CPS 2022 RFP – FINAL PROJECT REPORT

Project Title:
A metagenomic approach to food safety risk mitigation in pears

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

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Objectives:

1. Identify culturable microbiological community members (yeasts, molds, and lactic acid bacteria) on conventional, whole, intact pears prior to storage.
2. Describe yeasts, molds, and lactic acid bacteria composition of marketable and unmarketable conventional, whole, intact pears under two different storage practices at three, six, and nine months in long-term controlled atmosphere cold storage to develop a metagenomic profile and track community composition.
3. Co-inoculate representative yeast, mold, and bacterial community members with *Listeria monocytogenes* on pears under industry-relevant conditions to characterize synergistic and antagonistic effects.

Funding for this project was provided partly through the CPS Campaign for Research.

FINAL REPORT

Summary of Findings and Recommendations

1. **No growth, but survival of *Listeria monocytogenes* on whole, intact pears**: *Listeria monocytogenes* did not grow under any tested condition but remained viable on intact pears for up to 7 months during controlled atmosphere (CA) storage. This highlights the importance of addressing pathogen persistence, not just growth.
2. **Investigated the effect of copper or ethoxyquin alternatives as an antimicrobial measure to reduce pathogen and decay organisms:** Antimicrobial wrapping significantly reduced *L. monocytogenes* levels (by >5 log CFU) and inhibited *Penicillium expansum* growth, demonstrating its potential as a practical dual-purpose intervention for food safety and quality.
3. **Co-inoculation with *Penicillium expansum* enhanced *Listeria* die-off**: When pears were co-inoculated with *P. expansum*, a plant pathogen, *Listeria* levels declined more rapidly. In contrast, *Aureobasidium pullulans* and *Bacillus thuringiensis* (potential biological control organisms) had little to no impact on *Listeria* survival.
4. **Packing line reduced microbial load on pear surfaces**: Pears sampled after passing through packing line showed significantly reduced total microbial load, confirming the value of postharvest handling.
5. **Storage practice and duration affected microbial profiles**: Psychrotolerant bacteria and fungi became more dominant over time. Pears stored in antimicrobial wrap showed lower microbial richness and diversity, compared to those stored in bulk.
6. **Generated the first long-term microbial profile of stored pears**: This study established a foundational dataset describing microbial communities on pears before and during storage, enabling future food safety risk assessments and intervention development.

Abstract

D'Anjou pears are routinely stored for up to nine months under controlled atmosphere (CA) conditions to meet market demands. While this practice maintains fruit quality, limited information exists on its food safety implications, specifically the survival of *Listeria monocytogenes* and the role of natural microbiota. This multi-institutional study investigated the microbiological dynamics of d'Anjou pears during long-term storage and evaluated how interactions with naturally occurring microorganisms influence *Listeria monocytogenes* survival under commercial conditions. First, researchers characterized microbial communities on pear surfaces at harvest and after the packing line (pre-storage). Postharvest processing significantly reduced total microbial load, although community structure at the genus level remained stable. Second, microbial communities were monitored throughout storage in both bulk and wrapped, and marketable and unmarketable pears using high-throughput 16S and ITS amplicon sequencing. Over time, psychrotolerant genera such as *Leucosporidium* and *Pseudomonas* became dominant. Wrapped pears exhibited lower microbial richness and diversity than bulk-stored pears, and marketability had a minor effect compared to time and packaging practice. Lastly, 1,620 pears were inoculated with *L. monocytogenes* alone or in combination with *Aureobasidium pullulans*, *Bacillus thuringiensis*, or *Penicillium expansum*. *L. monocytogenes* did not grow under any tested condition but remained detectable for up to seven months on intact, unwrapped pears. Wrapping with tissue containing copper and ethoxyquin significantly enhanced *L. monocytogenes* die-off—up to 5.4 log CFU/pear—and suppressed decay organisms like *P. expansum*. Co-inoculation with *P. expansum* accelerated *L. monocytogenes* die-off, while *A. pullulans* and *B. thuringiensis* had minimal impact. This

project generated the first longitudinal metagenomic profile of pears in CA storage and demonstrated that while current storage practices inhibit *L. monocytogenes* growth, the pathogen may persist in the absence of targeted controls. Findings support continuation of GAPs and GMPs and highlight the potential of antimicrobial wrapping materials as a potential postharvest intervention.

Background

In 2014, the tree fruit industry experienced its first known foodborne illness outbreak linked to intact tree fruit, when caramel-coated apples were implicated in a multistate listeriosis outbreak. This event prompted interest in the food safety risks during long-term storage of tree fruit commodities. While substantial research has since been conducted on apples, little is known about pears, particularly d'Anjou pears, which are routinely stored under controlled atmosphere (CA) conditions for up to nine months. Unlike apples, most existing pear research focuses on postharvest quality (e.g., disorders, decay), with little attention given to the microbiome or its influence on food safety. Pears may be stored in bulk bins or individually wrapped in antimicrobial untreated or treated copper-impregnated paper within 40-lb cartons. These differing practices could influence microbial dynamics and pathogen persistence, yet no evaluation has previously been conducted. Recent studies suggest that environmental microorganisms including yeasts, molds, and lactic acid bacteria may impact the survival or inactivation of *Listeria monocytogenes*, a pathogen of concern in cold-stored products. However, it is not yet known whether the changes in microbial communities during CA storage help reduce or inadvertently support the survival of foodborne pathogens like *L. monocytogenes*. Furthermore, the interaction between storage-associated microbiota and potential biological control organisms (e.g., *A. pullulans*, *B. thuringiensis*) had not been evaluated in pears. Thus, this project addressed those gaps using a metagenomic and culture-based framework.

Research Methods and Results

Objective 1: Identify culturable microbiological community members (yeasts, molds, and lactic acid bacteria) on conventional, whole, intact pears prior to storage*. Specific questions include "is there a difference between culturable and total microbiome samples (more for methods development)" and "is there a difference between pears that have just been harvested and those that have just passed over the packing line prior to entering cold storage"?*

Methods:

To evaluate the culturable microbial community present on pears prior to long-term storage, conventional, whole, intact d'Anjou pears were sampled at harvest and after postharvest processing (packing line). Three lots (n = 15 pears/lot) were collected in Washington State for microbiological analysis. To collect surface microbiota, five pears per treatment were submerged and washed sequentially in a buffer solution to dislodge reversibly attached microorganisms. The wash solutions were homogenized and split for total microbiome and culturable fraction analyses. For the culturable community, 1-mL aliquots were plated in duplicate on Tryptic Soy Agar (TSA) for bacteria and Potato Dextrose Agar (PDA) for yeasts and molds. Plates were incubated at 35°C for 24–48 h. After incubation, PBS was used to flood and scrape plates to recover microbial lawns. These recovered suspensions were pooled, concentrated, and stored at –20°C for downstream DNA extraction. Un-plated portions of the original wash were concentrated and stored as the "total community/total rinse" reference. DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen), and amplicon libraries targeting the V3–V4

region of the 16S rRNA gene and the ITS1 region were generated and sequenced on an Illumina MiSeq platform. High throughput sequencing was performed on the Illumina MiSeq sequencer at the UGA Center for Food Safety, generating paired 300bp reads for each amplicon. To target bacterial communities the V3-V4 region of 16S rRNA was sequenced, while the first internal transcribed spacer (ITS1) of the Eukaryotic ribosomal cistron was targeted for the fungal communities. Primers, PCR parameters for both V3-V4 16S and ITS amplicon sequencing were obtained from Illumina application notes. All bioinformatics and statistical analyses, apart from sequence alignment and phylogenetic inference were performed in R. Sequence alignment was performed using Muscle 5.1 and phylogenetic trees were inferred using FastTree on a Linux server. The R package DADA2 was used to infer amplicon sequence variants largely following the tutorials outlined on the website of the authors (https://benjjneb.github.io/dada2/tutorial_1_8.html). Phyloseq was used for further microbiome analyses (same analyses workflow between Objective 1 and 2).

Results:

The packing process significantly reduced overall microbial load on pear surfaces, as confirmed across three commercial lots (**Table 1**). However, amplicon sequencing of the 16S rRNA and ITS1 regions revealed minimal shifts in microbial community structure at the phylum and genus levels between harvest and post-packing pears (**Figures 1.1** and **1.2**). No significant differences in fungal or bacterial diversity were observed across lots. While bacterial (16S) read quality met sequencing standards, fungal ITS reads often failed to meet the 99.9% base call accuracy threshold, particularly in low-biomass, stress-exposed samples, indicating the need for improved sample preservation methods. Despite these limitations, baseline microbial community data were established for pears going to storage (Objective 2) and for downstream comparisons (Objective 3).

Objective 2: Describe yeasts, molds, and lactic acid bacteria composition of marketable and unmarketable conventional, whole, intact pears under two different storage practices at three, six, and nine months in long-term controlled atmosphere cold storage to develop a metagenomic profile and track community composition. *Specific questions include "how does the pear microbiome change during storage", "how does the storage method (bulk vs. wrapped) change the microbiome", and "is the microbiome different on marketable pears compared to unmarketable pears"?*

Methods:

Pears were shipped to the Center for Food Safety at 3, 6 and 9 months after harvesting. Pears were either received in bulk or individually wrapped; for both categories pears were marked as 'marketable' or 'unmarketable' by industry collaborators. Marketable and unmarketable pears were collected for each time point and type of pear, except for bulk 9-month pears, for which only marketable fruit was obtained. Total pear numbers and type are listed in **Table 2**. Three pears per lot of the same marketability type were sonicated with 250 mL of wash solution comprising 1X Tris-EDTA buffer (G-Biosciences, Saint Louis, MO, USA) supplemented with 2% Tween 80 (Research Products International, Mount Prospect, IL, USA) in a stomacher bag. To dislodge microorganisms from the surface of the samples, the stomacher bag with the product sample was sonicated for 5 minutes using a sonicator (VWR International, LLC, Radnor, PA, USA). During sonication, samples were securely contained within sterilized sampling bags to maintain aseptic conditions. The resulting wash solution, likely containing dislodged microorganisms, was divided into seven aliquots of 30–35 mL in sterilized conical centrifuge tubes. These aliquots were centrifuged at 3900 rpm for 15 minutes at 4°C. Following centrifugation, the clear supernatant was carefully removed, leaving a dense pellet in each aliquot. The pellets from all aliquots were combined into a single tube and centrifuged again at 3900 rpm for 15 minutes at 4°C.

After discarding the supernatant, the consolidated pellet was transferred to a microcentrifuge tube and subjected to a final centrifugation at 14,000 × g for 10 minutes. The remaining concentrated pellet served as the sample for DNA extraction. DNA was extracted from the pellet using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. High throughput sequencing was performed on the Illumina MiSeq sequencer at the UGA Center for Food Safety, generating paired 300bp reads for each amplicon. To target bacterial communities the V3-V4 region of 16S rRNA was sequenced, while the first internal transcribed spacer (ITS1) of the Eukaryotic ribosomal cistron was targeted for the fungal communities. Primers, PCR parameters for both V3-V4 16S and ITS amplicon sequencing were obtained from Illumina application notes. All bioinformatics and statistical analyses, except for sequence alignment and phylogenetic inference were performed in R. Sequence alignment was performed using Muscle 5.1 and phylogenetic trees were inferred using FastTree on a Linux server. The R package DADA2 was used to infer amplicon sequence variants largely following the tutorials outlined on the website of the authors (https://benjjneb.github.io/dada2/tutorial_1_8.html). Phyloseq was used for further microbiome analyses, largely following the workflow outlined in Callahan et al. 2016. Overrepresented taxa in pairwise comparisons (e.g., month 3 versus month 6, marketable versus unmarketable) were inferred using the DeSeq2 R package, while capscale analyses in the vegan R package were performed to infer which parameters were significantly influencing beta-diversity in the microbial communities.

Results:

Alpha diversity. Alpha diversity of amplicon sequence variants (ASVs) was estimated using the Choa1 statistic. Estimated alpha diversity of ITS1 ASVs for fungal communities was notably lower (average 18.3, CI 15.5 – 21.1) than estimated alpha diversity of 16S ASVs in bacterial communities (average 166.4, CI 136.2 – 196.6). The diversity observed in the culturable communities was significantly (< 0.01, Welch two sample t-test) lower than total rinse communities for both fungi (ITS1) and bacteria (16S). Total bacterial communities showed an average of 298 ASVs, while bacterial communities recovered from cultures showed an average of 32 ASVs. The average number of fungal (ITS1) ASVs was 25 for DNA isolated from the total rinse, and six for DNA isolated from cultures.

No significant differences in alpha diversity of fungal communities were found when fungal communities were compared from bulk and individually wrapped pears, however, bacterial communities did show a significant difference ($P < 0.05$; Welch T-Test). No significant differences in Chao1 richness indices were observed when marketable versus unmarketable pears were compared for both fungal and bacterial communities.

Using the total rinse richness estimates we tested for significant differences in richness between the 3-, 6- and 9-month time points using a one-way ANOVA and Tukey Honest Significant Differences test as post hoc test. Bulk and wrapped pears were tested separately. For the fungal communities in bulk pears, we saw no significant change in the Chao1 index between the 3- and 6-month time point, while a significant ($P < 0.05$) decrease was observed from the 6 to 9-month time-points. Wrapped pears showed a different pattern; a significant increase in ASV richness was observed between the 3-month and 6-month time points, followed by a significant decrease between the 6-month and 9-month timepoints. For bacterial communities in bulk pears a significant ($P < 0.05$) decrease in richness of ASVs was observed between the 3-month and 6-month time points, while a significant ($P < 0.05$) increase was observed between the 6-month and 9-month time points. For bacterial communities on wrapped pears, we saw a pattern that resembled the pattern observed for fungal communities on wrapped pears; a significant increase in richness between the 3-month and 6-month time points, and a significant decrease between the 6-month and 9-month time points.

Beta diversity. To compare the composition (beta diversity) of the fungal and microbial communities found on the pear surfaces Principal Coordinate Analyses (PCoA) were performed based on weighted UniFrac distances. To test which parameters (e.g., marketability, bulk versus wrapped) were significantly associated with beta diversity we used a capscale analysis in the vegan package. Beta diversity analyses were only performed for the 'total rinse' samples, as the datasets of culturable organisms only represent small subset of the total diversity. No distinct clusters could be found for 3- and 6-month fungal communities, irrespective of marketability or being bulk or wrapped. Fungal communities of 9-month marketable bulk pears and unmarketable wrapped pears are distinct from the 3- and 6-month pears (Fungal PCoA, **Figure 2.1**). A capscale analysis based on both bulk and wrapped pears showed that marketability and pears being individually wrapped had a highly significant effect on the beta diversity ($P < 0.01$), while time of sampling had a significant effect ($P < 0.05$). When individually wrapped pears and bulk pears were analyzed separately, time of sampling was still highly significant, while marketability was borderline significant for bulk pears ($P = 0.05$), while marketability was not significant for individually wrapped pears.

The PCoA analyses of the bacterial communities showed tight clustering by time-point for the individually wrapped pears, irrespective of their marketability (Bacterial PCoA, **Figure 2.2**). Nine-month marketable bulk pears cluster with the 9-month individually wrapped pears, while 3-month and 6-month bulk pears form very loose clusters, which are mainly separated on the Y-axis, with no clear clustering by marketability. Capscale analyses confirm the same pattern, showing that both time and being either individually wrapped or bulk have a highly significant ($P < 0.01$) effect on the beta diversity of samples, while marketability is not significant ($P > 0.05$).

Composition of fungal and bacterial communities associated with the surface of pears. Bacterial communities (barplot 16S, **Figure 2.3**) consisted of a mixture of genera found in food processing environments and other build environments such as Acinetobacter, Pseudomonas, bacteria associated with the microbiota of plants (e.g., Frondihabitans, Erwinia) and sugar rich environments (e.g., Gluconobacter). The fungal communities (Barplot ITS, **Figure 2.4**) were dominated at the 3- and 6-month time points by Aurobasidium and Penicillium species, however these genera were virtually absent at the 9-month time point. At all time-points we found a large variety of psychrophilic and psychrotrophic yeasts (e.g., Mrakia, Leucosporidium, Cutaneotrichosporon, Tausonia). Additionally fungal genera associated with fruit spoilage were found, such as Botrytis and Mucor.

Enrichment of fungal and bacterial ASVs between time points. DeSeq2 analyses for individual time points were performed separately for bulk and individually wrapped pears. For bulk pears ASVs associated with the bacterial genera Gluconobacter, Rhanella, Leuconostoc and Pantoea were significantly enriched in 6-month communities compared to 3-month communities, while Pseudomonas were significant reduced. In the 9-month versus 6-month comparison ASVs associated with Pseudomonas, Acinetobacter, Microbacter and Rhodococcus were significantly enriched, while Gluconobacter and Rhanella were reduced. For fungal communities in bulk pears ASVs associated with Neonectria, Niesslia, Penicilllium fuscoglaucum and Fusarium were significantly enriched in 6-month communities as compared to 3-month communities, while ASVs associated with Penicilllium fimorum, Aureobasidium, Mrakia, Botrytis and Candida were significantly reduced. ASVs associated with Leucosporidium were significantly enriched in 9-month fungal communities compared to 6-month communities, while ASVs associated with Aureobasidium, Penicillium fuscoglaucum and Neonectria were significantly reduced.

For individually wrapped pears, ASVs associated with the genera Williamsia, Pseudomonas, Frondihabitans, Rhodococcus and Curtobacterium were significantly enriched in 6-month bacterial communities, while ASVs associated with Pseudomonas, Stenotrophomonas and Carnobacterium were

reduced. In the 9-month versus 6-month comparison, ASVs associated with Pseudomonas, Acinetobacterium, Microbacterium and Rhodococcus, while Rhanella and Gluconobacter associated ASVs were significantly reduced. In fungal communities in individually wrapped pears, ASVs associated with Cadophora, Mrakia, Cladosporium and Vishniacozyma were significantly enriched in 6-month communities, and only one ASV associated with the yeast genus Metschnikowia was significantly reduced. In 9-month communities compared to 6-month communities, only one ASV associated with the genus Filobasidium was enriched, while ASVs associated with Aureobasidium, Vishniacozyma, Cutaneotrichosporon, and Rhodotorula were among the genera associated with reduced ASVs.

Objective 3: Co-inoculate representative yeast, mold, and bacterial community members with *Listeria monocytogenes* on pears under industry-relevant conditions to characterize synergistic and antagonistic effects. *Specific questions include "does L. monocytogenes grow (if yes, how long)", "does decay or postharvest disease make food safety (L. monocytogenes) worse"?*

Methods:

Pears and inocula preparation. Whole, fresh d'Anjou pears were harvested in Washington State in Fall 2023 and shipped under refrigeration (3°C) to the University of Georgia. Four strains of *Listeria monocytogenes*, previously linked to foodborne outbreaks, were cultured and combined into a four-strain cocktail (~9.5 log CFU/mL). Commensal organisms included *Bacillus thuringiensis* (BTNow®), *Aureobasidium pullulans* (Botector®), and a laboratory-cultured *Penicillium expansum* isolate. Each co-inoculum was prepared following manufacturer instructions or standard microbiological protocols.

Experimental design and inoculation. A total of 1,620 pears were randomized across 12 treatment combinations based on co-inoculation type (Lm only or co-inoculated with one of three microbes), condition (intact or mechanically wounded), and storage format (bulk or wrapped). Prior to inoculation, pears were surface-marked, and wounds were introduced to half the samples using sterile scalpels. Each pear was spot-inoculated with 100 µL of the Lm cocktail, followed by an additional 100 µL of the respective co-inoculum once dry. Pears designated for wrapping were enclosed in tissue paper containing copper carbonate (1.3%) and ethoxyquin (0.1%) prior to storage.

Storage conditions. Pears were stored under conditions simulating commercial practices: regular air (RA) at 3°C for up to 84 days, followed by controlled atmosphere (CA) storage (2–3% O_2, 1% CO_2, 3°C) through day 270. Wrapped pears were individually enclosed, while bulk-stored pears were grouped in bins or boxes by treatment. Samples were collected at 15 timepoints across the 9-month study.

Microbial enumeration. At each timepoint, nine pears per treatment were rinsed in buffered peptone water with Tween 80. Rinsates were plated on selective media: CHROMagar Listeria for Lm, MYP agar for *B. thuringiensis*, and DRBC agar for *A. pullulans* and *P. expansum*. When Lm counts fell below detection (2.67 log CFU/pear), a three-tier MPN assay with BLEB enrichment and CHROMagar confirmation was used.

Statistical analysis. Microbial populations were log-transformed and analyzed using JMP Pro 17 in a mixed model–repeated measures design. Fixed effects included co-inoculation, wounding, storage time, and their interactions. Significance was determined at $P < 0.05$ using Tukey's post-hoc comparisons.

Results:

D'Anjou pears (n = 1,620) were inoculated with *Listeria monocytogenes* only or with *L. monocytogenes* and one of the representative commensal community members identified in Objective 2, including bacterial (*Bacillus thuringiensis),* fungal *(Aureobasidium pullulans),* and one of the major causes of pear fungal postharvest losses *(Penicillium expansum).* The survival of *L. monocytogenes* on d'Anjou pears co-

inoculated with *Bacillus thuringiensis*, *Aureobasidium pullulans*, and *Penicillium expansum* stored in refrigerated controlled atmosphere during 9 months under industry-relevant conditions was determined (**Figure 3.1**). Industry-relevant conditions evaluated included the use of bulk storage or wrapping with tissue paper impregnated with 1.3% copper carbonate and 0.1% ethoxyquin. Pears were kept intact or intentionally damaged mechanically using scalpels to simulate potential wounding that can occur during pre/post-harvest handling. Storage conditions included the use of refrigerated regular atmosphere (RA) on pears stored for 3 months, while CA chambers (2-3% O_2 and 0.3% CO_2) (LabPod; Storage Control Systems Inc., MI) were used to store pears for 4 to 9 months. A certified biosafety level II refrigerated storage room (3°C) was used for this objective due to the use of *L. monocytogenes.*

Populations of *B. thuringiensis*, *A. pullulans*, and *P. expansum* were enumerated throughout 9 months of storage using selective and differential agar plates (**Figure 3.2**). Antagonistic effects of co-inoculum over *L. monocytogenes* were identified. These effects were dependent on the condition of storage (intact, mechanically damaged, or wrapped pears). For instance, populations of *L. monocytogenes* were similar on intact pears regardless of co-inoculation. On mechanically damaged pears, an antagonistic effect of *P. expansum on L. monocytogenes* was observed. A significant increase in *P. expansum* population caused *L. monocytogenes* to fall below the limit of detection after 3 months of storage. No effect of *B. thuringiensis* or *A. pullulans* on *L. monocytogenes* growth was identified, regardless of condition. Pears wrapped with tissue paper containing copper carbonate (1.3%) and ethoxyquin (0.1%) showed significantly lower populations of *L. monocytogenes* compared to intact (unwrapped) pears throughout storage time. The greatest *L. monocytogenes* reduction (~2.0 log) on wrapped pears occurred during the first 84 days of storage across all co-inoculation treatments. *P. expansum* growth was significantly reduced on wrapped pears compared to intact (unwrapped) pears throughout storage time.

In summary, significant contributions show industry practices evaluated do not support the growth of *L. monocytogenes* on d'Anjou pears regardless of co-inoculation or wounding throughout 9 months of CA storage. However, in some conditions, levels of *L. monocytogenes* remained constant for up to 7 months of storage, demonstrating *L. monocytogenes* long-term survival on pears even under low oxygen and refrigerated conditions. Additionally, the efficacy of chemically treated tissue paper to simultaneously improve food safety and *P. expansum* rot decay on pears was demonstrated.

Outcomes and Accomplishments

Objective 1:

- Demonstrated that postharvest handling (i.e., the packing line) significantly reduces total microbial load on the surface of d'Anjou pears across multiple commercial lots.
- Confirmed that microbial diversity decreases following pre-sorting, even though community structure at the genus level remained relatively stable.
- Established a methodological framework for capturing both culturable and total microbiota from intact pear surfaces using rinse-based and agar-plated recovery.
- Identified limitations in fungal (ITS) sequence quality, particularly in low-biomass samples, emphasizing the need for improved sample preservation and processing protocols for future metagenomic analyses.
- Generated a baseline microbial profile of pears immediately prior to long-term storage, enabling subsequent longitudinal comparisons throughout CA storage.
- Highlighted the importance of integrating metadata (e.g., orchard, lot, harvest time, storage practice) to aid interpretation of microbial ecology data in fresh produce research.

Objective 2:

- No significant differences were found between the bacterial and fungal communities on marketable or unmarketable pears, even though we found several fungal organisms associated with fruit spoilage (e.g., Botrytis, Mucor and Penicillium).
- Storage practices, in this study individually wrapped pears versus bulk pears had a significant effect on the composition and storage over time of both fungal and bacterial communities.
- For fungal communities, the highest (alpha) diversity was observed at the 6-month time point, followed by a decrease in diversity at 9 months. Bacterial communities from individually wrapped pears showed a similar trend in alpha diversity over time, while bulk pears only showed a significant decrease in diversity between the 6- and 9-month time points.

Objective 3:

- Whole d'Anjou pears did not support the growth of *L. monocytogenes.*
- *Listeria monocytogenes* persisted for up to 7 months of storage in some conditions.
- Copper and ethoxyquin paper wrap significantly reduced *L. monocytogenes* over time.
- *L. monocytogenes* fell below the detection limit as *P. expansum* population increased.
- *P. expansum* growth was significantly reduced on wrapped pears compared to intact (unwrapped) pears throughout storage.
- *B. thuringiensis* and *A. pullulans* persisted during 9 months of storage.
- Emphasized the importance of continuous vigilance across the supply chain by reinforcing preharvest food safety practices (e.g., Produce Safety Rule, Good Agricultural Practices) to prevent initial contamination and postharvest GMPs and prerequisite programs to minimize cross-contamination risks during long-term storage.

APPENDICES

Publications and Presentations **Presenter (all authors reviewed presentations/posters/works)*

Publications:

Ruiz-Llacsahuanga, B, Raad, R, Greenbaum, H, Daniel, J, Appolon, C, Burnett, A, Murphy, CM, Den Bakker, H, Hamilton, AM, Strawn, LK, and Critzer, F (2024). Survival of *Listeria monocytogenes* on d'Anjou pears co-inoculated with *Bacillus thuringiensis, Aureobasidium pullulans, and Penicillium expansum* during nine months of long-term storage. Journal of Food Protection. Manuscript in preparation.

Presentations & Articles:

Fruit Growers News. (2023, January 30). *Assessing food safety of pears in storage*. https://fruitgrowersnews.com/news/assessing-food-safety-of-pears-in-storage/

Strawn, LK*, AM Hamilton*, H Den Bakker, F Critzer, and LK Strawn. Storage wars: How exploring the pear microbiome can help improve safety and quality outcomes during storage. Seminar presentation to Washington State tree fruit industry, USDA AMS researchers, and Washington State University graduate students. 2023 April 3; Wenatchee, WA.

Strawn, LK*, AM Hamilton, H Den Bakker, and F Critzer. Washington Tree Fruit Research Commission Pear Committee: A metagenomic approach to food safety risk mitigation in pears. Seminar presentation to Washington Tree Fruit Research Commission Pear Committee. 2023 April 4; Yakima, WA.

Hamilton AM, H Den Bakker, F Critzer, and LK Strawn*. Evaluating the Long-term Storage of Pears-Poster. Center for Produce Safety Annual Meeting. 2023 Jun 21; Buckhead, GA.

Redacción. (2024, January 3). *Two projects look at effects of pear storage on microbes*. Postharvest.biz. https://www.postharvest.biz/two-projects-look-at-effects-of-pear-storage-on-microbes

Murphy, CM*, AM Hamilton, H Den Bakker, F Critzer, and LK Strawn. Northwest Horticultural Council Food Safety Committee Annual Meeting. Research Update: A metagenomic approach to food safety risk mitigation in pears. 2024 Feb 14; Yakima, WA.

Strawn, LK, A. Hamilton, I. Reynoso*. "A Metagenomic Approach to Risk Mitigation in Pears"-Poster. Virginia Tech FST Graduate Poster Competition. 2024 Apr 19; Blacksburg, VA.

Hamilton AM*, H Den Bakker, F Critzer, and LK Strawn. Washington Tree Fruit Research Commission Pear Committee: Food safety risk mitigation in pears using microbiome. Seminar presentation to Washington Tree Fruit Research Commission Pear Committee. 2024 May 21. Yakima, WA.

Strawn, LK*, A. Hamilton. "A Metagenomic Approach to Food Safety Risk Mitigation in Pears-Poster and Short Talk". Center for Produce Safety Annual Meeting. 2024 Jun 19; Denver, CO.

Ruiz Llacsahuanga*, B., Raad, R., Greenbaum, H., Daniel, J., Appolon, C., Burnett, A., Hamilton, A., Strawn, L., den Bakker, H., Critzer, F. Survival of *Listeria monocytogenes* on d' Anjou pears co-inoculated with *Bacillus thuringiensis, Aerobasidium pullulans,* and *Penicillium expansum* during 70 days of cold storage – Poster. International Association for Food Protection Annual Meeting. 2024 July 24; Long Beach, CA.

LK Strawn*, CM Murphy, AM Hamilton, H Den Bakker, and F Critzer. Northwest Horticultural Council Food Safety Committee Annual Meeting. A metagenomic approach to food safety mitigation pears: A CPS Project. 2025 Mar 20; Yakima, WA.

Budget Summary

This project was awarded $337,187 in research funds. The majority of funds were spent. The remaining travel budget will be spent for PI Strawn and Co-pi Hamilton to attend the CPS Research Symposium in La Jolla, CA. Some funds remaining were due to having supplies from another project in the Strawn Lab (so less was ordered).

Tables and Figures (see below)

Table 1 Quantities of bacteria between pears that had just been harvested and those that had just passed over the packing line prior to entering cold storage

Lot	Harvest	Packing
1	3.79 Aa[a]	3.13 Ba
2	3.51 Aa	3.09 Ba
3	4.01 Aa	3.40 Ba

[a] Different capital letters denote significant differences between treatments (within a row) while different lowercase letters denote significant differences within a treatment (within a column)

Table 2 Collection dates of d'Anjou pears used in this study

Wrapped or bulk	3 months	6 months	9 months
bulk	January 2024	April 2023	June 2023
wrapped	December 2024	March 2025	June 2023

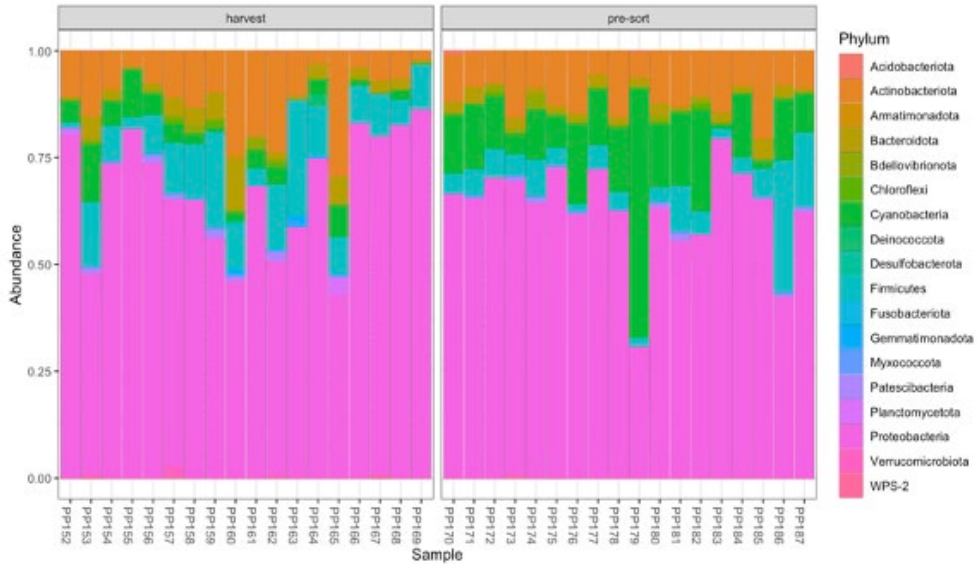

Figure 1.1 Relative abundance plot (phylum level) of the microbial communities for pears that had just been harvested and those that had just passed over the packing line prior to entering cold storage.

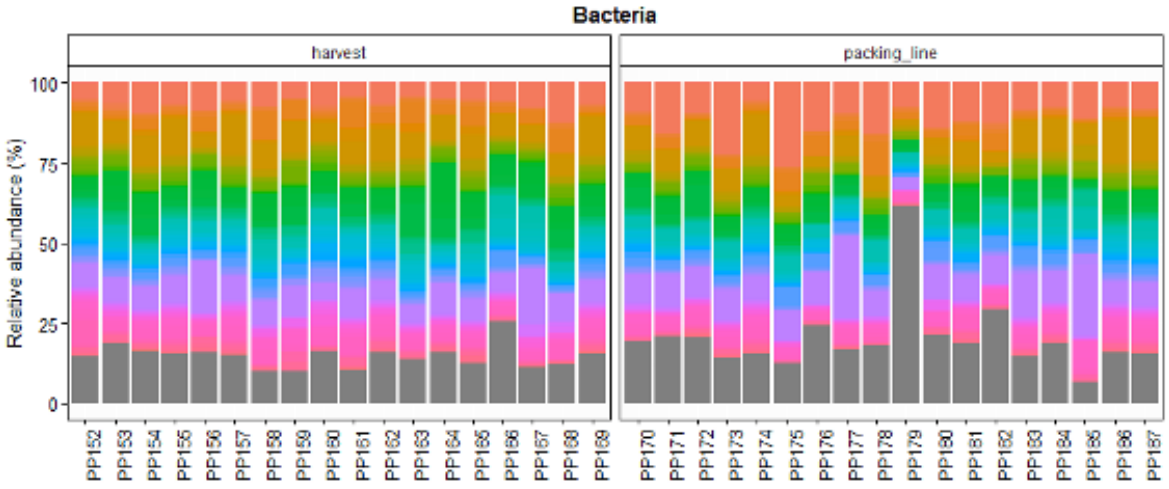

Figure 1.2 Relative abundance plot (genus level) of the microbial communities for pears that had just been harvested and those that had just passed over the packing line prior to entering cold storage. (Note: Genus legend not shown due to number of organisms and color pixels. Available upon request.)

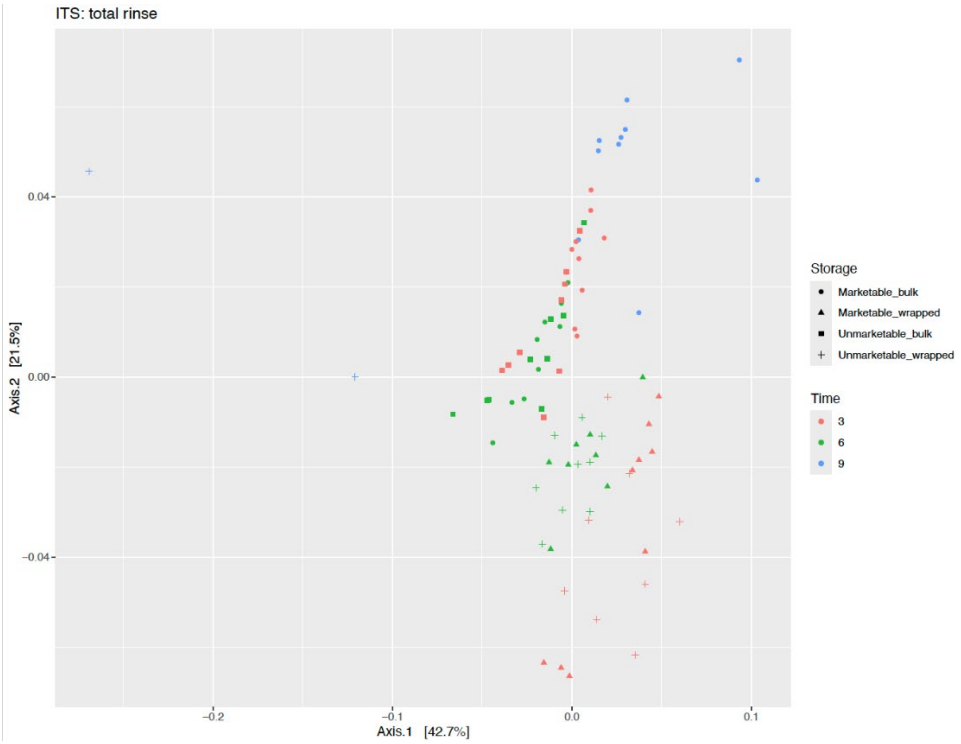

Figure 2.1 Fungal PCoA using the ITS region (total rinse samples) from pears in long term storage (3, 6, and 9-month samples) that represent marketable (bulk and wrapped) and unmarketable (bulk and wrapped).

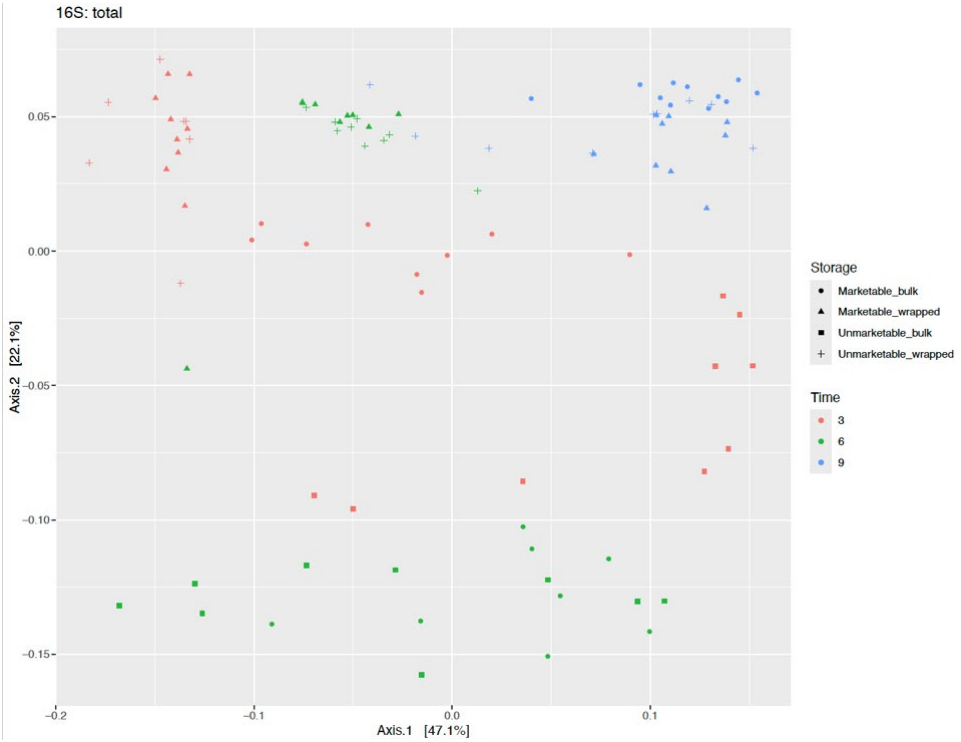

Figure 2.2 Bacterial PCoA using 16S (total rinse samples) from pears in long term storage (3, 6, and 9-month samples) that represent marketable (bulk and wrapped) and unmarketable (bulk and wrapped).

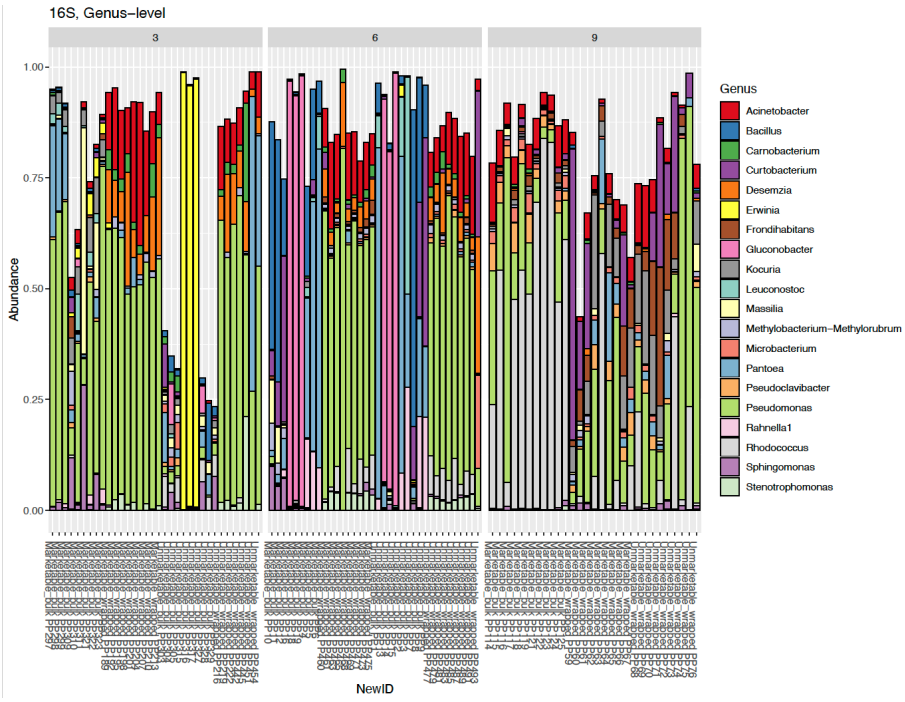

Figure 2.3 Relative abundance plot (16S, genus level) of the microbial communities for pears in 3, 6, and 9 months of storage under different conditions.

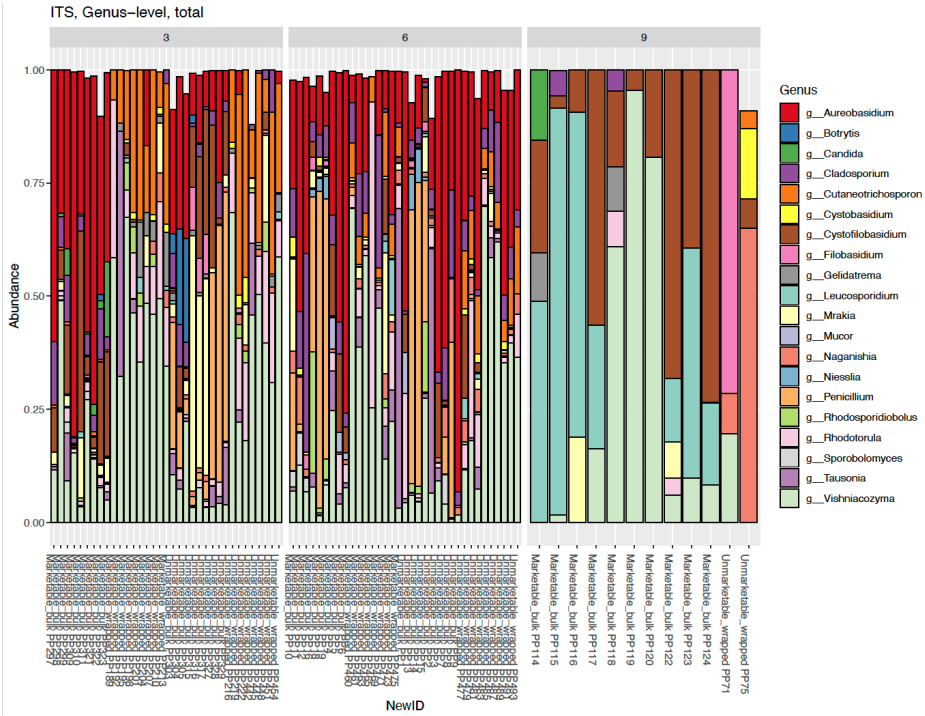

Figure 2.4 Relative abundance plot (ITS, genus level) of the microbial communities for pears in 3, 6, and 9 months of storage under different conditions.

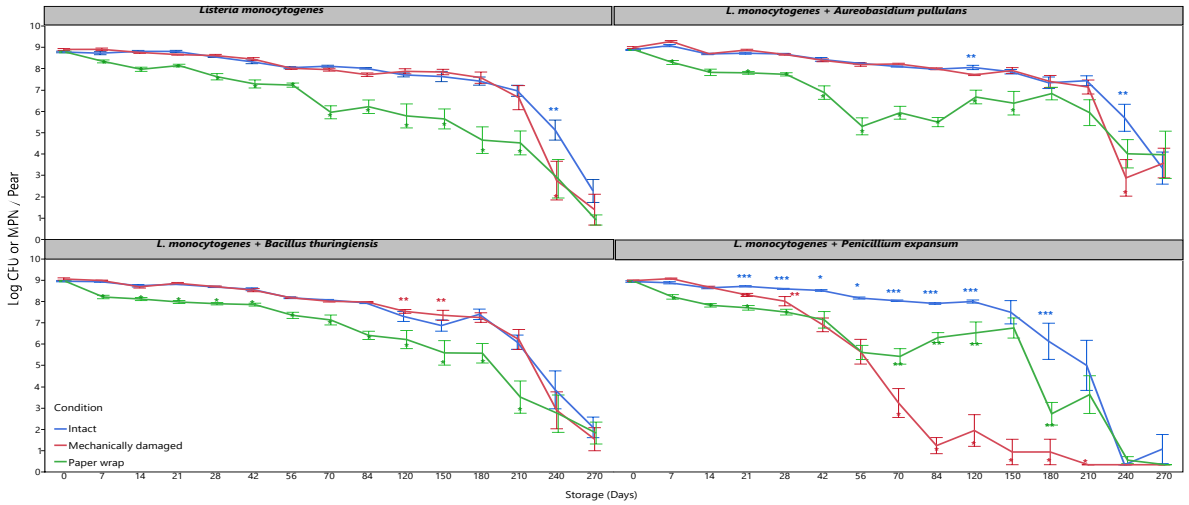

Figure 3.1 Survival of *Listeria monocytogenes* on co-inoculated d'Anjou pears throughout 270 days of refrigerated controlled atmosphere (Log CFU or MPN/pear) (Mean ± std error). Significant differences are indicated across condition treatments within sampling point ($p < 0.05$). Asterisks of each color (blue, red, and green) represent intact, mechanically damaged and paper wrap condition treatments, respectively. At each time point, the presence of only one asterisk (*) represents the significant different treatment, with no significant differences between the remaining two treatments. The presence of (**) and (*) represents a significant difference between the two treatments containing different number of asterisks; the treatment that does not contain an asterisk is statistically similar to the other two treatments. The presence of (***)(**)(*) denotes significant differences among the three condition treatments.

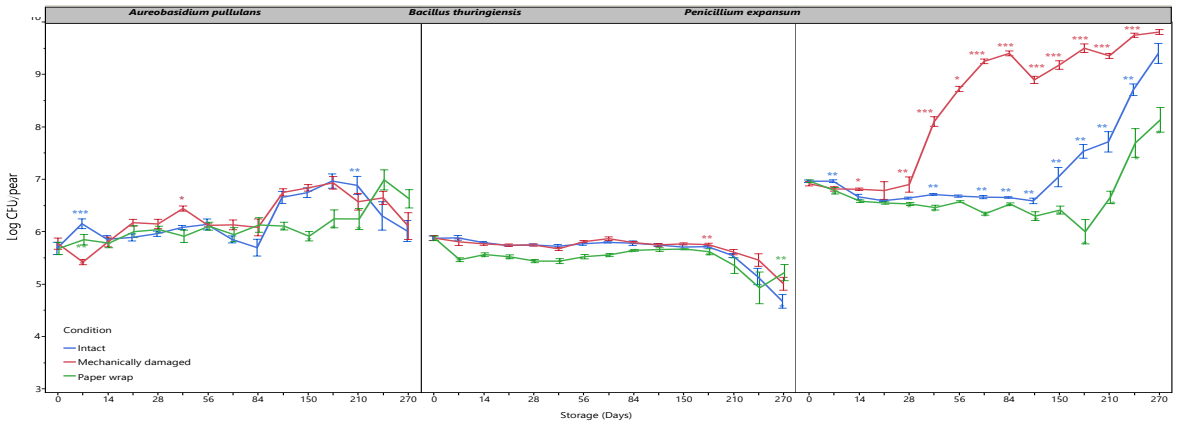

Figure 3.2 Survival of *Aureobasidium pullulans, Bacillus thuringiensis* and *Penicillium expansum* on d'Anjou pears inoculated with *L. monocytogenes* (Log CFU/pear) throughout 270 days of refrigerated controlled atmosphere (Mean ± std error). Significant differences are indicated across condition treatments within sampling point ($p < 0.05$). Asterisks of each color (blue, red, and green) represent intact, mechanically damaged and paper wrap condition treatments, respectively. At each time point, the presence of only one asterisk (*) represents the significant different treatment, with no significant differences between the remaining two treatments. The presence of (**) and (*) represents a significant difference between the two treatments containing different number of asterisks; the treatment that does not contain an asterisk is statistically similar to the other two treatments. The presence of (***)(**)(*) denotes significant differences among the three condition treatments.