Washington Blueberry Commission Research Proposal Format

Title: Inactivation of *Listeria monocytogenes* on Berries by Water-Assisted Ultraviolet light

Current Year: 2023 **Year Initiated:** 2024 **Terminating Year:** 2024

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Justification and Background:

While no documented *Listeria monocytogenes* outbreaks have been linked to blueberries, numerous recalls of blueberries and other berries due to *L. monocytogenes* contamination highlights that this foodborne pathogen is a known and foreseeable hazard for blueberries *(2-4)*. The majority of *L. monocytogenes* recalls are related to frozen blueberries. Unlike those going to the fresh market, frozen blueberries normally undergo a washing step prior to freezing.

Water use is often a necessary part of postharvest handling; however, it also represents a significant potential source of microbial contamination. Cross-contamination from hazards that may be introduced into the water can lead to widespread pathogen proliferation and contamination of all subsequent produce that contacts the water. To combat this risk, producers rely heavily on adding and maintaining sanitizers to minimize the potential for cross-contamination of produce. The use of chlorine and peracetic acid to reduce the microbial load in production water and prevent cross-contamination is widespread. However, these chemical strategies exhibit reduced antimicrobial effectiveness in the presence of organic matter and have raised concerns regarding their impact on environmental and worker health. Furthermore, the addition of a sanitizer to postharvest water is not meant to clean produce, instead, it can only be used to prevent cross-contamination from the water to the produce and limit the build-up of pathogens in the water.

In contrast, Ultraviolet light (UV-C; 254 nm) has proven capable of inactivating a wide spectrum of microorganisms in production water and is an FDA-approved non-thermal physical decontamination approach for produce surfaces. UV-C systems are relatively cost-effective and the use of UV-C on produce surfaces does not cause damage to the produce *(6)*. Nevertheless, UV-C's shallow penetration depth on opaque surfaces (e.g., blueberries) necessitates any bacteria on a food surface be directly exposed to the UV-C lamp for inactivation. Water-assisted UV systems present a solution to this limitation and are a promising option for reducing *L. monocytogenes* during frozen blueberry production. By immersing blueberries in moving or agitated water, their surfaces receive more uniform UV-C exposure. Furthermore, as UV-C effectively penetrates clear liquids, any bacteria dislodged from the surface into the water can also be efficiently inactivated.

In a recent study on strawberries, water-assisted UV-C treatment was able to reduce *Listeria innocua* and *Salmonella* Typhimurium populations by 4.5 ± 0.3 and 3.7 ± 0.5 log CFU/strawberry, respectively, with no significant difference in reduction based on contact time (1 minute and 5 minute) or number of UV-C lights (2 or 4) *(5)*. Furthermore, water-assisted UV-C treatment was effective at minimizing microorganisms remaining in wash water and did not significantly impact the color, firmness, antioxidant activity, organic acid, anthocyanin, vitamin C, or total phenolic content of

strawberries *(5)*. The objective of the research outlined in this proposal is to evaluate the decontamination efficacy of water-assisted UV-C against *L. monocytogenes* inoculated onto the surface of blueberries to understand its viability as a risk reduction strategy.

Relationship to WBC Research Priority(s):
The research outlined in this proposal addressed the following 2024 WBC Research Priority: "Food safety including the development of kill steps for fresh and processed blueberries, including water quality."

Objectives:
The objective of the research outlined in this proposal is to evaluate the decontamination efficacy of water-assisted UV-C against *L. monocytogenes* inoculated onto the surface of blueberries to understand its viability as a risk reduction strategy. Experimental parameters to be examined include treatment time, water turbidity, and distance from the UV-C source. In parallel, dry UV-C treatment will be conducted to enable a comparative analysis of efficacies.

Procedures:
A five-strain cocktail of *L. monocytogenes* strains with different subtypes (e.g., 1/2a, 1a/b, 4b) and origins (e.g., environmental, clinical) will be used to create the inoculum (~8-9 log CFU/mL) in the outlined study. Fresh blueberries (10g) will be spot inoculated with *L. monocytogenes* in weigh boats and dried in a biological safety hood at room temperature until visibly dry (starting concentration of ~ 6-7 log CFU/g). After inoculation and drying, the starting concentrations of *L. monocytogenes* on untreated inoculated control samples will be determined and verified by plate count onto tryptic soy agar (TSA) and Modified Oxford agar (MOX).

The 10g samples of inoculated blueberries will either undergo treatment of UV-C directly (dry UV-C) or water-assisted UV-C treatment in order to enable a comparative analysis of efficacies. All UV-C treatments will be conducted in a biological safety hood with UV-C lights at 254 nm within a UV-C chamber. UV intensity will be measured and verified using a UVA/UVC Light Meter/Datalogger right above the surface of blueberry samples. Experimental parameters to be examined include treatment time (e.g., 30 seconds, 1 minute, and 2 minutes), water turbidity (e.g., 0, 40, and 80 NTU), and distance from the UV-C source. Exact times, turbidities, and distances are subject to change based on stakeholder input. Two independent replicate experiments will be conducted. For each of the two replications, triplicate blueberry samples will be analyzed for treatment combination.

For dry UV-C treatments, weigh boats containing 10g of inoculated blueberries will be placed under the UV-C lights at the designated distance from the source for the allotted time. For water-assisted UV-C treatments, 10g of inoculated blueberries will be immersed in agitated sterile, room temperature (22 °C) deionized water in a glass beaker containing a stir bar during the UV-C treatment. Preliminary trials will be conducted to determine the amount of water and agitation speed that is needed to ensure that blueberries can rotate freely in the water. After treatments, the blueberry and wash water samples will immediately undergo microbial analyses.

Immediately following treatment blueberries will be placed into a sterile Whirl-Pak bag and hand massaged using a 30-s rub, 30-s shake, 30-s rub method with 0.1% peptone to remove any residual *L. monocytogenes* from the berry surface. As needed, serial dilutions will made in 0.1% peptone water and surface plated (0.1 mL) in duplicate onto TSA and MOX. To lower the limit of detection, 1 mL from the Whirl-Pak bag will be plated onto four plates each (0.25 mL per plate) in duplicate onto both agars. Additionally, 1 mL of wash water will be collected immediately after water-assisted UV-C treatments

and surface-plated (0.25 mL per plate) in duplicate on TSA and MOX. TSA and MOX will be incubated for 48 h at 35°C and 30°C, respectively. After 48 hours colonies will be counted, and *L. monocytogenes* population reductions will be determined.

Describe how this research will benefit Washington blueberry growers:

According to the USDA's National Agricultural Statistics Service's 2019 blueberry statistics, Washington State ranked as the 3rd largest state in terms of harvested acreage (16,700) and 2nd for value of utilized production ($153,224,000) *(8)*. Therefore, blueberry production is essential to the farm gate within the state of Washington. Thus, any lapse in food safety during the production of blueberries that results in an outbreak or recall can have devastating effects on Washington growers, regardless of the outbreak or recall origin. The economic consequences of outbreaks associated with produce are substantial and have potentially devastating effects on individual businesses, industry sectors, and agricultural production regions. The extensive costs and losses faced by the industry include those associated with product recalls, the disposal of unmarketable produce, lost production time, litigation, and long-term damage to the company's reputation. Previous produce-related outbreaks in the US have resulted in anywhere from $6-25 million in losses of sales revenues *(1)*. An outbreak or recall can be damaging to an entire segment of the produce industry or to a production area, rendering the commodity unmarketable. For example, the 2018 and 2019 *Escherichia coli* O157:H7 outbreaks linked to romaine lettuce resulted in sustainable decreases in retail sales of any product containing romaine and significant net loss to society *(7)*. Therefore, research aimed at collecting data on strategies for inactivating *L. monocytogenes* on the surface of blueberries that do not contribute to product loss or pose a risk to worker health will help reduce the burden of foodborne illnesses and maintain the economic viability of blueberries.

References:

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2. Desai, A. N., A. Anyoha, L. C. Madoff, and B. Lassmann. 2019. Changing epidemiology of *Listeria monocytoge*nes outbreaks, sporadic cases, and recalls globally: A review of ProMED reports from 1996 to 2018. *International Journal of Infectious Diseases*. 84:48-53.
3. Food and Drug Administration. Date, 2023, Townsend Farms Inc. Voluntarily Recalling Specific Frozen Fruit Products Because of Possible *Listeria monocytogenes* Contamination. Available at: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/townsend-farms-inc-voluntarily-recalling-specific-frozen-fruit-products-because-possible-listeria. Accessed November 28, 2023.
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8. United States Department of Agriculture National Agricultural Statistics Service New Jersey Field Office. Date, 2020, 2019 Blueberry Statistics. Available at: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.nass.usda.gov/Statistics_by_State/New_Jersey/Publications/Blueberry_Statistics/NJ%202019%20Blueberry%20Summary.pdf. Accessed December 1, 2023.

Budget:

	2024
Salaries	-
Time-Slip[1]	$5,100
Operations (goods & services)[2]	$13,000
Travel	-
Meetings	-
Other	-
Equipment[3]	$1,400
Benefits[4]	$514
Total	**$20,014**

[1] hourly employee
[2] $3,000 for disposable supplies (e.g., petri dishes, pipet tips), $10,000 for microbiology media supplies (e.g., modified oxford agar)
[3] Equipment - UVA/UVC Light Meter/Datalogger
[4] Benefits for hourly employee at 10.1%

Additionally Funding and Support:

- To perform this research on both blueberries and raspberries, a grant of $19,614 has been submitted to the Washington Red Raspberry Commission
- Master's student who will oversee the project and hourly employee will be funded off of C. Murphy's startup package.