2003 MAFMA Final Report

Project Title	**Microbial Standards and Reduction Strategies for Highbush Blueberries**
PI (s)	**Ewen C.D. Todd, Elliot T. Ryser, Annemiek Schilder, Eric Hanson**
Academic Institution	**Michigan State University**
Award Date	**8/1/2003**

1**. Objective Summary**

The objectives of this MAFMA proposal formed part of a larger grant that was funded through the United States Highbush Blueberry Council. The overall goals of the project were to:

1. Determine baseline levels of microbial contamination (total plate counts, *Enterobacteriaceae*, coliforms, staphylococci, *Escherichia coli*, *Listeria*, yeast, molds) for blueberries at key points during harvesting, cleaning, processing, sorting, and packaging that impact the microbial quality of the final product.
2. Compare the efficacy of sodium hypochlorite, chlorine dioxide gas, and other antimicrobial agents for inactivating yeasts, molds, mesophilic aerobic bacteria and pathogens on blueberries that will extend product shelf-life and satisfy the needs of both consumers and clients.
3. Plan and facilitate a workshop on microbial reduction strategies and standards for Midwest blueberry growers and other stakeholders

2. Objective Accomplishments

Objective 1. Determine baseline levels of microbial contamination (total plate counts, Enterobacteriaceae, coliforms, staphylococci, Escherichia coli, Listeria, yeast, molds) for blueberries at key points during harvesting, cleaning, processing, sorting, and packaging that impact the microbial quality of the final product.

Concerns regarding blueberry spoilage, safety, and development of microbiological standards prompted a 2003-2004 survey in which highbush blueberries were collected from 18 different Michigan fields before harvest and quantitatively examined for mesophilic aerobic bacteria (MAB), coliforms, *Escherichia coli*, yeasts and molds. Thereafter, blueberries from these same fields were harvested and similarly assessed at different points during processing (e.g., after harvest, blower exit, after washing, and before packaging for freezing) at six facilities along with environmental samples (e.g., blower and filler conveyor belts, chlorinated wash water). Duplicate blueberry (100 g), wash water (50 ml) and environmental swab samples (~10 x 10 cm) were analyzed for MAB, coliforms, *E. coli*, yeasts and molds by plating on tryptic soy agar containing 0.6% yeast extract and cyclohexamide, Petrifilm^{TM} *E. coli*/coliform plates, and potato dextrose agar containing streptomycin and ampicillin, respectively.

Numbers of MAB, yeasts, molds, coliforms and *E. coli* at pre-harvest ranged from 1 to 3.41 log CFU/g of fruit for the different field locations with highest populations seen near the end of the harvest season. Microbial populations increased 0.6 to 1.5 logs from pre-harvest to post-harvest with this difference statistically significant ($P < 0.05$). Only washing of the fruit significantly reduced ($P < 0.05$) microbial populations with no statistically significant differences ($P > 0.05$) seen elsewhere during processing. Microbial levels on fruit at pre-packaging and pre-harvest were similar for yeasts, molds and *E. coli*, but were significantly higher ($P < 0.05$) for bacteria and coliforms.

During processing, populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts entering the blower area increased 1.29, 1.00, 1.58, 1.39 and 0.97 log CFU/cm^2, respectively, and on conveyer belts entering the pre-packaging area 1.17, 1.15, 1.12, 1.06 and 0.66 log CFU/cm^2 respectively, with all of these increases being statistically significant ($P < 0.05$). Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tanks increased 0.56, 0.74, 0.86, 0.41 and 0.32 log CFU/ml, respectively, during processing with these increases also statistically significant ($P < 0.05$).

After 3 months of frozen storage at -20°C, populations of yeasts, molds, and *E. coli* on processed berries decreased by 0.44, 0.46, and 0.68 log CFU/g, respectively, with all of these reductions being statistically significant ($P < 0.05$). No further reductions in microbial levels were seen between 3 and 6 months of frozen storage.

In summary, average MAB, yeast and mold counts on blueberries were 3.49, 3.81 and 3.35 at pre-harvest, increasing to 4.95, 4.37, and 4.06 at post-harvest, and decreasing to 4.21, 3.86 and 3.52 logs CFU/g after washing, respectively. Coliform and *E. coli* counts increased 0.64 and 0.16 logs from pre-harvest to after washing, respectively. Microbial populations were highest on the blower and filler belts and lowest in the chlorinated wash water. Overall, MAB populations increased ~1.5 logs between harvest and processing (4 to 18 h) with chlorinated wash water (~10 to 200 ppm chlorine) reducing populations <1 log. Thus, improved storage strategies before processing and more effective microbial reduction strategies during processing are needed to enhance the microbial quality of blueberries.

Objective 2. Compare the efficacy of sodium hypochlorite, chlorine dioxide gas, and other antimicrobial agents for inactivating yeasts, molds, mesophilic aerobic bacteria and pathogens on blueberries that will extend product shelf-life and satisfy the needs of both consumers and clients.

In preliminary work, the efficacy of chlorine (100, 200 and 400 ppm), aqueous chlorine dioxide (ClO_2) (3 and 5 ppm), organic fatty acid A (OA) (caprylic acid, Emsorb 6915, and mineral oil) at concentrations of 0.3%, 0.6%, and 0.9 % (v/v), and OA at 0.3% + organic fatty acid B (OB) (glycolic acid, caprylic acid, and Emsorb 6915) at 0.15% (v/v) were assessed in an aqueous model system using a 5-minute exposure and on blueberries inoculated with three strains each of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* as well as yeasts (*Aureobasidium* sp., *Bullera* sp., *Cryptococcus* sp., *Sporidiobolus* sp., and *Filobasidiu* sp.) and molds (*Colletotrichum* sp., *Epicoccum* sp., *Cladosporium* sp., *Phoma* sp., and *Alternaria* sp.).

Overall, chlorine dioxide was the least effective sanitizer for all microbial categories. Concentrations of 3 to 5 ppm ClO_2 reduced MAB, yeast and mold populations by 1.61, 1.16 and 1.83 log CFU/ml respectively, with these reductions only statistically significant ($P < 0.05$) for MAB, and molds. Chlorine and the organic fatty acids were more effective, with significantly ($P < 0.05$) greater reductions than 5 ppm ClO_2 for MAB and molds, and significantly ($P < 0.05$) greater reductions than 3 ppm ClO_2 for all microbial categories. These two sanitizers decreased populations >4 logs with no greater reductions seen at higher concentrations.

The two organic fatty acids were the most effective sanitizers tested on inoculated blueberries. OA at a concentration of 0.9% decreased MAB, yeast, and mold populations by 2.52, 3.77, and 3.72 logs CFU/g, respectively. Increasing the OA concentration from 0.3 to 0.9%

resulted in significantly ($P < 0.05$) greater reductions for MAB and yeasts. Chlorine at 400 ppm was the next most effective sanitizer, reducing populations of MAB, yeast, and mold by 0.91, 1.28, and 2.14 logs CFU/g, respectively. Similar microbial reductions were seen when the Cl concentration was decreased from 400 to 100 ppm. Although 3 and 5 ppm ClO_2 were least effective, these sanitizers were not significantly ($P > 0.05$) different from Cl for MAB and molds, and sterile distilled water (SDW) for MAB and yeast. Exposing inoculated blueberries to SDW for 5 minutes reduced populations of MAB, yeast and mold by 0.24, 0.15 and, 0.49 log CFU/g, respectively. In subsequent sensory work, the only statistically significant differences between the sanitizer treatments were observed for 0.9% OA and 0.3% OA +0.15% OB (OA+OB) for all sensory attributes. OA 0.9% compared with OA+OB treated fruit was also significantly ($P <0.05$) lower in terms of texture, flavor, and overall acceptability.

In response to increasingly stringent microbial specifications being imposed by purchasers of blueberries, gaseous chlorine dioxide (ClO_2) generated by a dry chemical sachet was also tested against three foodborne pathogens as well as five yeasts and molds known for spoilage. Initially, five fresh blueberry samples (100 g) were separately inoculated with *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7 (3 strains each), and yeasts and molds (5 genera each) to contain ~10^6 CFU/g and exposed to ClO_2 (4 mg/L, 0.16 mg/g) for 12 h in a sealed 20 liter container (99.9% RH) at ~22^{o}C (3 replicates). After gassing, blueberries (25 g) were diluted 1:5 in neutralizing buffer, pulsified for 1 min and plated using standard procedures to quantify survivors. This treatment yielded reductions of 3.94, 3.62, 4.25, 3.10, and 3.17 log CFU/g for *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, yeasts and molds, respectively. Thereafter, 30 lugs of uninoculated blueberries (~9.1 kg/lug) were stacked on 4 x 4 ft pallets (5 lugs/level x 6 levels) (6 replicates), tarped, and exposed to ClO_2 (18 mg/L, 0.13mg/g) for 12 h. After gassing, significant ($P < 0.05$) reductions of 2.33, 1.63, 0.48, 1.47, and 0.52 logs CFU/g were seen for mesophilic aerobic bacteria (MAB), yeasts, molds, coliforms, and *E. coli*, respectively, compared to ungassed controls. No significant differences ($P > 0.05$) in microbial inactivation were seen between lug levels and, with one exception (MAB), between the bottom and top surface of individual lugs. Based on these findings, ClO_2 sachets may provide a simple, economical and effective means of enhancing the microbial shelf-life and safety of fresh blueberries.

Objective 3. Plan and facilitate a workshop on microbial reduction strategies and standards for Midwest blueberry growers and other stakeholders

The results obtained through this grant were presented at two half-day workshops in Fennville, MI in May of 2004 and 2005. Approximately approximately 20 individuals comprised of local blueberry growers, processors and members of the Michigan Blueberry Growers Association attended each workshop and expressed considerable interest in our findings.

3. Unexpected findings, if any: None

4. Practical impacts of research efforts.

a. Short Term Impacts: Our work with gaseous chlorine dioxide generated considerable interest at both workshops, largely due to the ease of application and the low cost with several growers/processors planning to gas some of their late-harvest blueberry pallets in 2006. Use of these chlorine dioxide sachets on late harvest blueberries will allow blueberry processors to reduce microbial contaminants to early harvest levels, thereby enhancing the quality and safety of the fully processed fruit.

b. Long Term Impacts: Present regulations dictate that blueberries treated with chlorine dioxide gas be subjected to a potable water rinse before consumption. However, after full approval, chlorine dioxide gas can likely be used to enhance the microbial shelf-life of blueberries destined for the fresh market.

5. Publications resulting from this research.

Published Abstracts

1. Popa, Iuliano, Eric J. Hanson, Annemiek Schilder, Ewen C.D. Todd, and Elliot T. Ryser. 2005. Inactivation of bacteria, yeasts and molds on palletized highbush blueberries using chlorine dioxide sachets. Ann. Mtg. Intern. Assoc. Food Prot., Baltimore, MD, Aug. 15-17.

2. Popa, Iuliano, Siva Sabaratnam, Eric J. Hanson, Annemiek Schilder, Ewen C.D. Todd, and Elliot T. Ryser. 2005. Levels of microbial contaminants in highbush blueberries before, during and after processing. Ann. Mtg. Intern. Assoc. Food Prot., Baltimore, MD, Aug. 15-17.

M.S. Thesis

1. Popa, Iuliano. 2005. Microbial levels and reduction strategies for Michigan Highbush blueberries. Michigan State University, East Lansing, MI, December 2005.