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INSIGHTS INTO FRESH BEEF PRESERVATION: DELAYING *PSEUDOMONAS* SPOILAGE THROUGH VACUUM PACKAGING FOR ENHANCED SHELF LIFE

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I. INTRODUCTION

Beef, a widely consumed high-protein food, is highly susceptible to microbial spoilage, leading to a resource waste and economic losses. Current preservation strategies primarily focus on post-packaging control (e.g., vacuum/modified atmosphere packaging), yet neglect microbial prevention during slaughtering-reducing initial spoilage microbiota is critical for shelf-life extension. This study proposes a **"front-end prevention and back-end inhibition"** synergy framework: 1) Profiling microbial communities at slaughtering to identify initial spoilage sources; 2) Innovatively integrating three packaging methods (air, vacuum, and modified atmosphere) with two storage temperatures (4°C and -1°C), combined with high-throughput sequencing and correlation modeling, to quantify temporal dynamics of spoilage microorganisms (e.g., *Pseudomonas*) and quality indices (pH, total viable count [TVC], total volatile basic nitrogen [TVB-N]); 3) Constructing a full-chain microbial network linking slaughtering to storage. Results demonstrate that *Pseudomonas*, strongly associated with slaughtering contamination, dominates spoilage. Vacuum packaging at -1°C delayed its growth by 5 days, extending shelf life to 21 days. This study bridges the gap between segmented control measures, advancing a "targeted microbial interception" system for meat preservation.

II. MATERIALS AND METHODS

Sample Preparation and Experimental Design: Top sirloin from six Simmental steers (2 years old, 625 kg average weight) was collected within 45 min post-slaughter, transported on ice to a 4°C laboratory refrigerator, and aged for 24 h. After trimming, each carcass was divided into 25 segments (350 g each), randomly assigned to six groups (**Table 1**) with three packaging methods (air, vacuum, modified atmosphere (55% CO_2/6.6% O_2/38.4% N_2)) and two storage temperatures (4°C and -1°C). Samples were collected at 0, 3, 9, 15, and 21 days. **Spoilage indices:** The pH [1], TVB-N (GB 5009.228–2016), and TVC (GB 4789.2–2022) were determined. **Microbiome:** DNA extracted using FastDNA® Spin Kit. V3-V4 regions amplified with primers 338F/806R (PCR: 95°C 3 min; 27 cycles of [95°C 30 s, 55°C 30 s, 72°C 45 s]; 72°C 10 min) [2]. Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina Nextseq2000 platform (Illumina, San Diego,USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). OTU clustering (97% identity), SILVA annotation, α/β diversity, and PCoA were analyzed via Majorbio Cloud. **Statistics:** Two-way ANOVA was conducted by SPSS 20.0. The co-occurrence networks were constructed based on significant Spearman correlations ($|\rho|>0.5$, $p<0.05$).

Table 1 – Overview of the experimental design.

Samples	Treatment	
	Packaging	Storage
A4	Air packaging (AP)	4°C
V4	Vacuum packing (VP)	4°C
G4	Modified atmosphere packing (MAP)	4°C
A1	AP	-1°C
V1	VP	-1°C
G1	MAP	-1°C

III. RESULTS AND DISCUSSION

Packaging systems directly affect bacterial physiology and, consequently, spoilage occurrence and dynamics [3]. While pH showed no intergroup differences, TVB-N served as a robust freshness biomarker, escalating with storage time. At 4°C, MAP and AP samples exhibited TVB-N surges post-day 9 ($P<0.05$), surpassing VP at day 15. VP suppressed TVB-N accumulation entirely ($P>0.05$), maintaining freshness for 21 days. Lower temperatures (-1°C) delayed but did not halt spoilage. TVC trends confirmed accelerated microbial growth at 4°C (**Fig. 1A**). VP's efficacy

at -1°C likely stemmed from hypoxia inhibiting *Pseudomonas* proteases, disrupting the TVB-N-TVC feedback loop [4]. PCoA (Bray-Curtis) and ADONIS revealed that packaging and storage temperature significantly drove bacterial community divergence. VP showed continuous and diverse community succession, while AP stabilized post-day 9, likely due to dominant colonizers. Proteobacteria (>65%) dominated, with *Pseudomonas* most abundant in AP (99%), confirming its spoilage role. VP suppressed *Pseudomonas* but enriched *Carnobacterium*, while MAP promoted *Serratia*, aligning with oxygen-control mechanisms. 4°C elevated LAB, revealing temperature-modulated microbial interactions for shelf-life extension (Fig.1B). Source tracking revealed that dominant taxa, particularly *Pseudomonas*, originated from slaughterhouse contamination (tools, environment, and processing surfaces) (Fig. 1C), confirming that slaughter hygiene is prerequisite to suppress *Pseudomonas* early proliferation and extend shelf life.

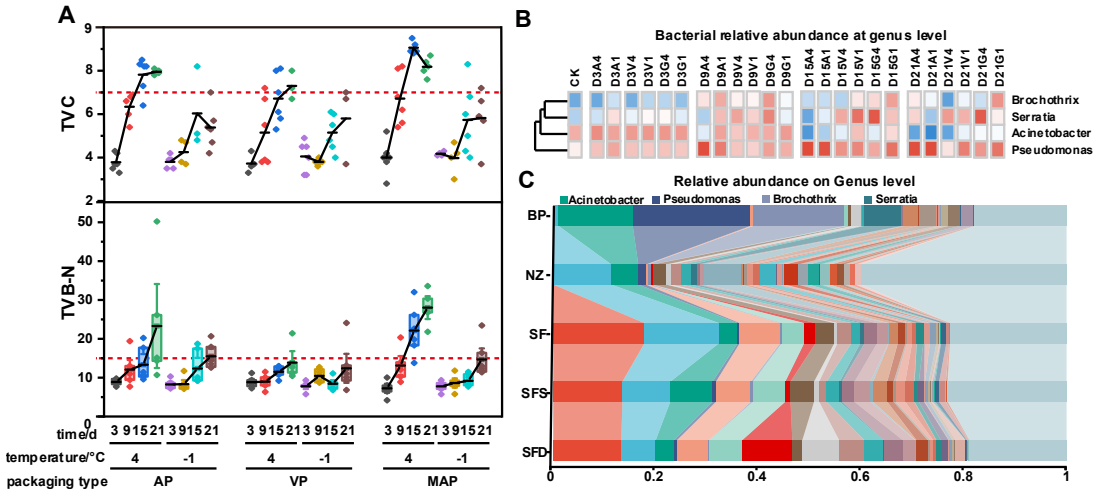


Figure 1. Effect of different packaging types combined with different temperature conditions TVC and TVB-N of packaging beef during 21 days storage (A); Microbial community compositions at genus levels(B), and microbial changes of cattle carcass surface, cutting tool and human hand surface during the slaughter process (C).

IV. CONCLUSION

This study deciphered the critical mechanisms of beef spoilage control across the full chain.
Time-dependent dominance of *Pseudomonas*: Early proliferation linked to slaughterhouse contamination (initial abundance >42%), driving TVB-N accumulation via proteases and forming a TVC-positive feedback loop.
Packaging-temperature synergy threshold: VP combined with -1°C extended the shelf life of beef to 21 days, whereas MAP at 4°C delayed spoilage by only 5 days due to limited CO_2 solubility.
Full-chain intervention imperative: Isolated measures (e.g., packaging alone) failed to fully inhibit spoilage, necessitating integrated strategies from slaughterhouse microbiota control to storage monitoring.
We recommend adopting VP with -1°C storage industrially and developing *Pseudomonas*-abundance-based shelf-life prediction models. Future studies should optimize slaughterhouse sanitation to reduce initial microbial loads.

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