CPS 2022 RFP – FINAL PROJECT REPORT

Project Title:
Interaction of resident microbiome and *Listeria* on pears during cold storage

Project Period:
January 1, 2023 – December 31, 2024 (extended to February 28, 2025)

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Objectives:

1. Examine the fate of *Listeria* and temporal changes of the resident microbiome on pears and their interactions during long-term cold storage, and further characterize the dominant and differential bacterial and fungal populations in the microbiome in the co-occurrence of *Listeria*.
2. Evaluate the impacts of organic practices on the microbiome community and persistence of *Listeria* on pears of the selected varieties during long-term storage.

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FINAL REPORT

Summary of Findings and Recommendations

The study investigated the survival of *Listeria monocytogenes* under simulated storage conditions and examined the dynamics of *L. innocua* and native microbiota on different pear varieties during long-term cold storage. It further evaluated microbiome composition and its interaction with *Listeria* on pears across pear varieties, agricultural practices, and storage regimes using metagenomic sequencing. Results show that pear surfaces are generally inhospitable to *Listeria*, with significant reductions over time regardless of pear variety, storage temperature, or production method. Bosc pears showed greater initial reductions during the first 24 hours but supported higher survival over time. Yeasts and molds increased during storage across all conditions. Each pear variety harbored a distinct microbiome, and conventional and organic pears exhibited unique bacterial communities. Inoculation with *L. innocua* reduced overall bacterial diversity and shifted community structure, increasing *Pseudomonas*, *Enterococcus*, and *Stenotrophomonas*, while decreasing *Pantoea*, *Curtobacterium*, and *Sphingomonas*. Microbial diversity and composition shifted over time across taxonomic levels. Low levels of *Listeria* were detected in uninoculated samples, suggesting a possible natural occurrence. The findings provide insights into how pear cultivar, production method, and storage conditions influence pathogen survival, and highlight potential links between *Listeria* contamination and microbiome, supporting the development of future postharvest safety strategies.

Abstract

Listeria monocytogenes can be found in a wide variety of natural and food production environments and has been implicated in multiple fresh produce-related listeriosis outbreaks. Washington leads pear production in the United States with a total of 347,000 tons in 2020. Though fresh whole pears have not experienced an incidence of listeriosis illness or outbreak, multiple recent large-scale recalls and high-profile outbreaks associated with other tree fruits due to potential *L. monocytogenes* contamination have prompted the pear industry to develop validated interventions and have proactive measures to minimize postharvest risks. Pear surfaces are colonized by diverse groups of microorganisms and further exposed to microbial contamination during production and processing. The associated resident microbial communities (microbiome) and those introduced at harvest or postharvest handling may influence the persistence of foodborne pathogens on the surface. There is a general lack of knowledge on the fate of *Listeria* on pears during storage, or the dynamic changes of the pear microbiome over long-term cold storage. The objectives of study were to evaluate temporal changes of microbiome and their interactions with *Listeria* on pears following different agricultural practices and storage regimes using a metagenomic sequencing approach. The study first examined the fate of *L. monocytogenes*, its surrogate, and temporal changes of the resident microbiota on pears during long-term cold storage, and further evaluated the impacts of organic practices on the microbiome community and persistence of *Listeria* on pears of the selected varieties during long-term storage. Findings provide insights into the interaction between *Listeria* and indigenous microbiota on pear surfaces.

Background

Listeria monocytogenes is listed by the U.S. Food and Drug Administration as a 'pathogen of concern' and has been singled out on both ready-to-wash and ready-to-eat produce due to its nature as a true environmental species, common prevalence in many produce-associated locales and operations (Ruiz-Llacsahuanga et al., 2021; Simonetti et al., 2021; Sullivan and Wiedmann, 2020), and high rate of mortality at 22.5% in produce outbreaks (Sheng and Zhu, 2021). *Listeria* spp. including *L. monocytogenes* have been

isolated from commercial tree fruit packinghouses (Ruiz-Llacsahuanga et al., 2021; Simonetti et al., 2021). The prevalence of *Listeria* spp. on food contact surfaces increased after 3 and 6 months of apple storage (Ruiz-Llacsahuanga et al., 2021). Additionally, *L. monocytogenes* can grow at cold temperatures. For example, *L. monocytogenes* grows well on apple slices (Conway et al., 2000), wound sites, or in the caramel-coated apple-surface microenvironment (Glass et al., 2015) at 7-10 °C. *L. monocytogenes* has been implicated in multiple fresh produce-related listeriosis outbreaks (CDC, 2012, 2015, 2020). Washington and Oregon lead pear production in the United States with a total of 347,000 tons in 2020 (NASS, 2021). Though fresh whole pears have not experienced an incidence of listeriosis illness or outbreak, multiple recent large-scale recalls (FDA, 2017, 2019, 2020) and a high-profile outbreak associated with other tree fruits (CDC, 2015) due to potential *L. monocytogenes* contamination indicate that proactive measures are needed to minimize postharvest risks.

The state of Washington produces a versatile variety of pears throughout the harvest season. Each variety has a unique surface structure, which is expected to affect the establishment of the microbiota, resulting in a unique microbiome. Pears typically involve short or long-term postharvest storage, undergoing dynamic changes during long-term cold storage. The associated resident microbial communities (microbiome) on the surface or introduced at harvest or postharvest handling may influence the persistence of contaminating *Listeria* or other foodborne pathogens. The resident microbiota showed antagonistic effects on foodborne pathogens including *Listeria* in fresh produce (Liao and Fett, 2001; Siroli et al., 2015). The presence of the native microbiota on the carrot surface substantially reduced the growth of *L. monocytogenes* in carrots during storage (Liao, 2007). A recent study also suggested that the packinghouse environment microbiome in tree fruit packinghouses was associated with the prevalence/ persistence of *L. monocytogenes* in packinghouse environments (Tan et al., 2019). These findings indicate a complex interaction between the resident microbiota, *Listeria*, apple variety, and storage conditions.

In recent years, organic pear production has increased steadily (Prengaman, 2021). Differences in management systems between organic and conventional orchards are also expected to affect resident microbiome composition and diversity. For example, the microbiome of apples cultivated under organic farming practices was significantly different from that of conventionally cultivated apples (Wassermann et al., 2019). Interestingly, the bacterial family of *Enterobacteriaceae*, which contains taxa responsible for foodborne outbreaks, was higher on conventional fruits than organic ones (Wassermann et al., 2019). Besides agricultural practice, other environmental factors such as geographic location can impact microbiome composition on tree fruit surfaces (Abdelfattah et al., 2021).

Up to now, information about the fate of *Listeria* on pears during refrigerated air (RA) and controlled atmosphere (CA) storage has remained unavailable. There is a lack of knowledge about the microbiome of different pear cultivars produced with different agricultural managements and their temporal changes over long-term commercial cold storage, as well as their interaction with *Listeria* on pear surfaces. Therefore, we pursued two specific objectives as outlined below.

Research Methods and Results

A. Research Methods

Objective 1: Examine the fate of *Listeria* and temporal changes of the resident microbiome on pears and their interactions during long-term cold storage, and further characterize the dominant and differential bacterial and fungal populations in the microbiome in the co-occurrence of *Listeria*.

1. Pear cultivar selection

Three popular pear varieties were used: Bartlett, d'Anjou, and Bosc. For each pear cultivar, fruits from conventional orchards were sampled from commercial harvest bins at the cooperating packinghouse, transferred to cold storage, and then delivered to the food microbiology lab within 48 hours.

2. Strain selection

L. monocytogenes: A panel of three outbreak strains consisting of serotype 1/2a (cantaloupe outbreak), 1/2b (stone fruit outbreak), 4b (stone fruit or apple outbreak) was used to prepare a 3-strain cocktail inoculum.

L. innocua: Three *L. innocua* isolates from processing plants (Bidart apple facility, avocado facility, and wheat flour processing plant) were used to prepare a 3-strain cocktail of *L. innocua* inoculum per our well-established method (Sheng et al., 2018; Sheng et al., 2022).

These strains were kept in a stock solution of trypticase soy broth supplemented with 0.6% (w/v) yeast extract (TSBYE) and 20% (v/v) glycerol at -80 °C until use.

3. Inoculum preparation and inoculation

Inoculum preparation: Each strain was growth-phase synchronized twice in TSBYE broth by consecutively culturing at 37 °C for 24h, then pelletized by centrifugation and re-suspended in 0.1% peptone water to achieve the target population. To prepare a 3-strain *L. monocytogenes* or *L. innocua* inoculum cocktail, each respective strain suspension was mixed in a 1:1:1 ratio.

Inoculation: Unwaxed conventional pears of selected varieties (Bartlett, D'Anjou, and Bosc) at commercial maturity were individually and separately inoculated with 3-strain *L. monocytogenes* or *L. innocua* at 1×10^6 CFU/pear per our established method. The inoculated fruits were held at room temperature (~22 °C/71.6 °F) for 24 h before being subjected to the respective storage. Inoculated fruits of each variety were randomly sampled at 0 h and 24 h, respectively, to confirm the initial inoculation level, the uniformity of inoculation, and the initial bacterial population before storage. An additional set of fruits were randomly sampled before inoculation to document initial levels of the natural resident microbiota. The microbial suspension of these samples was further collected and used for microbiome sequencing as described below.

4. Lab simulated storage and sampling

Lab simulated storage: The *L. monocytogenes*-inoculated pears were randomly aligned on fiber fruit trays 24 h after inoculation, with each tray holding pears from all inoculation batches, and stored at 0 ℃, 10 ℃, or 22 °C (room temperature, RT), representing the typical commercial RA storage temperature, temperature abuse setting, and RT, respectively (Freed et al., 2022; Prange and Wright, 2023). The temperatures were monitored daily during storage.

Sampling: Sets of 10-12 pears were sampled at selected time points from each temperature treatment to enumerate *L. monocytogenes* and resident yeasts and molds (YM). For short-term storage (0 ℃, 10 ℃, and RT), inoculated pears were sampled at 1, 4, 7, 14, and 28 days. For long-term storage (0 ℃), sampling occurred at 1, 2, 4, 8, 12, 16, and 20 weeks over two consecutive years.

5. Commercial cold storage and sampling

Commercial cold storage: The inoculated (24h post-inoculation) and uninoculated pears of the selected varieties were wax coated manually, wrapped, and randomly packed into boxes for commercial storage practices. The boxed inoculated or uninoculated pears were randomly separated into two groups and half of them subjected to RA (0.6 °C) or CA (0.6 °C, 2.0% O2, 1.0% CO2) for up to 9 months in semi-commercial RA/CA rooms ($297m^2$/3200 ft^2) with a relative humidity of 85-90% in our cooperator's commercial facility per commercial packing facility practices. For Bartlett pears, an additional set of inoculated pears were subjected to dynamic controlled atomsphere (DCA) storage for up to 12 weeks.

Sampling: Fruits were sampled right after inoculation, right before storage, and at 3, 6, 12, 24, and 36 weeks of respective storage. Four replicates of 16 fruits each were used for each variety on each sampling day at each storage condition.

6. Surviving *Listeria* analysis

At each sampling day, fruits under the respective storage conditions were sampled for each variety. *Listeria* survival on pear surfaces was also analyzed immediately or within 24h, per our previous published method with tree fruit (Sheng et al., 2018; Sheng et al., 2022). To evaluate an alternative microbial detachment method, a peeling enumeration approach was compared with the hand rubbing-stomaching method. Briefly, unrubbed pears were peeled using a sterile stainless-steel vegetable peeler, with the stem and calyx areas removed following the same procedure described above. The peels, along with the stem and calyx areas, were transferred to a sterile stomacher bag containing 10 mL of neutralizing buffer, then homogenized under the same conditions. If survival of *Listeria* on pear fruits was below the limit of detection (LOD), the suspension was enumerated for Presence/Absence after 48h enrichment in Buffered *Listeria* Enrichment Broth (BLEB) and streaked onto a selective CHROMagar *Listeria* agar plate. Presumptive positive colonies were further confirmed by PCR (FDA, 2015).

7. Resident microbiota enumeration

At each sampling day, four sets of 16 uninoculated pears per variety under the respective storage conditions were analyzed for the selected resident microbiota enumeration using a plating method. Total native bacteria were enumerated on trypticase soy agar supplemented with 0.6% (w/v) yeast extract (TSAYE) plates and incubated at 30 °C for 48h. Yeast/mold counts were enumerated on potato dextrose agar (PDA) plates supplemented with 100 µg/ml chloramphenicol and incubated at room temperature (~22 °C) for 5 days.

8. Microbial DNA extraction

Microbial detachment from the surface of pears with or without *L. innocua*: At each sampling day, after plate enumeration of respective bacteria, microbial suspensions of 16 pears were pooled to form one composite sample (inoculated or uninoculated) at each storage condition of the selected variety. Four composite samples (replicates) of the selected variety were prepared per storage condition and storage time combination for both inoculated and uninoculated fruits.

DNA extraction and purification: Genomic DNA was extracted from each microbial composite sample using the commercial DNeasy PowerSoil Pro Kit (Qiagen, Valencia, CA) per our established method. The concentration of DNA was measured using Nanodrop spectrometry (Thermo Scientific), while the quality of DNA was monitored using DNA agarose gels.

9. Bacterial 16S rRNA sequencing and bioinformatics analysis of sequencing data

Sequencing analysis of the bacterial community was processed and analyzed with the ZymoBIOMICS® Targeted Sequencing Service for Microbiome Analysis (Zymo Research Corporation) using Illumina NextSeq sequencing (2 x 300) targeting the V3-V4 region of the bacterial 16S rRNA gene. Raw DNA sequence reads were processed by bioinformatics at Zymo Research Corporation. Unique amplicon sequence variants were inferred from raw reads using the DADA2 pipeline (Callahan et al., 2016). Chimeric sequences were also removed with the DADA2 pipeline. Taxonomy assignment was performed using Uclust from Qiime v.1.9.1 with the Zymo Research Database, a 16S database that is internally designed and curated, as a reference.

10. Bacterial diversity analysis

Shannon alpha diversity, along with beta diversity, encompassing Bray-Curtis' distance and Jaccard distance, were further analyzed using R-Studio.

11. Assess fruit quality of pears during RA or CA storage

Fruit quality analyses were assessed at harvest, and separate sets of uninoculated pears were stored at RA and CA for nine months. Fruit quality measurements, including firmness (kg), total soluble solids (TSS, % Brix), titratable acidity (TA, g/ml), and assessment of external and internal storage disorders, were evaluated following Washington pear industry standards and recommendations provided by our cooperators.

Objective 2. Evaluate the impacts of organic practices on the microbiome community and persistence of *Listeria* on pears of the selected varieties during long-term storage.

1. Pear cultivar selection

Bartlett and d'Anjou were selected for Objective 2 studies. For each pear cultivar, fruit from conventional and organic orchards of our industry cooperators in the same region was collected from harvest bins at our cooperator's packing facility, transferred to cold storage, and then delivered to the food microbiology lab within 48 hours.

2. Strain selection and inoculum preparation

The same 3-strain *L. innocua* cocktail was used as described in Objective 1.

3. Inoculum preparation and *inoculation*

Inoculum preparation and pear inoculation were conducted the same as Objective 1 studies.

4. Cold storage and sampling

Cold storage: The inoculated and uninoculated pears of the selected varieties were wrapped with wax paper and randomly packed into boxes to simulate commercial storage practices. The boxed inoculated or uninoculated pears were randomly separated into two groups and subjected to RA (0.6 °C) or CA (0.6 °C, 2.0 % O2, 1.0 % CO2) for up to 9 months in semi-commercial cold storage rooms (3200 ft^3) with a relative humidity of 85-90% in our cooperator's commercial facility.

Sampling: Fruits were sampled right after inoculation, right before storage, and at 3, 6, 12, and 24 weeks of storage. Four replicates of 16 fruits were used on each sampling day at each storage condition.

5. Surviving *Listeria* analysis

Enumeration of *L. innocua* on pear surfaces was conducted the same as described in the Objective 1 studies.

6. Resident microbiota enumeration

Total native bacteria and yeast/mold counts were enumerated as described in Objective 1.

7. Microbial DNA extraction

Microbial detachment from the pear surfaces, collection, microbial DNA extraction and purification were conducted the same as described in the Objective 1 studies.

8. Bacterial 16S rRNA sequencing and bioinformatics analysis of sequencing data

The 16S rRNA amplicon and library preparation and Illumina sequencing were conducted as described in Objective 1.

9. Bioinformatics analysis of sequencing data

Bacterial sequencing data bioinformatics analysis was conducted as described in Objective 1.

10. Assess fruit quality of pears stored under RA or CA storage

The quality of pear fruits under storage was analyzed as described in Objective 1.

B. Results

1. Dynamics of *L. monocytogenes* across pear varieties during simulated storage

1.1 Attachment behavior of *L. monocytogenes* on pear surfaces

Immediately after inoculation, the initial levels of *L. monocytogenes* on pears were ~ 6.7 log CFU/pear, regardless of variety (Figure 1A). After 24 h of establishment at RT, *L. monocytogenes* levels on pear surfaces decreased ($P < 0.05$), particularly on Bosc pears, resulting in the levels of 6.23 ± 0.03, 6.30 ± 0.01, and 5.12 ± 0.01 log CFU/pear on Bartlett, d'Anjou, and Bosc pears, respectively (Figure 1A). The visual appearance of three pear varieties post-inoculation is displayed in Figure 1B.

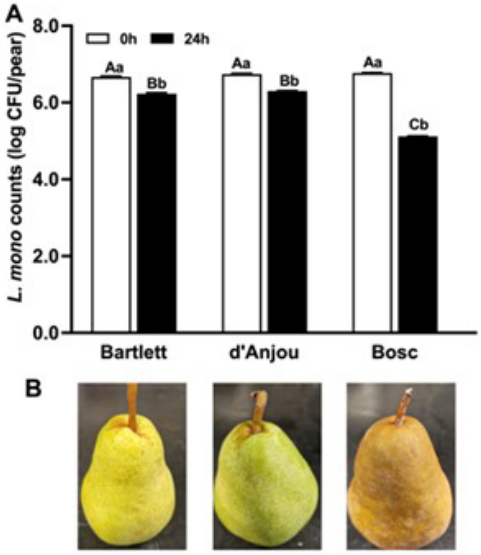

Figure 1. Attachment behavior of *L. monocytogenes* on different pear varieties. A. *L. monocytogenes* populations at 0 and 24 h post-inoculation. B. Representative image of Bartlett, d'Anjou, and Bosc pears. (A–C) indicate significant differences between varieties at each sampling point ($P < 0.05$). Different letters (a, b) within the same variety indicate significant differences over time ($P < 0.05$). Mean ± SEM, n = 30.

To evaluate an alternative microbial detachment method, a peeling enumeration approach was further tested. However, the peeling approach for microbial detachment resulted in *L. monocytogenes* levels that were 60-80% lower ($P < 0.05$) compared to the rubbing-stomaching method, regardless of pear varieties (Figure 2). Consequently, the rubbing-stomaching method was selected for subsequent pear storage.

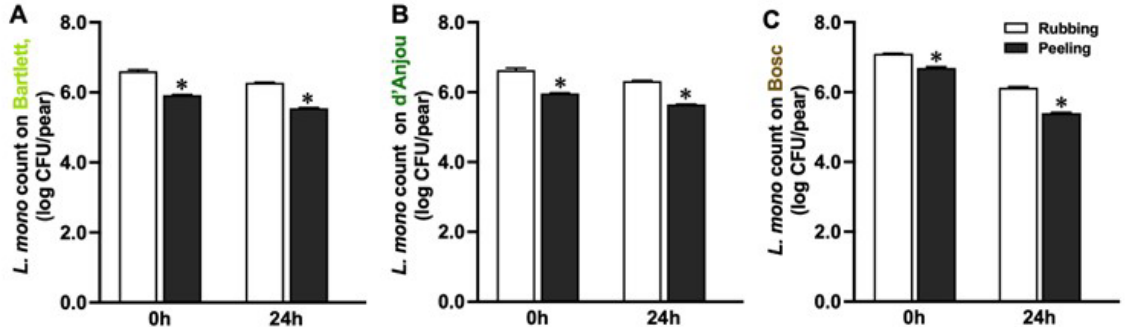

Figure 2. Detachment of *L. monocytogenes* from fresh Bartlett (A), d' Anjou (B), and Bosc (C) pears using rubbing-stomaching and peeling methods**.** Mean ± SEM, n = 30. * indicates a significant difference ($P < 0.05$) between peeling and rubbing methods at 0 and 24 h post-inoculation.

1.2 *L. monocytogenes* survival on pears under different storage temperatures

L. monocytogenes populations gradually decreased on pears over 28 days of storage, regardless of the storage temperature, with the most significant reductions occurring in the first 14 days (Figure 3). After 14-day storage at 0 and 10 °C, the populations of *L. monocytogenes* decreased ($P < 0.05$) to ~ 4.30, 5.04, and 3.87 log CFU/pear on Bartlett, d'Anjou, and Bosc pears, respectively. During the remaining 14 days of storage, populations remained relatively stable, ranging from 3.75-4.89 log CFU/pear (Figure 3). Increasing the storage temperature to RT led to a significant reduction compared to the other temperatures; the population of *L. monocytogenes* maintained at 3.63-4.54 log CFU/pear by the end of 28-day storage at 22 °C, regardless of the pear variety (Figure 3). *L. monocytogenes* gradually decreased on pears during long-term storage at 0 °C, exhibiting similar survival patterns across pear varieties. After 20-week storage at 0 °C, *L. monocytogenes* decreased by 2.65, 2.86, and 2.83 log CFU/pear on Bartlett, d'Anjou, and Bosc pears, respectively, in year 1, and 3.84, 3.13, and 2.82 log CFU/pear, respectively, in year 2 (Figure 4).

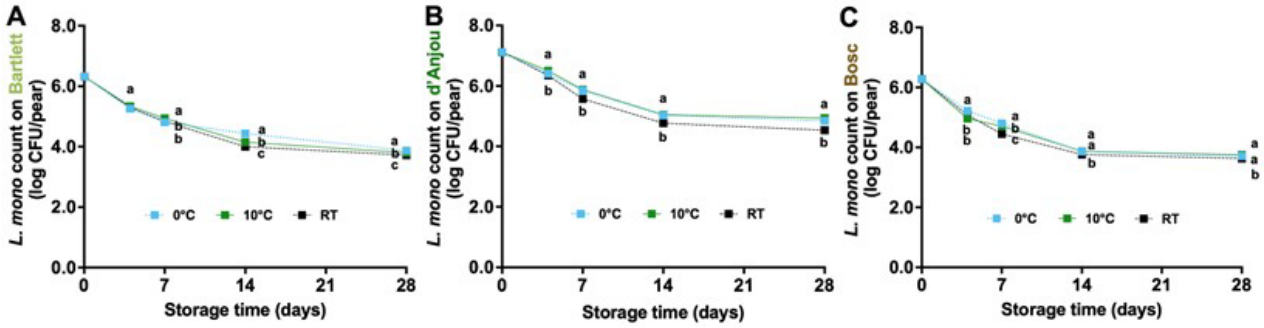

Figure 3. Survival of *L. monocytogenes* on Bartlett (A), d'Anjou (B), and Bosc (C) pears during 28 days of storage at 0 °C, 10 °C, and RT (~22 °C). Mean ± SEM, n = 10-12. Different letters (a–c) indicate significant differences between temperatures at each sampling point ($P < 0.05$).

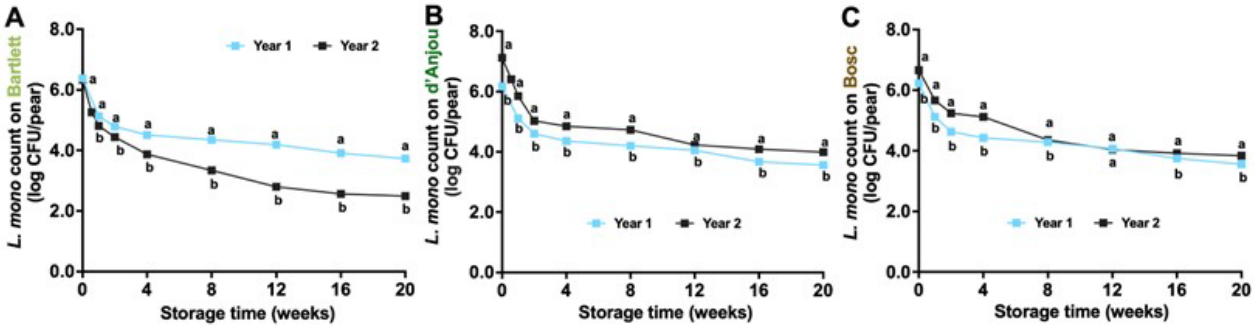

Figure 4. Survival of *L. monocytogenes* on fresh Bartlett (A), d'Anjou (B), and Bosc (C) pears during 20 weeks of storage at 0 °C across two crop years. Mean ± SEM, n = 10-12. Different letters (a, b) indicate significant differences between years at the same sampling point ($P < 0.05$).

2. Survival of *L. innocua* across pear varieties during commercial cold storage

The initial *L. innocua* population immediately after inoculation was 5.50-6.11 log CFU/pear. The levels decreased ($P < 0.05$) to 5.19-5.24 log CFU/pear across all pear varieties after 24 h of establishment (Figure 5A). During 36 weeks of RA or CA storage, *L. innocua* gradually decreased on pears, demonstrating distinct ($P < 0.05$) survival dynamics among pear varieties, with Bosc recording higher survival, regardless of storage conditions and duration. Due to quality issues, Bartlett pears were stored for up to 12 weeks, showing a similar trend with d'Anjou in the first 12 weeks (Figure 5B, C).

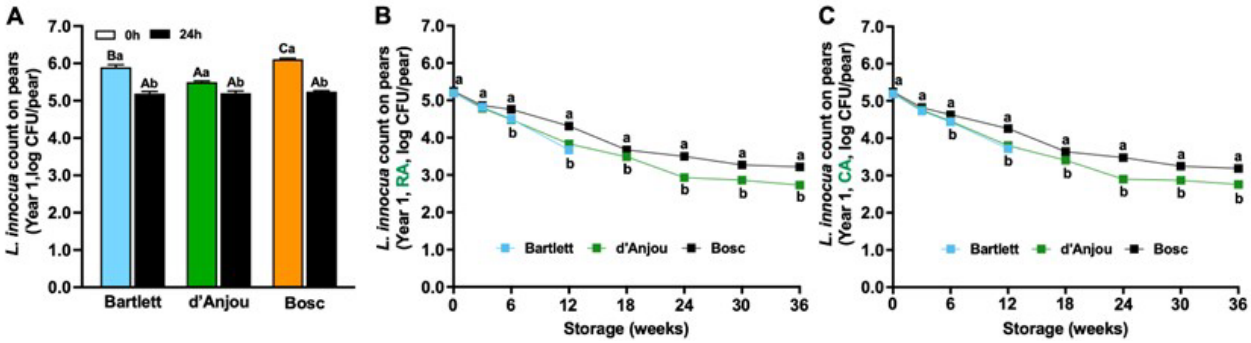

Figure 5. Survival of *L. innocua* on pears during commercial cold storage. A. Initial *L. innocua* levels on pears at 0 and 24 h post-inoculation; Different letters (A-C) indicate significant differences between pear varieties at 0 h/24h, and different letters (a, b) indicate significant differences between 0 h and 24 h ($P < 0.05$). (BC) *L. innocua* count on pears stored under rRA (B) or CA (C) for up to 36 weeks Mean ±SEM, n= 64. Different letters (a, b) indicate significant differences among pear varieties at each time point ($P < 0.05$).

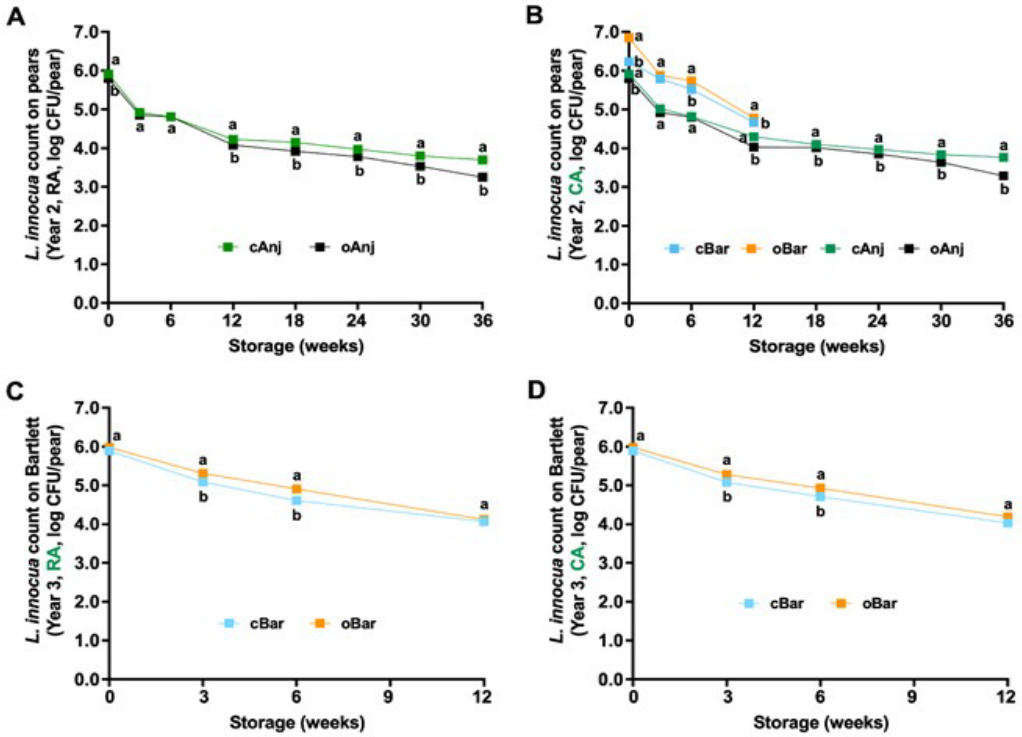

Figure 6. Impact of agricultural practices on *L. innocua* survival on Bartlett and d'Anjou during up to 36 weeks of commercial storage. (AB) *L. innocua* on conventionally grown Bartlett (cBar) and d'Anjou (cAnj) versus organically grown Bartlett (oBar) and d'Anjou (oAnj) pears over up to 36 weeks of RA (A) or CA (B) conditions in Year 2. (CD) *L. innocua* count on cBar and oBar during 12 weeks of RA (C) and CA (D) storage in Year 3. Mean ± SEM, n = 64. Different letters (a, b) indicate significant differences ($P < 0.05$) between conventional and organic pears of the same variety at each time point.

3. Impact of agricultural practices on *L. innocua* survival during 36 weeks of commercial cold storage

The population of *L. innocua* decreased on pears throughout storage, regardless of variety and agricultural practice. Organic d'Anjou exhibited lower survival than conventional ones ($P < 0.05$), regardless of RA or CA (Figure 6A, B). For Bartlett, the storage duration was limited to 12 weeks in Year 2 under CA conditions due to quality loss. To address this limitation, the study was repeated in Year 3 under both RA (Figure 6C) and CA (Figure 6D) conditions. Overall, *L. innocua* survival on conventional and organic Bartlett showed a similar trend across both years (Figure 6B, C, D).

4. Resident microbiota over commercial cold storage: influence of variety and agricultural practices

4.1 Resident bacteria and yeasts & molds on pears of selected variety during commercial cold storage

Resident bacteria on pears across variety were comparable before storage (Figure 7A, B), and gradually declined during storage. During the initial 12 weeks of storage, resident microbial loads remained relatively stable across varieties. However, as storage progressed, d'Anjou pears exhibited a significantly greater reduction compared to Bosc, particularly at later time points ($P < 0.05$) (Figure 7A, B). The initial YM level on Bartlett, d'Anjou and Bosc in the Year 1 study was comparable (Figure 7C, D). Unlike TPC, YM levels gradually increased throughout storage across varieties, regardless of storage conditions, with Bosc pears consistently exhibiting the highest YM counts. After 12-week storage, YM levels increased by ~0.69 log CFU/pear on Bartlett, and by the end of the 36 weeks, YM increased by ~1.22 log CFU/pear on d'Anjou and ~1.58 log CFU/pear on Bosc, regardless of storage practice (Figure 7C, D).

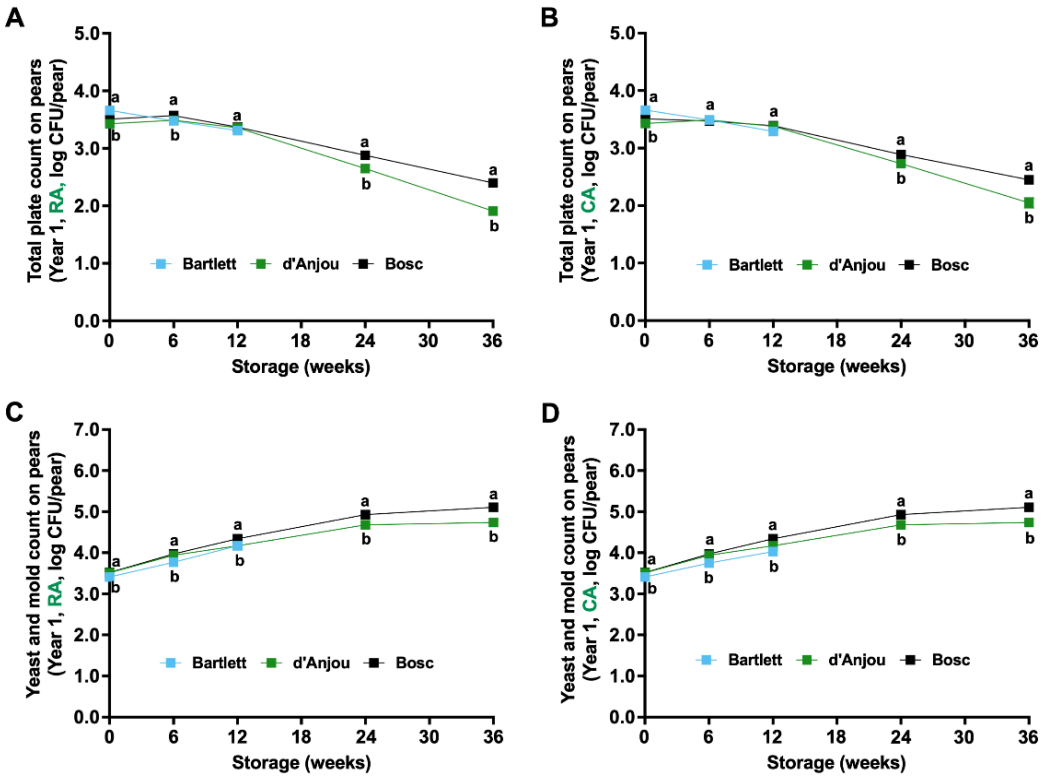

Figure 7. Resident microflora on Bartlett, d'Anjou and Bosc pears during up to 36 weeks of commercial cold storage in Year 1. (A, B) Total plate count on pears during 36 weeks of refrigerated atmosphere (RA; 0.6 ℃) (A) and controlled atmosphere (CA; 0.6℃, 2% O_2, 1% CO_2) (B) conditions. (C, D) Yeast and mold count on pears under RA (C) and CA (D) conditions. Mean ±SEM, n= 64. Different letters (a, b) at each time point indicate statistically significant differences ($P < 0.05$) among pear varieties.

4.2 Resident microbes on conventionally and organically grown Bartlett and d'Anjou pears during commercial cold storage

The microbial dynamics on conventionally and organically grown Bartlett and d'Anjou pears across the 36-week storage period under different storage conditions were evaluated. Initially, TPCs were comparable between conventional and organic fruits within each variety. Bartlett pears had significantly higher initial TPC compared to d'Anjou pears ($P < 0.05$) (Figure 8A, B). Over time, TPC decreased significantly under both RA and CA conditions (Figure 5A, B).

The initial YM population on conventional and organic Bartlett pears was comparable at 4.42 log CFU/pear, whereas organic d'Anjou had a higher YM count than conventional d'Anjou. Unlike TPC, YM levels increased throughout storage under both RA and CA conditions. By the end of 12 weeks of CA storage, YM levels increased by ~1.39 log CFU/pear on both conventional and organic Bartlett pears. By the end of the 36-week storage period, YM counts increased by ~1.35 and 0.75 log CFU/pear on conventional and organic d'Anjou pears (Figure 8C, D), respectively.

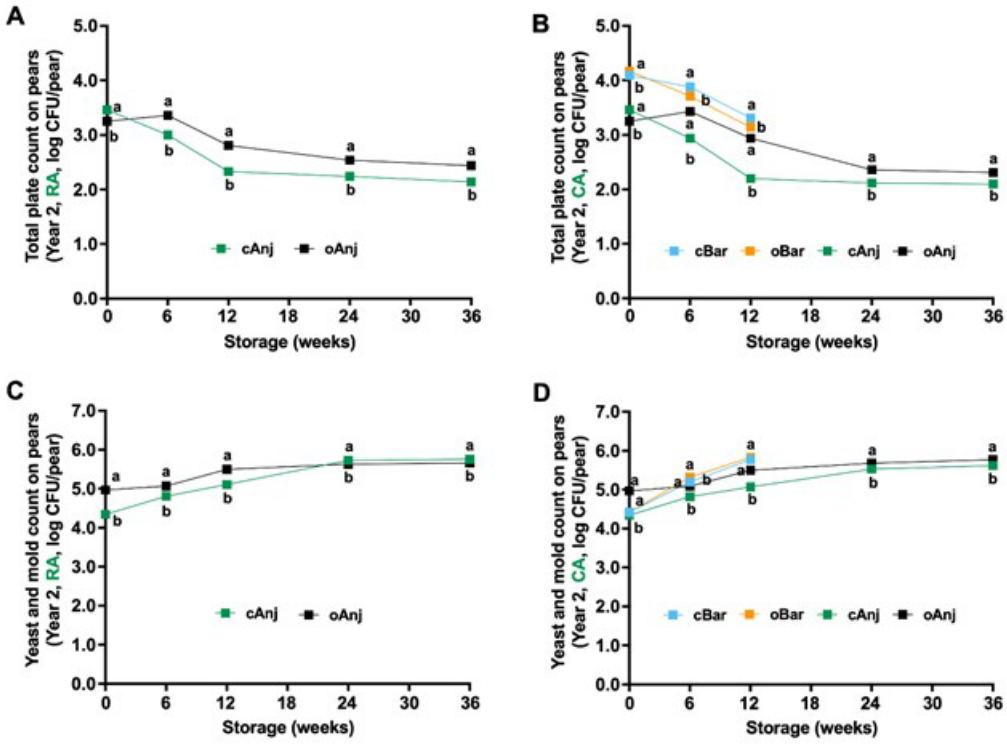

Figure 8. Resident microflora on Bartlett, d'Anjou and Bosc pears during up to 36 weeks of commercial cold storage in Year 1. (A, B) Total plate count on pears under RA (A) and CA (B) conditions. (C, D) Yeast and mold count on pears under RA (C) and CA (D) conditions. Mean ±SEM, n= 64. Different letters (a, b) at each time point indicate statistically significant differences ($P < 0.05$) among pear varieties.

5. Fruit quality of pears after 36 weeks of cold storage

Bosc pears had higher firmness than d'Anjou before storage, and both varieties lost firmness after 36 weeks, with d'Anjou showing a significantly greater reduction ($P < 0.05$, Table 1). In Year 1, conventional d'Anjou pears under CA maintained higher firmness than those under RA, whereas Bosc pears stored under RA retained comparable firmness to those under CA. In Year 2, conventional and organic d'Anjou had similar initial firmness, but after 36 weeks, organic d'Anjou pears under CA maintained higher firmness than those under RA ($P < 0.05$, Table 1).

Fruit weight remained stable over 36 weeks, though variation occurred due to different fruit sets used at 0 and 36 weeks. In Year 1, TSS of d'Anjou and Bosc remained unchanged during 36 weeks of storage, with no significant differences between varieties or storage conditions. In Year 2, TSS declined in both conventional and organic d'Anjou after 36 weeks ($P < 0.05$), with no difference between CA and RA (Table 1). TA values significantly decreased across all treatments after 36 weeks ($P < 0.05$). Bosc had significantly lower TA than d'Anjou ($P < 0.05$), and the TA values of d'Anjou pears in Year 2 were significantly higher than in Year 1 ($P < 0.05$). No statistical difference in TA was observed between conventional and organic pears before or after storage (Table 1).

Table 1: Fruit quality attributes of d'Anjou and Bosc pears under RA and CA storage

Variety	Storage	Weight (g)		Firmness (kg)		TSS (%Brix)		TA (%malic acid)[1]	
		0 week	36-week	0 week	36 -week	0-week	36 -week	0 -week	36 -week
Year1									
Conventional d'Anjou	RA	206.00 ± 6.20^{A}	199.00 ± 5.90^{aA}	5.84±0.11^{A}	1.98±0.11^{aB}	13.10 ± 0.20^{A}	12.90 ± 0.10^{aA}	0.30 ± 0.02^{A}	0.19 ± 0.02^{aB}
	CA		220.00 ± 5.80^{bB}		2.46±0.14^{bB}		13.40 ± 0.20^{aA}		0.20 ± 0.01^{aB}
Conventional Bosc	RA	243.00 ± 7.00^{A}	213.00 ± 13.20^{aB}	6.66±0.06^{A}	5.01±0.14^{aB}	12.80±0.10^{A}	11.90 ± 0.50^{aA}	0.16 ± 0.01^{A}	0.08 ± 0.01^{aB}
	CA		199.00 ± 13.60^{bB}		4.97±0.14^{aB}		12.20 ± 1.60^{aA}		0.06 ± 0.00^{aB}
Year2									
Conventional d'Anjou	RA	126.58±4.91^{A}	130.40±7.70^{aA}	6.45±0.10^{A}	2.35±0.12^{aB}	13.90±0.20^{A}	12.30±0.20^{aB}	0.43±0.02^{A}	0.25±0.02^{aB}
	CA		129.25±6.01^{aA}		2.28±0.20^{bB}		12.80±0.25^{aB}		0.25±0.02^{aB}
Organic d'Anjou	RA	145.08±5.94^{A}	154.20±14.60^{aB}	6.11±0.08^{A}	2.24±0.13^{aB}	14.10±0.20^{A}	12.80±0.25^{aB}	0.42±0.00^{A}	0.25±0.02^{aB}
	CA		152.35±6.77^{aB}		3.34±0.09^{bB}		13.80±0.21^{aB}		0.20±0.01^{aB}

[1] The percentage of malic acid was reported by grams of malic per 100 g of fresh weight of pears.
a,b Means within a column for the same variety and cropping year that do not share a common letter differ significantly ($P < 0.05$).
A,B Means of individual quality parameters that do not share a common letter differ significantly between 0-week and 36-week ($P < 0.05$).
TSS: Total soluble solids; TA: Titratable acidity; RA: Refrigerated air; CA: Controlled atmosphere, Values represent Mean ± SEM, $n = 40$.

6. Microbiome profiling on pears of the selected varieties during long-term storage

6.1 Overall comparison of microbiome diversity among pear varieties during 36 weeks of storage

Beta diversity analysis revealed significant differences in the bacterial community composition among the pear varieties at harvest. Bartlett (green), d'Anjou (purple), and Bosc (orange) displayed a distinct microbiome profile in principal coordinate analysis (PCoA) plots (Fig. 9). Fig. 10 shows PCoA plots of the microbiome over 36 weeks of storage. Within d'Anjou, beta diversity shows a shift over storage, but storage environment (RA or CA) had a minor influence compared to the storage period (Fig. 10A). This trend is less distinct for Bosc pears (Fig. 10B). The Shannon alpha diversity is not different statistically among varieties at harvest (data not shown). The Shannon alpha diversity of the selected variety decreased over storage under both RA and CA storage (Fig. 11).

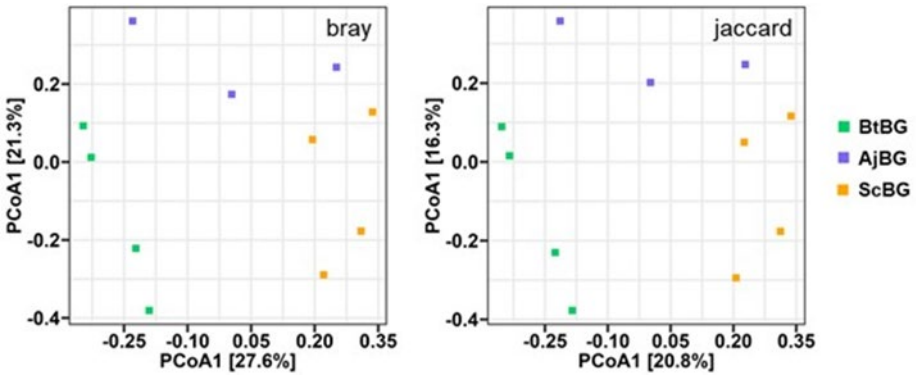

Fig. 9. Beta diversity (Bray-Curtis and Jaccard) of pears before storage. Each point represents a microbial profile from a single sample within a given pear variety. $N = 3–4$, each with 16 pears.

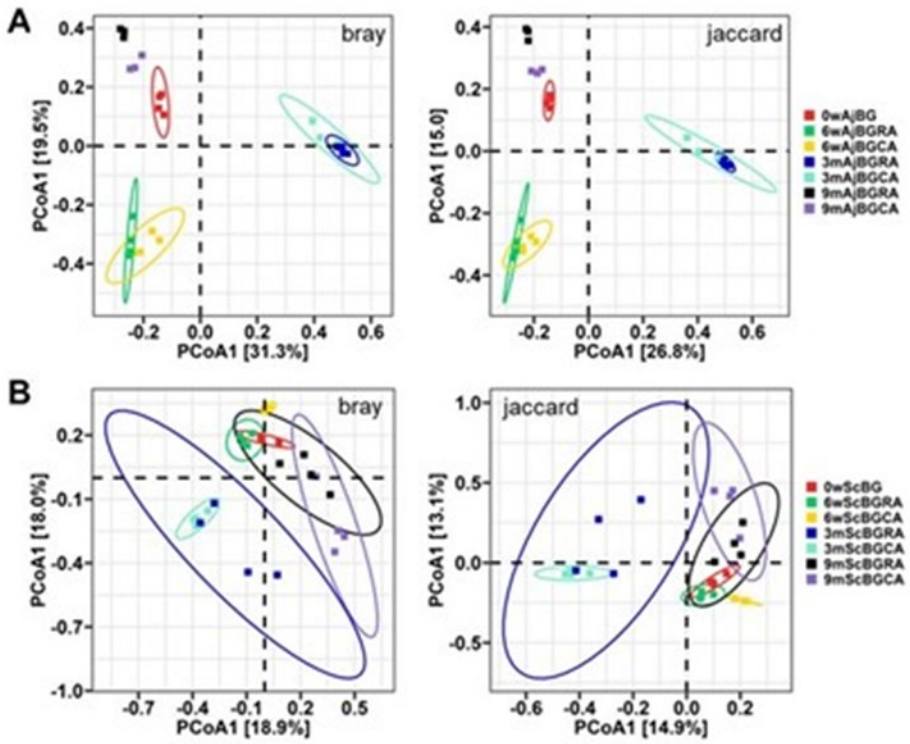

Fig. 10. Beta diversity (Bray-Curtis and Jaccard) of d'Anjou (Aj) and Bosc (Sc) pears over 36 weeks of storage. Each point represents a microbial profile from a single sample within a given pear variety. N = 3–4, each with 16 pears. RA: Refrigerated air; CA: Controlled atmosphere.

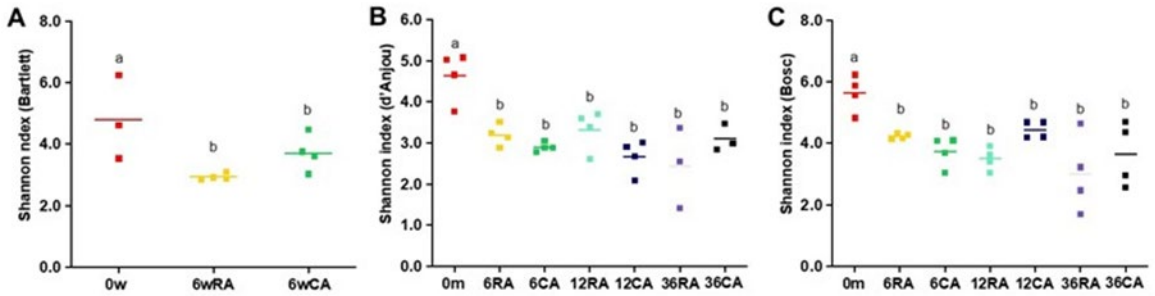

Fig. 11. Alpha diversity Shannon index across pear varieties before storage. A. Bartlett; B. d'Anjou; C. Bosc. [a-b]Mean at each sampling point without common letter differs significantly ($P < 0.05$). N = 3–4, each with 16 fruits. RA: Refrigerated air; CA: Controlled atmosphere.

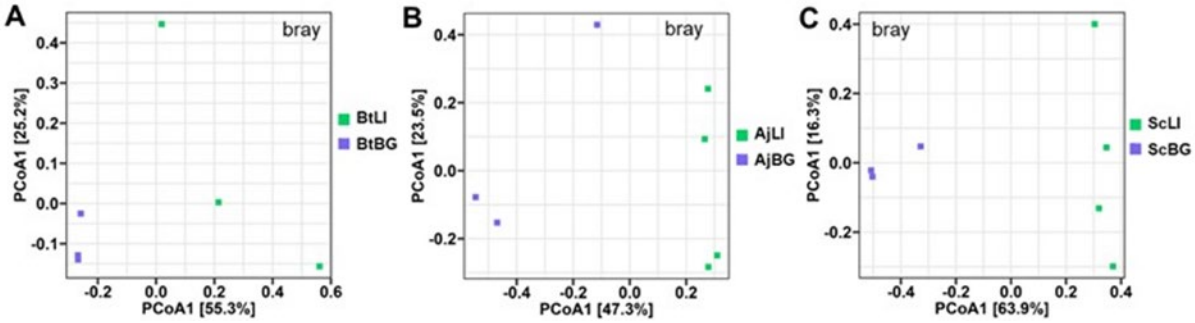

Fig. 12. Beta diversity across pear varieties with (green) or without (purple) *L. innocua* inoculation. Each point represents a microbial profile from a single sample within a given pear variety. N = 3 – 4, each with 16 pears. Bt: Bartlett; Aj: d'Anjou; Sc: Bosc.

6.2 Overall comparison of microbiome diversity of pear before and after inoculation and throughout storage

PCoA plots reveal distinct microbial community patterns between inoculated and uninoculated fruits, consistent across all three pear varieties (Fig. 12). Similar to uninoculated fruit, the beta diversity of the microbiome on inoculated pears shifts significantly over the storage period, regardless of varieties, with storage duration appearing to have a greater influence than storage conditions. The Shannon alpha diversity was significantly higher in inoculated fruit compared to uninoculated fruit across all pear varieties before storage (Fig. 13), while the alpha diversity within each variety remained stable throughout the storage period.

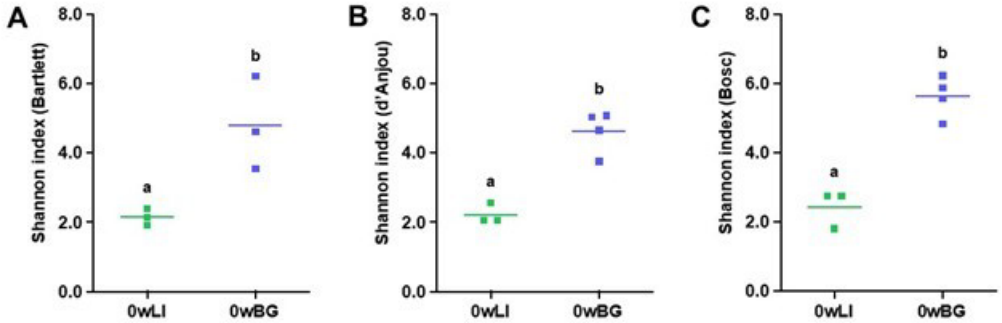

Fig. 13. Alpha diversity Shannon index in microbiome of inoculated (0wLI) and uninoculated (0wBG) pears across different pear varieties before storage. [a-b]Mean at each sampling point without common letter differs significantly ($P < 0.05$). N = 3-4, each with 16 fruits.

6.3 Overview of bacterial community among pear varieties with and without *L. innocua* inoculation over 36 weeks of commercial storage

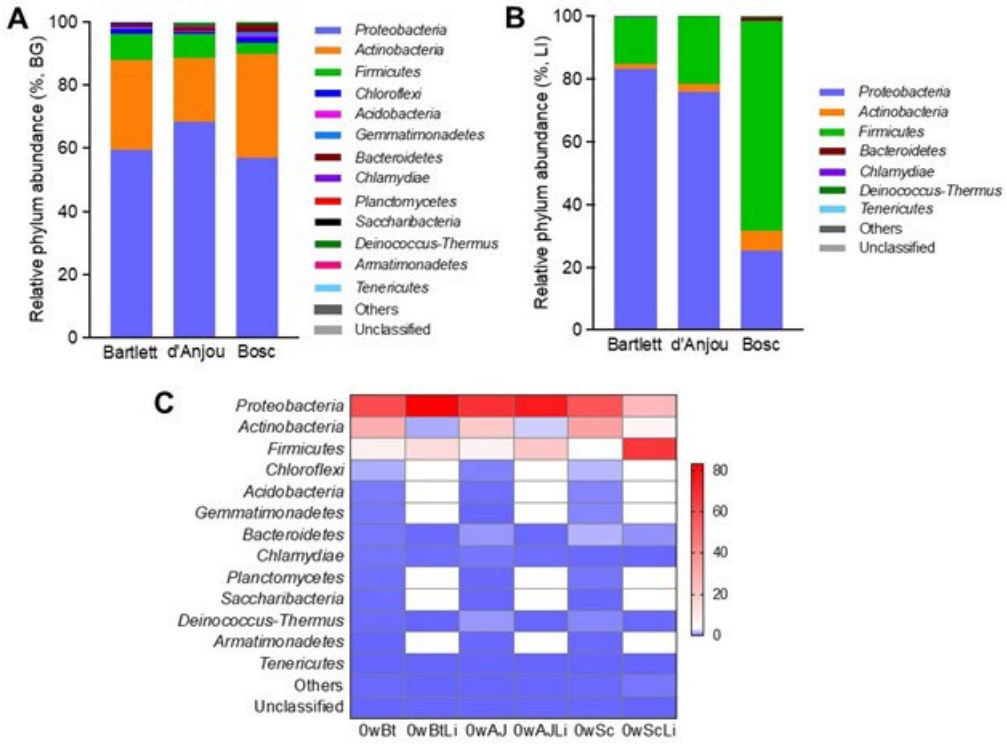

Fig. 14. Bacterial phyla across pear varieties before storage. (A) Uninoculated fruits. (B) Inoculated fruits. (C) Heatmap showing the relative abundance of bacterial phyla in pear varieties with and without inoculation. Color intensity indicates relative abundance. Bt: Bartlett, Aj: d'Anjou; Sc: Bosc. N = 3-4, each with 16 fruits.

Prior to the introduction of *L. innocua*, over 13 bacterial phyla were detected across pear varieties, with *Proteobacteria* being the most dominant, followed by *Actinobacteria* and *Firmicutes* (Fig. 14A). Following inoculation, bacterial phyla diversity decreased markedly, with only seven phyla identified: *Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Chlamydiae, Deinococcus-Thermus*, and *Tenericutes* (Fig. 14B). Similar to the uninoculated fruits, *Proteobacteria* remained the most dominant phylum in both Bartlett and d'Anjou pears, though its relative abundance was significantly higher in the inoculated samples. Relative abundance of *Firmicutes* showed a significant increase in all inoculated pear varieties compared to their respective uninoculated counterparts, with the most notable increase observed in Bosc pears (Fig. 14B). The heatmap in Fig. 14C illustrates the distribution and relative abundance of these phyla across different pear varieties and treatment groups.

Throughout 36 weeks of RA or CA storage, bacterial phyla on both d'Anjou and Bosc pears exhibited dynamic changes (Fig. 15A–B). Notably, *Proteobacteria* showed a marked increase in week 6, followed by a gradual decline over time in both varieties (Fig. 15A–B). The extent and pattern of these changes varied by variety and storage conditions. In parallel, the relative abundance of *Actinobacteria* decreased at week 6 across all varieties and storage conditions (Fig. 15A–C). Similar to uninoculated pears, the composition of bacterial phyla exhibited variety- and storage-specific shifts over the storage period. *Proteobacteria* remained the dominant phylum across all varieties and storage, except Bosc pears. *Firmicutes* remained consistently abundant in inoculated fruits throughout storage.

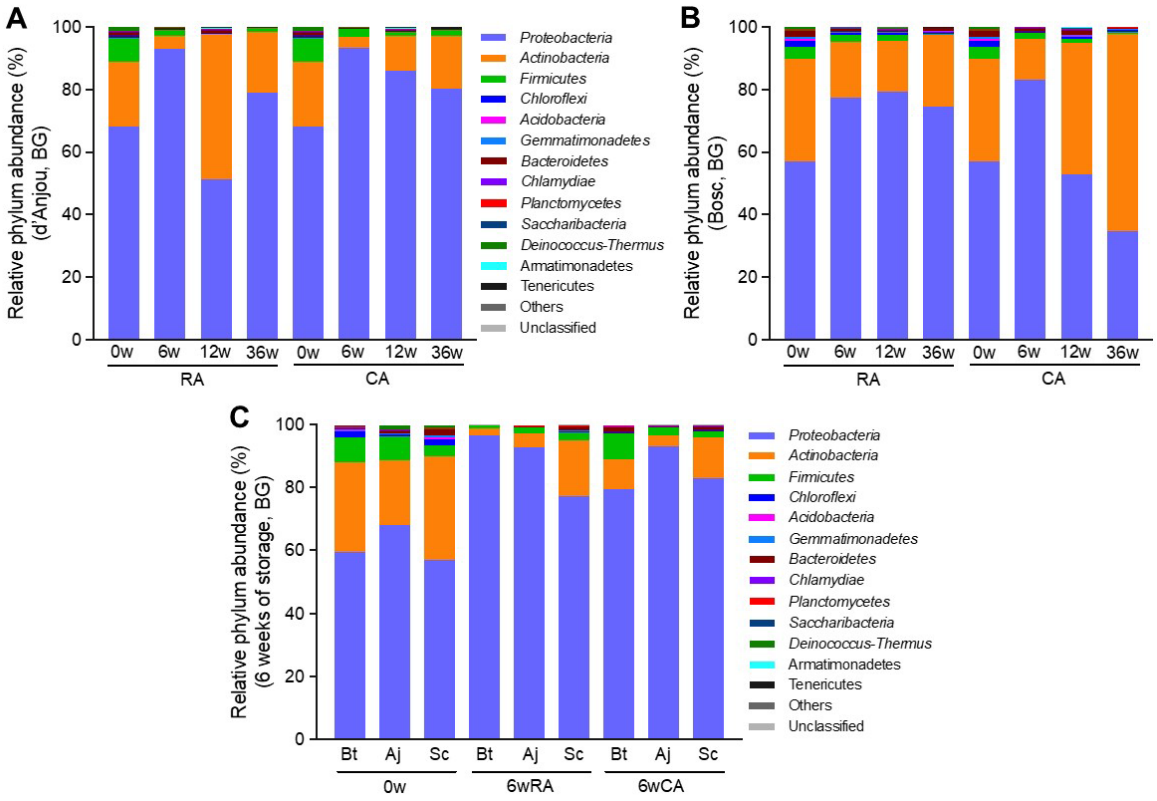

Fig. 15. Relative abundance of bacterial phyla on pears from different varieties during 36 weeks of storage: (A) d'Anjou (Aj), (B) Bosc (Sc). (C) Comparison of bacterial phyla composition among three pear varieties after 6 weeks of storage: Bartlett (Bt), d'Anjou (Aj), Bosc (Sc). N = 3-4, each with 16 fruits.

6.4 Bacterial genera among pear varieties with and without *L. innocua* inoculation over 36 weeks of commercial storage

Fig. 16 illustrates the relative abundance of dominant bacterial genera across different pear varieties,

with and without *L. innocua* inoculation. Clear shifts in microbial profiles at the genus level were observed between uninoculated and inoculated samples, as well as among pear varieties. The introduction of *L. innocua* notably reduced bacterial diversity. Among the dominant genera, *Pseudomonas* remained prevalent across nearly all samples, with particularly high abundance in inoculated Bartlett and d'Anjou pears. Its abundance significantly increased following *L. innocua* inoculation (Fig. 16).

As expected, *Listeria* was strongly enriched in inoculated samples, especially in Bosc pears. *Listeria* was also detected at low abundance in uninoculated samples, suggesting its natural occurrence in fruit surfaces. Inoculation with *L. innocua* also influenced the overall bacterial community structure (Fig. 16). Furthermore, the bacterial genera exhibited significant shifts over the 36 weeks of storage. Notably, the relative abundance of *Pseudomonas* remained high in all the samples. *Listeria* remained prominent in inoculated samples, particularly in Bosc pears, at 36 weeks. In contrast, its relative abundance in uninoculated fruits declined to a very low level or below the detection level.

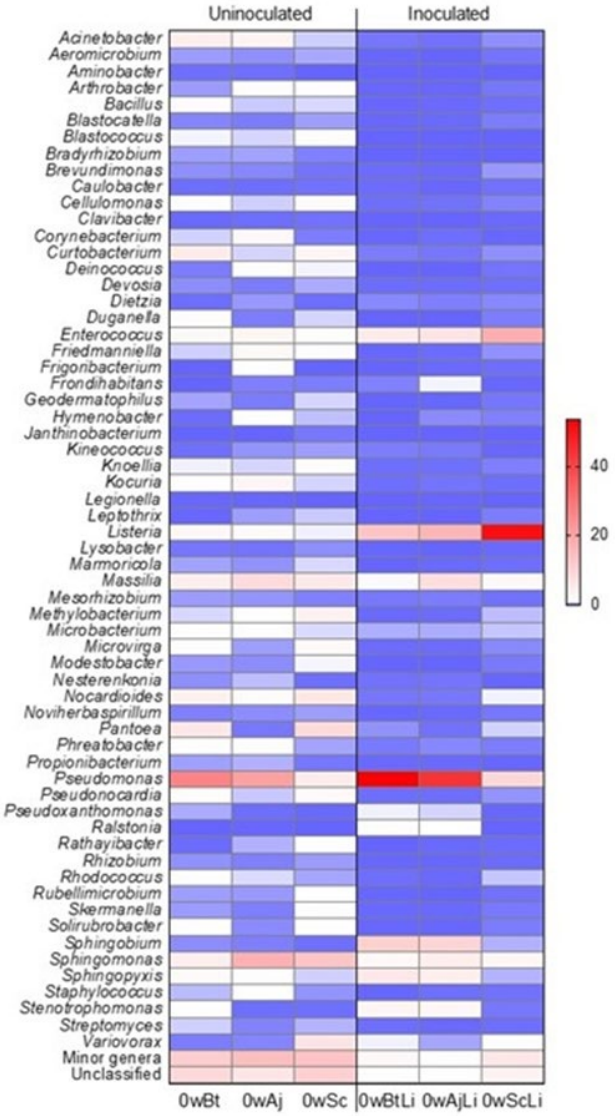

Fig. 16. Heatmap showing the relative abundance of bacterial genera on pears with or without *Listeria innocua* contamination across three pear varieties prior to storage. *N* = 3–4, with each replicate consisting of 16 fruits. Bt: Bartlett; Aj: d'Anjou; Sc: Bosc; Li: pears inoculated with *L. innocua*.

7. Impact of agricultural practice on microbiomes on pears of the selected varieties at harvest and during long-term storage

Bray-Curtis beta diversity revealed significant differences in the bacterial community composition between conventional and organic pears of the selected variety before storage, as shown in the distinct clustering on the PCoA plot (Fig. 17). Inoculated and uninoculated fruits also exhibited distinct microbial profiles, independent of agricultural practice or variety (Fig. 17). Additionally, the microbiome of pears inoculated with or without *L. innocua* shifted significantly over the storage period, regardless of whether pears were conventionally or organically grown (Fig. 18). Conventional and organic pears exhibited distinct microbial profiles that shifted over time across taxonomic levels.

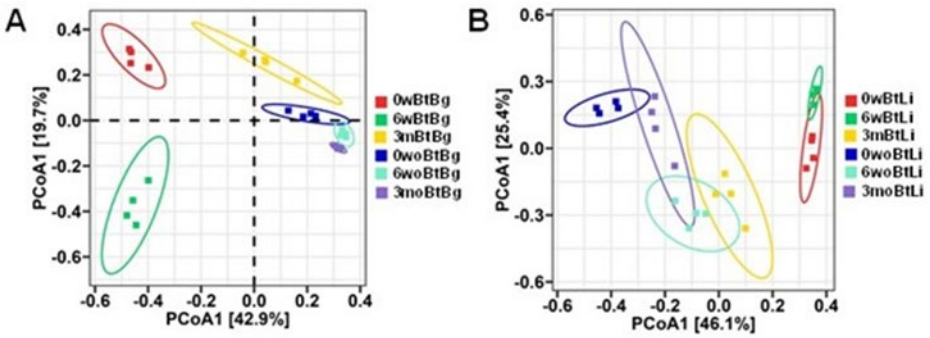

Fig. 17. Bray-Curtis beta diversity of bacterial communities on conventional and organic pears before storage. Each point represents a microbial profile from a composite sample within a given pear variety. *N* = 3–4, each with 16 pears.

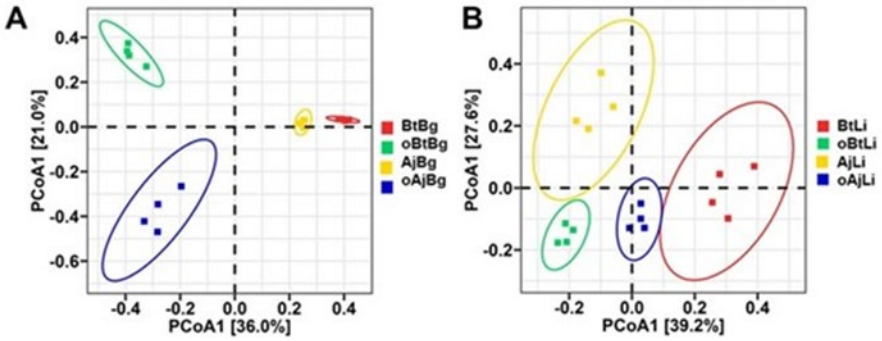

Fig. 18. Bray-Curtis beta diversity of bacterial communities on conventional and organic pears over 3 months of storage. Each point represents a microbial profile from a composite sample within a given pear variety. *N* = 3–4, each with 16 pears.

Outcomes and Accomplishments

1. Pear surfaces are generally inhospitable to *Listeria*, which declined across all varieties and storage conditions.
2. Storage temperature had a limited impact on *L. monocytogenes* survival, and *L. innocua* exhibited similar behaviors on pears as *L. monocytogenes*.
3. Storage atmosphere and agricultural practices had minimal impact on the fate of *L. innocua* on pears.
4. *L. innocua* showed greater reduction on Bosc pears during the initial 24-hour attachment but higher survival thereafter.
5. Yeasts and molds increased over time, with Bosc pears consistently showing higher yeast and mold levels.
6. Each pear variety harbored a distinct microbiome. *L. innocua* inoculation significantly reduced bacterial diversity and altered composition – *Pseudomonas*, *Enterococcus*, and *Stenotrophomonas* increased while *Pantoea*, *Curtobacterium*, and *Sphingomonas* decreased.
7. Low-level *Listeria* was detected in uninoculated samples, suggesting possible natural contamination.
8. Conventional and organic pears exhibited unique bacterial communities, as shown by distinct PCoA clustering.
9. Microbial diversity and composition shifted over time across taxonomic levels, revealing temporal dynamics of pear surface microbiomes
10. This study provides insight into the relationship between pear cultivar, *Listeria* contamination, microbiome dynamics, and commercial cold storage conditions, supporting future postharvest safety strategies.

APPENDICES

Publications and Presentations

Hang, M., E. L. Afari, X. Shen, Y. Su, M. Mendoza, I. Hanrahan, and M.-J. Zhu. 2025. Population dynamics of *Listeria monocytogenes* and yeast and mold levels across pear varieties during simulated storage. *Foods* 14: 1701. https://doi.org/10.3390/foods14101701

Afari, E. L., M. Hang, Y. Su, H. Zi, X. Shen, B. Thapa, M. Jo, J. M. Deavillar, M. Mendoza, T. Chiu, X. Zhang, I. Hanrahan, and M. J. Zhu. Fate of *Listeria innocua* on pears during long-term commercial cold storage: as impacted by variety, agriculture and storage practices. Submitted to *Postharvest Biology and Technology*.

Two meeting abstracts have been published and additional manuscripts are in preparation for submission to scientific journals.

Budget Summary

This project was awarded $382,433 in research funds. Most of the grant funds awarded have been spent, with a small portion reserved to cover sequencing and data analysis costs, as well as to support attendance at the 2025 CPS Symposium.

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