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Assessing the microbiome of a poultry burger processing line: A combined approach using culturing techniques and metabarcoding

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ABSTRACT

Culture-dependent techniques have been traditionally employed to characterize the microbiota of food products and processing environments. However, culture-independent techniques, such as metabarcoding, are increasingly used as they provide a more comprehensive and accurate understanding of the microbial communities. This study integrated culturing techniques and metabarcoding to provide complementary insights into the microbiome of a poultry burger processing line. Samples included chicken thighs, burgers, expired burgers, and different work surfaces. Metabarcoding indicated that the predominant genera were *Pseudomonas, Psychrobacter, Brochothrix, Acinetobacter*, *Carnobacterium* and *Lactobacillus*. This was consistent with culturing results, which showed *Pseudomonadaceae* as the predominant family, followed by lactic acid bacteria (LAB). Metabarcoding revealed a significant shift in the microbial composition of burger samples after the shelf-life period, characterized by an increase in the relative abundance of LAB, a change missed by culturing techniques. Food-contact surfaces showed a different microbiome composition compared to poultry products and were not considered a source of food contamination. Notably, metabarcoding highlighted the significant influence of seasonality (winter vs. summer) on the microbiome composition. The insights provided by metabarcoding underscore the importance of incorporating culture-independent methods into the microbiome characterization of food products and food processing environments.

1. Introduction

Meat constitutes a vital component of the daily diet and its consumption has increased over the last 50 years. According to the Food and Agricultural Organization (FAO, 2023), 363.9 billion kg of meat were produced worldwide in 2023, with a per capita consumption of 45 kg. In particular, poultry was the most produced and consumed meat worldwide and this sector showed a production of 142.7 billion kg and a consumption of 14.9 kg/per capita (OECD, 2021). However, its high nutritional content, high water activity, and moderate pH make meat a highly perishable food commodity susceptible to the growth of both spoilage and pathogenic bacteria (Dave & Ghaly, 2011). This not only results in organoleptic alterations, food waste and economic losses, but also in serious public health problems.

Microorganisms responsible for food spoilage are typically unavoidable contaminants in raw materials that are not effectively inactivated during processing. Consequently, the microbiological quality (i.e. the acceptable levels of microorganisms to ensure food safety and limit spoilage) and shelf-life of food products are largely influenced by their initial microbial composition and load, and storage conditions (Møretrø & Langsrud, 2017). Generally, cooling temperatures and low oxygen modified atmosphere packaging (MAP) have been employed in the storage of poultry meat to prevent or delay microbial proliferation (Höll et al., 2016).

Additionally, the implementation of different processing steps and the contact with different surfaces, equipment or utensils can also lead

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to contamination of food products during processing. In fact, food processing environments (FPE) are recognized as potential sources of contamination for food products (Møretrø & Langsrud, 2017): the lengthy production cycles, extensive areas for microbial growth, and nutrient availability in food processing lines create an ideal environment for the persistence of microorganisms (Lindsay & von Holy, 2006; Yuan et al., 2021).

Knowledge of the microbiota that limits the shelf-life of poultry products, tracking their origins, and monitoring microbial composition changes throughout the processing line are crucial for designing and implementing effective preventive and control measures. Traditionally, microbial characterization of food products and FPE have been conducted using culture-dependent techniques, which allow for the absolute quantification of specific microbial groups. However, culture-independent techniques, such as metabarcoding (based on the amplification and sequencing of the 16S rRNA gene), are increasingly used as they provide a more comprehensive and accurate understanding of the microbial communities (Jagadeesan et al., 2019). Nevertheless, metabarcoding has notable limitations, including the inability to determine the absolute abundance of the identified taxa, and the uncertainty regarding the viability or activity of these taxa.

Several investigations have employed metabarcoding to comprehensively describe the microbial communities in fresh poultry products or those stored under different storage conditions (Dourou et al., 2021; Kim et al., 2019; Lauritsen et al., 2019; Lee et al., 2019; Min et al., 2023; Rouger et al., 2018). However, the vast majority of metabarcoding studies assessing the microbiome of poultry FPE have focused on farms or slaughterhouses (Chen et al., 2020a; Chen et al., 2020b; Lauritsen et al., 2019; Park et al., 2023; Wang et al., 2019, 2020), with much less attention given to processing plants. To the best of our knowledge, a limited number of studies have used metabarcoding to trace the microbial communities of chicken samples (including live birds, carcasses, and meat products) and their associated FPE throughout the chicken meat supply chain, from farm to market, including processing plants (Park et al., 2023).

Different environmental factors, such as seasonality, have a strong influence over the microbial compositions of broilers (Marmion et al., 2021). Understanding these seasonal variations is crucial, as they can affect the microbial load and diversity at various stages of the poultry supply chain, potentially impacting food safety and shelf-life. Although the seasonal influence on the microbial composition of poultry birds, carcasses, and meat has been previously evaluated using metabarcoding (Kim et al., 2019; Schofield et al., 2022), the impact of seasonality on the microbial composition of poultry FPE remains largely unexplored.

In this context, the aim of this study was to integrate culturing techniques and metabarcoding to study a) microbial dynamics throughout the processing line and the storage period; b) the influence of microbial communities from FPE on the microbial communities of poultry products; and c) the influence of seasonality on the microbial communities of poultry products and FPE.

2. Material and methods

2.1. Sampling strategy

This study was conducted in a chicken burger processing line of a poultry processing plant. Different poultry products and food-contact surfaces were sampled during processing across three separate production days during both winter (December 2019) and summer (July 2020) seasons. Poultry products included chicken thighs (previously processed at the slaughterhouse), freshly packaged burgers stored in a modified atmosphere (MAP) containing 70% N_2 and 30% CO_2, and, only in summer season, expired packaged burgers. Food-contact surfaces included different equipment: formula table, grinder, kneader, forming hopper and conveyor belt.

2.2. Sample collection

Five chicken thighs and two burger packages were collected per production day using sterile plastic bags. One burger package stored in MAP was maintained under cooling conditions (0–4 °C) until its sell-by date (totaling 22 days) to obtain the expired packaged burgers. Food-contact surfaces were swabbed horizontally, vertically, and diagonally, turning the swab around in between, using sterile sponge swabs (Nasco Whirl-Pak Speci-Sponge, Wisconsin, USA) pre-moistened with 20 mL of sterile buffered peptone water (BPW) (Oxoid, Basingstoke, United Kingdom). The sampled area depended on the subsequent study: 100 cm^2 was sampled for the culturing method and 300 cm^2 for metabarcoding. Once sampled, sponge swabs were placed inside sterile plastic bags, the air inside the bags was manually removed, and the bags were sealed. Once collected, all sample bags were transported to the laboratory in a cooling box containing ice packs.

It is worth noting that appropriate protective clothing was used during the sampling process, and that gloves were changed between different samples to avoid cross-contamination.

2.3. Sample processing

Once in the laboratory, samples were processed to carry out culturing and metabarcoding. For poultry products, 25 g of each sample were mixed with 225 mL of BPW in blender bags (VWR, Pennsylvania, United States) and then homogenized at 230 revolutions per minute (rpm) for 1 min in a peristaltic homogenizer (Stomacher 400 Circulator, Seward, New York, United States). For food-contact surfaces, each sponge swab was mixed with 100 mL of BPW in filter bags and then homogenized at 230 rpm for 30 s. After the homogenization step, a stock dilution of each sample was obtained.

2.4. Culturing techniques

To assess the microbiota present throughout the burger processing line, the following microbial groups were cultured according to UNE-EN ISO standards: mesophiles, *Pseudomonadaceae*, Enterobacterales, lactic acid bacteria (LAB). Briefly, Plate Count Agar was used for mesophiles and plates were incubated at 30 °C for 72 h; *Pseudomonas* CFC/CN agar enriched with Cephalothin, Fucidin, Cetrimide (CFC) selective supplement was used for *Pseudomonadaceae*, and plates were incubated at 25 °C for 48 h; Violet Red Bile Glucose Agar (VRBG) was used for Enterobacterales, and plates were incubated at 37 °C for 24 h; and De Man Rogosa Sharpe Agar (MRS) was used for LAB, and plates were incubated for 30 °C/3–5 days under anaerobic condition. A more detailed description of the culturing method of these microbial groups can be found in a published book chapter (Merino et al., 2021).

GraphPad Prism software (v.8.4.2.679, Boston, Massachusetts, USA) was used to generate microbial counts bar charts as well as to compare sample types via analysis of variance (ANOVA) followed by *post-hoc* Tukey test; differences were considered statistically significant if p value ≤ 0.05.

2.5. Metabarcoding

2.5.1. DNA extraction

DNA extraction was carried out from stock dilutions of the different poultry products and surfaces (section 2.3.). The first step was the centrifugation of stock dilutions at 3345×g for 10 min at 4 °C. The supernatants were discarded, and the pellets were collected for DNA extraction, which was performed following the manufacturer's instructions of the PowerSoil Pro Kit (Qiagen, Hilden, Germany), but performing a double elution step with 25 μL to improve the DNA yield. Then, DNA concentrations were measured using a Qubit fluorometer and following the Qubit 1x dsDNA HS assay kit protocol (Thermo Fisher Scientific, Massachusetts, United States).

2.5.2. Amplicon sequencing

DNA samples were sent to Novogene (Cambridge, United Kingdom) for amplification and sequencing of the V3-V4 hypervariable region of the bacterial 16S rRNA gene. PCR reactions were carried out with 15 μL of Phusion High - Fidelity PCR Master Mix; 0.2 μM each of forward (341F: CCTAYGGGRBGCASCAG) and reverse (806R: GGACTACNNGGGTATCTAAT) primers and, and about 10 ng template DNA. The thermal cycling protocol included an initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, and a final elongation step at 72 °C for 5 min. Amplicon (466 bp in size) sequencing was carried out on the Illumina NovaSeq X Plus platform, generating 250 bp paired-end reads. After completion of the run, sequences were demultiplexed according to their unique barcodes and truncated by cutting off the barcode and primer sequences to obtain the raw reads.

2.5.3. Taxonomic analysis

After checking the quality of the raw paired-end reads with FastQC (Andrews, 2010), taxonomic classification of the sequences was performed using the QIIME2 pipeline, v2024.2 (Bolyen et al., 2019). Raw paired-end reads were imported into the pipeline, trimmed with the cutadapt plug-in, and filtered, denoised and merged using the DADA2 plug-in to produce high-resolution amplicon sequence variants (ASVs). These ASVs were taxonomically classified using a pre-trained Naive Bayes classifier based on the SILVA 138 database (Quast et al., 2012). Table S1 presents the number of raw reads, high-quality used reads (after the filtering process), and the percentage of reads that were taxonomically assigned and unassigned. The resulting taxonomic data in the form of an ASVs table and a taxonomy table were imported from QIIME2 to the R 4.3.2 environment for further data analysis using the phyloseq v1.46.0 R package (McMurdie & Holmes, 2013). Relative abundances of microbial genera were generated after filtering out the instances of mitochondria and chloroplast, glomming the data with automatic pruning at the genus level, transforming genus abundance data into relative counts, and merging all genera with a cumulative count below 5% across all seven sample groups into the “Others” category. For the alpha and beta diversity estimates, chloroplast and mitochondria sequences were filtered out from the original phyloseq object, which was then rarefied to the minimal sequencing depth of 15,701 reads per sample to facilitate comparison across samples. The plot showing the rarefaction curves was done with the vegan package v.2.6.6.1 (Oksanen et al., 2022). Alpha diversity was assessed using the phyloseq package, employing the metrics ’Observed’, ’Shannon’, and ’Inverse Simpson’. For the beta diversity analysis, Principal Coordinates Analysis was performed on the Bray-Curtis dissimilarity matrix using the ordinate(a) function of phyloseq. PERMANOVA (Permutational Multivariate Analysis of Variance) was used to compare beta diversity between groups. The analysis was performed using the adonis(A) function from the vegan R package, with Bray-Curtis dissimilarity as the distance metric. The significance of group differences was assessed based on 999 permutations. Differential abundance analysis was performed with the R package microbiomeSeq v0.1 (Ssekagiri et al., 2017) on absolute count data, based on DESeq2 implementation (Love et al., 2014). DESeq2 (Differential Expression analysis for Sequence data 2) is considered a well-suited method for differential abundance analysis in microbiome studies because it models overdispersed count data using a negative binomial distribution, handles sparse data effectively, and incorporates estimators to control false discovery rate (Jonsson et al., 2016; Weiss et al., 2017). All graphs were plotted using ggplot2 v3.5.1 (Wickham, 2011). The package rstatix v0.7.2 (Kassambara, 2023) was used for the incorporation into the alpha diversity graphs of statistical results from multiple pairwise comparisons. These comparisons between alpha diversity indices were done using the Wilcoxon rank-sum test because the normality assumption required for parametric tests was not met, as determined by the Shapiro-Wilk test. All statistical analyses were performed in the R environment, and statistical significance was assessed at $p \leq 0.05$. When necessary, p-values adjusted for multiple comparisons according to the Benjamini and Hochberg method (Benjamini & Hochberg, 1995) were taken.

3. Results and discussion

3.1. Culture-dependent approach

Culturing techniques were utilized to determine the absolute abundance of different cultivable microbial groups in different samples collected throughout the poultry burger processing line across both seasons (Fig. 1). *Pseudomonadaceae* was the predominant microbial group in chicken thigh and burger samples across both seasons (mean value of 4.77 log CFU/g), followed by LAB (mean value of 3.97 log CFU/g), and then Enterobacterales (mean value of 3.04 log CFU/g). No significant differences ($p > 0.05$) were detected in the microbial counts between thigh (Fig. 1A and D) and burger samples (Fig. 1C and F), suggesting that the addition of ingredients and contact with surfaces (Fig. 1B and E) did not contribute to an increase in the bacterial load of the microbial groups studied. In fact, even during processing, the microbial levels of food-contact surfaces remain low and comparable to clean surfaces in other industries (Kim & Yim, 2017).

Additionally, a complementary shelf-life experiment was conducted during the summer season to identify variations in microbial counts of burger samples before and after the shelf-life period. No great differences were observed between freshly produced burgers (Fig. 1F) and expired burgers (Fig. 2), with *Pseudomonadaceae* remaining the most represented group, followed by LAB and Enterobacterales. This differs from previous investigations where poultry products stored under similar conditions were dominated by LAB genera (Nieminen et al., 2012; Merino et al., 2024; Wang et al., 2017). Although microbial counts did not increase during processing, additional strategies such as the inclusion of natural antimicrobial compounds (e.g., essential oils, organic acids) or the use of advanced packaging technologies (e.g., active packaging incorporating antimicrobial agents) could be explored to extending shelf-life of poultry burgers under low oxygen conditions (Vilela et al., 2018).

When seasonal influence was assessed, we only found relevant differences at surfaces, with summer food-contact surfaces (Fig. 1E) exhibiting slightly higher microbial counts (~1 log cycle) than surfaces sampled in the winter (Fig. 1B).

3.2. Metabarcoding approach

Metabarcoding was utilized to determine the taxonomic profile (Fig. 3) as well as alpha diversity (Fig. 4) and beta diversity (Fig. 5) across the poultry burger processing line.

3.2.1. Taxonomic profile

The predominant genera in thigh and burger samples were *Pseudomonas*, *Psychrobacter*, *Brochothrix*, *Acinetobacter*, *Carnobacterium* and *Lactobacillus* (Fig. 3; Table S2), with differences in their predominance and relative abundance depending on the sample type and season. These findings were consistent with previous studies which also used metabarcoding to describe the taxonomic profile of different poultry products. For example, Park et al. (2023), Dourou et al. (2021), Min et al. (2023) and Song et al. (2021) highlighted the predominance of *Pseudomonas*, *Psychrobacter* and *Acinetobacter* in various poultry pieces. In addition to these genera, Rouger et al. (2018) and Lee et al. (2019) also identified *Brochothrix* and *Carnobacterium* as part of the predominant microbiota present on poultry.

All these genera have been previously involved in poultry spoilage. *Pseudomonas*, the predominant genus in our study, is considered the main spoilage genus of poultry meat stored under chilling and aerobic conditions (Dourou et al., 2021), inducing slime formation and off-odors by metabolizing nitrogenous compounds once its primary substrates

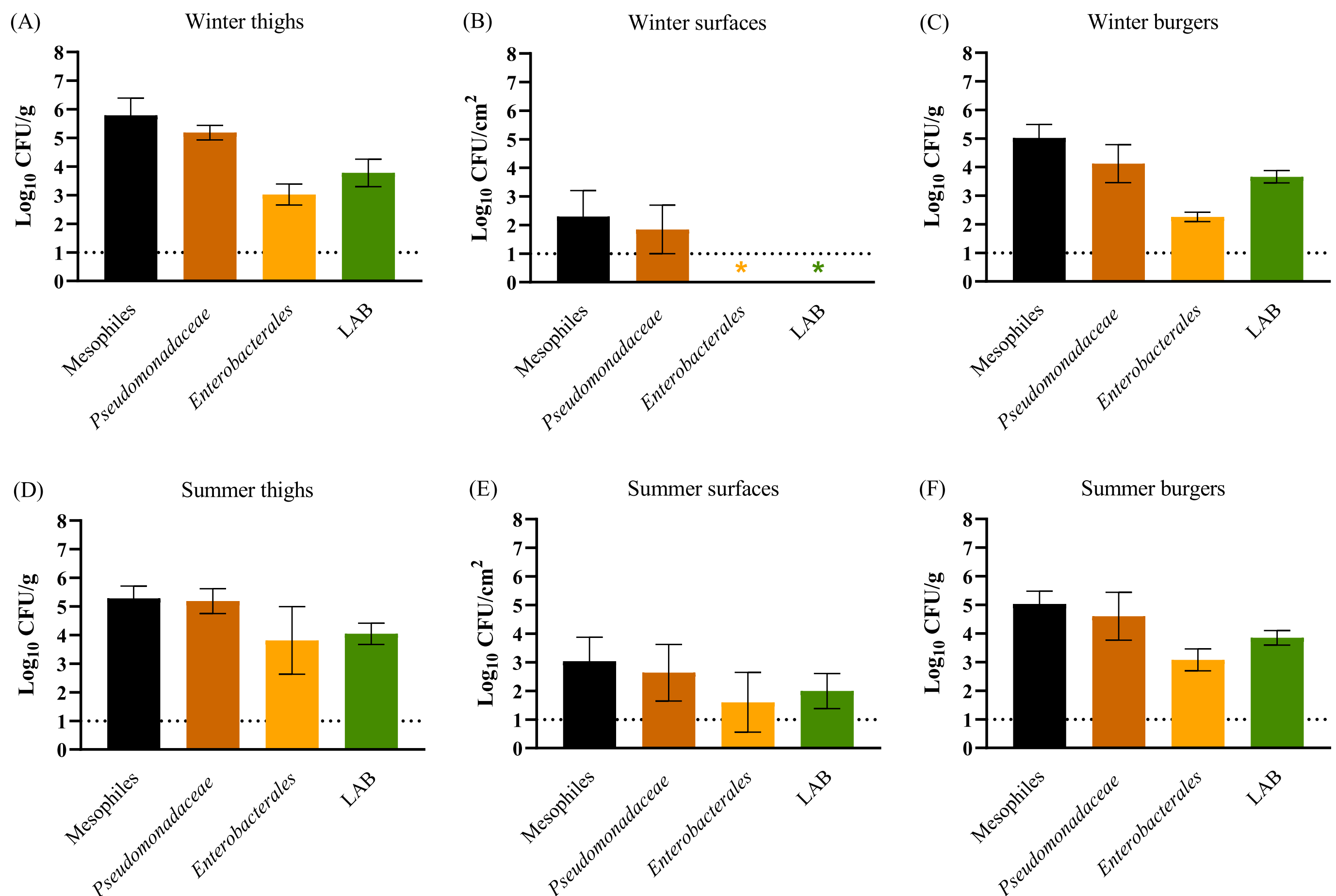

Fig. 1. Microbial counts throughout the burger processing line. Counts of mesophiles (■), *Pseudomonadaceae* (■), *Enterobacterales* (■), and LAB (■) in thigh samples (A, D), surface samples (B, E), burger samples (C, F); collected in winter (A, B, C) and in summer (D, E, F). The bars on the top of the columns represent the standard deviation. Horizontal dotted line represents the limit of detection.
* counts below the detection limit.

(glucose and lactate) are depleted (Nychas et al., 2008). *Brochothrix* is frequently linked to the spoilage of fresh meats (Ercolini et al., 2011; Pennacchia et al., 2011), characterized by cheese, buttery, and sour off-odors rather than putrefaction (Holley, 2014; Nychas & Drosinos, 2014). This genus can become predominant in meats stored under MAP or vacuum conditions when sufficient oxygen is present, but it can also shorten the shelf-life of meat stored under aerobic conditions (Stanborough et al., 2017). Both *Psychrobacter* and *Acinetobacter* have been also identified in poultry meat stored under cooling and aerobic conditions, although their spoilage potential is lower than that of the previously described genera (Cerveny et al., 2009; Dourou et al., 2021).

Unlike culturing techniques, metabarcoding revealed that expired burger samples (SEB) exhibited a different taxonomic profile (Fig. 3) compared to summer burger samples (SB). This difference was characterized by a significant increase ($p < 0.05$) in *Lactobacillus*, and a significant decrease ($p < 0.05$) in *Psychrobacter* during the shelf-life period (Table S3_A). These changes are likely due to the low oxygen conditions during the storage period (70% N_2 and 30% CO_2), which inhibit the growth of *Psychrobacter*, while promoting the proliferation of facultative anaerobic microorganisms such as *Lactobacillus*. In fact, a previous study also identified *Lactobacillus* as the predominant microorganism in broiler fillet strips stored under cooling and low oxygen conditions, with *Carnobacterium* also present in a significant abundance (Nieminen et al., 2012).

The comparison between poultry products (thighs and burgers) and food-contact surfaces revealed greater taxonomic differences in the winter season compared to the summer season. During the winter season, *Pseudomonas*, *Carnobacterium*, *Shewanella* and *Serratia* were significantly less abundant ($p < 0.05$) in food-contact surfaces (WS) compared to poultry products (WT and WB) (Table S3_B); while *Acinetobacter* and *Brochothrix* were more prevalent on food-contact surfaces (WS) compared to poultry products (WT and WB) (Fig. 3). The high prevalence of *Acinetobacter* on food-contact surfaces, probably due to contamination from thighs, was expected due to its biofilm-forming ability, its resistance to cleaning and disinfection (C&D) protocols, and its ability to survive in low temperatures and low nutrient availability. In fact, this genus is prevalent in fish and meat processing environments (Møretrø & Langsrud, 2017). Indeed, cross-contamination events of *Acinetobacter* spp. from mechanical pickers to poultry carcasses have been documented (Hinton et al., 2004). *Brochothrix* also exhibits a high resistance to extremely low temperatures (1 °C), allowing it to proliferate in FPE (Møretrø & Langsrud, 2017). Stellato et al. (2016) detected this genus on food-contact surfaces from various large and small meat processing plants after C&D protocols, comprising more than 10% of the resident bacteria.

In contrast, during the summer season, the predominant genera (*Pseudomonas* and *Psychrobacter*) on poultry products (ST and SB) were also the predominant genera on food-contact surfaces (SS) (Fig. 3). This result was consistent with a previous investigation where *Pseudomonas* and *Psychrobacter* were also the predominant genera on food-contact and non-food-contact surfaces from different slaughterhouses and meat industries (Alvarez-Molina et al., 2023). Nevertheless, significant differences in the relative abundance of certain specific genera were also observed. *Carnobacterium* was significantly less abundant ($p < 0.05$) on

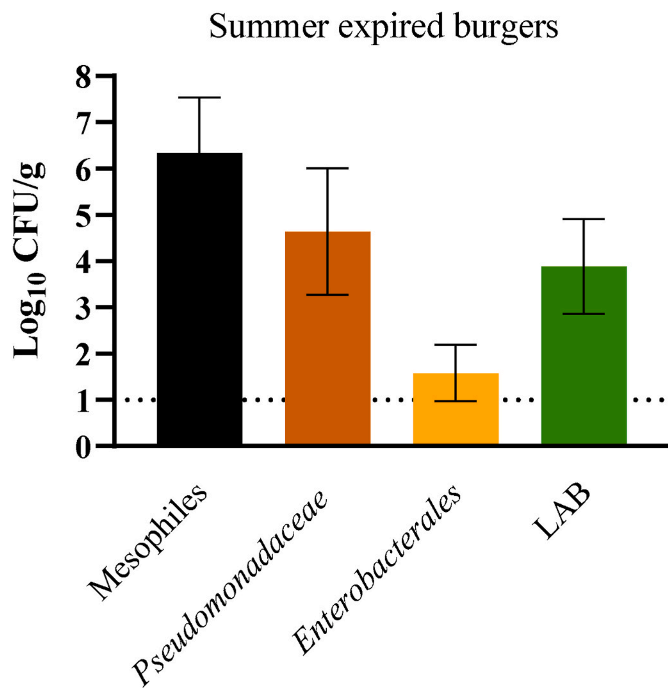

Fig. 2. Microbial counts after the shelf-life period. Counts of mesophiles (■), *Pseudomonadaceae* (■), *Enterobacterales* (■), and LAB (■) in expired burger samples collected during the summer season. The bars on the top of the columns represent the standard deviation. Horizontal dotted line represents the limit of detection.
* counts below the detection limit.

food-contact surfaces (SS) than in poultry products (ST and SB), while *Brochothrix*, *Proteus* and *Enterococcus* exhibited significantly higher relative abundances ($p < 0.05$) on food-contact surfaces (SS) than in poultry products (ST and SB) (Table S3_C). The significantly higher prevalence of *Proteus* on food-contact surfaces can be attributed to the resilience of some members of the Enterobacterales order to low temperatures, limited nutrient availability, and exposure to detergents and disinfectants (Møretrø & Langsrud, 2017).

Notably, the most significant taxonomic differences measured by metabarcoding were influenced by seasonal variation. Winter samples showed significantly higher abundance of *Acinetobacter*, *Brochothrix*, and *Lactococcus* ($p < 0.05$), whereas summer samples exhibited a significantly higher abundance of *Pseudomonas*, *Psychrobacter* and *Enterococcus* ($p < 0.05$) (Table S3_D). These seasonal variations in the taxonomic profile of poultry samples have been previously documented by other researchers (Kim et al., 2019; Song et al., 2021), although the predominant genera in each season differ from those observed in our study. Conversely, another study did not find seasonal differences in the microbiomes of poultry samples (Li et al., 2020).

The comparison of each sample group (thighs, surfaces or burgers) between the two seasons (winter and summer) allowed for a more detailed analysis and revealed greater differences in the taxonomic profiles. For example, the higher abundance of *Pseudomonas* in summer samples was specifically attributed to their increased presence ($p < 0.05$) on food-contact surfaces (SS) (Table S3_E). Additionally, *Enterobacter*, *Shewanella* and *Carnobacterium* exhibited significantly higher abundance ($p < 0.05$) in thigh samples during the winter season (WT) than during the summer (ST) (Table S3_F). However, *Shewanella* and *Carnobacterium* were significantly more abundant on surface samples during the summer season (SS) in comparison to the abundance in winter (WS) (Table S3).

3.2.2. Alpha diversity analysis

Rarefaction was performed to normalize sequencing data by subsampling to a uniform read depth across all samples (Fig. S1). This ensured that observed differences in microbial community diversity were not artifacts of unequal sequencing depths. Alpha diversity indexes were calculated using both rarefied and non-rarefied data to assess the

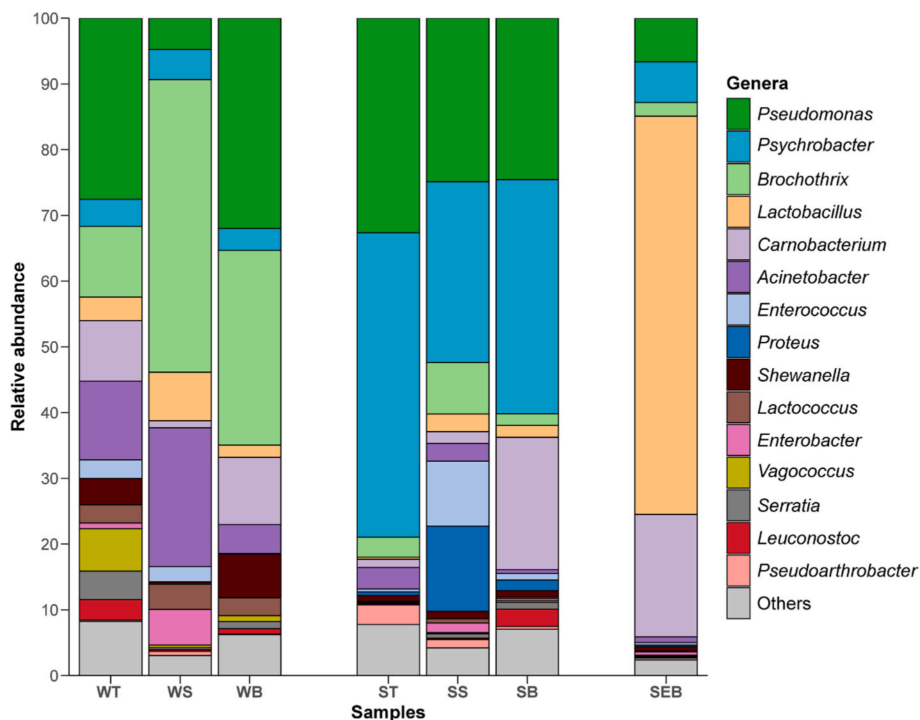

Fig. 3. Microbial composition throughout the poultry burger processing line. Relative abundances (%) of ASVs annotated at the genus level in each sample group throughout the poultry burger processing line [winter thigh samples (WT), winter food-contact surface samples (WS), winter burger samples (WB), summer thigh samples (WM), summer food-contact surface samples (WS), summer burger samples (WB), summer expired burger samples (SEB)].

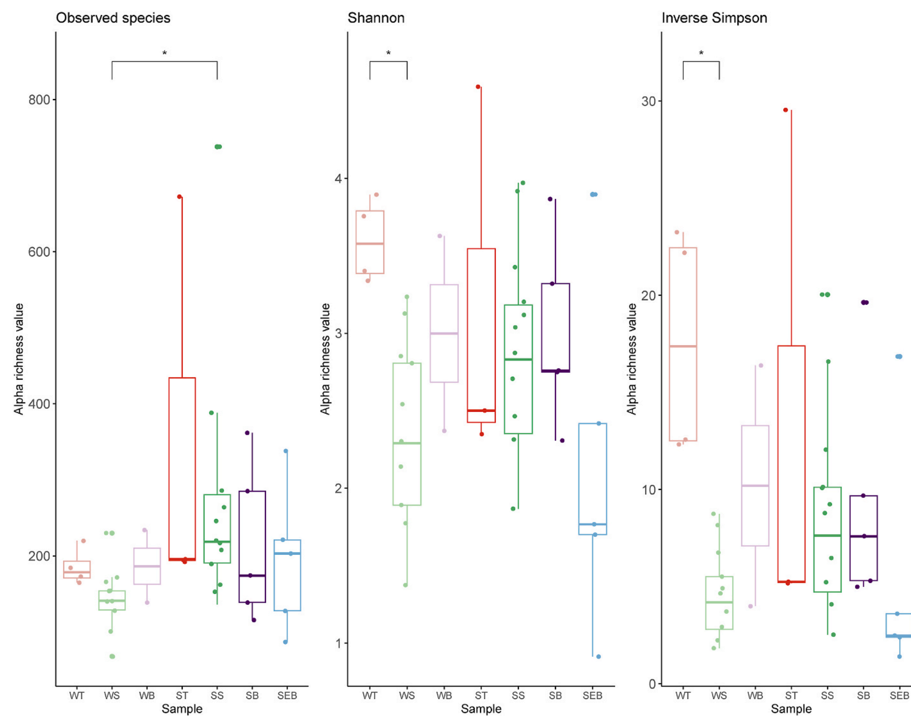

Fig. 4. Alpha diversity analysis of the microbial communities of the different sample groups throughout the poultry burger processing line. Boxplots of the Observed species, Shannon and Inversed Simpson indexes performed on the genus-level bacterial profiles of the different sample groups [winter thigh samples (WT), winter food-contact surface samples (WS), winter burger samples (WB), summer thigh samples (ST), summer food-contact surface samples (SS), summer burger samples (SB), summer expired burger samples (SEB)]. Each boxplot illustrates the distribution of the resulting richness data from the minimum to the maximum value, with horizontal lines indicating the 25th, 50th (median), and 75th percentiles. The bars on the top of the columns represent the standard deviation. The * indicates that the means of the two samples are significantly different (Wilcoxon rank-sum test, $p \leq 0.05$).

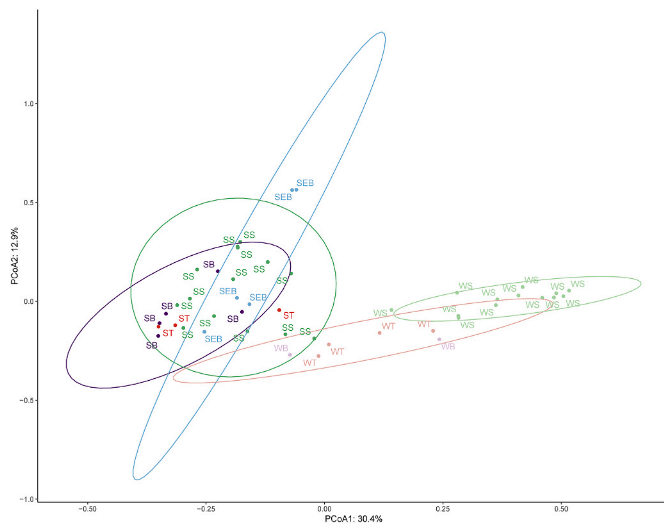

Fig. 5. Beta diversity analysis of the microbial communities of the different sample groups throughout the poultry burger processing line across both seasons. PCoA plot based on Bray-Curtis dissimilarities of all the samples performed on genus-level bacterial profiles. Outlined ellipses represent the 95% confidence area for winter thigh samples (WT, pink), winter surface samples (WS, light green), summer surface samples (SS, dark green), summer burger samples (SB, dark purple) or summer expired burger samples (SEB, blue). Winter burger (WB, light purple) and summer thigh (ST, red) samples could not be clustered.

effect of rarefying on diversity measures (Table S4). Substantial differences in the Observed Species index were only observed in a single surface sample collected during the summer (specifically, sample SSF3) (Table S4). This finding was consistent with its rarefaction curve (Fig. S1), which suggests that only for that sample a higher sequencing depth would have been required to accurately estimate the number of observed species.

Regarding alpha diversity analysis using rarefied data, no significant differences ($p > 0.05$) were observed in any of the alpha diversity indexes between thigh and burger samples in either season (Fig. 4; Tables S4 and S5), contrasting with the study conducted by Wang et al. (2019), which reported an increase in bacterial diversity in poultry carcasses after the chilling step, indicating that this particular processing step may be a potential source of contamination.

In contrast, when comparing the alpha diversity indexes of summer burger (SB) and expired burger (SEB) samples, a noticeable decrease in bacterial diversity after the shelf-life period was evident. Specifically, there was a reduction in the bacterial evenness of the microbial communities after the shelf-life period, indicated by lower values of the Shannon and Inverse Simpson indexes in expired burger samples (SEB) (Fig. 4; Table S4) with median values of 1.77 and 2.48, respectively. This reduction could be attributed to the significant predominance of *Lactobacillus* and *Carnobacterium* at the end of the shelf-life. This aligns with the study carried out by Dourou et al. (2021), which also reported a significant decrease in bacterial diversity after the storage period.

The comparison of alpha-diversity indexes between poultry products and food-contact surfaces only revealed significant differences ($p < 0.05$) in the winter season, showing higher evenness of microbial communities in thigh samples (WT) (Shannon median value of 3.58 and Inverse Simpson median value of 17.38) compared to surface samples (WS) (Shannon median value of 2.29 and Inverse Simpson median value of 4.20) (Fig. 4; Tables S4 and S5). The lower evenness of microbial communities in food-contact surfaces could be attributed to the substantial predominance of *Acinetobacter* and *Brochothrix*, which together represent over 60% of the relative abundance.

Finally, seasonality also influenced bacterial richness, with summer

samples showing higher values of the Observed Species index than winter samples collected from the same sample type. Specifically, the most significant seasonal differences in alpha diversity were observed among food-contact surfaces (WS and SS), where summer surfaces (SS) exhibited a significantly higher number of observed species (median value of 219) compared to winter surfaces (WS) (median value of 141) ($p \leq 0.05$) (Fig. 4; Tables S4 and S5). These findings could be attributed to two different factors. Firstly, the challenge in maintaining the cooling temperature in the processing plant during the summer season could promote the growth of a wide variety of microorganisms. Secondly, the longer operational period of the facility by the summer season (July 2020), compared to its initial operation during the winter season (December 2019), could have allowed for greater microbial colonization of surfaces over time. In fact, Cobo-Díaz et al. (2021) also observed an increasing bacterial diversity over time in a pork processing plant.

3.2.3. Beta diversity analysis

The taxonomic differences among the sample groups described earlier are visually represented in Fig. 5. As expected, the most pronounced distinction was between winter and summer samples ($p < 0.05$). Additionally, for the winter season, food-contact surfaces (WS) formed a separate cluster from poultry product samples (WT and WB), a separation that was not evident for the summer.

4. Conclusions

The integration of culturing techniques and metabarcoding offered a comprehensive view of the microbiota along the entire poultry burger processing line. The culture-dependent approach delivered data on the absolute abundance of viable microorganisms; whereas the metabarcoding approach offered a more detailed analysis of bacterial composition and diversity, enabling the identification of genus-level differences in each sample comparison.

Pseudomonas, Psychrobacter, Brochothrix, Acinetobacter and LAB bacteria were identified as the predominant microorganisms in poultry products. Unlike culturing techniques, metabarcoding detected shifts in the bacterial composition of burgers after reaching the expiration date. Furthermore, metabarcoding revealed distinct microbiota on food-contact surfaces compared to poultry products, although the contact with surfaces does not appear to contribute to an increase in the bacterial load of the microbial groups studied. Additionally, metabarcoding provides insights into how seasonal variations shape the composition of the microbiota of poultry products and poultry-contact surfaces. This understanding will guide implementation of C&D protocols suited to different seasons, potentially enhancing the safety and shelf-life of poultry products.

It should be noted that the comparison of results obtained with both techniques are often subjected to different biases, such as the selective growth conditions of culturing techniques and the amplification biases (Wu et al., 2010) or sequencing errors inherent in metagenomics (Hinton et al., 2004), which should be taken into account in the interpretation of microbial taxonomic profiling.

In conclusion, the detailed insights provided by metabarcoding underscore the importance of incorporating this technique, or other culture-independent methods, into the microbiome characterization of food products and FPE.

CRediT authorship contribution statement

Natalia Merino: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Espina:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Elisa Pagán:** Methodology, Investigation. **Hera Vlamakis:** Writing – review & editing, Conceptualization. **Laura Grasa:** Writing – review & editing. **Daniel Berdejo:** Writing – review & editing. **Rafael Pagán:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Diego García–Gonzalo:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Data availability statement

Raw sequencing reads are available in the Sequence Read Archive of the National Center of Biotechnology Information (NCBI) under the BioProject accession number PRJNA1182963. Additionally, the microbiome dataset can be accessed at https://doi.org/10.5281/zenodo.14621413.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 4 in order to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117392.

Data availability

Metabarcoding data have been uploaded to National Center of Biotechnology Information (NCBI) under the accession number PRJNA1182963.

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