around 6 Log CFU.g^{-1} at the end of storage. At D08, LAB, bioluminescent bacteria and *Brochothrix* count were below the enumeration threshold (1.7 Log (CFU.g^{-1}) as well as Enterobacteriacea counts (0.7 Log (CFU.g^{-1})). They reached their maximum level at D22, with respectively, 3.7 Log (CFU.g^{-1}) for *Enterobacteriaceae*, 2.2 Log (CFU.g^{-1}) for *Brochothrix*, 5.3 Log (CFU.g^{-1}) for LAB and 5.4 Log (CFU.g^{-1}) for bioluminescent bacteria. At the end of the storage, these concentrations present a small decrease of 0.3 to 0.7 Log (CFU.g^{-1}) except for *Brochothrix* count.

In processing plant L2 samples, TPVC concentration already reached 6.5 Log (CFU.g^{-1}) at D08 and rise at a maximum level of 8.3 Log (CFU.g^{-1}) at the end of storage, LAB level increase from 4.4 Log (CFU.g^{-1}) at D08 to 8.3 Log (CFU.g^{-1}) at D29. *Brochothrix* count also increased from 3.7 to 6.1 Log (CFU.g^{-1}) at the end of storage. *Enterobacteriacea* present a maximum concentration of 2.2 Log (CFU.g^{-1}) at D22. Bioluminescent bacteria counts were below the enumeration threshold (1.7 Log (CFU.g^{-1}) all along the storage.

For processing plant L3, TPVC also reached around 6.5 Log (CFU.g^{-}1) after 8 days of storage and decrease a little to a level of 6 Log (CFU.g^{-1}) at the end of storage. Bioluminescent bacteria present >6 Log (CFU.g^{-1}) at D08 and decrease also to reach around 5 Log (CFU.g^{-1}) at the end of storage. During the storage, LAB and *Enterobacteriacea* increased respectively from 3.4 and 2.1 Log (CFU.g^{-1}) at D08 to a maximum of 5.5 Log (CFU.g^{-1}) and 3 Log (CFU.g^{-1}) at D22. At D29, their level decreased and *Brochothrix* count were below the enumeration threshold of 1.7 Log (CFU.g^{-1}) until D22 but stays under 2.5 Log (CFU.g^{-1}) until the end of storage.

3.4. Microbiota

Illumina-based amplicon sequencing of the V3-V4 region of the 16S rRNA yielded a total of 1,811,766 reads, of which 98 % (1,759,605) were retained after quality filtering with an average of 48,881 ± 21,335 reads per sample. From these high-quality reads, 468 ASVs (amplicon sequence variants) were inferred and taxonomically assigned: 387 (83 %) at the genus level. The repartition of the ASVs among the processing plants showed that 383 ASVs are only specific to one processing plant with respectively, 224 ASVs specific to L1, 52 ASVs to L2 and 107 ASVs to L3. On the opposite, 85 ASVs are commonly shared by several processing plants, with respectively 54 ASVs shared between two processing plants and 31 ASVs present in the three plants. The alpha diversity based on the Shannon diversity index presented the lower values for L1 and L3 samples with respectively 1.63 and 1.61 and L2 samples showed a slightly higher (not significant) index value of 1.90.

Overall at the genus level (Fig. 3), the composition of the bacterial communities within the L1 and L3 processing plants is mainly dominated by *Photobacterium* (on average 77 %) and *Aliivibrio* (on average 18 %). For these two processing plants, the taxonomic profile does not vary according to the time of storage. For L2 samples, early storage samples (D08) are also dominated by *Photobacterium* (average abundance >99 %) but from D15 a taxonomic composition switch is observed in favour of three LAB genera such as *Carnobacterium*, *Latilactobacillus* and

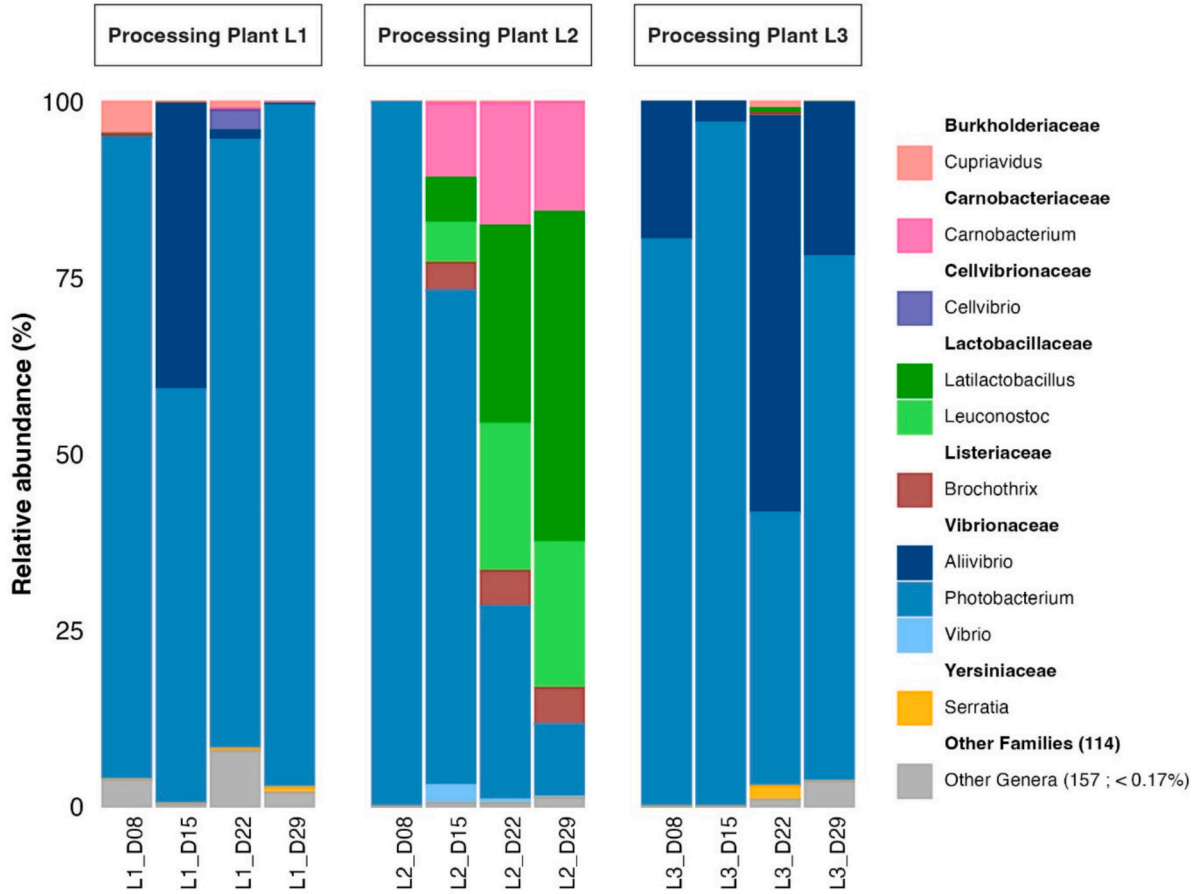

Fig. 3. Relative abundance of the ten dominant bacterial genera associated with the microbiota of CCS samples from different processing plant during refrigerated storage. For each condition, the replicates were merged as no significant differences were observed between them (PERMANOVA, p-value = 0.923). Other Genera (grey bar) represent 157 genera with a relative abundance <0.17 %.

Leuconostoc which became dominant at D22 and D29. *Brochothrix* also appears in all L2 samples from D15 to the end of storage. It is also worth noting the presence of *Vibrio* among the top 10 dominant genera identified. This genus is completely absent in L1 samples and appears only at D29 in L3 samples (relative abundance <0.1 %). In L2, *Vibrio* is present at all storage times but in low abundance (<0.5 %) at D08, D22, and D29. In contrast, at D15, it represents approximately 3 % of the community.

3.5. Volatile profile

The 97 semi-quantified volatile organic compounds (VOC) were present in all the samples but with various quantities (Supplementary data 1). Fifty-two volatile compounds were identified among which 33 were already identified in smoked salmon in the literature (Supplementary data 2). The volatile compounds belong to several chemical classes such as phenolic compounds, alcohols, alkanes, terpenes, furans, cyclopentanones etc. Phenolic compounds are the most numerous and

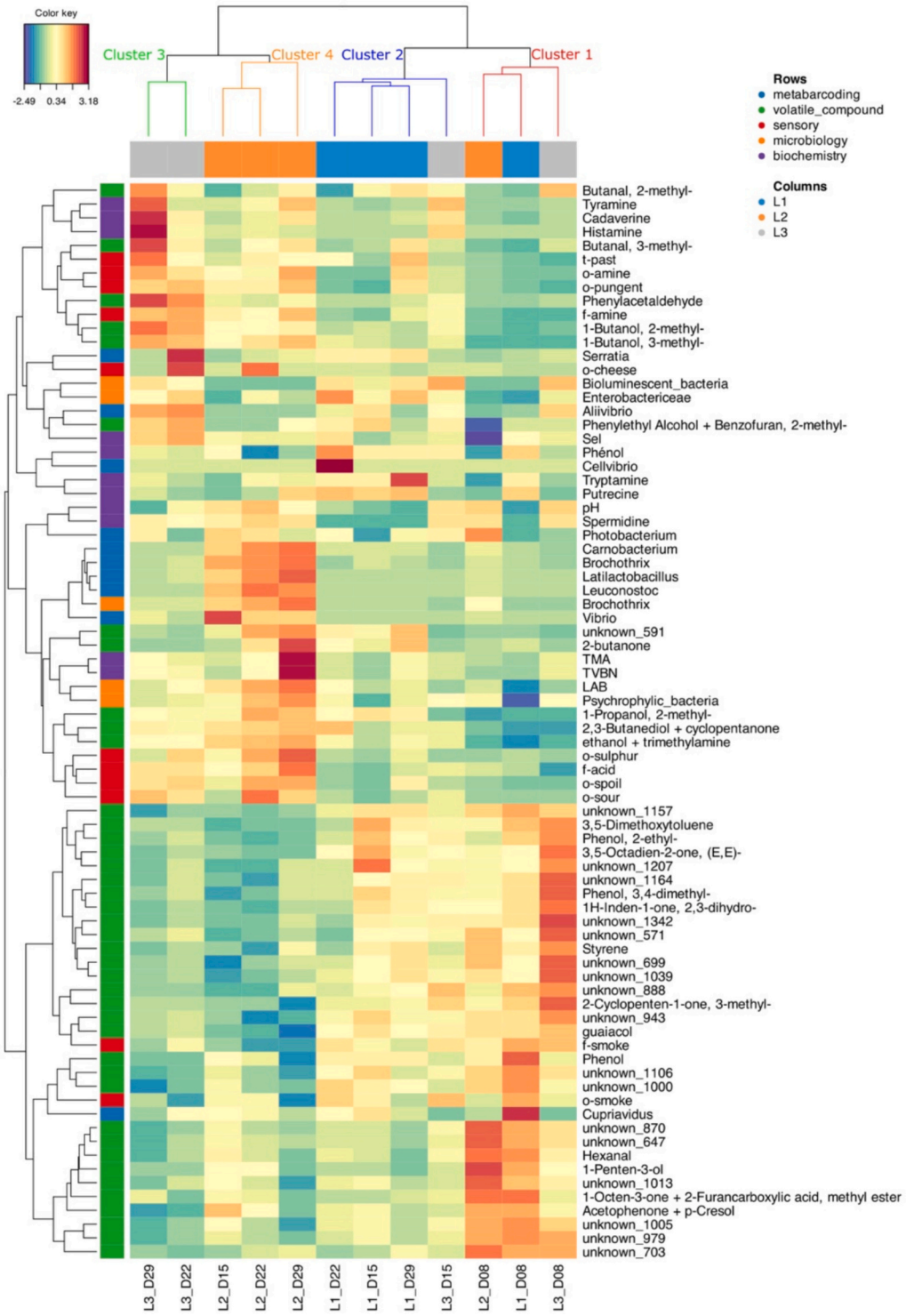

Fig. 4. Clustered Image Maps (CIMs) representing the multi-omics signature profile, with each discriminant feature (in rows) for each sample (in columns).

represent approximately 30 % of the total quantity of volatile compounds. Among the 52 identified compounds in the samples, 14 are phenols including phenol, 2-methylphenol (=o-cresol), 4-methylphenol (=p-cresol), guaiacol, 2,5-dimethylphenol, 2-ethylphenol, 3,4-dimethylphenol, 4-methylguaiacol, 4-ethylguaiacol, 2-methoxy-4-vinylphenol (= 4-vinylguaiacol), 2,6-dimethoxyphenol (= syringol), eugenol, 2-methoxy-4-propylphenol (= 4-propylguaiacol) and (*E*)-isoeugenol. They are the most important odorant phenolic compounds of smoke (Varlet et al., 2006).

In total, L1 and L3 have similar profiles whereas L2 is slightly different. L2 has a lower total quantity of VOCs and particularly a lower quantity of phenolic compounds than L1 and L3. These differences gave their specific characteristics to the smoked salmons produced in each processing plant.

An evolution of the volatilome is observed for the 3 processing plants during storage. The total quantity of alcohols and amines tended to increase during storage whereas the quantity of phenolic compounds decreased, particularly for L3. Carbonyls compounds varied depending on the compound. More in detail, the quantity of ethanol + trimethylamine; 2-methyl-1-propanol; 3-methyl-1-butanol; 2-methyl-1-butanol; 2,3-butanediol + cyclopentanone; 2-butanone; 3-methylbutanal; 2-methylbutanal; phenylacetaldehyde and tridecane increased with storage time in all plants. On the opposite, the quantity of 1-penten-3-ol; 2-furanemethanol; styrene; hexanal; 1-octen-3-one +2-furancarboxylic acid; methyl ester; phenol; acetophenone + p-cresol; guaiacol; 2-ethylphenol; 4-ethylguaiacol; 2,6-dimethoxyphenol; eugenol; 2-methoxy-4-propylphenol; (E)-isoeugenol decreased with storage. All these volatile compounds may be odorants depending on their concentration in the samples (Table X identif), thus the modifications observed during storage may affect the global aromatic quality of the smoked salmon.

3.6. Multi-omics integration analysis

Using the mixOmics R package and the DIABLO framework, all dataset types were integrated, revealing strong correlations, especially between metabarcoding data and volatile compound data (r = 0.74). This DIABLO model enabled the identification of distinct clusters of biological and molecular features based on plant type and sampling time (Fig. 4). A first cluster emerged, consisting of D08 samples (irrespective of their processing plant origin), and is primarily defined by the presence of specific volatile compounds associated with phenols or unidentified substances. With extended storage times, each plant developed into distinct biological and molecular clusters. A second cluster gathered three samples from plant L1 (D15/22/29) as well as D15 samples from L3. The biological and molecular characteristics of this cluster closely resembled those observed at D08, with slightly lower levels of phenolic compounds, the emergence of biogenic amines, and sensory attributes such as a pungent amine-like aroma in some samples. In contrast to L1, the samples from L3 with longer storage times (D22 and D29) formed a third distinct cluster, defined by features specific to these samples, including high abundances of the genus *Aliivibrio*, as well as elevated levels of tyramine, cadaverine, and histamine along with specific volatile compounds such as phenylacetaldehyde, 2-methylbutanal, 3-methylbutanal, 2-methyl-1-butanol and 3-methyl-1-butanol. The fourth cluster identified included samples from L2, from D15 to D29, and was characterized by high abundances of the genera *Carnobacterium*, *Brochothrix*, *Leuconostoc*, and *Latilactobacillus*, the presence of *Vibrio*, elevated levels of TMA and TVBN, and high concentration of 2-butanone, 1-propanol-2-methyl and 2,3-Butanediol + cyclopentanone. This cluster also exhibited low concentrations of phenolic compounds and strong sulfurous and pungent odours.

We next investigated the relationship between the different taxa and others features according to plant and visualized the correlation in a

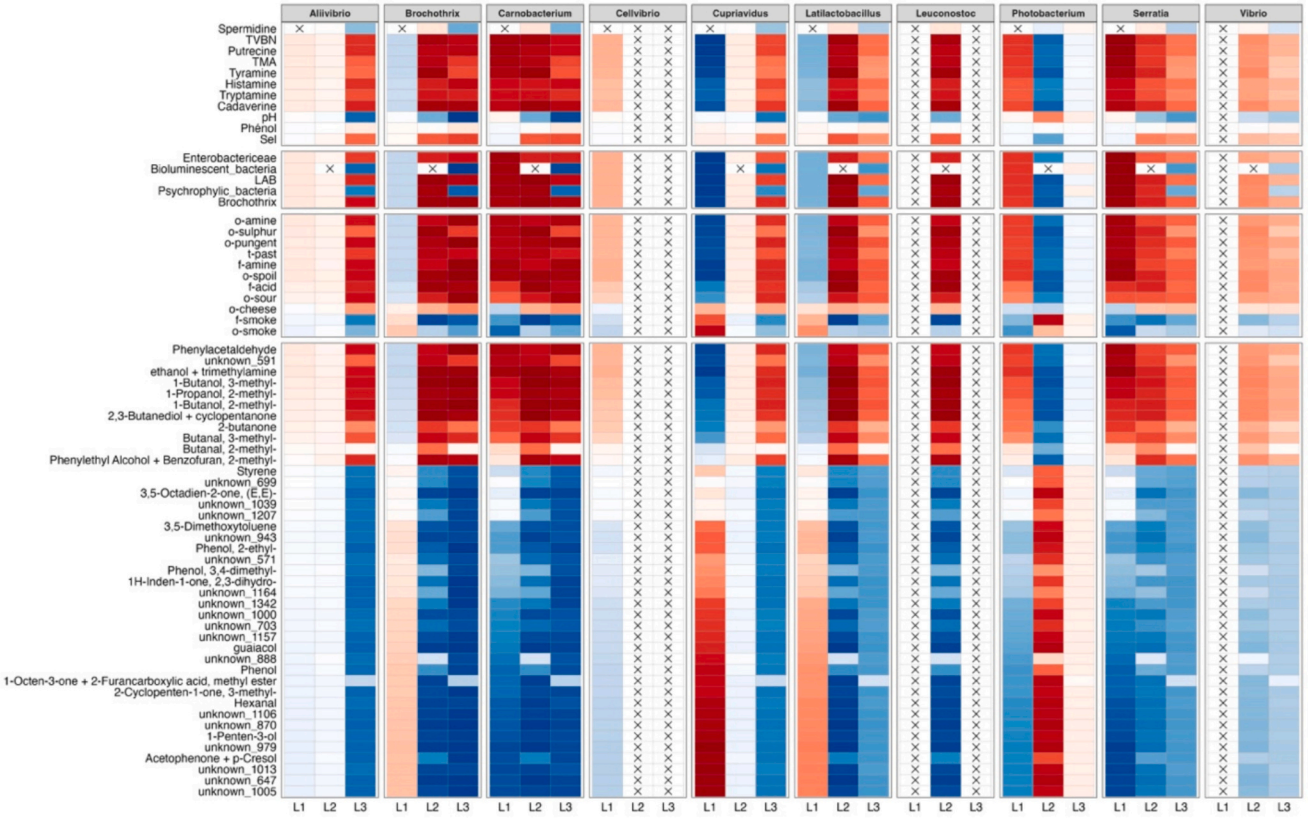

Fig. 5. Heatmap of correlations between major bacterial genera (columns) and discriminant features (rows) in each plant. The crosses indicate the absence of correlation due to the absence of one of the two components involved in the correlation.

heatmap (Fig. 5). The results revealed significant behavioral differences across bacterial genera in relation to distinct discriminatory features in each plant. Regardless the processing plant, certain bacterial genera such as *Aliivibrio*, *Carnobacterium*, and *Serratia* consistently show positive correlations of varying strengths with spoilage-related characteristics, including high concentrations of TVBN, TMA, ethanol + trimethylamine, 2-methyl-1-butanol, and strong sulfurous and pungent odours. For other genera, different or even opposite correlations with various features were observed depending on the plant. *Brochothrix* and *Latilactobacillus* genera show negative correlations with spoilage-related features in L1, whereas in L2 and L3 these genera appear to be strongly associated with the spoilage features (elevated levels of TMA, TVBN, Enterobacteriaceae, nauseating odours, Phenylacetaldehyde, ethanol + trimethylamine). This profile is also found for *Cupriavidus* even if the correlations are very low in L2. Conversely, the behavior of the genus *Photobacterium* is the opposite of these observations with positive correlations with L1 spoilage features and negative for L2 and L3. The genera *Cellvibrio* and *Vibrio* exhibit similar correlations, but only in the absence of one another. Specifically, *Cellvibrio* shows a positive association with spoilage products in L1, where *Vibrio* is absent. Conversely, comparable correlations are observed between *Vibrio* and the same products, but only in L2 and L3, where *Cellvibrio* is absent. Finally, the last genus *Leuconostoc* show positive correlations with spoilage products solely in L2 where it is present.

4. Discussion

Many factors can influence the organoleptic quality of CSS. Some are related to the fish itself (fish flesh, age, seasonal variations, feed quality etc.), some are due to the process applied such as salting (salt quantity) or smoking (wood type, temperature, time, humidity etc.), some concern the storage conditions after processing (type of packaging, temperature, time, etc.) (Jónsdóttir et al., 2008; Leroi and Joffraud, 2000; Mikš-Krajnik et al., 2016; Sivertsvik et al., 2002; Wiernasz et al., 2021) and others the product microbiota and its bacterial interaction.

In this paper, multi-omics integration analysis was carried out to evaluate the links between different types of omics data within a CSS spoilage framework. The major novelties in our data analysis approach with the R package "*mixOmics*" consisted in correlating different data blocks while considering the particularities in each block by appropriate statistical pre-treatment. A similar type of approaches was use on spoiled meat product to quantify the importance of causality relationships determined a priori between each type of responses as well as to identify important responses involved in spoilage (i.e., off-odour profiles, COVs) (Luong et al., 2021).

This novel approach enables the identification of four distinct clusters of biological and molecular features, referred here as "multi-omics signatures": one cluster representing unspoiled samples (D08 of all different plants) and three others corresponding to CSS products from each processing plants during storage. These three multi-omics signatures appeared from D15 and represent the spoilage evolution of each processing plants so, it further confirms the existence of a "plant signature". Recently, by comparing 16S rRNA-based microbiota profiles from CSS processed on three different processing plants, specific bacterial signatures have been highlighted for each processing environment (Maillet et al., 2021). As previously mentioned, CSS is a lightly preserved product. While smoking, drying, and salting initially reduce the microbial load, other steps in the process likely facilitate the introduction of bacteria from the plant environment. For instance, slicers and trimming tables are known to harbour high microbial loads and can act as sources of contamination, transferring bacteria from the plant environment to the CSS (Løvdal, 2015). Here the processing plant signature were not only correlated with bacterial genera but with all other biological, organoleptic or chemical features.

The first specific multi-omics signature identified corresponds to unspoiled products from all processing plants at D08, representing a distinct time-related signature. This unspoiled signature profile was not linked with specific bacterial genera presence but rather with smoke odours and flavours as well as phenolic compounds which are the most important VOCs in CSS. These compounds are mainly produced during thermal degradation of wood through lignin pyrolysis. The content of phenolic compounds in smoked fish depends on the type of wood used during the smoking process (Jónsdóttir et al., 2008). Phenolic compounds generally have low odour threshold which explains their important contribution to the smoky notes of CSS. Except thymol, all the main odorant phenolic compounds identified by (Varlet et al., 2006) were present in our samples whatever the processing plant. The quantity of phenolic compounds decreased during storage. In addition to phenolic compounds, furans are also related to the smoking process. In fact, these compounds are present in wood smoke or can be generated through Maillard and Strecker reactions occurring between wood smoke and fish flesh during the smoking process (Varlet et al., 2006; Wiernasz et al., 2021).

Regarding the three distinct signatures associated with the processing plants, the multi-omics signature of L1 during storage (from D15 to D29) shows minimal differences compared to that identified on D08. It remains similar to an unspoiled product signature profile (phenolic compound) with in addition a correlation with bioluminescent bacteria, Enterobacteriaceae and putrescine. This phenolic compound signature is also observed on D15 for L3. However, despite a similar taxonomic composition of the bacterial communities between L1 and L3 with *Photobacterium* as a prominent genus in both processing plant, L3 samples exhibit higher bacterial counts from the onset of storage spoilage. This accounts for the greater spoilage observed in L3 samples, as metabolite production is closely linked to bacterial spoilers concentration (Dalgaard, 2005). Thus, starting from D22, a distinct signature specific to L3 was observed and characterized by abundant biogenic amine (tyramine, cadaverine, and histamine), bioluminescent bacteria and *Aliivibrio* genus. This luminous bacterium (Yoshizawa et al., 2010) is not typically known as a seafood spoiler, but it belongs to the same *Vibrionaceae* family as *Vibrio* and *Photobacterium*, both of which can produce histamine (Engevik et al., 2024). In our study, the high level of aldehydes like 2-methylbutanal, 3-mehylbutanal, phenylacetaldehyde, furan (benzofurane, 2 methyl), alcohol like 2-methyl-1-butanol and 3-methyl-1-butanol are correlated to L3 multi omics signature. Alcohols are mainly produced by the microbial activity (Wiernasz et al., 2021). Some bacteria such as *P. phosphoreum*, *B. thermosphacta* and *S. liquefaciens* are able to produce alcohols such as ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-methyl-2-butanol, 1-penten-3-ol, 2,3-butanediol (Macé et al., 2013; Shumilina et al., 2016; Wiernasz et al., 2021). These compounds contribute to the spoilage off-flavour of CSS (Jónsdóttir et al., 2008). Aldehydes could be products of lipid oxidation, microbial degradation or Strecker degradation (Jónsdóttir et al., 2008; Varlet et al., 2006). Some aldehydes could be indicators of food degradation such as 3-methylbutanal, hexanal, 2,4-heptadienal, (Z)-4-heptenal, propanal, nonanal (Gómez-Estaca et al., 2018; Jónsdóttir et al., 2008). Aldehydes contribute to the development of rancid, fatty, sweet, sour, fruit spoilage odour (Jónsdóttir et al., 2008) which can be linked with cheesy odours characterized in L3-D22 sample. On fresh salmon fillets, *Photobacterium* is able to produce 2-methylbutanal, 3-mehylbutanal and other aldehydes like isobutyraldehyde and benzaldehyde (Macé et al., 2013) According to Wiernasz et al. (2021), several bacteria like *Pseudomonas*, *Acinetobacter* and *Serratia* genera, are able to metabolize furane like furfural and hydroxymethylfurfural into 2-furanmethanol and 3-furanmethanol., which corroborate with L3-D22 sample where both the concentration of 2-methylbenzofuran and the abundance of the *Serratia* genus were elevated. Moreover, a recent genome mining study indicate that key enzymes of seafood spoilage metabolites were found in strains of *S. liquefaciens* and *S. proteamaculans* and could allow this strain to produce spoilage odorous metabolites (methanethiol, dimethyl sulfide, TMA, acetyl-pyrroline) or biogenic amine putrescine and cadaverine (Begrem et al., 2021).

In contrast to the L1 and L3 processing plants, the L2 multi-omics signature profile shows a notable divergence as early as D15. This distinct profile is marked by a high abundance of LAB and the presence of strong sulfurous, pungent/acidic, and sour odours, which are hallmarks of advanced spoilage. Furthermore, it is associated with elevated levels TMA, the only amine compound identified in our samples. This compound is one of the most important indicator of fish spoilage and has a great impact on the organoleptic properties of CSS with the development of an unpleasant "fishy" odour (Shumilina et al., 2016). TMA has two main production pathways. It could result from the bacterial reduction of trimethylamine oxide (TMAO) or from the breakdown of choline or other trimethylalkylammonium compounds, such as carnitine or betaine, during fish spoilage (Shumilina et al., 2016). The quantity of TMA increases all along the storage time, reflecting the degradation of CSS. Other abundant metabolites were associated with L2 like TVBN, 2-butanone, 1-propanol-2-methyl, 2,3-butanediol + cyclopentanone, and ethanol + trimethylamine. Ketone production is widely recognized as being closely associated with the anaerobic metabolic pathways of LAB, which involve the degradation of proteins and lipids in salmon. These byproducts alter the organoleptic properties of the salmon, with ketones like 2-butanone serving as reliable spoilage markers under controlled storage conditions. (Jónsdóttir et al., 2008). This compound (2-butanone) has also been related with presence of *Lactobacillus* strains and *Carnobacterium* (Stohr et al., 2001) and 2,3 butanedione (diacetyl) and 2,3 pentanedione in presence of *Carnobacterium* strains inoculated on sterile blocks of cold-smoked salmon ((Joffraud et al., 2001). *Photobacterium* is also able to produce ketone like 3-hydroxybutanone (Macé et al., 2013). Alcohols, and particularly ethanol, 3-methyl-1-butanol and 2,3-butanediol, are known to be ones of the main spoilage indicators of CSS, explaining their increased quantity during storage (Jónsdóttir et al., 2008; Kuuliala et al., 2019; Wiernasz et al., 2021). The dominance of LAB in L2 profile does not solely explain the high levels of TMA and TVBN. Indeed, LAB are not known to be primary drivers of these compounds' production, as they are less efficient at reducing TMAO than other bacterial genera such as *Shewanella* or *Photobacterium*. However, they can play a modulatory role within the spoilage ecosystem by creating conditions that are more favorable for other microorganisms, thereby enhancing their metabolic and enzymatic activities.

5. Conclusion

This study highlighted different multi-omics signature associated to the beginning of the storage products and then an evolution of the samples to processing plant-specific signatures. This study confirmed that both microbiota composition and bacterial concentration play a key role in product spoilage. While Specific Spoilage Organisms (SSOs) concept (Dalgaard, 2000; Gram and Huss, 1996) suggested that only a subset of bacteria in a food product are responsible for spoilage, the process is more complex and cannot be attributed to a single species. Bacterial interactions occurring in the whole microbiome influence the production of spoilage metabolites production and contribute to the release of off-odours. These interactions must be further studied to better understand the mechanisms driving spoilage and identify the genes involved in metabolic pathways. Understanding the bacterial metabolic pathways that lead to the formation of VOCs or other spoilage metabolite present a significant challenge, especially when identifying spoilage indicators. Future research should focus on metagenomic analyses to complement multi-omics signatures by pinpointing key genes and metabolic pathways responsible for spoilage, paving the way for more precise and sustainable solutions in seafood preservation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2025.111233.

CRediT authorship contribution statement

Sabrina Macé: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Cécile Rannou:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Marc Jérôme:** Methodology, Formal analysis. **Frédérique Chevalier:** Methodology, Formal analysis. **Laetitia Kolypczuk:** Methodology, Formal analysis. **Claire Donnay-Moreno:** Methodology, Formal analysis. **Françoise Leroi:** Writing – review & editing, Methodology, Funding acquisition. **Mireille Cardinal:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **Cyril Noël:** Writing – review & editing, Writing – original draft, Validation, Software, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. There is no financial and no personal interest or belief that could affect their objectivity.

Acknowledgements

This study was carried out as part of the SALMONICS project (PULSAR 2020-2023) financially supported by the Pays de la Loire region (Nantes, FRANCE). We thank the Pôle de Calcul et de Données Marines (PCDM; https://wwz.ifremer.fr/en/Research-Technology/Research-Infrastructures/Digital-infrastructures/Computation-Centre, accessed on 15 November 2024) for providing access to the DATARMOR computing and storage resources. We also extend our gratitude to the Ifremer Bioinformatics Core Facility (SeBiMER; https://sebimer.ifremer.fr/) for their technical support in data management.

Data availability

I have share my data in the manuscript through a public link

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