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Microbiological Analysis of Nova Scotia Wild Lowbush Blueberries

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Abstract

The province of Nova Scotia produces over 65 million lbs. of wild lowbush blueberries (*Vaccinium angustifolium*) each year but the vast majority of this yield is individually quick frozen (IQF) and sold at commodity pricing. Presently, only about 1.5 million lbs. of berries are designated to be carefully harvested to target the more profitable fresh fruit market. Growers wish to divert more of this large crop to the lucrative fresh market, but the microbiological risks have not been thoroughly assessed. Therefore, we have conducted a microbiological survey of Nova Scotia blueberries where general microbial loads, expressed as total plate counts of aerobic bacteria (TPC) and yeast and moulds (YMC), were determined as well as the frequency and level of coliforms and *Escherichia coli* present on the berries as related to harvesting practices. In addition, samples that tested positive for *E. coli* were further examined for the presence of other enteric pathogens. Overall, berries collected during the early half of the harvest season had TPC (6.0 $\log_{10}$ CFU/g) and YMC (4.8 $\log_{10}$ CFU/g) levels that were greater by 1.2 log and 0.5 log respectively, than those acquired late in the season. Microbial loads varied significantly ($P<0.05$) as did the presence of coliform bacteria, by farm (location) and the type of harvest practices employed. In general, blueberries carefully harvested by our Perennia team (PH) by using sanitized hand-rakes consistently had lower TPC and YMC values than those harvested by farm crews. However, the so-called gentle harvesting (GH) methods (hand-raking or modified mechanical harvester) that were implemented by these crews still showed significantly reduced levels of microorganisms on the blueberries compared to those obtained using a traditional mechanical harvester (MH). For example, TPC levels on MH berries from Farm#1 were 0.7 log greater than when GH methods were employed. The frequency of detection of coliforms on the berries was also impacted by the harvesting methods. Here, detection rates for PH, GH and MH berries were 25%, 68% and 84%, respectively. However, levels were less than 2.4 $\log_{10}$ CFU/g for both PH and GH berries, significantly higher on the MH berries (3.6 $\log_{10}$ CFU/g). Although ~50% of all blueberry samples collected (n=350) contained coliforms, only 12 contained *E. coli* and none of these tested positive for STEC or *Salmonella* spp. Furthermore, a subset (n=34) of fully processed berries were also analyzed, which overall showed a substantial reduction in microbial numbers from the berries entering the line as well as a lower frequency and level of coliforms, with no pathogens being detected. However, environmental testing (ATP swabs) of surfaces along the processing line identified "hot spots" where microbial activity remained after cleaning. In addition, monitoring dirty conveyor belts and berry totes at different times demonstrated a potential risk for the development of pathogen biofilm as 4 of 61 samples were positive for *Listeria monocytogenes*, albeit at very low levels (not detected without enrichment).

Impact: Results of this work serve to validate benefits of implementing gentle harvesting practices for wild blueberries. Lower microbial loads achieved through minimized transfer of soil particulates and other debris should positively impact the shelf-life of berries with fewer spoilage organisms present as well as a reduced probability of carriage of human pathogens on fresh packed products. Moreover, environmental sampling identified potential problem areas along the processing line where improvements can be made that could help reduce the risk of the formation of persistent biofilms that could harbour human pathogens such as *Listeria monocytogenes.*

Introduction

Canada is the world's largest producer and exporter of lowbush blueberries or wild blueberries (*Vaccinium angustifolium* Ait.), with the majority of production being in Atlantic Canada and Quebec. Wild blueberries are Nova Scotia's most valuable agricultural export commodity with over 65 million lbs. grown and processed annually, making the province Canada's largest producer (Canadian Wild Blueberries, 2022). Since traditional mechanized harvesting practices can result in damage to the berries, most of the annual yield is sold as frozen product, where following harvest, they are winnowed to remove large debris, washed and then individually quick frozen (IQF). Moreover, washing prior to freezing can also remove the natural surface bloom on the berries which may compromise their appearance and integrity, leaving them more susceptible to damage during frozen storage and less appealing to consumers when thawed. Consequently, IQF berries do not garner the same retail return as those sold as "fresh", as would be the case for so-called "highbush" cultivated blueberries (*Vaccinium corymbosum* L.). Still, in a given year a fraction (1.5 million lbs.) of these berries are harvested by alternative methods (e.g. hand raked or other gentle harvesting methods) and sold as fresh-packed or "fresh-frozen" berries; for both of these products debris is removed using blowers and berries are sorted in a dry state instead of a wash step and either marketed as fresh or frozen.

Presently, stakeholders (i.e. Wild Blueberry Producers Association of Nova Scotia; WBPANS) are exploring alternative ways to add value to their product through novel approaches for harvesting, processing and packaging wild blueberries to minimize damage and extend shelf-life. Ultimately, the goal is to channel a greater portion of berries to the more lucrative fresh and/or fresh-frozen markets. Though promising results are being attained with some new approaches, questions remain regarding the microbiological safety of wild blueberries sold as RTE products, since no risk assessment data are available. This represents a serious knowledge gap for the industry moving forward. To become competitive in the fresh product market, both domestically and internationally, consumer confidence regarding the safety of wild blueberries must be achieved. Therefore, there is a vital need to establish baseline data to assess the risk through a science-based approach. While several surveillance type studies have been carried out for a variety of foodborne pathogens on other fruit commodities including frozen berry crops such as strawberries and raspberries (Canadian Food Inspection Agency, 2019), limited research has been conducted on fresh wild blueberries (Quansah et. al, 2019), and certainly no such data exist pertaining specifically to blueberries grown in Nova Scotia or to the alternative novel harvesting/ processing practices currently being evaluated.

Agriculture and Agri-Food Canada microbiologists at the Kentville Research and Development Centre (KRDC) along with Perennia Food and Agriculture specialists have conducted a study to provide baseline data to ascertain the risk associated with pathogen carriage on wild blueberries in the field and after processing. Since wild blueberries do not receive manure-based fertilizers, nor are they generally irrigated, pathogen contamination events in the field can mainly be attributed to contact with wildlife or due to weather events such as those resulting in runoff from adjacent agricultural landscapes following heavy rainfalls. However, during harvest and processing, the hygiene of workers and proper sanitation of equipment would also contribute to this risk. Therefore, the research undertaken here aimed to bridge the knowledge gap by conducting a comprehensive surveillance study on the microbiological status of wild blueberries. Specifically, we aimed to determine the presence of fecal contamination, specific pathogens and general microbiological load on berries sampled directly from the field, following harvest (via different methods), and after processing. We also examined the efficacy of cleaning practices for controlling contamination through environmental sampling of contact surfaces along the production line (i.e. conveyors and totes). Results obtained from this work to date are presented herein.

Material & Methods

Blueberry sample collection: A total of 384 individual berry samples were collected in duplicate during the 2021 wild blueberry harvest season. An individual sample constituted a one-pint vented polyethylene-terephthalate PET clamshell pack. Three sample types were partitioned according to the method of harvest (i.e. PH, hand-raked/ harvested by Perennia; GH, gently harvested by hand-raking, walk-behind harvester or modified mechanical harvester; MH; mechanically harvested by traditional mechanical harvester). A fourth sample type was acquired to represent berries designated for fresh pack that had already been processed (PRO) but not packaged. Sample types were further delineated by farm (five operations), fields and time of the season (early, middle, or late). Table 1 gives a breakdown of the number of samples collected prior to processing.

Table 1. Sampling scheme for used for microbiological assessment of wild lowbush blueberries

Sample Type	Season	Fields sampled per farm						Total fields or lots*	Samples per field or lot*	Total samples
		Farm #1	Farm #2	Farm #3	Farm #4	Farm #5	Farm #6			
Field (Perennia; hand-harvested)	Early	6	4	0	2	2	2	16	5	80
	Late	6	4	0	2	2	2	16	5	80
									Total	160
Harvested by farm (walk-behind,	Early	6	4	0	2	2	0	14	5	70
hand raked, modified mechanical	Late	6	4	0	2	2	0	14	5	70
									Total	140
Traditional mecahically harvested	Early	2	2	0	0	0	1	5	5	25
(control for comparison)	Late	2	2	0	0	0	1	5	5	25
									Total	50
TOTAL Samples										**350**

Field samples hand-raked by our team were taken from five random locations in each selected field. Rakes were surface sterilized by misting with a chlorinated (100 ppm) disinfectant spray and then wiped dry with a clean paper towel before harvesting each sample. To remove leaf litter and stems, raked berries were sieved through a grate which was held over a fan, allowing berries to pass while blowing off larger debris such as leaves and twigs. Berries harvested by farm personnel (GH) either by hand-raking or gentle modified harvesters were obtained by collecting samples off the blower on the sorting line. Mechanically harvested berries were collected directly from plastic totes in the fields where machines were operating or upon arrival at packing sheds. In the case of the latter, # Fields sampled / # Samples taken samples were collected from totes at different times during the day to ensure a wide range of diversity in terms of field location. Once clamshell containers were filled with berries, they were immediately placed in coolers containing ice packs. Coolers were moved to a walk-in cold room (4-5°C) at the end of each sample collection day before being transferred to the laboratory. Processing of all samples occurred within 48 h of their harvest collection. Duplicate sample packs were immediately placed in a freezer upon arrival at the laboratory.

Sample processing: For each individual sample (i.e. clamshell pack), 25 g of berries were placed in a 400 g filtered stomacher bag and diluted 10-fold in pre-chilled (4°C) buffered peptone water (BPW) using a DiluFlow gravimetric diluter (Interscience Inc., Woburn, MA). Samples were homogenized for two minutes on the normal speed setting in a Seward stomacher blender (West Sussex, UK) and then immediately placed in a cooler to limit the growth of resident microorganisms.

Microbiological analysis

General quality and spoilage potential: Traditional microbiological methods were employed to enumerate total aerobic bacteria (TPC, total plate count) along with yeasts and moulds (YMC); including heat tolerant (HTM moulds). For all PH, GH and MH berries, composite samples were prepared for each field or lot by combining 5 ml of homogenate from each of the five associated samples in a sterile disposable 50 mL conical centrifuge tube. For example, the five early season samples taken from field #1 of Farm #1 were combined. Composite samples (70 in total) were thoroughly vortexed, serially diluted accordingly in BPW and then spiral plated (Don Whitley Scientific, UK) onto tryptic soy agar (TSA) for TPCs and onto potato dextrose agar (PDA) supplemented with 100 µg/ ml chloramphenicol, for YMCs. The plates were incubated at 30°C for 48 h and 25°C for 5 days for TPC and YMC, respectively. However, for berries that were collected post-processing, analyses were carried out on each individual sample (i.e. no composites).

Fecal contamination and carriage of bacterial human pathogens

Coliforms and Escherichia coli: In order to assess the potential risk for carriage of human pathogens, coliform and generic *Escherichia coli* counts were used as indicators for general hygiene and fecal contamination. All individual sample homogenates from above were screened using 3M™ Petrifilm™ E. coli/Coliform Count Plates (3M Canada, London ON). One mL of initial berry homogenate or appropriate dilutions were applied to the film according to the manufacturer's directions and the films were incubated as recommended. Samples testing negative for the coliforms were then re-plated on 3M™ Petrifilm™ High-Sensitivity Coliform Count Plates using the recommended protocol provided by the manufacturer.

Screening for specific pathogens

All samples that tested positive for the presence of *E. coli* were further screened for shiga-toxin producing *E. coli* (STEC) and *Salmonella* spp. Additionally, all composite samples for PH, GH and MH as well as all PRO samples were also screened for the presence of *Listeria monocytogenes*. For STEC, 25 g of sample were homogenized as described above except modified tryptic soy broth (mTSB; Oxoid Canada, Nepean, ON) supplemented with 20 µg/mL novobiocin was used in place of BPW. Stomacher bags containing the homogenates were incubated at 41.5°C for 22 h. Following incubation, 10 µL aliquots were streaked onto cefixime tellurite sorbitol-MacConkey agar (CT–SMAC; Oxoid) and plates were incubated at 35°C for 24 hours. To screen for *Salmonella* spp. 25 g of berries were pre-enriched in 3M™ S*almonella* enrichment broth at 41.5°C for 24 hours. An aliquot of 0.1 mL of pre-enrichment culture was transferred to 10 mL of Rappaport-Vassiliadis R10 (R-V R10;) broth which was again incubated for 24 hours at 41.5°C. Following incubation, 10 µL of culture was streaked onto hydrated 3M™ Petrifilm SALX Plates according to the manufacturer's directions and incubated at 41.5°C for 24 hours. For detection and isolation of *Listeria monocytogenes* in processed berry samples, the International Standards Method ISO 11290-1:2017 was used. Briefly, 25 g of berries were weighed directly into a filtered stomacher bag

along with 225 mL of half Fraser broth (Oxoid). The sample was homogenized in a stomacher blender as before and then incubated for 25 h at 30°C. After incubation, 0.1 aliquot of culture was transferred to 10 mL of full Fraser broth which was in turn incubated for 24-48 h at 35°C. Presumptive positive cultures showing blackening of medium were then streaked onto RAPID L'mono agar (RLM: BioRad Canada, Mississauga, ON) and modified Oxford agar (Oxoid) and then incubated for 24 h at 37°C.

Environmental Sampling

General sanitary assessment

As a measure to assess the cleaning efficacy of contact surfaces along the blueberry processing chain, a two-pronged approach was used. First, as an indicator for the general cleanliness of conveyor belt surfaces after routine cleaning, ATP (adenosine triphosphate) levels were measured using the 3M™ CleanTrace™ system. Surfaces were sampled using 3M™ UXL100 swabs. ATP activity on the activated swabs was measured using a 3M™ Clean-Trace™ Luminometer and the output was recorded as Relative Light Units (RLU). Samples were taken at different time points during the harvest season.

Detection / enumeration of Listeria monocytogenes

Since *L. monocytogenes* is known to be problematic as a persistent colonizer of surfaces across the RTE food industry, its presence on food contact surfaces within the participating blueberry processing facilities was determined. Here, 3M™ sponge-sticks were used to sample conveyor belts and totes (before routine cleaning). The pre-wetted sponges were rubbed along test surfaces as directed by the manufacturer and then returned to their zip-locked bags. The samples were placed in portable coolers containing ice packs and then transported to the laboratory. To process samples, sponges were released from the stick holders directly into an 80 mL stomacher bags containing 40 mL of phosphate buffered saline (pH 7.2). Samples were homogenized in a compact IUL Masticator blender (IUL S.A., Barcelona, Spain) for 1 minute at room temperature. The sponge was raised above the surface level of buffer within the stomacher bag and then squeezed to ensure maximum recovery of liquid. After removal of the sponge from the bag 30 mL of the homogenate was transferred to a 50 mL conical centrifuge tube. To this, sterile glycerol was added and then mixed to achieve a final concentration of 20% w/v. The tube was then stored at -80°C. Detection of *Listeria* was carried out using the ISO 11290-1:2017 method with a slight modification. Specifically, 90 mL of pre-warmed (30°C) half Fraser broth was added to the stomacher bag containing the remaining 10 mL of sample homogenate, gently mixed and then incubated for 24 h at 30°C. After incubation, the ensuing steps of ISO 11290-1:2017 were followed.

Enumeration of Listeria monocytogenes

For enrichment samples that tested positive for *L. monocytogenes*, the preserved homogenates previously stored at -80°C were thawed. It was anticipated that samples would contain low levels of *Listeria* since the development of characteristic black precipitate during enrichment was slow. To accommodate the anticipated low level of listeriae in the samples, 200 µL of undiluted homogenate was directly spiral plated onto each of five plates of modified Oxford agar. The plates were then incubated at 35°C for 48 h.

Results

Total aerobic bacteria, yeast and moulds

For TPCs and YMCs, composite samples (n = 70) were prepared by combining equal portions of the homogenates of five individual samples taken from the same field, while all 34 individual samples of processed berries were also assessed. Results for counts of total aerobic bacteria versus yeasts and moulds delineated by season (early or late) and sample type are shown in Figure 1.

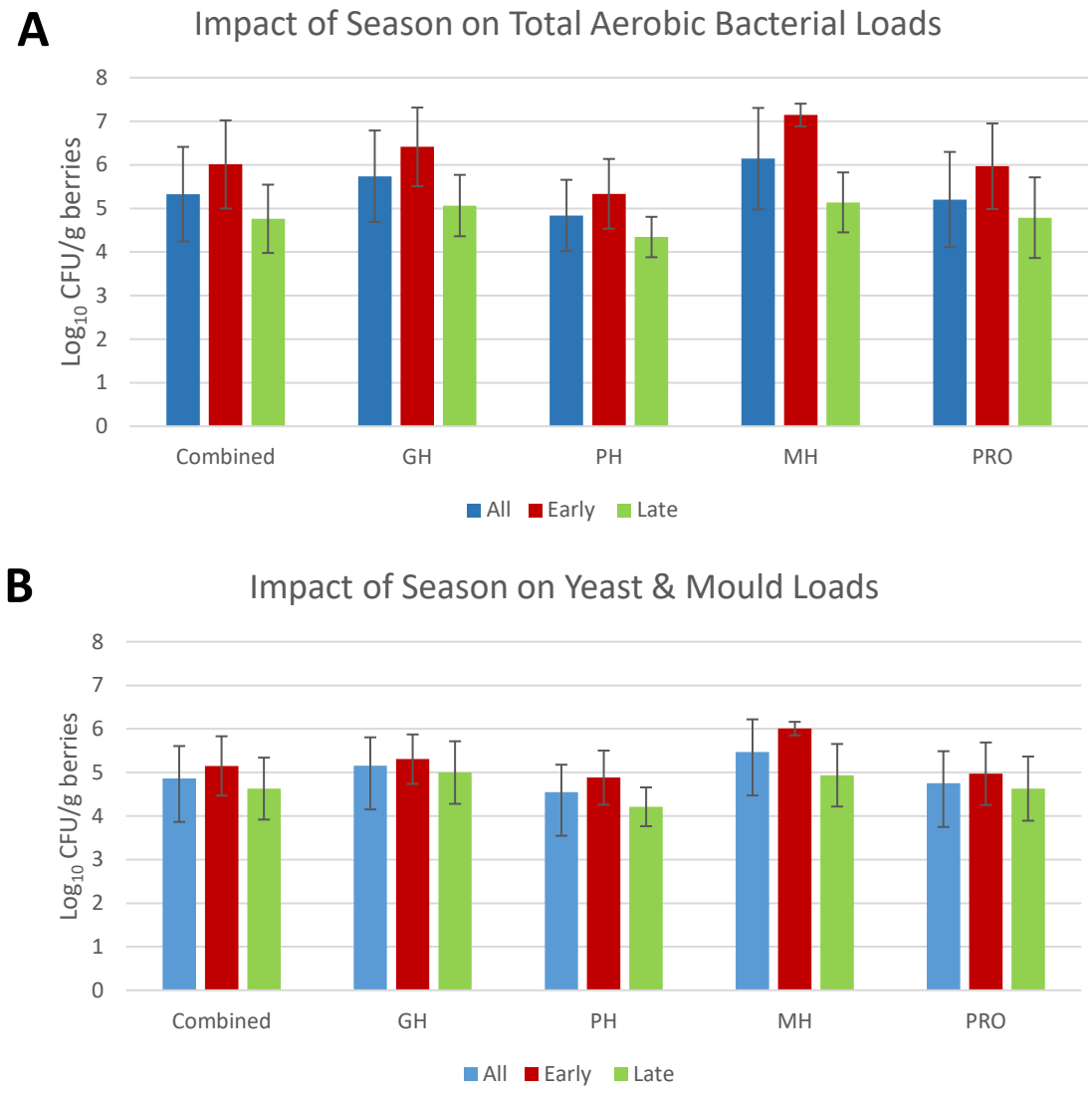

Figure 1. Microbial loads A) total plate counts of aerobic bacteria (TPC), and B) total yeast and mould counts (YMC) present on blueberries. Berries were assessed according to harvest season (early vs. late) and sample type (harvesting method). Sample types: GH = gently harvested (hand raked or modified harvester); PH, Perennia harvested (hand-raked by research team): MH, mechanical harvested (traditional); PRO, fully processed (GH berries). Error bars represent the standard deviations.

In general, berries collected during the early part of the harvest season had significantly greater ($P < 0.05$) numbers of aerobic bacteria as well as yeast and moulds. TPC values for all sample types combined were more than 1.2 log greater for early samples (6.01 $\log_{10}$ CFU/g) than those collected later in the season (4.76 $\log_{10}$ CFU/g) (Fig. 1A). For YMCs, values were ~ 0.5 log higher for the early season berries over late season samples (5.15 $\log_{10}$ CFU/g versus 4.63 $\log_{10}$ CFU/g) (Fig. 1B). The type of method used to harvest the berries also had a significant impact on TPC and YMC values. This was noticeable by visual inspection of the berries upon arrival at the late as berries collected from traditional mechanized harvesters (MH) contained debris (leaves, stem, soil, etc.). As such, early season samples from MH had TPCs of ~7 $\log_{10}$ CFU/g; however, these numbers were 2 log lower for those gathered during late season (Fig. 1A). Similarly YMCs for MH berries during early and late season were 6.01 $\log_{10}$ CFU/g and 4.94 $\log_{10}$ CFU/g, respectively (Fig. 1B). In all cases, berries harvested by Perennia staff (PH) had the lowest TPC and YMC values relative to all other field samples. Early season values of 5.33 $\log_{10}$ CFU/g and 4.88 $\log_{10}$ CFU/g were observed for TPCs and YMCs, respectively. These values were further reduced to 4.34 $\log_{10}$ CFU/g and 4.21 $\log_{10}$ CFU/g, respectively for late season TPCs and YMCs. Berries harvested by so-called gentle harvest methods (i.e. hand raked by farm crews, modified mechanical harvesters or walk-behind harvesters) produced TPC and YMC values intermediate to those obtained for MH and PH berries. Processed berries had counts trending towards those from berries carefully hand raked by Perennia staff. Early season berries had TPCs of 5.97 $\log_{10}$ CFU/g and YMCs of 4.97 $\log_{10}$ CFU/g, while for late season samples numbers decreased to 4.97 $\log_{10}$ CFU/g and 4.63 $\log_{10}$ CFU/g for TPCs and YMCs, respectively (Fig. 1A & 1B).

TPC and YMC values were also delineated by farm number and harvesting method (Figure 2). For Farm #1 the overall trend of TPC and YMC values was MH > GH > PRO > PH. The TPC average value for MH samples was just under 7.0 $\log_{10}$ CFU/g while the YMC was 5.77 $\log_{10}$ CFU/g (Fig. 2A & B). The alternative GH methods (mainly hand-raked) reduced TPC numbers to 6.23 $\log_{10}$ CFU/g but less so for YMCs (5.63 $\log_{10}$ CFU/g). Again cleaning of the berries (i.e. processed samples) reduced these numbers further as PRO samples had values of 5.93 $\log_{10}$ CFU/g and 5.29 $\log_{10}$ CFU/g for TPCs and YMCs, respectively. Samples collected by Perennia staff (PH) displayed the lowest values with TPCs of 5.07 $\log_{10}$ CFU/g and YMCs of 4.71 $\log_{10}$ CFU/g. For Farm #2, the TPC values for berries harvested by MH (6.10 $\log_{10}$ CFU/g) were not significantly different ($P > 0.05$) than those harvested using a modified harvester (GH; 5.99 $\log_{10}$ CFU/g).

Similarly, YMC values for MH (5.07 $\log_{10}$ CFU/g) and GH (5.21 $\log_{10}$ CFU/g) berries were not statistically different ($P>0.05$). Traditional mechanically harvested samples were not collected from Farm #4 or Farm #5 (Fig. 2A & B). For both operations GH berries (hand-raked or walk behind harvester) had higher TPC and YMC values than those hand-raked by Perennia staff (PH). Specifically, counts obtained from Farm #4 for total aerobic bacteria on GH berries were 5.21 $\log_{10}$ CFU/g and 5.63 $\log_{10}$ CFU/g for PH berries. YMC values were 4.78 $\log_{10}$ CFU/g and 4.45 $\log_{10}$ CFU/g for GH and PH, respectively. Processed berries from this operation had similar TPC values (5.73 $\log_{10}$ CFU/g) to the PH collected berries, but slightly lower YMC values (4.21 $\log_{10}$ CFU/g). Overall, berries collected from Farm #5 displayed the lowest TPCs and YMCs for all GH, PH and PRO samples. GH samples had TPCs and YMCs of 4.15 $\log_{10}$ CFU/g and 3.90 $\log_{10}$ CFU/g, respectively, while PH samples had TPCs of 3.93 $\log_{10}$ CFU/g and YMCs of 3.75 $\log_{10}$ CFU/g. These numbers were further reduced on processed berries as the average TPC was 3.64 $\log_{10}$ CFU/g and the YMC was 3.55 $\log_{10}$ CFU/g. Lastly, for Farm #6, only MH berries along with those collected by our team (PH) were obtained. TPC values for MH samples were 6.31 $\log_{10}$ CFU/g while counts for PH samples

were significantly lower at 4.65 log_{10} CFU/g. Similarly YMC values for MH samples (5.66 log_{10} CFU/g) were significantly greater ($P < 0.05$) than those obtained by careful hand-raking (PH) (4.61 log_{10} CFU/g).

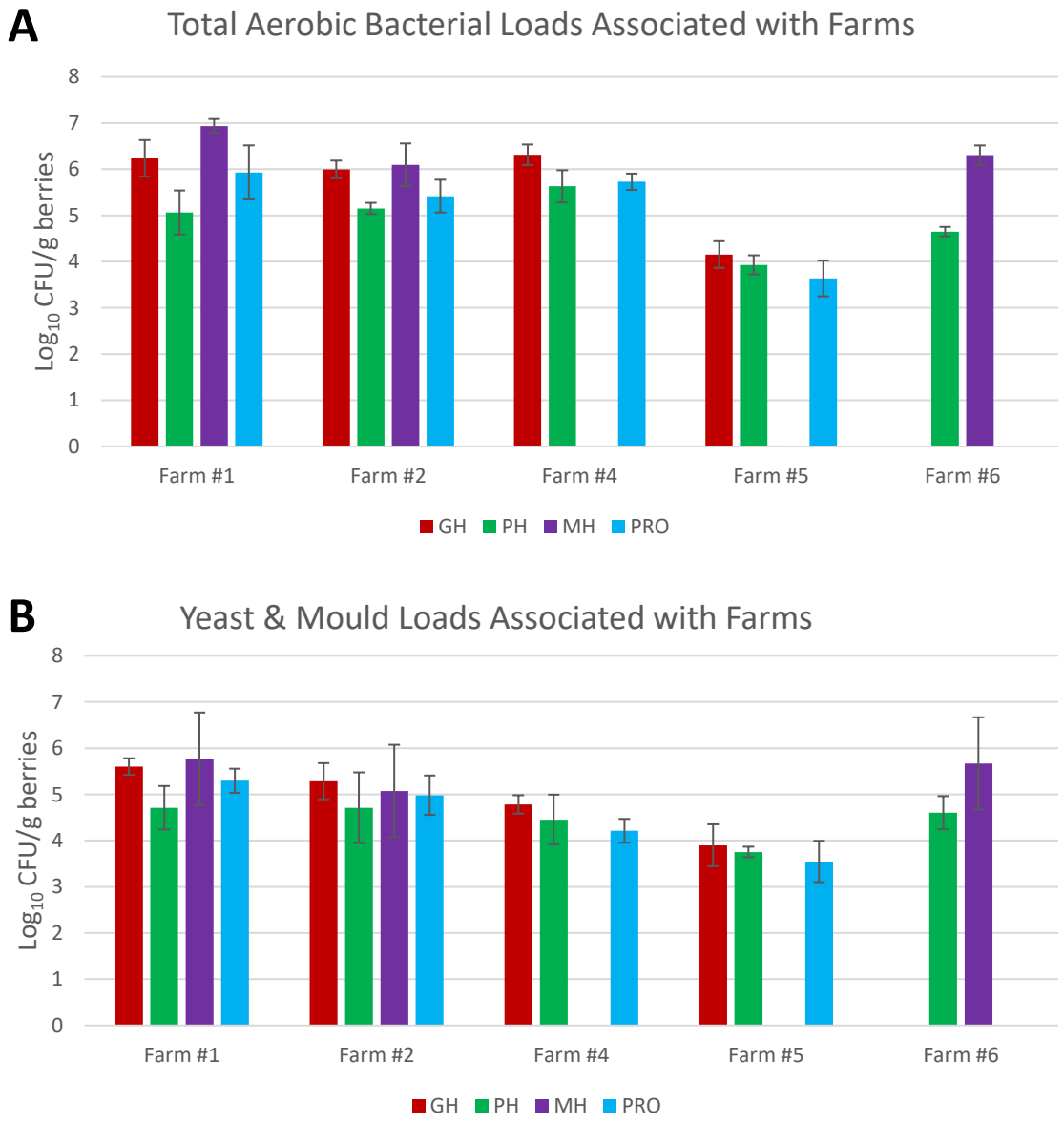

Figure 2. Microbial loads A) total plate counts of aerobic bacteria (TPC), and B) total yeast and mould counts (YMC) present on blueberries. Berries were assessed according to farm location and sample type (harvesting method). Sample types: GH = gently harvested (hand raked or modified harvester); PH, Perennia harvested (hand-raked by research team): MH, mechanical harvested (traditional); PRO, fully processed (GH berries). Error bars represent the standard deviations.

Presence of coliforms, generic E. coli, STEC and Salmonella spp.

All individual wild blueberry samples (n=384) were screened for the presence of coliforms and more specifically, *E. coli* as indicators of general hygiene and possible fecal contamination, respectively. For these assessments, 3M™ Petrifilm™ E. coli/Coliform Count Plates and/ or 3M™ Petrifilm™ High-Sensitivity Coliform Count Plates were used. For hand-raked samples harvested by Perennia staff (PH), 25% of all collected samples tested positive for coliforms and the average count for these samples was 2.02×10^2 CFU/g (Table 2). There were also more early season harvest samples that tested positive for coliforms (35%) with the average counts being 3.17×10^2 CFU/g. Positive samples during this period varied by field site from 50%, 45%, 43% and 20% for Farm #5, Farm #2, Farm #1 and Farm #4, respectively. Coliform counts for all 10 samples collected from Farm #6 were below the detectable limit (i.e. <2 CFU/g). Similar to the observed TPC and YMC results, the trend of lower microbial numbers continued for coliform counts associated with the late harvest samples. In fact only 15% of PH berries were found to be positive for the presence of coliforms during this period, which was down from 35% in the early season. Moreover, no coliform colonies were detected among any of the 20 samples that were collected from Farms #2 or #5. Of the 12 late season samples that were coliform-positive, eight of these came from Farm #4, three from Farm #1 and a single positive sample was from Farm #6. The average coliform concentration for all of these samples combined was only 4.88×10^2 CFU/g (Table 2). None of the PH samples tested positive for *E. coli*.

Table 2. Frequency of coliform and pathogen presence in hand-raked wild lowbush blueberry samples collected in Nova Scotia by staff of Perennia Food & Agriculture during the 2021 harvest season.

Hand-Raked Field Samples (Perennia Staff)							
Season	Farm #	# Fields sampled / # Samples taken	Positive Coliforms (%)	Mean Coliforms CFU/g	*E. coli* Positive	STEC Positive	*Salmonella* spp. Positive
Early	1	6 / 30	13 (43%)	86.2	0	0	0
	2	4 / 20	9 (45%)	728.9	0	0	0
	4	2 / 10	2 (20%)	225.0	0	0	0
	5	2 / 10	5 (50%)	228.0	0	0	0
	6	2 / 10	0	< 2	0	0	0
TOTALS		16 /80	28 (35%)	317.0	0	0	0
Late	1	6 / 30	3 (10%)	40.0	0	0	0
	2	4 / 20	0	< 2	0	0	0
	4	2 / 10	8 (80%)	96.3	0	0	0
	5	2 / 10	0	< 2	0	0	0
	6	2 / 10	1 (10%)	10.0	0	0	0
TOTALS		16 / 80	12 (15%)	48.8	0	0	0
CUMULATIVE		32/160	40 (25%)	202.0	0	0	0

The frequency of coliform presence on wild blueberries collected by gentle harvesting (GH) methods was significantly greater than observed for berries harvested by Perennia staff (PH) (Table 3). Coliforms were detected on 68% of GH berries. Of these, 74% of samples coming from the early harvest season were positive. Although a far greater percentage of samples (relative to PH samples) tested positive for coliforms, their actual numbers remained low with the average concentration of coliforms in GH samples being 2.47×10^2 CFU/g. Coliform-positive sample numbers varied widely by the farm from which they were acquired as well as the time of harvest season. For example, all 10 samples collected from Farm #4 were found to be coliform-positive during the early season (4.21×10^2 CFU/g). Positive samples ranged from 95% for Farm #2 during late season (9.95×10^1 CFU/g), 83% for Farm #1 for early season (2.62×10^2 CFU/g) and 80% for Farm #4 during late season (8.0×10^1 CFU/g). Lower percentages were obtained for Farm #2 early season samples (60%, 7.58×10^1 CFU/g), Farm #1 late season samples (53%, 1.98×10^2 CFU/g), and Farm #5 early season samples (50%, 5.38×10^2 CFU/g). During late season sampling none of the 10 samples obtained from Farm #5 were found to be positive for the presence of coliform bacteria (Table 3). Three of the samples that were positive for coliforms also tested positive for *E. coli*. Two 3M™ Petrifilm™ E. coli/Coliform Count Plates from the 1/10 sample dilution (berry homogenate) presented a single *E. coli* colony while two colonies were present on a third plate also from the original homogenate. All of these samples were from Farm #1 during the early season harvest. Further enrichment of these samples for the detection of STEC and *Salmonella* spp. gave no indication of the presence of these human pathogens.

Table 3. Frequency of coliform and pathogen presence in wild lowbush blueberries that were gentle harvested (hand-raked or modified harvesters) by participating farms in Nova Scotia during the 2021 harvest season.

Gentle Harvester (e.g. hand-raked, modified mechanical harvester, walk behind harvester)							
Season	Farm #	# Fields sampled / # Samples taken	Positive Coliforms (%)	Mean Coliforms CFU/g	*E. coli* Positive	STEC Positive	*Salmonella* spp. Positive
Early	1	6 / 30	25 (83%)	262.0	3	0	0
	2	4 / 20	12 (60%)	75.8	0	0	0
	4	2 / 10	10 (100%)	420.8	0	0	0
	5	2 / 10	5 (50%)	538.0	0	0	0
TOTALS		14	52 (74%)	324.2	0	0	0
Late	1	6 / 30	16 (53%)	198.1	0	0	0
	2	4 / 20	19 (95%)	99.5	0	0	0
	4	2 / 10	8 (80%)	80.0	0	0	0
	5	2 / 10	0	< 2	0	0	0
TOTALS		14	43 (61%)	125.9	0	0	0
CUMULATIVE		28 / 140	95 (68%)	246.6	3	0	0

Berry samples harvested by traditional mechanized harvesters generally had significantly higher ($P < 0.05$) levels of aerobic bacteria as well as yeasts and moulds as compared to samples harvested by other methods (Fig. 1 and Fig. 2). Therefore, it is not surprising that 84% of MH samples collected were

positive for coliforms (Table 4). Moreover, the average number of coliforms in these samples was also significantly greater ($P < 0.05$) with counts of 4.11×10^3 CFU/g. Twenty one of 25 samples (84%) collected from farms during both the early season and late season were positive form coliforms. However, mean coliform counts for the early season samples (7.72×10^3 CFU/g) were significantly greater ($P < 0.05$) than those observed during the late season (9.56×10^2 CFU/g). All MH samples came from three Farms (#1, #2, and #6). One hundred percent of all samples (both early and late season) collected from Farm #1 and Farm #6 were positive for coliforms, whereas MH samples from Farm #2 for both seasons were 60% positive for coliforms. Of the 50 MH samples collected, nine were positive (18%) for *E. coli*. Five of the positive samples came from Farm #6 during early harvest while the four others were from Farm #1 late season. The mean *E. coli* count from the positive Farm #6 samples was 5.36×10^2 CFU/g which is not surprising as the total coliform count was 1.7×10^4 CFU/g. As for the *E. coli* counts for the Farm #1 late season samples, the average was 8.5×10^1 CFU/g from samples with an average coliform counts of 2.16×10^3 CFU/g. All *E. coli* positive samples were enriched for STEC and *Salmonella* spp. but neither pathogen was detected.

Table 4. Frequency of coliform and pathogen presence in mechanically harvested wild blueberry samples collected in Nova Scotia during the 2021 harvest season

Mechanical Harvester (Traditional)							
Season	Farm #	# Fields sampled / # Samples taken	Positive Coliforms (%)	Mean Coliforms CFU/g	*E. coli* Positive	STEC Positive	*Salmonella* spp. Positive
Early	1	2 / 10	10 (100%)	3371.0	0	0	0
	2	2 / 10	6 (60%)	1100.0	0	0	0
	6	1 / 5	5 (100%)	17000.0	5*	0	0
TOTALS		5 / 25	21 (84%)	7270.4	5	0	0
Late	1	2 / 10	10 (100%)	2156.0	4**	0	0
	2	2 / 10	6 (60%)	381.7	0	0	0
	6	1 / 5	5 (100%)	330.0	0	0	0
TOTALS		5 / 25	21 (84%)	955.9	4	0	0
CUMULATIVE		10 / 50	42 (84%)	4113.1	9 (18%)	0	0

*Mean 5.36×10^2 CFU/g

**Mean 8.50×10^1 CFU/g

Berry samples collected after processing (PRO) and prior to package were also collected to assess their potential for carriage of human pathogens. Twenty two of 34 samples (64.7%) were positive for coliforms. All 12 samples collected from the processing line of Farm #2 were positive, while coliforms were detected in five of six samples from Farm #4 and nine of 12 samples from Farm #1 nine of twelve. Only four samples were collected from Farm #5, and none had detectable coliforms. The overall mean coliform count for all positive samples combined was relatively high (5.26×10^4 CFU/g) but this value was inflated by a single sample from Farm #1 with coliform counts exceeding 7.86×10^4 CFU/g. This was also the only processed berries sample to test positive for *E. coli* and levels of these bacteria were

exceptionally high (7.4×10^3 CFU/g). It should be noted that when the duplicate clamshell pack for this sample was tested, coliform were still present but counts were only 2.74×10^2 CFU/g and no *E. coli* was detected. The sole *E. coli* positive sample was tested for STEC and *Salmonella* spp. but neither pathogen was detected. All processed berry sample (n = 34) were also tested for *L. monocytogenes* but were all negative.

Table 5. Frequency of coliform and pathogen presence in wild lowbush blueberry samples processed for fresh or fresh-frozen markets in Nova Scotia during the 2021 harvest season

Processed Berries								
PRO	Farm #	Time Points	# Samples / Positive Coliforms	Mean Coliforms CFU/g	*E. coli* Positive	STEC Positive	*Salmonella spp.* Positive	*Listeria monocytogenes* Positive
Early	1	4	4 / 3	130.0	0	0	0	0
Middle	1	4	4 / 4	87.5	0	0	0	0
Late	1	4	4 / 2	39750.0	1*	0	0	0
Early	2	4	4 / 4	840.0	0	0	0	0
Middle	2	4	4 / 4	27.5	0	0	0	0
Late	2	4	4 / 4	30.0	0	0	0	0
Early	4	4	4 / 4	1200.0	0	0	0	0
Late	4	2	2 / 1	10.0	0	0	0	0
Middle	5	2	2 / 0	<2	0	0	0	0
Late	5	2	2 / 0	<2	0	0	0	0
TOTALS		34	34 / 22	5259.4	1	0	0	0

**E. coli* level was 7.4×10^3 CFU/g

Environmental surface testing

Sanitary Assessment: ATP swabs were taken from various conveyor belt surfaces along the food processing line following cleaning. Specifically, lines were cleaned and sanitized by the site crews in the evening and swabbing was done the next morning before the start of operations. In general, ATP activity was found to be relatively low for most areas along the line after cleaning (Figure 3). The combined average reading from all three lines for swabs taken from the inspection belt was 329 RLUs. Interestingly, similar average readings (i.e. 344 RLUs) were observed for swab samples taken at the receiving belt where the raw berries entered the line. Here, one would expect higher microbial numbers. Readings from swab samples collected from the tilt conveyor belts were also in a similar range (i.e. 377 RLUs). However, significantly higher RLU readings were recorded for swabs taken from the sizer belts at two of the three sites. For Farm #4, the readings were extremely high at >26,000 RLUs while values for Farm #5 were >3200 RLUs. Conversely, the sizer belt at Farm #2 had low RLU readings that were similar to other belt surfaces along their processing line. Also noted, of the three farms/

processors, Farm #4 had the highest RLU readings at all four belt sampling positions, albeit the RLUs for the sizer belt swabs was the only position of concern.

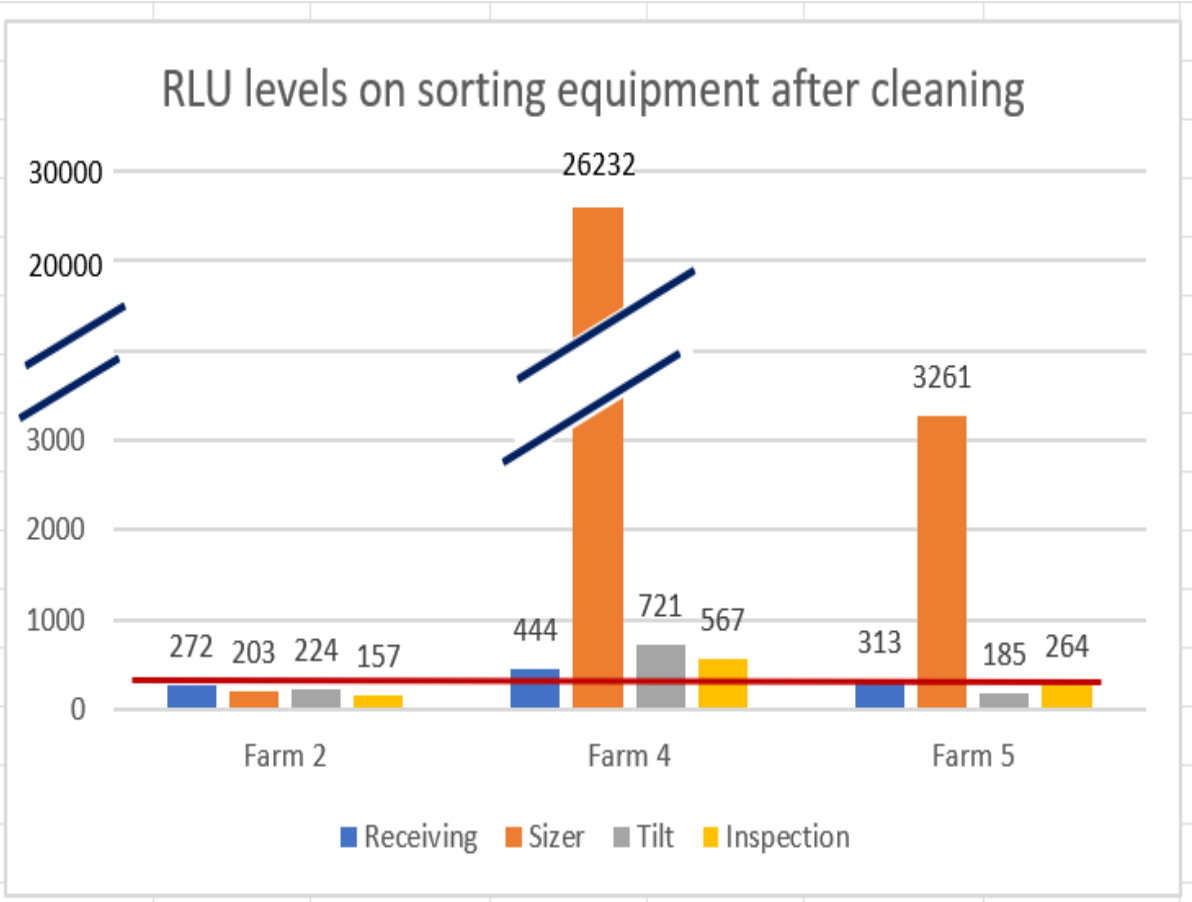

Figure 3. Residual microbial contamination of conveyor belts on blueberry processing lines following sanitation procedures. Conveyor belt cleanliness at different junctures of the processing line was assessed by taking surface swabs and measuring ATP activity using the 3M™ CleanTrace™ system. ATP activity is expressed as relative light units (RLUs).

A total of 61 surfaces from processing line conveyor belts and blueberry totes were swabbed to assess their general cleanliness and carriage of *L. monocytogenes*. Following Fraser broth enrichments and plating onto modified Oxford selective agar, four swabs' samples (3 belts, 1 totes) revealed colonies characteristic *L. monocytogenes* (Table 6). All three positive belt swabs were from the same farm (Farm #1), two of which taken during early harvest season while the third was a mid-season sample. However, since the pathogen could only be detected through enrich and not by direct plating, it implies that it is present are very low level. As for the totes, only a single sample recovered from Farm #4 tested positive for *L. monocytogenes* following enrichment but again, numbers were too low to enumerate via direct plating. Confirmation of presumptive *L. monocytogenes* colonies picked from modified Oxford agar was made via PCR.

Table 6. Detection of *Listeria monocytogenes* on blueberry processing contact surfaces during the 2021 harvest season

Environmental Sponges					
Farm #	Season	Belts	*L. monocytogenes* Positive	Totes	*L. monocytogenes* Positive
1	Early	4	2*	3	0
	Middle	3	1*	3	0
	Late	3	0	3	0
2	Early	3	0	3	0
	Middle	1	0	1	0
	Late	2	0	0	0
4	Early	2	0	4	0
	Middle	0	-	0	0
	Late	3	0	3	1*
5	Early	4	0	3	0
	Middle	3	0	3	0
	Late	3	0	3	0
TOTALS		31	3	30	1

*No *Listeria* detected by direct plating of 1 mL of homogenate onto modified Oxford agar.

Discussion

Wild lowbush blueberries have long been marketed for their high nutritional value and overall healthful goodness. In addition to providing an excellent source of fiber (~2.5 g per 100 g of berries) blueberries are also a good source of manganese, vitamin C and niacin (Dróżdż et al., 2018). Perhaps most notably, wild blueberries have been promoted for their high levels of polyphenols such as anthocyanins and proanthocyanidins, which are thought to improve cardiovascular health (Kalt et al., 2020), while results from other studies have implied that wild blueberries may have a protective capacity against oxidative damage to DNA (Johnson et al., 2017).

Despite these healthful benefits, few studies have examined the microbiological risks associated with wild lowbush blueberries. In the current study, the microbiologic status of these berries was established during the 2021 harvest season. Here, the general microbial load (total aerobic bacteria, yeasts and moulds) was determined as a measure for the shelf-life potential of the product, while coliform counts and the detection of *E. coli* served as indicators of overall hygiene and the possible presence of fecal contamination, respectively. In general, microbial counts observed on berries collected during the early season harvest had significantly higher levels for both TPCs and YMCs ($P < 0.05$), than those from late season (Figure 1). This observation held true for all sample types (GH, MH, PH, PRO) and collectively the difference in mean CFU counts/g for aerobic bacteria was more than 1.2 log and

approximately 0.5 log for yeast and molds. Not surprising, there was a significant difference ($P < 0.05$) in microbial levels depending on how the samples were harvested as those obtained at early harvest via traditional mechanical harvesters were found to carry as much as 10^7 CFU/g and 10^6 CFU/g of aerobic bacteria and yeast and moulds, respectively. It should also be noted that for GH berries, sample collection occurred at the "blower" stage along the processing lines; hence, these samples did not receive maximal cleaning where additional debris, unripe and soft berries would be removed at the tilt belt, sizer and sorting conveyors (by hand or electronically). In contrast, fully "processed berries" (PRO) were sampled at the end of the processing line. Therefore, the elevated numbers are likely due to soil particulates and other debris contacting the berries, as well as soft or damaged berries that would be expected to harbor elevated microbial counts due to leakage of juices that would serve as nutrients for the microorganisms present. For IQF berries, Hazen et al. (2001) reported counts of 4.39 $\log_{10}$ CFU/g for aerobic bacteria on finished product; however MH berries entering the processing line had levels approximately 2.5 log greater. Recently, Holland and co-workers (2021) demonstrated that various surfaces on mechanical harvesters harboured elevated levels of total aerobic bacteria, yeasts and moulds. The authors also indicated evidence that passage through the harvesters translates to statistically significant higher levels of microorganisms on the berries entering the processing lines.

In comparison, for berries that were carefully harvested by our team using sanitized hand-rakes average levels for both TPC and YCM were around 5 $\log_{10}$ CFU/g during early harvest and approximately 1 log lower later in the season. Those berries "gently" harvested by crews at the participating farms were found to have microbial loads intermediate to the MH and PH berries. The reason for this early versus late season difference is not clear, but we speculate that the high microbial loads may be related to the slow or inefficient removal of field heat in the berries harvested earlier. It was noted that daytime temperatures and humidity were excessively high during the first two weeks of harvest. As a result, field heat in clamshell packed berries placed in coolers did not dissipate quickly and samples remained at relatively warm temperature for an extended period before being transferred to the laboratory. Moreover, berries collected from totes in the field (mechanically harvested) were often left sitting in the field for extended periods before being transported to processing facilities. In another study conducted in Georgia, berries destined for fresh market had mean counts for aerobic bacteria 3.89 $\log_{10}$ CFU/g, while yeast and mold counts were 4.45 $\log_{10}$ CFU/g (Quansah et al., 2019). These values are similar to those observed in the present work for PH berries and those that were fully processed. Also, it was noted that in the Georgia study, berries collected at noon had significantly higher counts than those obtained either early morning or late in the day, which supports our theory regarding field heat. Previously, Jackson et al. (1999) showed that minimalizing delays in cooling blueberries after harvested resulted in reduced numbers of spoilage microorganism during refrigerated storage.

When partitioning the total microbial loads present on the berries by farm operation, significant differences ($P<0.05$) can be observed, and these can be further delineated by harvesting practice (Figure 2). Again those berries collected directly from the field by our research team consistently showed lower counts than berries harvested by farm crews whether handpicked or by machinery. For three farms employing so-called "gentle harvesting" methods TPCs were around 6 $\log_{10}$ CFU/g and average YMCs ranged from 4.78 to 5.6 $\log_{10}$ CFU/g. However, berries harvested with sanitized rakes were found to have significantly lower ($P<0.5$) counts. For example, TPCs for Farms #1 and #2 were approximately 1 log lower and YMCs were 0.6 to 0.9 1 log fewer. Blueberries collected from one farm (#5) consistently had the lowest microbial loads relative to all other farms. In fact, TPC and YMC levels recorded for GH berries

on this farm were not significantly different (P>0.05) than those observed for PH berries. For this farm, GH berries were exclusively hand-raked. Clearly, the harvest method impacts the microbiological status of the berries entering the plant. Quansah et al. (2019) also observed significant differences in microbial counts among the six packing houses that participated in their survey.

While total microbial loads can serve as good predictors for the shelf-life potential of a product, total coliforms and fecal coliforms (*E. coli* specifically) can be used to assess the hygienic quality of foods (Leclerc et al. 2001). The application of these as indicator microorganisms was initially used for testing water quality but have also been applied to other foods. Coliforms are a large group of bacteria that can be found in soil and water; however, their presence in large numbers in water would indicate possible issues with surface runoff or another feeder source into a water system. Fecal coliforms are a subset of this group, and they are specifically associated with the intestinal tracts of warm-blooded animals, including humans. *Escherichia coli* is the main bacterium in this group and is part of the gut community in healthy individuals. Since they are generally present in high numbers in fecal matter they are easily detected and as such are used as indicators for fecal contamination. Detection of generic *E. coli* does not necessarily mean pathogenic microorganisms are present in a sample, but instead serves as a warning that there is a potential since pathogens are more difficult to detect as they are most often present in low numbers.

In the present study a total of 160 individual wild blueberry samples were hand-raked by our team. Collectively, 25% of these samples contained coliform bacteria but the mean counts were about 2 log CFU/g. Again, seasonal differences were observed with 35% (mean ~2.5 $\log_{10}$ CFU/g) detection in samples during early harvest and only 15% (mean ~2.5 $\log_{10}$ CFU/g) for late harvest. None of the samples were found to contain *E. coli*. Conversely, a dramatic increase was noted for berries harvested by farm crews, either by hand or with machinery. When “gentle” harvesting methods were employed the frequency of samples with coliform increased to 68%, with early harvest (71%) and late harvest (61%) again displaying differences. Despite the increased number of positive samples, the actual counts remained low (mean ~2.5 $\log_{10}$ CFU/g). Guidelines for acceptable levels of coliform bacteria vary depending on food commodity and country. For a number of ready to eat (RTE) foods, the limit for total coliforms is ≥1000 CFU/g; however, fresh fruits and vegetables are exempt from this rule as high numbers are expected due to the nature of the product (Health Canada, 2010). Since lowbush blueberries grow in close proximity to the ground it is not surprising that bacteria from the soil can be deposited on berries through wind action or splashing of rain. Machine harvesters also exacerbate this issue since the topography of fields is often uneven. This was especially demonstrated with traditional mechanical harvesters as 84% of 50 samples recovered tested positive for total coliforms and the average counts exceeded the 1000 CFU/g limit. However, berries harvested by these means are destined for IQF and thereby will be washed which effectively aids in the significant reduction of the microbial load (Hazen et. al, 2001). Moreover, employing innovative washing methods have been shown to inactivate pathogens (Pangloli and Hung, 2013).

In contrast to the ambiguity regarding acceptable levels of coliforms on fresh produce, limits for generic *E. coli*, when detected, tend to be more decisive (Health Canada, 2010). In this case satisfactory, marginal and unsatisfactory levels are <10, <100 and ≥100, respectively. Three of the 30 samples collected from Farm #1 tested positive for *E. coli*, but in all cases the levels were well below 100 CFU/g. Not surprisingly, nine MH samples were found to contain *E. coli*. Specifically, five early harvest samples from Farm #6 contained generic *E.coli* at levels of 2.7 $\log_{10}$ CFU/g (Table 4). This correlated with the

exceptionally high coliform numbers (4.3 $\log_{10}$ CFU/g) found in the samples. Generic *E. coli* was also detected in four MH samples from Farm #1, but counts were low at only 1.9 $\log_{10}$ CFU/g. Although, these samples contained generic *E. coli*, none exceeded the specified limits. Even if higher levels were found in these berries, they are not the finished product as washing would likely reduce or eliminate the observed levels. Since evidence of fecal contamination was observed in 12 in GH and/or MH samples, we proceeded to test for other enteric pathogens, specifically these were STEC and *Salmonella* spp. No samples were found to contain either pathogen, despite the presence of generic *E. coli*. Where all samples testing positive for *E. coli* displayed relatively low levels this likely indicates the amount of fecal material in contact with the berries was small or was not a recent occurrence. Therefore, the presence of enteric pathogens is unlikely. The few other studies examining microbiology of wild blueberries provided similar results in that the carriage of human pathogens appears to be sporadic (Quansah et al., 2019; Holland et al., 2022).

Microbiologic analysis of the blueberry samples taken directly from the field provided a measure of the carriage of natural microflora and possible environmental contamination and/or that associated with harvesting practices. These samples were analyzed prior to the berries going through any cleaning process. We also collected post-processing berries that were destined for the fresh-packed market to determine the level of effectiveness of processing regimens at reducing microbial loads. For this process, washing is avoided and instead blowers are implemented to remove field debris, while the combination of sizers, tilt belts and plant personnel (or electronic sorters) remove unripe, split, decayed or bruised berries. In total, 34 post-processing samples were collected from four of the participating farms/ processors. In all cases, processing reduced TPCs and YMCs in comparison to levels observed for pre-processing GH and/or MH berries from the same farm (Figure 2). Coliforms were detected in 64.7% of all processed samples collected which is not unexpected since 68% of GH the berry samples contained coliforms prior to processing (Tables 3 & 5). Total coliform counts were generally low with the exception of one late season sample collected from Farm #1 which had coliform levels of 4.6 $\log_{10}$ CFU/g with *E. coli* making a significant contribution (3.9 $\log_{10}$ CFU/g) to these high counts However, the implied fecal contamination observed was an anomaly as sampling a of a second 25 g portion from the same clamshell pack or duplicate clamshell from the sample lot produced total coliform counts more in line with the other processed samples (i.e. < 3.0 $\log_{10}$ CFU/g) with no *E. coli* detected. It is possible that a localized fecal contaminant (e.g. rabbit or deer droppings) did not get removed during processing and was picked up in the sample. Another study showed that microbial numbers on blueberries can vary depending on which point along a processing line samples have been acquired (Gazula et al., 2019). The same work also demonstrated the presence of coliform bacteria at ever point along the processing line, but levels remained low (i.e. < 2.0 $\log_{10}$ CFU/g). It should be noted that no human pathogens were found in the processed berry samples.

Finally, environmental surface swabs were used to assess the efficacy of cleaning protocols at participating sites. ATP readings provided a general measure of microbial activity on conveyors at strategic locations along the processing line while the presence of *L. monocytogenes* was used as a barometer for the potential of pathogen-harbouring biofilms to develop on conveyors systems and berry totes if proper cleaning practices are not employed. It was demonstrated that the conveyor belts associated with the sizer for two processors appears to be a problem area where cleaning may not be as stringent. This was not a problematic area for Farm A, nor did this appear to be an issue for processors

involved in the study by Gazula et al. (2019). Future work needs to be carried out to elucidate the reasons for these observations.

Although, ATP monitoring provides a good indication of general cleanliness along the processing line, we also wanted to gauge the potential for pathogen biofilms if Good Manufacturing Practices are not followed. Therefore, environmental swabs of belts and totes were collected before cleaning from four farms at various points over the season. The opportunistic intracellular pathogen *L. monocytogenes* is widespread in nature and known for its ability to survive and persist for long periods in food production areas (Colagiorgi et al., 2017). In total we collected 61 environmental sponge/swab samples (31 belts and 30 totes) and enriched for *L. monocytogenes* using ISO 11290-1:2017. Since pathogens are generally expected to be present in low numbers relative to other bacteria in the sample, enrichment in a *Listeria*-selective growth medium promotes its growth while suppressing the background microflora. Four samples were found to contain *L. monocytogenes*; three from belts and one from a dirty tote. All three belt samples were from Farm #1 while the contaminated tote was from Farm #4. It is worth noting that two of the three positive belt samples were taken at different times during the same day (i.e. early morning and noon) early in the season, while the third came from swabbing belts during mid-season. It is highly possible that the two early season samples from the same day are the same *L. monocytogenes* strain. The four samples that tested positive after enrichment were then subjected to direct plating on *Listeria* selective agar to determine its viable cell numbers. However, no *Listeria* colonies were detected which indicated that its numbers were low. In Canada, the policy for *L. monocytogenes* in RTE foods is two tiered. That is to say that for foods that support the growth of *L. monocytogenes* its presence alone regardless of numbers would dictate a recall. However, for foods that do not support the growth of *Listeria* or are stored in such a way that does not allow growth (e.g. freezing), a limit of 100 CFU/g is permitted (Health Canada, 2011). Since the acidic nature of wild blueberries would prevent the growth of *L. monocytogenes* the latter category applies. Although growth is not expected *L. monocytogenes* can survive on blueberries during refrigerated storage or when frozen (Sheng et al., 2017). Our direct plating results confirmed its levels on the belt were well below that needed to cross contaminate berries with substantial numbers.

Conclusions

This surveillance study examines the microbiological status of Nova Scotia lowbush blueberries. The microbial load associated with a particular sample of blueberries can vary widely between fields and even within the same field. Moreover, the time within the harvest season appears to play a large role in the number of microorganisms present on the berries. This could be related to slow removal of field heat prior to processing. Jackson et al. (1999) demonstrated that delays in cooling before packaging berries resulted in increases in spoilage bacteria during refrigerated storage. The low proximity of the plants to the ground inherently subjects berries to increased probability of relatively high microbial loads and this can be exacerbated by the method used to harvest the berries. This was evident for pre-processed berries as samples carefully collected by our team consistently had lower counts for total aerobic bacteria, yeast and molds as well as total coliforms. Unfortunately, the technique used here is not feasible for commercial harvesting as hand-rakes were sanitized between every sample in order to avoid cross contamination. The other extreme was seen for blueberries mechanically harvested by traditional machinery as in some case total counts for total aerobic bacteria exceeded 7.0 $\log_{10}$ CFU/g.

However, these berries are destined for IQF processing for which the winnowing and washing steps can significantly reduce these numbers (Hazen et al. 2010). Generally, the gentle harvesting methods employed by the participating farms in this study did not restrict levels of microorganisms on the blueberries to the same degree as our hand-raking method (with the exception of Farm #5). However, microbial loads were still reduced in most cases. For the most part, lower microbial numbers on GH berries entering the processing lines coupled with cleaning (i.e. blowers and sorting techniques) often resulted in final products with total microbial levels similar to those acquired using our sanitary raking method. However, where our method excelled was in regard to a significant reduction in total coliform levels. Still, only four GH samples contained evidence of fecal contaminants as did a single processed sample. None of the GH samples contained *E. coli* levels of concern which was evidenced by no detection of actual enteric pathogens. As the lone processed sample appeared to contain substantially high levels of *E. coli*, this appeared to be a very localized contaminant as a second sample did not reveal the same level of contamination. Overall, from results obtained here the apparent risk for carriage of enteric pathogens on the blueberries appears to be low. Similarly,

Recommendations and Future work

Although results obtained from this work demonstrate a low incidence of fecal contaminants on berries entering processing lines for fresh packed RTE berries data suggests that there are a number of areas for improvement both in the field and on the processing line:

1) Early season samples had significantly higher microbial loads than those obtained later in the season. We attribute this observation to possible differences in the dissipation of field heat by the berries. It was noted that berries placed in coolers during the hot and humid sampling days during this period did not cool sufficiently which likely contributed to the observed increase in microbial numbers. Improving the removal of field heat would be an area for improvement for all operations. This may not so much be a food safety issue as pathogen numbers are not likely to increase, but delays in reducing the temperature will enhance the growth of spoilage microorganisms, thereby reducing shelf-life of the fresh pack berries.

2) Gentle harvesting methods clearly impacted the levels of viable microbes on the harvested berries, as evidenced by the results from samples harvested by our team. However, this level of vigilance would not be practical for commercial harvests. Nonetheless, modified harvesters or walk-behind devices were shown to produce berry yields with lower microbial loads relative to traditional mechanical harvesters. In fact, at one location (Farm #5) where a walk-behind harvester was employed, the produced however, to completely avoid contact with uneven field surfaces is not possible and soil contaminants get transferred to berry contact surfaces. Regular cleaning and sanitization of this equipment could help reduce microbial numbers since biofilms can build on unattended surfaces; thereby increasing the potential for dissemination of problematic microorganisms. In a recent study, Holland et al. (2022) identified a number of areas on mechanical harvester that possessed high levels of environmental microorganisms. They also pointed out a relationship between high microbial counts for berries passaging through these harvesters. Therefore, increased focus on cleaning and sensitizing of these surfaces would be warranted.

3) Environmental swab sampling of contact surfaces (conveyor belts and totes) revealed some potential problem areas along the blueberry production/processing continuum. High levels of microbial activity (as indicated by elevated RLUs for ATP tests) were observed on sizing belts in two of the processor

operations appeared to be hotspots for ATP activity. This indicates the need for a revised cleaning protocol focused on this area. Insufficient cleaning can leave food residues on surface which serve as a nutrient source for microorganisms to grow. The majority of these microbes are harmless in regard to human health, but bacterial pathogens can he harboured in these communities as demonstrated from the results of the detection assays for *Listeria monocytogenes*.

4) Isolation of *Listeria monocytogenes* from four of 61 environmental swab samples demonstrates the potential risk associated with this pathogen. Known for its persistence in the environment it can become established in hard-to-clean (HTC) places in processing facilities which can cross-contaminate food products. The pathogen has most often been associated with recalls of RTE deli meats and dairy products (soft cheeses) but over the last decade there has been increasing frequency of its detection in other products including a variety of fruit (Zhang et al., 2020). We are in the process of typing the isolated strains by pulsed field gel electrophoresis (PFGE; Gold Standard typing method). To date we used PCR to place the isolates into serotype groups (data not shown). All three isolates from the same processing line (two on the same day and the other two weeks later) are in the same serotype group while the isolate from the contaminated tote on another farm is different. If PFGE shows the isolates from the same farm are the same strain, this would indicate a potential *Listeria* persistence issue since the isolates were acquired at different time points. If this is the case, a revision to cleaning practices would be necessary to mitigate long term issues with this pathogen.

Given the results of this survey future work in this area would be best aimed in two areas: 1) improved methods to lower the initial microbial loads on incoming berries. Frequent cleaning of harvesters could help reduce these levels. Also as part of this strategy more attention could be paid to ways of reducing field heat to limit microbial growth. 2) Improved cleaning/ sanitation programs within the processing facilities should be examined not only to benefit by reducing spoilage microorganisms on processed RTE berries but also mitigate against the potential persistence of foodborne pathogen biofilms.

References

Anonymous, Canadian Wild Blueberries. 2020, Wild blueberries have been an important fruit in Nova Scotia since the 1880s. https://canadianwildblueberries.ca/our-story/nova-scotia/. Accessed 2022-02-28

Canadian Food Inspection Agency, 2019. Bacterial Pathogens, Viruses and Parasites in Various Food Commodities - April 1, 2016 to March 31, 2018; Food microbiology - Targeted surveys - Interim report. https://inspection.canada.ca/food-safety-for-industry/food-chemistry-and-microbiology/food-safety-testing-bulletin-and-reports/bacterial-pathogens-and-indicators-viruses-and-par/eng/1617204353546/1617204354140

Colagiorgi, A., Bruini, I., Di Ciccio, P. A., Zanardi, E., Ghidini, S., Ianieri, A. (2017). *Listeria monocytogenes* biofilms in the wonderland of food industry. Pathogens (Basel, Switzerland), 6(3), 41. https://doi.org/10.3390/pathogens6030041

Dróżdż, P., Šėžienė, V., & Pyrzynska, K. (2018). Mineral composition of wild and cultivated blueberries. Biological trace element research, *181*(1), 173–177. https://doi.org/10.1007/s12011-017-1033-z

Gazula, H., Quansah, J., Allen, R., Scherm, H., Li, C., Takeda, F., Chen, J. (2019). Microbial loads on selected fresh blueberry packing lines. Food Control, 100: 315-320, 10.1016/j.foodcont.2019.01.032

Hazen, R. A., Bushway, A. A., Davis-Dentici, K. (2001) Evaluation of the microbiological quality of IQF processed Maine wild blueberries, Small Fruits Review, 1:3, 47-59, DOI: 10.1300/J301v01n03_05

Health Canada (2010). Health Canada Microbial Guidelines for Ready-to-Eat Foods: A Guide for the Conveyance Industry and Environmental Health Officers Health Canada Microbial Guidelines for RTE Foods. http://publications.gc.ca/collections/collection_2014/sc-hc/H164-167-2013-eng.pdf

Health Canada (2011). Policy on *Listeria monocytogenes* in Ready-to-Eat Foods. https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/policy-listeria-monocytogenes-ready-eat-foods-2011.html

Holland, R.M., Chen, J., Gazula, H., Scherm, H. (2022) Environmental and fecal indicator organisms on fruit contact surfaces and fruit from blueberry mechanical harvesters. Horticulturae, 8: 20. doi: 10.3390/horticulturae8010020

Jackson, E.D., Sanford, K.A., Lawrence, R.A., McRae, K.B., Stark, R. (1999). Lowbush blueberry quality changes in response to prepacking, delays and holding temperatures. Postharvest Biology and Technology 15: 117-126.

Johnson, S.A., Feresin, R. G., Navaei, N., Figueroa, A., Elam, M. L., Akhavan, N. S., Hooshmand, S., Pourafshar, S., Payton, M. E., Arjmandi, B.H. (2017). Effects of daily blueberry consumption on circulating biomarkers of oxidative stress, inflammation, and antioxidant defense in postmenopausal women with pre- and stage 1-hypertension: a randomized controlled trial. Food Function. 25; 8(1):372-380. doi: 10.1039/c6fo01216g. PMID: 28059417.

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., Zamora-Ros, R. (2020) Recent research on the health benefits of blueberries and their anthocyanins, Advances in Nutrition, Volume 11, Issue 2, Pages 224–236, https://doi.org/10.1093/advances/nmz065

Leclerc H, Mossel D, Edberg S, Struijk C (2001) Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. Annual Reviews in Microbiology 55: 201–234.

Pangloli, P., Hung, Y.C. (2013). Reducing microbiological safety risk on blueberries through innovative washing technologies. Food Control, 32:621-625, 10.1016/j.foodcont.2013.01.052

Quansah, J.K.; Gazula, H.; Holland, R.; Scherm, H.; Li, C.; Takeda, F.; Chen, J. (2019) Microbial quality of blueberries for the fresh market. Food Control, 100:92–96. https://doi.org/10.1016/j.foodcont.2018.12.034

Sheng, L., Tsai, H.-C., Zhu, H., & Zhu, M.-J. (2019). Survival of *Listeria monocytogenes* on blueberries post-sanitizer treatments and subsequent cold storages. Food Control, 100, 138– 143. https://doi.org/10.1016/j.foodcont.2019.01.019

Zhang, H., Yamamoto, E., Murphy, J., Locas A., 2020. Microbiological safety of ready-to-eat fresh-cut fruits and vegetables sold on the Canadian retail market. International Journal of Food Microbiology, 335: 108855. ttps://doi.org/10.1016/j.ijfoodmicro.2020.108855