

RESEARCH ARTICLE

Microbial Profile of traditional Dry-cured Meat: Metagenomic Analysis of Microbial Community of Kastamonu Pastrami Compared to Industrial Counterparts

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Abstract

This study provides the first metagenomic characterisation of Kastamonu pastrami, a traditional Turkish dry-cured meat, and compares its microbial diversity with industrially produced alternatives. The findings are evaluated regarding product quality, fermentation characteristics, and food safety. We analyzed 15 sirt pastrami samples (*M. longissimus thoracis*) from traditional producers A–D (n=12) and industrial producer E (n=3). The 16S rRNA V3–V4 region was sequenced on Illumina MiSeq and processed with standard quality-filtering/denoising and taxonomy-assignment workflow. Diversity metrics included alpha diversity (Shannon) and beta structure (PCoA, hierarchical clustering); group differences were evaluated with multiple-comparison procedures. Traditional products (A–D) showed higher alpha diversity overall than the industrial group (E) (p<0.05). Shannon values were highest in A and C with medians around ~2.10 and ~2.06 respectively; B was intermediate (~1.88–1.90); D and E were lowest (~1.71 and ~1.65 respectively). Community composition reflected these patterns; traditional samples contained producer-specific lactic acid bacteria (LAB) consortia—*Lactobacillus*, *Lactococcus*, *Weissella*, and *Leuconostoc*—while A and C also harbored hygiene/spoilage-associated genera (e.e., *Pseudomonas*, *Staphylococcus*, *Psychrobacter*, *Bacteroides*, and *Photobacterium*). The industrial group (E) exhibited low diversity with *Lactobacillus* dominance (~80% relative abundance) and minimal non-LAB representation, consistent with controlled fermentation and sanitation. Findings indicate a diversity–safety trade off: traditional processing preserves microbial richness and typicity but may elevate contamination risk without robust GMP/HACCP, whereas industrial processing enhances microbiological safety at the expense of diversity. These results provide a practical basis for targeted hygiene interventions that improve safety while preserving the distinctive character of traditional pastrami.

Keywords: Food safety, Kastamonu pastrami, Lactic acid bacteria, Metagenomics, Traditional meat products

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INTRODUCTION

Pastrami is a dry-cured meat produced from whole muscles obtained from specific regions of cattle and buffalo carcasses, classified as an intermediate-moisture food (Dışhan et al 2021, Suncak et al 2025). It has been made in Türkiye since at least the 12th century and remains the country's most popular dried meat product (Kaya et al 2022). Although a traditional Turkish product, pastrami is also produced and consumed in parts of the Middle East, Central Asia, and several Mediterranean and European countries.

A wide range of cured meats—both heat-treated and

non-heat-treated—are produced globally, including pastrami, bacon, Bündnerfleisch (an air-dried beef from the Swiss canton of Graubünden), and ham. While these products share key processing factors and microbial drivers, they differ substantially in composition and intended eating quality (Güngören 2025). Typical dry-cured processes involve curing, drying, heating, smoking, and fermentation; however, pastrami is traditionally produced without a heating or smoking step.

Pastrami is commonly made from beef or buffalo, but it may also be prepared from turkey and, experimentally, from fish or chicken. The process involves drying



suitable muscle cuts and coating the surface with a fenugreek-based paste. Production generally takes 10–15 d, depending on muscle size. Up to 26 distinct pastrami cuts can be obtained from a mature bovine carcass. Premium pastrami typically uses loin, trunk, leg, and dorsal muscles. After trimming visible fat, the meat rests at ambient temperature for 4–8 h, is portioned and salted, and then held under controlled conditions for ~24 h. Salting—an ancient preservation step—modulates microbial growth primarily via water-activity reduction and ionic/osmotic stress; however, its efficacy is dose-dependent and genus-specific, with halotolerant taxa showing variable resilience. After curing, the meat is rinsed to remove surface salt and air-dried. The dried surface is then coated with a spice paste (çemen) containing garlic, red pepper, and fenugreek (*Trigonella foenum-graecum*) powder, followed by 1–2 d of additional drying (Kilic 2009, Yildirim et al 2017). Çemen imparts a distinctive flavor and exhibits antimicrobial activity, which can reduce the risks of mold and bacterial contamination (Güngören 2025).

Kastamonu pastrami is traditionally produced by small-scale enterprises under largely natural environmental conditions, relying on artisanal know-how passed down through generations (Türker et al 2019). During preparation, meat cuts are suspended in open air and subjected to natural drying for ~30–45 d. The final product typically exhibits a deeper color and softer texture than industrial variants. Historically, fatty meat from local black-cattle breeds was preferred; contemporary consumer preferences, however, favor leaner raw materials. The aging/drying season is colloquially known as the “pastrami summer,” occurring mainly between October and November. A further hallmark of Kastamonu pastrami is its very thin slicing with exceptionally sharp knives (Anadolu Lezzet Envanteri 2016). Its distinctive flavor is attributed to locally sourced meat and the use of Taşkoprü garlic. Kastamonu pastrami can be produced with or without a çemen; the non-fenugreek variant is widely used for “pastrami bread,” a notable item in local cuisine, and pastrami is commonly consumed at breakfast in paper-thin slices.

Metagenomics—defined as the study of genetic material recovered directly from environmental samples—was first articulated by Handelsman et al (1998). By sequencing nucleic acids directly, metagenomics provides profiles of complex microbial populations without the need for culture, enabling both taxonomic and functional insights (Leite et al 2022). It has growing applications in food safety, public health, and clinical microbiology (Forbes et al 2017).

Although metagenomic studies exist for pork-based cured meats from China and Europe (Yi et al 2017, Qu et al 2023), no metagenomic data are available for Turkish pastrami, including the Kastamonu style. Moreover, no study has compared genus-level microbiotas of traditional and industrially produced pastrami.

Accordingly, this study aims to (i) characterize the genus-level microbial composition of traditional Kastamonu back-cut pastrami, (ii) compare it with an industrial counterpart, and (iii) evaluate implications for product quality, fermentation traits, and food safety. To our knowledge, this is the first metagenomic investigation of Kastamonu pastrami, providing a scientific basis for improving hygiene practices while preserving its distinctive sensory attributes.

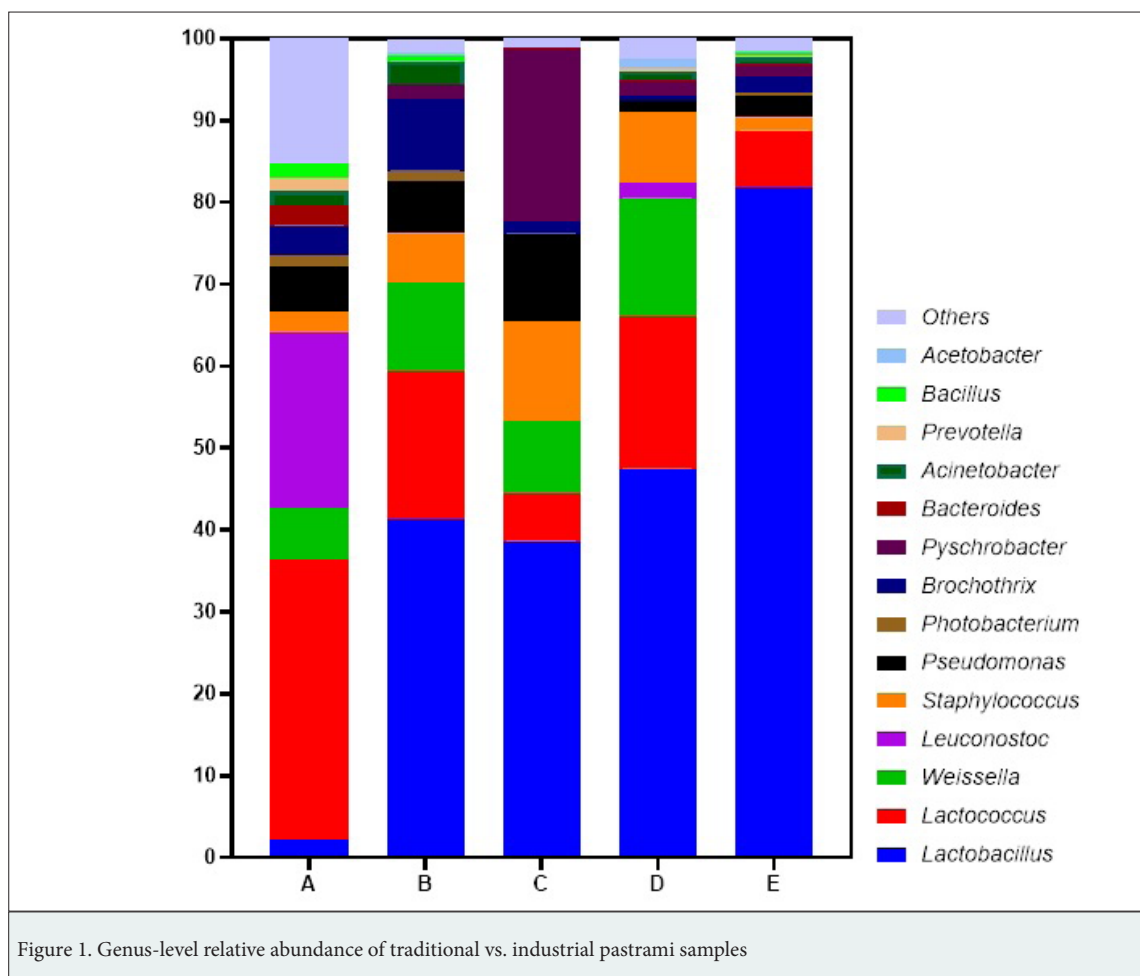
MATERIAL AND METHODS

Collection of pastrami samples

A total of 15 sirt pastrami samples (*M. longissimus thoracis*) were collected between October and November 2024, corresponding to the traditional “pastrami summer” drying period in Kastamonu, Türkiye. Twelve samples were obtained from four traditional producers (coded A–D; three samples each), and three samples were collected from an industrial producer (coded E) (Table 1). Traditional samples were purchased either un packaged or vacuum-packed from local retail markets, whereas industrial samples were obtained in their original commercial packaging. All samples were transported to the laboratory in insulated containers at 4±1°C within 4 h of purchase and stored under refrigeration for <24 h until analysis. Aseptic handling was applied throughout to prevent cross-contamination (Telli et al 2022).

Table 1. Distribution of traditional and industrial pastrami samples by production method and sample code (n = 15).

Production Type	Producer Code	No. of Samples
Conventional	A	3
	B	3
	C	3
	D	3
Traditional	E	3
Total		15



DNA extraction and quality control

Approximately 10 g of each sample was homogenized in 90 mL peptone water using a stomacher (BagMixer® 400, Interscience, France) for 2 min. Homogenates were transferred to sterile 2 mL microtubes and centrifuged at $13,000 \times g$ for 3 min at room temperature. Resulting pellets were used for DNA extraction with the DNeasy PowerFood Microbial Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). DNA integrity was verified by electrophoresis on a 1% agarose gel containing 0.15% ethidium bromide in TAE buffer, run at 120 V for 60 min, and visualized under UV illumination.

16S rRNA gene amplification and sequencing

Metagenomic analysis was performed on an Illumina MiSeq platform. The V3–V4 region of the 16S rRNA gene was amplified using primers Forward 5'-CCTACGGGNGGCWGCAG-3' and Reverse 5'-GACTACHVGGGTATCTAATCC-3' (Klindworth et al 2013). Index barcodes and adapter sequences were added to enable multiplexing and attachment of PCR products

to instrument oligos. Indexing was performed with the Nextera XT kit under PCR conditions of 95°C for 3 min; 8 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s; and a final extension at 72°C for 5 min. Libraries were cleaned with magnetic beads prior to pooling to ensure balanced representation, loaded onto the MiSeq flow cell, and clustered by bridge amplification. Sequencing used Illumina's sequencing-by-synthesis chemistry. Raw data were processed with MiSeq Control Software v2.2 and Real-Time Analysis v1.18, and base calls were exported as FASTQ files for downstream bioinformatics.

Bioinformatics analysis

Paired-end Illumina reads (2×250 bp) were imported into the QIIME 2 environment (Bolyen et al 2019). All samples exceeded 100× sequencing depth; no samples were excluded. Quality trimming, chimera detection, and denoising were performed with the DADA2 plugin (q2-dada2) (Callahan et al 2016), and bases with Phred < Q30 were trimmed. Amplicon sequence variants (ASVs) were taxonomically assigned against the SILVA 138 database (Schloss 2021). A phyloseq object (R version 4.1; R Core Team, 2016) was created from QIIME 2 artifacts for downstream analyses.

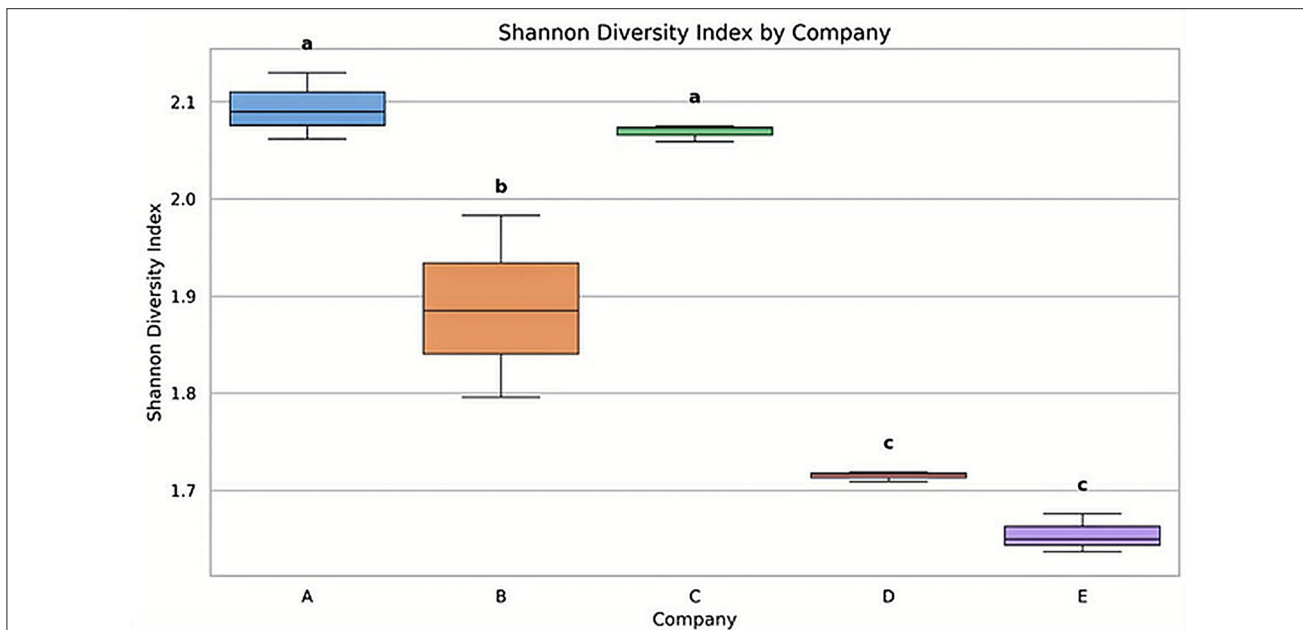


Figure 2. Shannon Diversity Index. There is no significant difference between groups that share the same letter. However, there is a significant difference between groups that share different letters.

Alpha diversity was assessed using Chao1, Shannon, and Simpson indices. P-values for group comparisons were calculated with the Kruskal–Wallis test (Kruskal and Wallis 1952). Beta diversity (between-sample differences) was computed using Jaccard, Bray–Curtis, and weighted/unweighted UniFrac distances. Differential abundance was tested with DESeq2 (R package) (Love et al 2014). LEfSe (Linear Discriminant Analysis Effect Size) was used to identify taxa with statistically significant differences between groups (Segata et al 2011).

RESULTS

We compared the genus-level microbiota of back-cut pastrami from four traditional producers in Kastamonu (A–D) and one industrial producer (E). Community composition per sample is shown in Figure 1, and diversity/ordination summaries are provided in Figures 2–4. Shannon alpha diversity differed among producers (Kruskal–Wallis $H = 13.03$, $p = 0.011$); pairwise Mann–Whitney U tests with Bonferroni correction indicated

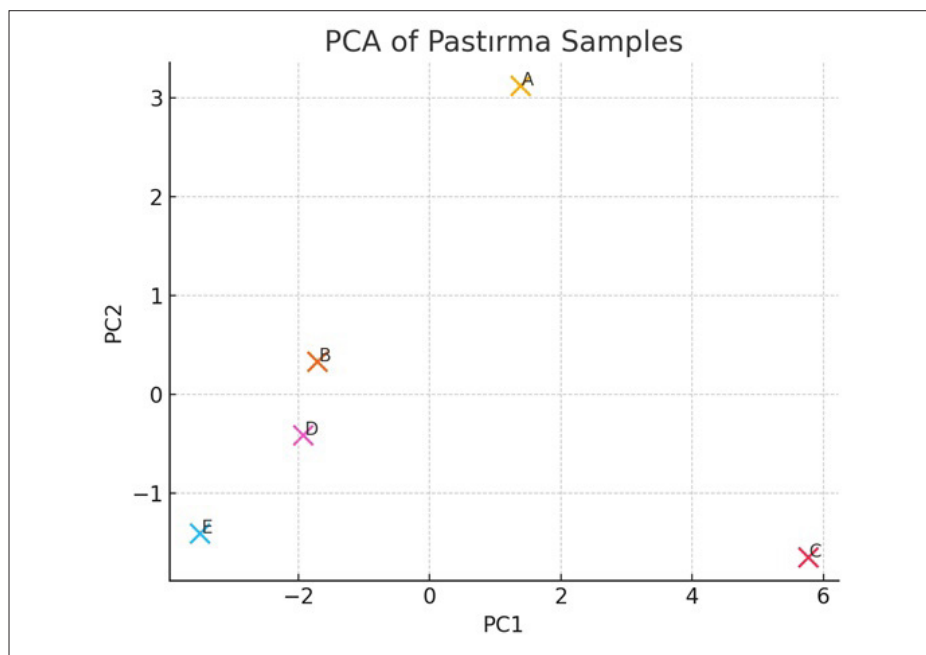
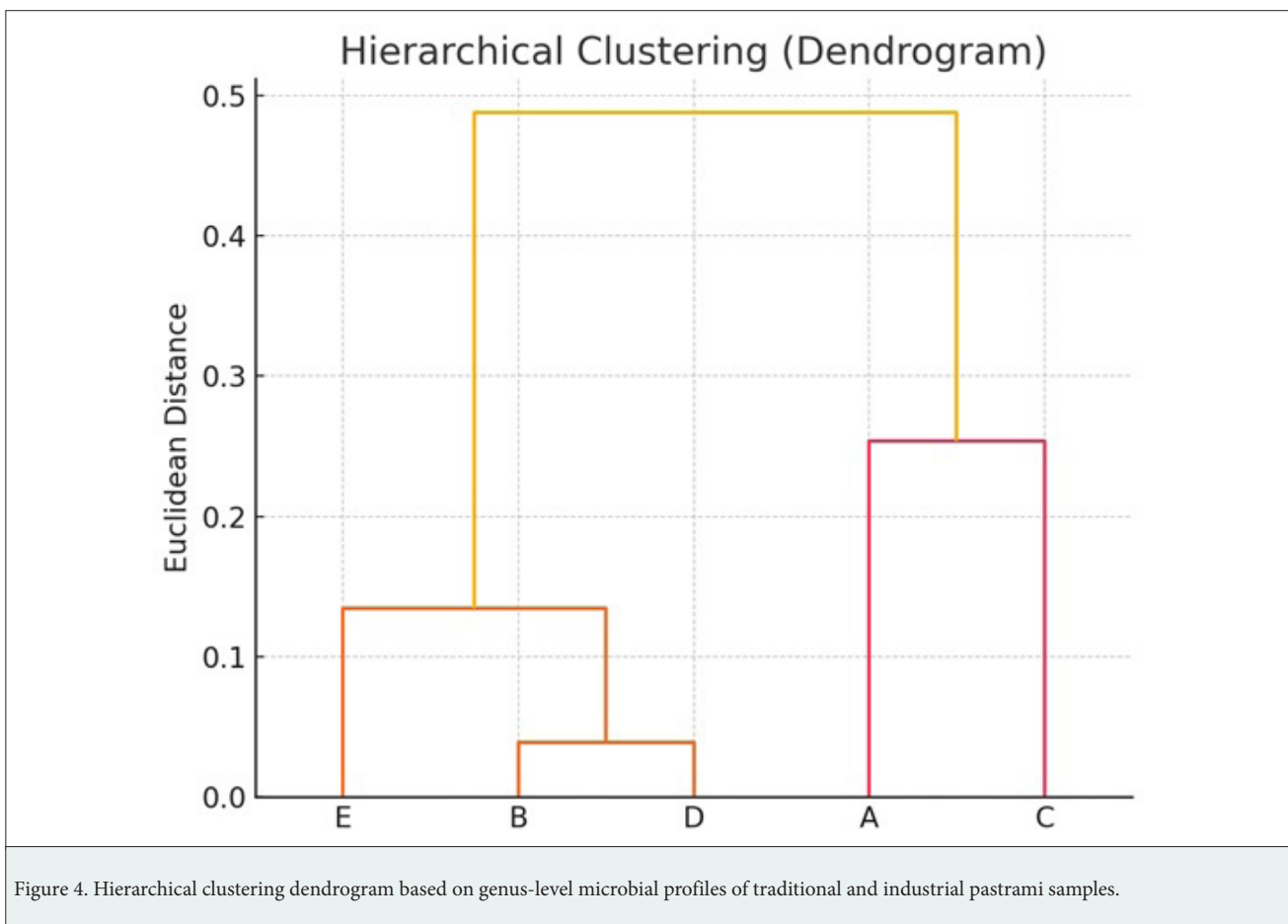


Figure 3. Principal coordinates analysis (PCoA) plot showing microbial community differences among pastrami samples based on genus-level composition



A and C > E ($p < 0.05$). Median Shannon values were approximately A ~ 2.10 , C ~ 2.06 , B ~ 1.88 – 1.90 , D ~ 1.71 , and E ~ 1.65 (Figure 2). At the genus level, traditional samples generally contained LAB (lactic acid bacteria) including *Lactobacillus*, *Lactococcus*, *Weissella*, and *Leuconostoc*: A showed LAB (64%) with *Pseudomonas*, *Staphylococcus*, *Photobacterium*, *Bacteroides*, *Brochothrix*, *Prevotella*, *Bacillus*, and *Acinetobacter*; B was predominantly *Lactobacillus* (41%) and *Lactococcus* (18%) with *Bacillus*, *Weissella*, *Staphylococcus*, *Pseudomonas*, *Acinetobacter*, *Acetobacter*, *Psychrobacter*, *Photobacterium*, and *Brochothrix*; C had higher *Psychrobacter* (21%), *Pseudomonas* (11%), and *Staphylococcus* (12%) with lower LAB (53%) (e.g., *Weissella*, *Lactococcus*, *Lactobacillus*) and detectable *Brochothrix*, and *Bacteroides*; D showed LAB (82%) with *Staphylococcus* and lower levels of *Pseudomonas*, *Brochothrix*, *Psychrobacter*, *Bacteroides*, *Acinetobacter*, *Prevotella*, and *Acetobacter*; and E (industrial) was *Lactobacillus*-dominant ($\sim 80\%$ relative abundance) with low non-LAB representation (e.g., *Staphylococcus*, *Pseudomonas*, *Photobacterium*, *Acinetobacter*, *Brochothrix*) (Figure 1). PCoA separated samples by community structure (Figure 3): E was distinct along PC1 (77.3%) and associated with *Lactobacillus* dominance, C separated along PC2 (21.0%), B and D clustered in proximity, and

A was positioned furthest overall. Hierarchical clustering resolved the data into two main groups, (A, C) and (B, D, E) (Figure 4). Overall, traditional samples (A–D) showed higher alpha diversity with producer-specific LAB consortia, whereas the industrial group (E) showed lower diversity and *Lactobacillus* dominance.

DISCUSSION

This study compared the genus-level microbial profiles of traditionally produced Kastamonu pastrami with those of an industrial counterpart, with implications for product quality and food safety. Overall, the results indicate that traditional processing supports a richer and more diverse community, whereas lapses in hygiene or temperature control can permit the occurrence of spoilage- and hygiene-associated taxa.

The detection of LAB—including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Weissella*—in Samples A–D confirms that traditional products underwent spontaneous fermentation. LAB are desirable in pastrami because they acidify the product, suppress pathogens, and contribute to flavor development (Kilic 2009, Öztürk 2015). *Leuconostoc*, in particular, can influence color and flavor through nitrate/nitrite reduction (Roberts and Dainty 1996).

In the industrial sample E, *Lactobacillus* dominated (~80%), consistent with controlled fermentation and a stable microbiological profile. This aligns with the role of process control in food safety (Forbes et al 2017).

By contrast, Samples A and C (traditional) contained hygiene/spoilage-associated genera such as *Pseudomonas*, *Staphylococcus*, *Photobacterium*, *Acinetobacter*, and *Bacteroides*. *Pseudomonas* can proliferate at refrigeration temperatures and reduce shelf life, while *Staphylococcus* includes strains capable of enterotoxin production (Yildirim et al 2017). The dominance of *Psychrobacter* in Sample C suggests prolonged refrigeration or cold-chain interruptions, a point that should be addressed as a critical control point under ISO 22000. The presence of *Bacteroides* is consistent with fecal contamination and underscores the need for stringent hygiene practices.

Our findings both converge with and diverge from prior metagenomic studies on cured meats. Using metagenomics, Yi et al (2017) reported higher abundances of *Staphylococcus*, *Sphingomonas*, *Pseudomonas*, *Enterobacteriaceae*, and *Leuconostoc* in bacon. Gong et al (2021) found *Staphylococcus*, *Acinetobacter*, and *Macroccoccus* to be dominant, with *Leuconostoc* and *Lactobacillus* at very low levels (~0.001%). Zhou et al (2021), analyzing Hui-style pastrami along production stages, observed early dominance of *Lactococcus* and *Pseudomonas*, shifting to *Staphylococcus*, *Salinivibrio*, and *Actinomycetes* by mid/end ripening. Wang et al (2021) reported *Staphylococcus* as most abundant in Sichuan pastrami, with geographically variable levels of *Pseudomonas*, *Brochothrix*, *Lactobacillus*, *Lactococcus*, and *Enterococcus*. Qu et al (2023) identified *Psychrobacter* and *Brochothrix* (with *Phoma* and *Trichoderma* among fungi) as dominant in Longxi pastrami, while Ning et al (2024) found *Staphylococcus*, *Psychrobacter*, and *Latilactobacillus* as key genera in Zhenba pastrami during curing/smoking. Li et al (2022) reported *Staphylococcus* as most dominant across western Hunan regions, also noting *Sphingomonas*. For Turkish pastrami, Özdemir et al (1999) used culture-based counts and described dominant flora comprising *Lactobacilli*, *Micrococci*, and *Staphylococci*.

In the present work, relatively high LAB levels in Kastamonu pastrami likely reflect traditional practices, including the fenugreek paste and extended open-air drying/maturation, which together shape the community structure. Notably, we found no prior genus-level metagenomic characterization of Turkish pastrami. Differences between our findings and studies from China may stem from raw-material species (beef vs pork), climate and geography, animal feeding, specific processing steps (e.g., smoking, additives, vacuum curing), and hygiene management—all of which can drive distinct community compositions and diversity.

CONCLUSION

This study demonstrates that traditionally produced Kastamonu pastrami exhibits considerably higher microbial diversity compared to its industrial counterpart. The presence of beneficial lactic acid bacteria—such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Weissella*—indicates spontaneous fermentation in traditional products. However, the concurrent detection of spoilage- and hygiene-associated genera (e.g., *Pseudomonas*, *Staphylococcus*, *Psychrobacter*, and *Bacteroides*) demonstrates that greater diversity is not uniformly advantageous and may reflect uncontrolled variability and suboptimal hygiene management. By contrast, the industrial sample exhibited a lower-diversity, LAB-dominant community consistent with controlled processing and enhanced microbiological safety.

Differences between traditional and industrial products likely arise from a combination of factors, including processing methodology and hygiene protocols, raw-material characteristics, climatic conditions, the application of fenugreek paste, and curing/drying duration. The scarcity of genus-level metagenomic data for Turkish pastrami—especially the Kastamonu style—underscores the novelty and relevance of these findings.

To strengthen safety without eroding typicity, we recommend: (i) targeted hygiene training for artisanal producers; (ii) broader implementation of GMP and HACCP (and alignment with ISO 22000 where feasible); and (iii) judicious use of controlled starter cultures in contexts where they do not compromise sensory identity. Collectively, these measures can reduce contamination risks while preserving the distinctive character of traditional Kastamonu pastrami. Finally, our results provide a foundation for future studies on the metagenomic characterization of traditional Turkish meat products and for the development of evidence-based standardization and risk-management strategies.

Limitations and Future Directions

This study was designed as an exploratory, real-world metagenomic survey rather than a confirmatory, hypothesis-testing trial. As such, the sample sizes were imbalanced across groups (traditional producers A–D: n = 12 total; industrial producer E: n = 3) due to seasonal availability, artisanal production volumes, and retail access during the October–November (pastrami summer) window. No a priori power calculation was performed; instead, we adopted a feasibility-driven sampling frame typical of pilot microbiome studies.

To mitigate the limitations imposed by small and unequal group sizes, we (i) used non-parametric statistics with multiple-comparison control (Kruskal–Wallis followed

by Bonferroni-adjusted Mann–Whitney tests), (ii) emphasized effect-oriented interpretation (ordination patterns, relative-abundance shifts) rather than dichotomous significance, (iii) ensured adequate per-sample sequencing depth (>100×) with uniform quality control (DADA2 denoising, chimera removal), and (iv) examined beta-diversity with metrics of complementary sensitivity (Jaccard, Bray–Curtis, weighted/unweighted UniFrac) to assess robustness of community-level differences.

We caution that generalizability is limited, particularly for the industrial group sampled from a single brand and for potential seasonality effects. Future studies should implement balanced sampling across producers, conduct a priori power analyses based on preliminary diversity estimates, include multi-brand industrial comparators, and consider longitudinal/seasonal replication to strengthen inference and support process standardization.

DECLARATIONS

Competing Interests

The authors declare that they have no conflict of interest regarding the publication of this article.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

According to the decision of the Kastamonu University Local Ethics Committee for Animal Experiments (HADYEK) dated 07.08.2025 (Ref. No: E-16498365-200-2500101078), the study titled “Metagenomic Profiling of Kastamonu Pastrami” does not involve the use of experimental animals and is therefore exempt from HADYEK approval requirements.

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Author Contributions

Motivation / Concept: YA; Design: YA, AG, AGU; Control/Supervision: YA, AGU; Data Collection and / or Processing: EFB, AG; Analysis and / or Interpretation: YA, EFB, AG; Literature Review: YA, EFB; Writing the Article: YA, AGU; Critical Review: YA, AG, AGU

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