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Microbiome-based study in wild-caught *Scomber scombrus* fish products at the end of the supply chain

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ABSTRACT

Fresh fish remain the dominant seafood forms and preservation technologies have enabled them to access ever more distant markets. In this study, we used metabarcoding of the 16S rRNA gene to generate gill microbiomes from *Scomber scombrus* bought at fishmonger's stores as fresh products but whose labels showed they had been harvested in the Atlantic or Mediterranean FAO fishing areas. Microbial data were analysed with the aim of evaluating their ability to maintain signals from their different geographical origins and the presence of taxa which can potentially act as spoilers, foodborne pathogens, or histamine producers. Results revealed that microbiota, at the end of the wild fish supply chain, had differences related to the two FAO fishing areas (Atlantic vs Mediterranean). Despite the presence of microbial genera potentially associated with spoilage, histamine-production or foodborne pathogens, their patterns confirmed that low-temperature storage is a traditional but effective method of preservation. However, the ongoing spoilage processes were more evident in *fresh non-local* specimens, dominated by psychrophilic Gram-negative bacteria, whereas *fresh local* specimens contained Planctomycetes taxa. Therefore, despite the current limitations mainly related to time and cost of the method, our study highlighted that microbiome-based applications are an emergent tool for food system transformation.

1. Introduction

The microbiome is a characteristic microbial community occupying a well-defined habitat with which it forms a dynamic micro-ecosystem that is integrated into a macro-ecosystem (Berg et al., 2020). The advent of High-Throughput Sequencing (HTS) technologies promoted microbiome studies and great attention has been paid to research focused on host-associated microbiomes, consisting in the characterization (composition and function) of communities associated or within higher organisms (humans, animals, and plants) (Johnston-Monje & Lopez Mejia, 2020; Michán et al., 2021; Quero et al., 2022; Zhang et al., 2019). Microbiome studies also play a crucial role in food systems (Olmo et al., 2022). Overall, food microbiota is affected by numerous factors (e.g., type of raw matrix, environment variables, human manipulation, geographical origin) and several microbiome-based studies have been applied to characterize microbial communities along the food chain and to identify existing and emerging risks in the food safety sphere (Ferrocino et al., 2022; Sequino et al., 2022; Yap et al., 2021). In fish and fish products, beyond the role in the host's physiology, microbiome studies play an important role especially in safety, since their application provides information about unculturable microorganisms, which make up the majority of fish microbiota (Sheng & Wang, 2021).

Even though international food traceability legislation has gone some way toward boosting consumer confidence in global trading of

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food products, the long, complex and not readily transparent seafood supply chain continues to favour a high global rate of mislabelling, with several consequences for consumer safety, fisheries sustainability and marine ecosystem conservation (Kroetz et al., 2020; Milan et al., 2019). In the event of mislabelling due to species substitution, DNA-based approaches are commonly and successfully employed both in cases of single-species foods (DNA barcoding or mini-barcoding) (Filonzi et al., 2021; Marchetti et al., 2020; Mottola et al., 2022; Panprommin & Manosri, 2022; Pardo & Jiménez, 2020) or in complex multispecies foods (metabarcoding) (Giusti et al., 2017; Klapper et al., 2023; Mottola et al., 2023; Piredda et al., 2022; Xing et al., 2019). In contrast, detection of fraud related to geographical origin poses a greater challenge, as the use of DNA tools to trace the origin of different stocks or specimens within a given commercial species is less frequent and more complex (Horreo et al., 2017; Pappalardo et al., 2011). This task often requires the implementation and standardization of dedicated panels of polymorphisms. Some studies have explored the feasibility of using spatial variations in a host's microbiome to trace authenticity of origin in seafood products, given that their microbial composition is closely linked to the initial microbiota of the product (Parlapani et al., 2018). In particular, distinct microbial profiles have been documented for benthic taxa such as soft-shell clams (Liu et al., 2020), sea cucumber (Feng et al., 2021) and oysters (Singh et al., 2022).

Given that fish is a highly perishable commodity, the trade in fresh product forms has traditionally been restricted to local or regional markets. Over the past four decades, however, the globalization of the fresh fish trade has led to dramatic increases, owing to advancements in storage and preservation technologies, as well as cheaper, more efficient transport. Furthermore, in accordance with Regulation (EC) 853/04, European countries permit the marketing of fish as *fresh* if it has been stored at a temperature equivalent to that of melting ice (about 0 °C) and has not undergone freezing from the time of harvest until it reaches the consumer. This means that in the market, under the umbrella definition of *fresh fish*, the local fish production of a species competes with populations of the same species from other parts of the country or even imported from abroad.

Atlantic mackerel (*Scomber scombrus*) is a pelagic, migratory, schooling planktivorous fish (McManus, 2017), mainly occurring on either side of the North Atlantic Ocean (FAO Fishing Area 27) but also present in the Mediterranean Sea (FAO Fishing Area 37). *Scomber scombrus* has huge importance for fisheries and is of considerable commercial value due to its desirable sensory and nutritional qualities. However, being fatty fish, all mackerel are highly perishable and, in common with several fish species from the Scombridae family, prone to causing histamine poisoning due to their high concentrations of the amino acid histidine. Thus, they must be carefully handled from the moment they are caught until they are sold. Indeed, the extension of shelf-life, guaranteed by constant and adequate refrigeration throughout the shipping and storage phases (Alice et al., 2020), does not kill or remove bacteria or stop spoilage mechanisms, but simply slows down the microbial development and deterioration processes (He et al., 2022).

In this framework, we have postulated that a microbiome-based approach for fresh fish intended for sale has the potential to provide a characterization of several critical aspects throughout the wild fish supply chain. To achieve this aim, a study was conducted in which metabarcoding of the 16S rRNA gene was used to generate gill microbiomes from specimens of Atlantic mackerel (*Scomber scombrus*) purchased from an Italian fishmonger as a '*fresh product*' but whose labels indicated they had been harvested in various FAO fishing Areas. Microbiome profiles were evaluated for such products at the end of the supply chain to determine three main aspects: (i) microbial signatures associated with distinct geographic FAO fishing areas; (ii) taxa which may act as spoilers, potential foodborne pathogens, or histamine producers; (iii) other taxa linked with other post-harvest actions.

2. Materials and methods

2.1. Sample collection

From February to March 2021, 16 specimens of fresh Atlantic mackerel (*Scomber scombrus*) were purchased from local fishmongers in the Apulia region (Italy). Sampling covered two different FAO fishing areas, with twelve specimens harvested in FAO area 27 (four from each of the FAO Subareas, i.e. 27.4, 27.7, 27.8) and four specimens from FAO area 37.2 (Table 1; Fig. 1). Each specimen was placed into a separate sterile plastic bag, stored at the temperature of melting ice, and immediately transferred to the laboratory for DNA extraction.

2.2. Labelling analysis

For each sample, the mandatory labelling requirements indicated by Council Regulation (EC) No. 1379/2013 (Art. 35) (i.e., commercial designation, scientific name, production method, geographical area, category of fishing gear used in the capture of the species and whether previously frozen) were verified.

2.3. DNA barcoding of Scomber scombrus

Conformity between the commercial designation 'Atlantic mackerel' and the scientific name *Scomber scombrus* declared on the labels was confirmed by amplifying a region of ~655 bp of the cytochrome oxidase subunit I (COI) mitochondrial gene using forward FISHF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′) and reverse FISHR1 (5′-AGACTTCTGGGTGGCCAAAGAATCA-3′) primers (Ward et al., 2005). Genomic DNA extraction and purification were carried out starting from 10 mg aliquots of muscle, using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). No added tissue was included as negative extraction control to verify the purity of the extraction reagents. The DNA concentration and purity were established by evaluating the A260 nm/A280 nm ratio using a BioPhotometer D30 filter (Eppendorf, Milan, Italy) and the PCR amplified products were verified in a 1.5% agarose gel, visualized with Green Gel Safe10,000 × Nucleic Acid Stain (5 μL/100 mL) (Fisher Molecular Biology, USA). Purification and sequencing reactions were performed by Eurofins Genomics s.r.l. (Ebersberg, Germany). Finally, for each DNA barcode generated, molecular identification was performed by analysis of similarity with a blast search against the Species Level Barcode Records database within BOLD SYSTEMS (http://www.boldsystems.org/index.php/IDS_OpenIdEngine).

2.4. DNA metabarcoding of gills

2.4.1. Sampling of gill mucosa

Microbial communities present in the gills were collected by swabbing, whereby gill microbiomes were sampled using a sterile cotton swab (Copan, Italy) rotated three times on both the anterior and posterior hemibranch of the entire right-hand side of the gill basket (Slinger et al., 2021). The swab tips were dissolved in 5 ml of sterile solution directly in the tube, ensuring that mucosal samples were suspended. Then, swab samples were vortexed at high speed, centrifuged at 8.000 rpm for 15 min, and subsequently stored at −80 °C until processed (Slinger et al., 2021).

2.4.2. DNA extraction, PCR amplification and sequencing

DNA extraction and purification were performed using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). Blank negative control (no added sample) was included to verify the purity of the extraction reagents. DNA concentration and purity were established by evaluating the ratio A260 nm/A280 nm using a BioPhotometer D30 filter (Eppendorf, Milan, Italy). The V3–V4 region of the 16S rRNA gene was amplified using the forward S-D-Bact-0341-b-S-17 (5′-

Table 1
Sampling details and labelling information for each *Scomber scombrus* specimen.

Sample ID	Sampling Date	Commercial designation[a]	Scientific name[a]	Production method	Category of fishing gear	Caught area
SG3	February 16	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.4
SG7	February 23	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.4
SG8	February 23	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.4
SG9	February 23	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.7
SG16	February 26	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.7
SG18	February 26	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 37.2
SG26	March 04	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 37.2
SG27	March 04	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 37.2
SG31	March 12	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.7
SG33	March 12	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.4
SG35	March 16	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.8
SG36	March 16	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.7
SG37	March 16	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.8
SG39	March 23	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.8
SG42	March 23	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 37.2
SG44	March 30	Sgombro	*S. sgombrus*	Caught	Trawls	FAO 27.8

[a] The commercial designation and the respective scientific name refer to Annex I of the Italian Ministry of agriculture, food sovereignty and forestry (Masaf) Decree dated September 22, 2017.

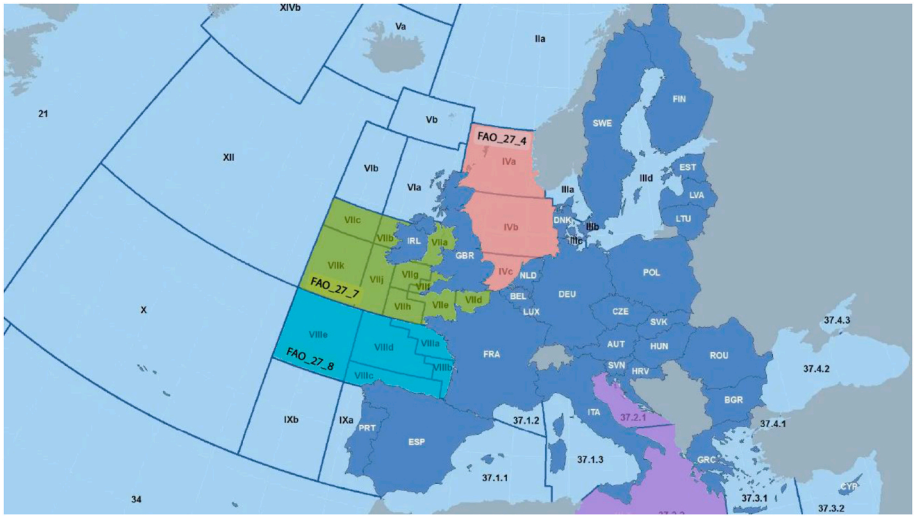

Fig. 1. Map of Geographic origins reported on the labels. The map shows the Atlantic FAO 27 and Mediterranean FAO 37 Fishing Areas and subareas shown on the labels of *Scomber scombrus* products.

CCTACGGGNGGCWGCAG-3′) and reverse S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) primers, described by Klindworth et al. (2013). PCR amplified products were purified using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Illumina paired-end sequencing (2 × 300 bp) was performed using the MiSeq platform by external company Eurofins Genomics s.r.l. (Ebersberg, Germany).

2.4.3. Data analysis

After sequencing, Illumina paired-end raw reads were pre-processed to generate Amplicon Sequence Variants (ASVs) using DADA2 R-package (Callahan et al., 2016). First, primers were removed, forward and reverse reads trimmed based on the Quality score plots (forward 280 bp, reverse 250 bp) and the reads filtered were then used to train the error model using machine learning approach. Then forward and reverse reads were dereplicated to generate unique sequences and denoised (collapsed) in amplicon sequence variants (ASVs) applying the trained error model. Finally, forward and reverse reads were merged and checked for chimera sequences. Representative sequences for each ASV were taxonomically assigned using the naive Bayesian classifier (Wang et al., 2007) against the SILVA database v132. ASVs assigned to chloroplast, mitochondria or "unknown" (i.e., that could not be classified at the kingdom level) were removed and excluded from analyses. The R packages "phyloseq" v1.32 (McMurdie & Holmes, 2013) and ggplot2 (Wickham, 2016) were used for the plots and to generate alpha diversity descriptors Richness (number of taxa) and Shannon Entropy index. Moreover, for beta diversity, Principal Coordinates analysis (PCoA) based on a Bray-Curtis dissimilarity matrix was performed.

Raw data were deposited in the Sequence Read Archive (SRA) under the BioProject PRJNA1010020.

3. Results

3.1. Labelling analysis

Labelling analysis revealed that all the labels reported the information required by Article 35 of EU Regulation No. 1379/2013 (commercial designation, scientific name, production method, caught area).

3.2. DNA barcoding

Specimen identifications by DNA barcoding confirmed, in all 16 specimens, the species (*S. scombus*) shown on the label, with 100% of similarity.

3.3. DNA metabarcoding

3.3.1. Preprocessing, alpha and beta diversity

Pre-processing steps of the 16 gill microbiomes, generated from each specimen, produced a clean dataset with 14,118 ASVs corresponding to 1,014,730 reads (Suppl. Table 1). After taxonomic assignment and normalization, at the second lowest number of reads, the final dataset includes 13.320 ASV (584,794 reads) (Suppl. Table 2). Alpha diversity exploration (richness and Shannon Index) showed higher values for Mediterranean specimens, with the exception of Atlantic SG35 (FAO 27.8) and SG16 (FAO 27.7) (Fig. 2; Suppl. Table 3). Beta diversity in PCoA (Fig. 3) showed a clear separation between gill communities from the Mediterranean (FAO 37) and the Atlantic (FAO 27). Moreover, Mediterranean communities were more homogeneous (except for sample SG18) in comparison with the Atlantic ones.

3.3.2. Taxonomic overview

A taxonomic overview of the total dataset revealed ten Phyla (Class for Proteobacteria) which accounted for 95% of the total reads. *Gammaproteobacteria* was the dominant taxa (48%), followed by *Planctomycetes* (15%), *Bacteroidetes* (10%), *Alphaproteobacteria* (5.2%), *Fusobacteria* (5.4%), *Actinobacteria* (3.7%), *Chlamydiae* (3.5%), *Deltaproteobacteria* (2.4), *Cyanobacteria* (1.9%) and *Firmicutes* (1%). Nevertheless, their distributions and abundances in the samples were very different (Fig. 4; Suppl. Table 4). *Gammaproteobacteria* were present in all specimens, but the lowest percentages were found in Mediterranean samples (SG27 and SG42 with 7%). In the Atlantic samples, all the specimens from FAO subarea 27.8 were dominated by *Gammaproteobacteria* with values between 58 and 83%, as well as in two specimens from FAO subarea 27.7 (SG26 98% and SG31 78%) and two from FAO 27.4 (SG33 80% and SG3 67%). By contrast, *Planctomycetes* were more abundant in Mediterranean samples (SG27 48%, SG42 50%, SG26 37%), while in Atlantic ones their abundance fell to below 3%, except for SG8 (48%), SG16 (26%) and two samples (SG 35 and SG31) showing about 9%. *Bacteroidetes* were almost absent in the Mediterranean samples but were present in some Atlantic samples SG8 (70%) and another four samples (SG7, SG16, SG37, SG39) with abundances below 30%. *Alphaproteobacteria*, present both in Mediterranean and Atlantic samples, always showed abundances lower than 10%, except for two samples, i.e. SG16 (10%) and SG26 (27%); *Fusobacteria* were quite abundant in two samples: Atlantic SG3 (32%) and Mediterranean SG18 (27%). *Actinobacteria* were abundant in one sample (Atlantic SG7 with 25%) and *Chlamydiae* were the dominant taxa in one Mediterranean specimen (SG18 43%). *Deltaproteobacteria* were homogenously present in the Mediterranean samples with abundances of around 10%, while *Cyanobacteria* were found with abundances lower than 1% with the exception

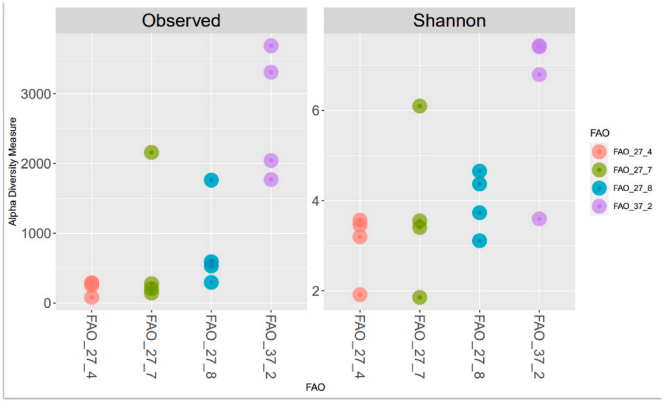

Fig. 2. Alpha diversity estimations of the microbiome profiles. Number of Observed ASVs (richness) and Shannon index calculated from gill microbial communities for each specimen from the different FAO subareas.

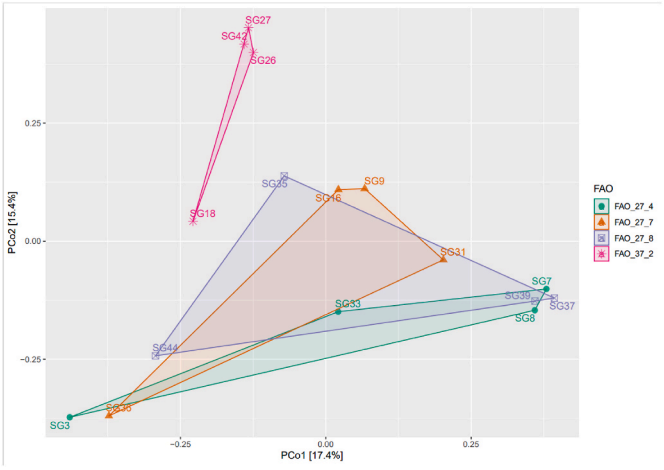

Fig. 3. Multivariate analysis of the microbiome profiles. Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity matrix calculated from gill microbial communities. Specimen codes were coloured and connected by convex hulls according to FAO subarea of origin.

of Atlantic specimens SG9 (24%) and SG31 (4%) (Fig. 4). At genus level (Fig. 5), the most abundant genera in the total dataset are psychrotrophic Gram-negative taxa *Psychrobacter* (14%), *Moritella* (12%), *Photobacterium* (8%), *Flavobacterium* (7%) and *Psychrilyobacter* (4.5%). However, their distribution in Atlantic FAO 27 specimens was quite scattered, while they were almost absent in specimens from Mediterranean FAO 37.2, where *Pirellulaceae* were the most abundant taxa.

3.3.3. Taxa with potential role of spoilers, foodborne pathogens, or histamine producers

Genera including potential spoilers were less abundant in the total dataset (Suppl. Table 4) even though some can reach higher values in specific specimens: *Shewanella* (1.6% in total dataset) reached 8% and 2% in the two Atlantic specimens SG35 and SG39 respectively, and 7% and 3.5% in the two Mediterranean SG18 and SG26; *Pseudoalteromonas* (0.7% in total dataset) reached 4% and 3.5% in the two Atlantic SG31 and SG37; *Pseudomonas* (0.4% in the total dataset) reached 2% and 1.5% in the two Atlantic SG44 and SG8; *Carnobacterium* (0.4% in total dataset) and *Brochothrix* (0.1% in total dataset) reached 5.6 and 1.4 respectively in the Atlantic SG7 specimens; *Chryseobacterium* (0.3% in total dataset) reached 3% and 1% in the two Atlantic SG37 and SG39; *Vagococcus* (0.03%) was only detected in trace amounts. The same was observed for the food-borne pathogen taxa: *Yersinia* (0.3% in total dataset) reached 5% in Atlantic SG44; *Myroides* (0.2% in total dataset) reached 3% in Atlantic SG44; *Vibrio* (0.2%), *Escherichia/Shigella* (0.1%), *Clostridium* (0.03%), *Staphylococcus* (0.01%) were always present in traces. Excluding *Photobacterium,* included in the list of abundant taxa, the other histamine-producing bacteria found in the dataset were *Acinetobacter* (0.2% in the total dataset) that reached 1.2% in the SG7 Atlantic specimen, and *Hafnia* (0.01%) found only in traces.

4. Discussion

In this study, HTS metabarcoding of the 16S rRNA gene was used to investigate the gill microbiomes of whole Atlantic mackerel (*Scomber scombrus*) purchased from a fishmonger and labelled as *'fresh'* but showing different FAO Fishing Areas. Thus, the microbiomes generated were not the result of an experiment with simulated and controlled storage conditions. Instead, they represented a snapshot of microbiome evolution observed at the end of the wild fish supply chain, while the temperature of melting ice should have always been maintained, but different times and heterogeneous activities affected the post-mortem processes, leading to several microbial profiles.

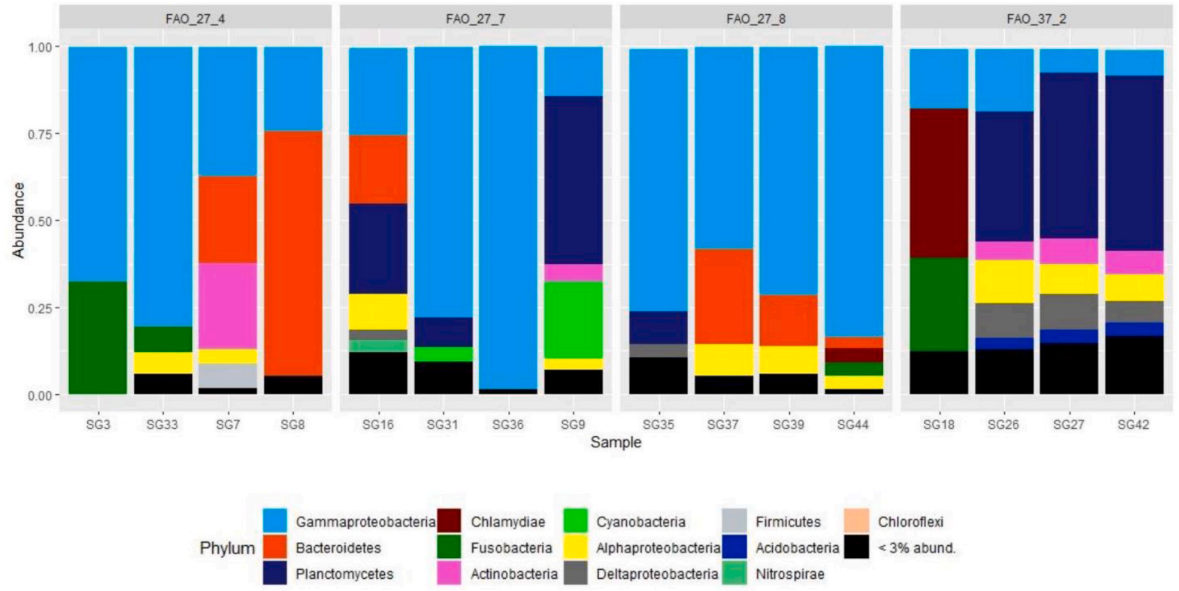

Fig. 4. Taxonomic overview at Phylum Level (Class for Proteobacteria). Barplots of relative abundances of taxa present in specimens grouped by FAO subarea. Taxa with abundances <3% in each specimen were collapsed together.

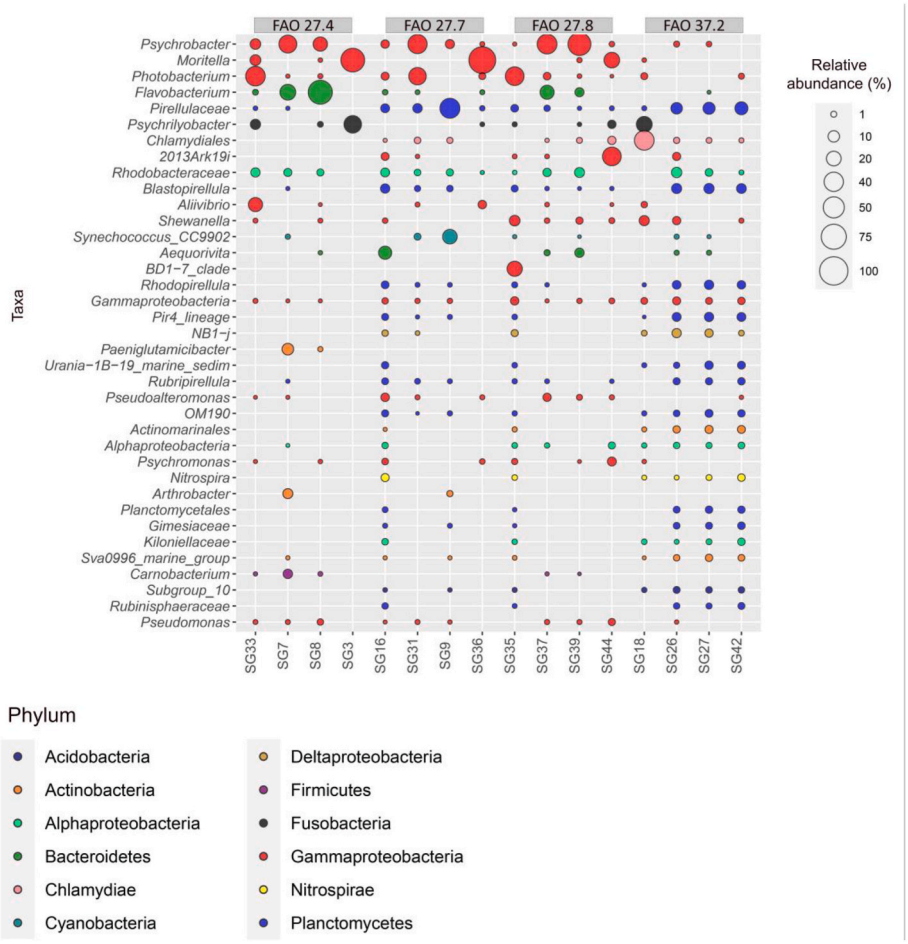

Fig. 5. Taxonomic overview at Genus Level. Bubble plot showing the relative abundances of the 37 most abundant taxa collapsed at genus level if available. Bubble colour corresponds to the Phylum to which they belong. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.1. Biogeographic patterns

Our results revealed a clear distinction in the microbial structures and compositions of gills in Atlantic mackerel specimens harvested in the two FAO fishing Areas (FAO 27 Atlantic vs FAO 37 Mediterranean). Indeed, Mediterranean specimens not only contained a higher number of evenly distributed species, but also a characteristic taxonomic profile even at Phylum level. Geographic patterns could be expected, since the microbial diversity of fresh live seafood among geographical regions had already emerged in several papers (see Table S1 in Parlapani, 2021). Indeed, the two FAO fishing areas (i.e., 27 and 37), shown on the labels, correspond to very different marine environments, the former holding Atlantic cold-temperate waters, where psychrophilic Gram-negative microbes are predominant, in contrast with Mediterranean subtropical waters harbouring a predominance of mesophilic taxa (Ashie et al., 1996). Despite that, post-mortem retention by gill microbiomes of the geographic origin of specimens cannot be taken for granted in commercial samples. Indeed, variations in pre and post harvesting activities, time differences between catching and selling, as well the rapid post-mortem microbial processes, could easily obscure the geographic signatures present in the gill microbiomes of living organisms. To the best of our knowledge, the potential of using microbiomes to trace different origins in uncontrolled conditions has not previously been investigated. However, our preliminary results with Atlantic mackerel are promising, suggesting that microbial diversity may be used to distinguish and authenticate different populations of the same species in the case of fresh fish products caught either in the Mediterranean or in the Atlantic.

Microbial profiles of the Mediterranean samples have shown that *Planctomycetes* is the most abundant phylum, with several taxa belonging to the aquatic family of *Pirellulaceae* already reported from the gill, skin and gut microbiomes of living organisms both in fresh and marine water environments (Kellogg et al., 2016; Parata et al., 2020). Little is known about the exact role of this family in the several contexts, including the evolution of seafood spoilage microbiota, but they have been reported to be chemoheterotrophic bacteria, able to adapt under aerobic and anaerobic conditions and playing an important role in the fermentation of carbohydrates (Elshahed et al., 2007; Kaboré et al., 2020). Most *Planctomycetes* are mesophilic organisms (Kaboré et al., 2020; Žure et al., 2017), so their presence in gill microbiomes from subtropical water harvested seafood is expected. However, their abundance could suggest that adequate refrigeration has not been constantly maintained throughout shipping or storage. Such a pattern could be related with the predominance in the Mediterranean basin of small-scale artisanal fisheries, characterised by short trips and small vessels with sole owners and no crew members involved on pre and post-harvesting activities (FAO, 2022). In this context, during initial storage in the fishing vessels and the transport of fish from landing sites to the local market, the refrigeration conditions may not have been able to ensure the strict temperature of melting ice, thus favouring the growth, or the survival, of mesophilic taxa that are expected to be part of the product's initial microbiota in tropical and subtropical seawater. Such a hypothesis is also supported by the presence of the *Deltaproteobacteria* phylum which includes primarily mesophilic anaerobes (Waite et al., 2020), and the family of *Thermoanaerobaculia (Acidobacteria* phylum) composed of anaerobic, thermophilic and chemoheterotrophic bacteria. Nevertheless, the evenness of the communities and the absence of taxa associated with spoilage indicated a short interval between fishing and retail for specimens caught in FAO 37, as would be expected in a short supply chain. Finally, the presence of *Actinomarinales* and *Microtrichales* taxa (*Actinobacteria)* previously found in benthic sediment communities (Miksch et al., 2021), is probably related to the use of pelagic trawls, the main gear targeting mackerel, herring and sprats in the Mediterranean basin. Indeed, although pelagic trawls are towed in mid-water with no intentional contact with the seabed and consequent damage to benthic communities, accidental impacts on the seabed environment could occur, leaving a mark on the gill microbiome in the Mediterranean samples.

In contrast with these findings, our results highlighted that gill microbiomes cannot be used to distinguish between specimens harvested within the three different FAO 27 Atlantic fishing subareas, which underlines that microbiome divergence is, to some extent, explained by the extent of geographical isolation (Ruuskanen et al., 2021). As shown in multivariate analyses, microbiomes grouped without clear signals related to Atlantic subareas and such inhomogeneity could be driven by different forces acting on microbial communities. On the one hand, the populations of Northeast Atlantic Mackerel (NEAM) are traditionally considered and managed as one single stock, because of seasonal and interannual migrations performed along the European continental shelf. Thus, the homogenizing effects of a shared environment and the physical interactions among individuals could lead to "microbiome convergence" (Härer & Rennison, 2023) among Atlantic specimens. On the other hand, the differences in post-harvest activities (e.g., transportation times, handling, and storage) also contributed to the evolution of the communities. By the end of supply chain, this leads to the establishment of microbiomes exhibiting casual variation in response to such differences (Ashie et al., 1996; Huang et al., 2021; Zhuang, Hong, Zhang, & Luo, 2021).

Analysis of the microbial structure of all Atlantic samples from FAO 27 revealed a general reduction in diversity and complexity, dominated by a limited number of taxa, as is to be expected during the spoilage process. Specimens were dominated by psychrophilic Gram-negative taxa, thus confirming both that Atlantic cold-water species harbour predominantly psychrophilic microbes and that chilled storage conditions promoted their growth. Such taxa may degrade food quickly, even at cold temperatures: indeed, it has been suggested that seafoods harvested from cold-temperate waters may spoil more quickly than tropical or warm-water ones because they are 'preinoculated' with psychrotrophic Gram-negative spoilage bacteria (Ashie et al., 1996). Five psychrophilic Gram-negative genera dominated the FAO 27 specimens, including *Psychrobacter, Moritella, Photobacterium, Flavobacterium* and *Psychrilyobacter*. Three of them (*Psychrobacter, Photobacterium* and *Flavobacterium*) were part of the bacterial profile of Atlantic mackerel described by Svanevik and Lunestad (2011) but also already reported as components of microbial profiles in studies on food ecosystems evaluated both with high throughput sequencing and conventional methods (Anagnostopoulos et al., 2022). The most abundant genus, *Psychrobacter*, dominated four Atlantic specimens. *Psychrobacter* species are always strictly aerobic, able to grow well at low temperatures, neutrophilic and tolerant of a wide range of salt concentrations. Most species have been isolated from cold saline environments as well in the normal surface of fish skin or marine animals, but *Psychrobacter* is also a component of the food microbiota due to the combination of psychrotolerance and osmotolerance and belongs to spoilage microbiota found on chilled proteinaceous foods, including seafood, meat products and even cheese and raw milk, stored in air or packaged under a modified atmosphere (MAP) (Zotta et al., 2019). Despite that wide range of foods, it is considered a relatively minor spoiler. Indeed, *Psychrobacter* members can break down lipids and hydrolyse amino acid spoilage producing a musty off-odour, usually after the fish has been stored in the cold for 7–10 days (Bowman, 2006), but it has also been reported that they do not produce significant amounts of volatile organic compounds (Broekaert et al., 2013).

Photobacterium, the dominant genus in two Atlantic samples, was almost absent in all the Mediterranean specimens, thus confirming that *Photobacterium* is rarely present in seafood from the Mediterranean Sea (Parlapani, 2021; Parlapani et al., 2020). The genus, reported in fish skin, gills and gut, includes aerobic and facultative anaerobic psychrophilic taxa, allowing *Photobacterium* taxa to be potent spoilers under both aerobic and anaerobic storage conditions. *Photobacterium* has been found in several chilled and packaged seafood products (*i.e.* cod, halibut and cold-smoked salmon), packaged fresh meat (Fuertes-Perez et al.,

2019; Parlapani, 2021) but it can survive at high CO2 so it is a typical SSO of fish stored under atmospheres containing high levels of CO2 or vacuum (Antunes-Rohling et al., 2019; Gornik et al., 2013; Parlapani et al., 2020). *Flavobacterium*, abundant in three FAO 27 samples, are aerobic, generally commensal bacteria, part of the normal bacterial flora in the mucus at the surface of fish and fish eggs, but also showing various degrees of pathogenicity for wild or ornamental fish and especially in intensive fish farming. *Flavobacterium* spp. have also been isolated from many chilled foods, in particular dairy products, fish, and meat and it uses both lipases and proteases to produce disagreeable odours.

Meanwhile, two of the five predominant genera in the FAO 27 samples have not often been reported to be part of the microbiota of fresh and spoiled seafood from sea- and freshwater. The genus *Moritella* is highly dominant in two Atlantic samples (70% in FAO 27.4 and 90% in FAO 27.7) and has been known to consist solely of psychrophilic species isolated from seawater, sediment, and fish samples collected from cold marine environments. Halophilic and facultatively anaerobic, this genus includes the animal pathogen *Moritella viscosa* that is known to be the causative agent of winter-ulcer disease in Salmonids and in some non-Salmonids such as cod (*Gadus morhua*) Lumpfish (*Cyclopterus lumpus*), and European Plaice (*Pleuronectes platessa*) (Gulla & Bornø, 2018, pp. 97–101; Lvoll et al., 2009). There are two studies highlighting the potential role of this genus in fish spoilage: the first found *Moritella* to be markedly more abundant in the spoilage microbiota of hake fillets stored under modified atmospheres (Antunes-Rohling et al., 2019) while the second found *Moritella* in microflora of spoiled Japanese horse mackerel (*Trachurus japonicus*) from Japan (Kyoui et al., 2022). However, such high abundance in our specimens could be generated by different scenarios. The first hypothesis relates to a potential spillover event from salmon farms to sympatric wild Atlantic mackerel. Indeed, the effects of aquaculture on wildlife raise concerns, in particular the risk of parasite and disease exchange between farmed and wild hosts, since seawater provides an ideal medium for the transportation of parasites, viruses, bacteria, and spores (Bouwmeester et al., 2021; Cantrell et al., 2020; Nekouei et al., 2018). The second hypothesis could relate to the piezophilic nature of members of the genus (optimal growth at a hydrostatic pressure equal to or above 10 MPa) and a post-harvest application of high-pressure processing (HPP). HPP applies pressure treatment, usually ranging from 100 to 600 MPa up to 1200 MPa and is gaining popularity in the seafood industry as it offers benefits such as shelf-life extension and safety assurance by inactivating pathogenic or spoilage bacteria and enzymes without compromising the sensory and nutritional quality of fresh and processed foods (Roobab et al., 2022). The application of such post-harvest activity could be hypothesized also in the light of a preliminary study by de Alba and co-authors (2019) which investigated the beneficial effect of high-pressure treatments on the quality of Mackerel (*Scomber* spp.) caught in the North Sea. Finally, the genus *Psychrilyobacter* is an obligate anaerobic halophile genus belonging to *Fusobacteria* phylum (Zhao et al., 2009) globally distributed with a preference for the guts of marine invertebrates. *Psychrilyobacter* has been found to be an abundant genus in the gut of free-living mussels (*M. chilensis*) from the Chilean coast (Santibáñez et al., 2022), in abalone (*Haliotis tuberculata*) and in oyster (*Crassostrea gasar*, *Crassostrea gigas*, *Rapana venosa*) and in a crab genus (*Austinograea* sp.) (Fernandez-Piquer et al., 2012; Horodesky et al., 2020; Pelikan et al., 2021) reported *Psychrilyobacter* as an important protein and/or amino acid degrader in marine sediments, but no study has established its role or metabolic functions on those species. Food studies have revealed it to be the dominant genus in the late-fermentation stage of traditional spontaneously fermented fish (stinky Mandarin fish) (Yang et al., 2020). However, further studies will be needed to elucidate the details of the origins as well the potential role of these two genera in seafood spoilage processes.

4.2. Safety and freshness

Long-term preservation of fatty fish such as Atlantic mackerel is a challenge for the seafood industry, mainly due to lipid degradation that can rapidly reduce fish quality. The specimens of mackerel used in our study, randomly chosen by the seller, were attractive for consumers and did not show any signals of deterioration, thus highlighting, on the one hand, that low-temperature storage is a traditional but effective method of preservation. However, microbial growth is not always easily detected by organoleptic evaluation (Semeano et al., 2018) and psychrotrophic pathogens can grow and proliferate without having any obvious sensorial impact (Brackett, 1992; Tavares et al., 2021). In addition to the potential spoilage taxa discussed above, the other taxa with potential role of spoilers, (*Psychromonas*, *Pseudomonas*, *Pseudoalteromonas*, *Carnobacterium* and *Vagococcus*), as well as potentially pathogenic ones (*Vibrio, Escherichia/Shigella, Clostridium, Staphylococcus*), were found in low abundance, suggesting that major spoilage bacteria genera in fish samples could become predominant just before the sensory rejection point. Efficiency of preservation was also confirmed by the limited abundance of potential histamine-producing bacteria. Among them, only *Photobacterium* sp. has been listed among the most abundant taxa confirming to be responsible for producing histamine in psychrophilic conditions (Oktariani et al., 2022) whereas other histamine-producing bacteria (*Acinetobacter*, *Vibrio*, *Hafnia*, *Morganella* and *Enterobacter*) were found in our samples as rare components.

In addition, this study shed light on the presence of fish pathogens in wild-caught specimens. The results confirmed that gill microbiota can serve as an indicator of both internal and external diseases, thereby providing valuable information about the overall health status of the fish (Legrand et al., 2018). Indeed, the presence of taxa from the *Chlamydiales* order, detected in one Mediterranean sample (43%), and the presence of B-proteobacteria 2013Ark19i (38%) in one specimen from FAO 27.8, could be related to the presence of gill epitheliocystis (Seth-Smith et al., 2016). Epitheliocystis is a disease of the skin and gills of marine and freshwater fish caused by a diverse range of bacteria from different phyla, with unknown effects on the health of wild fish but causing mortalities, especially in cultured juvenile fish (Blandford et al., 2018). Moreover, another sample from FAO 27.4 revealed the presence of the *Aliivibrio* genus (19%), a major component of the *Vibrionaceae* family in marine environments, which includes some taxa that are pathogenic to aquaculture species (Klemetsen et al., 2021). Interestingly, no other studies have reported the presence of this taxa in *Scomber scombrus*, but it could be related, as in the case of *Moritella* discussed above, with the risk of parasite and disease exchange between farmed and wild hosts in both the Mediterranean and the Atlantic.

4.3. Sustainability

Our results suggest that a microbiome-based approach could be a useful tool for fighting against geographic fraud, by distinguishing between Atlantic and Mediterranean stocks of '*fresh*' *Scomber scombrus*. This could help protect biodiversity in the Mediterranean basin, one of the most vulnerable marine ecosystems to climate change (Giorgi & Lionello, 2008; Rosenzweig et al., 2007). Moreover, knowledge regarding stock assessment for the main commercial species and monitoring of fisheries is often incomplete and fragmented at national or subregional levels. Yet, commercially important species can be considered at different levels of risk on the various IUCN lists at global or local scale, as is the case for *Prionace glauca*, considered NT (Near Threatened) at global scale but CR (Critically Endangered) in the Mediterranean Sea or for *Lamna nasus* considered VU (Vulnerable) globally, but CR in the Mediterranean Sea. Thus, the possibility to authenticate Mediterranean population could be important also for other commercial fish species and should be tested in dedicated studies.

From a sustainability perspective, the sale of *fresh local* and *fresh non-local* fish products from the same species will result in varying impacts.

Improved transportation and logistics coupled with better storage and preservation have created new opportunities to access the global markets, opening up competition among producers located thousands of miles away from the fresh fish markets traditionally served only by local fishermen. In general, more distant harvest sites have greater impact since chilled fish is usually road transported in refrigerated vehicles, so the environmental footprint of *fresh* products is clearly related to fishing area. This highlights the recognised impact of food transportation on environmental pollution and greenhouse gas emissions (European Union, 2020). However, it also raises a potential ambiguity in consumers' assessment of sustainability. Indeed, the term *'fresh fish'* can be misleading to the average seafood shopper, and the product form of *whole fresh fish* may wrongly suggest to consumers that it comes from local or regional fisheries. Although indication of geographic origin is mandatory on labels of fresh seafood products under current legislation Reg. (UE) 1379/2013 also other data such as the date of catch, date and port of landing could be important. In addition, the legislation does not take into account any of the land-based activities involved in the commercial seafood supply chain, such as means of transport or storage and preservation technologies (Weeratunge et al., 2010). Indeed, it is crucial to incorporate the environmental impact of the whole product supply chain into the labelling requirements. This would promote a more transparent policy and raise awareness among consumers about their choices. The use of Environmental Footprint methods for products and organisations is recommended by the European Commission (https://green-business.ec.europa.eu/environmental-footprint-methods_en), and in line with the Farm to Fork Strategy launched in 2020 as part of the European Green Deal, supporting the transition toward a food system that is fair, healthy and environmentally friendly.

5. Conclusions

This study shows that gill microbiome profiles generated by an HTS approach can provide insights into the traceability of geographical area of catches. Although such an approach has often been used to identify unculturable microorganisms, our results highlight the potential of microbiome-based applications to provide a holistic understanding linking retail fish products with the previous status of living organisms, their ecosystems and post-harvest activities. Nevertheless, at this moment the metabarcoding approach cannot be considered mature for routine application at the end of the supply chain because of limitations mainly related to time and cost of the method. However, metabarcoding or other microbiome-based applications have the potential to drive innovation in food science and research as well as promote transformation in food systems by facilitating integration between various actors and actions. Such importance is acknowledged in the EU policy Food 2030, which identifies the *microbiome world* as one of the 10 action pathways (https://research-and-innovation.ec.europa.eu/research-area/environment/bioeconomy/food-systems/food-2030_en#main-goals).
Although more data are needed, our findings suggest that the gill microbiome could help to authenticate the geographic origin (Atlantic FAO 27 vs Mediterranean FAO 37 fishing Areas) of *Scomber scombrus* sold as whole fresh fish. Despite having the same commercial definition and the improved storage and preservation technologies, the Mediterranean specimens (FAO 37) showed safer microbial profiles without the presence of psychrophilic taxa, confirming that, in fresh-product form, a shorter chain reduces food deterioration risk and enhances local economies and environmental sustainability. Currently, information on post-harvest activities in the seafood supply chain is unavailable to consumers, but voluntarily including Product Environmental Footprint (PEF) and more details of supply chain on labels, could help consumers distinguish between apparently identical seafoods, promoting truly sustainable products not only for marine ecosystems but for the whole biosphere.

CRediT authorship contribution statement

Roberta Piredda: Methodology, Software, Formal analysis, Writing – original draft, Writing – review & editing. **Anna Mottola:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Lucilia Lorusso:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Lucia Ranieri:** Writing – review & editing. **Gaetano Catanese:** Writing – review & editing. **Giulia Cipriano:** Writing – review & editing. **Roberto Carlucci:** Writing – review & editing. **Daniele Anaclerio:** Writing – review & editing. **Angela Di Pinto:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The manuscript entitled '*Microbiome-based study in wild-caught Scomber scombrus fish products at the end of the supply chain*' has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115264.

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