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**MICROBIAL LEVELS AND REDUCTION STRATEGIES FOR
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Iuliano Dumitru Popa

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MS

degree in

**Food Science and Human
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**MICROBIAL LEVELS AND REDUCTION STRATEGIES FOR MICHIGAN
Highbush Blueberries**

By

Iuliano Dumitru Popa

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTERS OF SCIENCE

Department of Food Science and Human Nutrition

2005

ABSTRACT

MICROBIAL LEVELS AND REDUCTION STRATEGIES FOR MICHIGAN Highbush BLUEBERRIES

By

Iuliano Dumitru Popa

Highbush blueberries were collected from 18 Michigan fields during 2003-2004 and assessed for mesophilic aerobic bacteria (MAB), coliforms, *Escherichia coli*, yeasts and molds during harvesting and processing (pre-harvest, post-harvest, blower-exit, water tank-exit, and pre-packaging for freezing) along with conveyor belt and chlorinated wash water samples from six processing facilities. Microbial populations generally increased ~1.5 log between pre- and post-harvest and decreased < 1 log after exiting the water tank (~10 to 200 ppm chlorine). Thus, microbial levels were higher after processing than before harvesting with populations significantly ($P < 0.05$) higher on conveyor belts and in chlorinated wash water during processing. Chlorine dioxide gas was also assessed as a pre-processing microbial reduction strategy for blueberries. In preliminary work using blueberries inoculated with several foodborne pathogens as well as spoilage yeasts and molds, 12 h of gassing (0.16 mg ClO_2 gas/g fruit) in a 20 L bucket decreased all microbial populations >3 logs. When 600 lb pallets of naturally contaminated blueberries were subsequently exposed to 0.13 mg ClO_2 gas/g fruit for 12 h, significant ($P < 0.05$) reductions of 2.12, 1.61, 0.72, 1.76, and 1.55 logs CFU/g were seen for MAB, yeasts, molds, coliforms, and *E. coli*, respectively, compared to ungassed controls. Gassing of additional blueberries with 0.19 mg ClO_2 gas/g for 12 h did not affect the appearance, aroma, texture, flavor or overall fruit acceptability.

To my dearest son Bogdan and wife Darclee for all of your love and support

ACKNOWLEDGMENTS

I would like to extend my sincere appreciation to my committee members Dr. Eric Hansone, Dr. Kirk Dolan, and to my project collaborators Dr. Annemiek Schilder, Dr. Ewen Todd.

I am grateful to my major professor Dr. Elliot Ryser for his excellent insight and his scientific guidance in preparing this dissertation and my carrier.

I am thankful to Dr. Zhinog Yan for his unforgettable help in the laboratory as well to all my laboratory colleagues.

Finally, I would like to thank my family for encouraging and supporting me. To my mom, thank you for all of your love and encouragement. You have helped this whole process to be a memorable experience that was worthwhile.

TABLE OF CONTENTS

LIST OF FIGURES.....	vii
LIST OF TABLES.....	ix
1. INTRODUCTION.....	1
2. LITERATURE REVIEW.....	5
2.1 BLUEBERRY INDUSTRY.....	5
2.1.1 CHARACTERIZATION OF BLUEBERRY.....	5
2.1.2 MICHIGAN BLUEBERRY INDUSTRY.....	6
A. Processed Market.....	8
B. Processed Blueberry Standards.....	10
C. Fresh Market.....	11
2.2 PROCUCE RELATED OUTBREAKS.....	11
2.2.1 SOURCES OF CONTAMINATION.....	12
2.2.2 BERRY- ASSOCIATED OUTBREAKS.....	13
2.3 BLUEBERRY MICROBIAL LIMITS.....	14
2.4 HUMAN PATHOGENS.....	16
2.4.1 <i>ESCHERICHIA COLI</i> O157:H7.....	16
A. Health significance of <i>E. coli</i> O157:H7.....	16
B. <i>E. coli</i> O157: H7 outbreaks in produce.....	17
2.4.2 <i>SALMONELLA</i>	18
A. Health significance of <i>Salmonella</i>	18
B. <i>Salmonella</i> outbreaks in produce.....	20
2.4.3 <i>LISTERIA MONOCYTOGENES</i>	21
A. Health significance of <i>Listeria monocytogenes</i>	21
B. <i>Listeria monocytogenes</i> outbreaks in produce.....	22
2.5 ECOLOGICAL FACTORS INFLUENCING HUMAN PATHOGENS ON BLUEBERRY.....	23
2.6. METHODS TO PREVENT AND ELIMINATE BLUEBERRY AND HUMAN PATHOGENS.....	24
2.7 SANITIZERS.....	30

2.7.1 CLOROX TM / SODIUM HYPOCHLORITE (NaClO).....	32
A. General characteristics.....	32
B. Antimicrobial performance.....	33
2.7.2 CHLORINE DIOXIDE	34
A. General characteristics.....	34
B. Antimicrobial performance.....	35
 3. MICROBIAL CONTAMINANTION IN Highbush blueberries before, during, and after processing.....	 38
3.1 INTRODUCTION.....	39
3.2 MATERIAL AND METHODS.....	40
3.3 RESULTS.....	44
3.4 DISCUSION.....	48
 4. EFFICACY OF CHLORINE DIOXIDE GAS SACHETS FOR ENHANCING THE MICROBIOLOGICAL QUALITY AND SAFETY OF BLUEBERRIES.....	 51
4.1 INTRODUCTION.....	52
4.2 MATERIAL AND METHODS.....	55
A. PILOT STUDY.....	55
B. PALLET STUDY.....	59
4.3 RESULTS AND DISCUSSION.....	63
A. PILOT STUDY	64
B. PALLET STUDY.....	66
4.4 CONCLUSIONS.....	70
5.CONCLUSIONS.....	72
APPENDIX A.....	75
APPENDIX B.....	86
APPENDIX C.....	97
APPENDIX D.....	102
APPENDIX E.....	138
REVERENCES.....	144

LIST OF FIGURES

FIGURE 3.1	Highbush Blueberry Processing and Sampling Diagram.....	42
FIGURE 3.2	Populations of MAB, Yeasts, Molds, Coliforms and <i>E. coli</i> on blueberries sampled at pre-harvest, post-harvest, blower exit, water tank exit and pre-packaging.....	44
FIGURE 3.3	Populations of MAB, Yeasts, Molds, Coliforms and <i>E. coli</i> on conveyor belts entering the blower (A) and pre-packaging areas (B), before and after fruit processing.....	46
FIGURE 3.4	Populations of MAB, Yeasts, Molds, Coliforms and <i>E. coli</i> in water samples before and after fruit washing.....	47
FIGURE 3.5	Populations of MAB, Yeasts, Molds, Coliforms and <i>E. coli</i> on blueberries before and after 3 and 6 months of storage at - 20° C.....	47
FIGURE 4.1	Chlorine dioxide gas systems used to treat inoculated blueberries in pilot study.....	58
FIGURE 4.2	Pallet study with gassed and un-gassed pallets	60
FIGURE 4.3	Schematic diagrams of a blueberry pallet and lug with sampling locations	62
FIGURE 4.4	Populations of <i>L. monocytogenes</i> , <i>Salmonella</i> , <i>E. coli</i> O157:H7, yeasts and molds on inoculated blueberries before and after exposure to ClO ₂ gas in a sealed bucket.....	65

FIGURE 4.5	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS, AND <i>E.COLI</i> RECOVERED FROM BLUEBERRIES INITIALLY AND FROM GASED AND UN-GASED FRUIT AFTER 12 H OF STORAGE.....	67
FIGURE 4.6	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS, AND <i>E.COLI</i> RECOVERED FROM GASED BLUEBERRY PALLETS AT DIFFERENT PALLET LEVELS (A) AND BETWEEN THE BOTTOM AND TOP SURFACE OF LUGS FROM THE SAME PALLET LEVEL (B).....	69
FIGURE 4.7	AVERAGE CONSUMER RATINGS FOR APPEARANCE, AROME, TEXTURE, FLAVOR, AND OVERALL ACCEPTABILITY OF TREATED AND UNTREATED BLUEBERRIES.....	70
FIGURE A 1	REDUCTIONS OF MAB, YEASTS, AND MOLDS AFTER A 5-MINUTE EXPOSURE TO VARIOUS SANITIZERS IN AN AQUEOUS MODEL SYSTEM.....	81
FIGURE A 2	REDUCTIONS OF MAB, YEASTS, AND MOLDS AFTER A 5-MINUTE EXPOSURE OF INOCULATED FRUITS TO VARIOUS SANITIZERS.....	83
FIGURE A 3	AVERAGE CONSUMER ACCEPTABILITY FOR FRESH BLUEBERRIES SUBJECTED FOR 5 MIN TO WASH TREATMENT WITH SODIUM HYPOCHLORITE, CHLORINE DIOXIDE, ORGANIC FATTY ACIDS AND SDW AS CONTROL.....	84
FIGURE D 1	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS AND <i>E.COLI</i> ON BLUEBERRY SAMPLES BEFORE AND AFTER FROZEN STORAGE IN INDUSTRIAL CONDITIONS.....	102
FIGURE E 1	GROWTH OF MAB, YEASTS, MOLDS, COLIFORMS, AND <i>E.COLI</i> ON NATURALLY CONTAMINATED BLUEBERRIES DURING STORAGE.....	141
FIGURE E 2	REDUCTIONS OF MAB, YEASTS, MOLDS, COLIFORMS, AND <i>E.COLI</i> ON INOCULATED AND UNINOCULATED BLUEBERRIES AFTER 12-H EXPOSURE TO ClO_2 GAS IN A SEALED BUCKET	142

LIST OF TABLES

TABLE 2.1	MICROBIAL LIMITS FOR MAB, YEASTS, MOLDS, COLIFORMS AND HUMAN PATHOGENS SET BY PURCHASERS OF BLUEBERRIES.....	15
TABLE 3.1	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS AND <i>E. COLI</i> ON BLUEBERRIES SAMPLED AT PRE-HARVEST, POST-HARVEST, BLOWER EXIT, WATER TANK EXIT AND PRE-PACKAGING.....	86
TABLE 3.2	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS AND <i>E. COLI</i> ON CONVEYER BELTS ENTERING THE BLOWER AND PRE-PACKAGING AREAS, BEFORE AND AFTER FRUIT PROCESSING.....	87
TABLE 3.3	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS AND <i>E. COLI</i> IN WATER SAMPLES TAKEN BEFORE AND AFTER FRUIT WASHING.....	88
TABLE 3.4	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS AND <i>E. COLI</i> ON BLUEBERRIES BEFORE AND AFTER 3 AND 6 MONTHS OF STORAGE AT - 20° C.....	89
TABLE 4.1	POPULATIONS OF <i>L. MONOCYTOGENES</i> , <i>SALMONELLA</i> , <i>E. COLI</i> O157:H7, YEASTS AND MOLDS ON INOCULATED BLUEBERRIES BEFORE, AFTER AND REDUCTIONS FROM EXPOSURE TO ClO ₂ GAS IN A SEALED BUCKET.....	90
TABLE 4.2	POPULATIONS OF MAB, YEASTS, MOLDS, COLIFORMS, AND <i>E. COLI</i> RECOVERED FROM BLUEBERRIES BEFORE AND FROM GASED AND UN-GASED FRUIT AFTER 12 H OF STORAGE AT 12 TO 14°C.....	91
TABLE A.1	REDUCTIONS OF MAB, YEASTS, AND MOLDS AFTER A 5-MINUTE EXPOSURE TO VARIOUS SANITIZERS IN AN AQUEOUS MODEL SYSTEM.....	93
TABLE A.2	REDUCTIONS OF MAB, YEASTS, AND MOLDS AFTER A 5-MINUTE EXPOSURE OF INOCULATED FRUITS TO VARIOUS SANITIZERS.....	94

TABLE E.1	GROWTH OF MAB, YEASTS, MOLDS, COLIFORMS, AND <i>E.COLI</i> ON NATURALLY CONTAMINATED BLUEBERRIES DURING STORAGE AT ROOM TEMPERATURE.....	95
TABLE E.2	REDUCTIONS OF MAB, YEASTS, MOLDS, COLIFORMS, AND <i>E.COLI</i> ON INOCULATED AND UNINOCULATED BLUEBERRIES AFTER 12-H EXPOSURE TO ClO ₂ GAS IN A SEALED BUCKET.....	96

1. INTRODUCTION

Consumption of fruits and vegetables in the United States has increased substantially, with per capita consumption of produce increasing 24% (from 573 lb in 1970 to 711 pounds in 1997) (Putnam and Allshouse, 1997). Moreover, new production and packaging technologies make possible year-around availability of numerous fresh fruits and vegetables that also are increasing in consumption. Fresh fruits and vegetables increased 3% in 2004 with a growth trend in consumption forecast by 2020 (Produce Marketing Association, 2005). Changes in dietary habits, with a higher per capita consumption of fresh and minimally processed fruits and vegetables, and increasing demand for salad bars and meals eaten outside the home have also amplified the number of foodborne outbreaks (Altekruse and Swerdlow, 1996). The median number of reported produce-associated outbreaks increased from 2 outbreaks per year in the 1970s to 7 per year in the 1980s, and to 16 per year in the 1990s. According to Sumathi et al. (2004), from 190 produce-associated outbreaks reported between 1973 through 1997, nine involved berries - 4 raspberry, 4 strawberry, and 1 blackberry.

Food safety concerns surrounding blueberries include a possible link to a 1984 outbreak of listeriosis in Connecticut after consumption of unwashed strawberries, blueberries or nectarines (Ryser, 1999), a 2002 confirmed outbreak of hepatitis A in New Zealand with domestic blueberries traced back to a single orchard that revealed multiple opportunities for contamination of blueberries by pickers (Calder et al., 2003), and a 1998 recall involving an undetermined quantity of frozen blueberries from California, Illinois and Australia that was contaminated with *Listeria monocytogenes* (FDA Enforcement Report, 1998). These blueberry and other berry-types outbreaks in the past with their

economical implications highlights the need for food safety programs in the berry fruit industry.

Although the domestic blueberry industry has not been negatively affected by any widespread outbreak with foodborne pathogens, microbial safety remains a critical concern for all segments of the blueberry industry that have indirectly affected producers and marketers in the form of buyer demands for microbial testing and increasing microbial specification.

The United States is the world's leading blueberry producer, and approximately one-third of the total national highbush blueberry crop is produced in Michigan. Most of Michigan blueberries are processed and frozen for later use (NASS/USDA, 2002). Some buyers of frozen berries now demand microbial testing for levels of spoilage bacteria, yeasts and molds in order to assure that the product does not exceed their specification and also have a 'zero tolerance' policy for human pathogens. However, microbial specifications for frozen blueberries vary considerably from buyer to buyer, in general reflecting different uses, and becoming increasingly stringent. One example are the pie manufacturers that need a low level of bacteria, yeast and molds in their product because most of these microbial categories isolated from blueberries are producing the enzyme amylase that reduce the viscosity of blueberry filing (Schilder Annemieck – unpublished data).

Blueberry producers have observed large variations in microbial levels that can not be correlated just to field conditions or harvesting procedures. A microbial assessment study of blueberries from different locations in Michigan during 2002 and 2003, revealed that populations of bacteria, yeasts and filamentous fungi varied widely

among field locations including those that were irrigated and non-irrigated although the same populations increased considerably on blueberry surface in time from green stage to the end of harvest (Schilder Annemieck – unpublished data).

A scientific understanding of the factors affecting variation in microbial levels, the risk associated with various microbial levels, and the steps needed to attain particular levels is needed in order to set microbial standards for blueberries. Understanding at which points in production, harvesting, processing and packaging contamination is likely to occur is clearly needed along with improved sanitizing and microbial reduction strategies in order to obtain a science-based uniform standard for microbial levels that are able to satisfy buyers and also be effective and affordable for growers and processors.

The emergence of new pathogens is a well-known fact. Pathogens that were previously less virulent have increased their virulence, being now considered of public health concern to certain categories of consumers. They acquired resistance to antibiotics, and environment conditions (low temperature, low pH) in which they were unable to survive before. The bacterial pathogens of greatest concern for the blueberry industry that can use the less acidic surface of blueberries as vectors to produce outbreaks include *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7. All these pathogens categories are included in this study in order to achieve the new microbial specifications that include the ‘zero tolerance’ for the human pathogens; however, there is a general lack of efficacy of aqueous chemical sanitizers in killing or removing these pathogens from the surface of raw fruits and vegetables (Beuchat, 1998). This fact can be attributed to difficulties in delivering aqueous chemical sanitizers to areas on the surface of fruits with a hydrophobic surface such as blueberries, where these human and also fruit

pathogens that produce the spoilage may be lodged (Burnet and Beuchat, 2001). The theory that hydrophobicity of microbial cells aids in their protection by inhibiting penetration of the disinfectants has also been proposed (Buck, 2003).

Alternative sanitizers such as gaseous chlorine dioxide have been explored as alternative to aqueous chemicals for sanitizing raw fruits and vegetables.

Studies have shown gaseous chlorine dioxide to be effective in microbial reductions including enteric pathogens on several fruits and vegetables in laboratory conditions. Gaseous chlorine dioxide has some advantages over chlorinated water that consist in removing phenolic tastes and odor from water, does not react with organic compounds, and has a greater oxidation and germicidal capacity including spores, viruses and protozoa that are resistant to chlorine. Gaseous chlorine dioxide is increasing in popularity by being used to effectively control the spread in molds in libraries (Weaver-Meyers et al., 1998), as a sanitizer for reducing yeast and mold populations in food processing plants on stainless steel surfaces (Han et al., 1999), and in the decontamination of buildings in the US after the 2001 anthrax attacks.

The goal of this research is to (1) assess the levels of microbial contamination at various points during blueberry harvesting, handling, cleaning, and packaging and (2) assess the efficacy of gaseous chlorine dioxide for inactivating *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, mesophilic aerobic bacteria, coliforms, *E. coli*, yeasts and molds on the surface of blueberries in industrial conditions before processing. These findings will eventually help to establish a science-based uniform standard for microbial levels and improve the actual microbial reductions strategies in blueberries that will satisfy the needs of buyers as well as growers and processors.

2. LITERATURE REVIEW

2.1 BLUEBERRY INDUSTRY

2.1.1 CHARACTERIZATION OF BLUEBERRY

The blueberry, which belongs to the genus *Vaccinium*, is native to North America. Native Americans took advantage of these fruits to consume fresh or to preserve for medicinal purposes and dyes (U.S. Highbush Blueberry Council, 2002). Although *Vaccinium* includes over 450 species of blueberries, the highbush blueberry (*V. corymbosum*) is economically the most important. This specie is grown in the Mid-Atlantic, Midwest and Pacific Northwest regions of the United States, and along the Pacific Northwest of Canada (Bowling, 2000).

Blueberry fruits are round to slightly flattened in shape with a diameter of about 0.5 inch. They have a crown-like structure termed the calyx on the bottom and a depressed ring on the top where the stem is attached. The blue-to-blue dark epidermal surface of the fruit is covered with a waxy bloom, giving the fruit a light blue appearance (Pritts, 1992). During the fruit expansion the total β -diketone per fruit increase from 191 to 909 $\mu\text{g}/\text{fruit}$. The hydrophobicity results from β -diketone arranged in a dense network of interlocking branched rodlets or closed tubes within this wax layer (Freeman et al., 1979). Possingham et al. (1976) suggested that the structural arrangement of wax on blueberries, together with the hydrophobic nature of the surface, controls water movement.

Blueberries have been important commercially in the northeastern United States and Canada since the 1880s. The Highbush blueberry industry, however, did not begin

until the early 1900s when Elizabeth White from New Jersey and Dr. Frederick Coville from Maryland initiated research for domestication of the wild highbush blueberry. Their work resulted in the development of blueberries that could be commercially grown by farmers (U.S. Highbush Blueberry Council, 2002).

2.1.2 MICHIGAN BLUEBERRY INDUSTRY

Development of the blueberry industry in Michigan is attributed to Stanley Johnston's work at Michigan State University during the 1930s (Bowling, 2000). Michigan's climate and soil conditions are ideal for blueberries that require sandy soils that are high in organic matter and very acidic (Hancock et al., 2001). Hence, they can thrive near the Lake Michigan shore, an area that was previously considered useless for agriculture (Bowling, 2000).

The United States is the world's leading blueberry producer with 55% of total production followed by Canada at 28% (ESR/ USDA, 2003). Approximately one-third of the total U.S. highbush blueberry crop comes from Michigan. The blueberry industry is composed of two market categories: fresh and processed blueberries. In the U.S. about 45 percent of all highbush blueberries are sold to the fresh market, with the remainder being further processed. Harvest begins in April in Florida, peaking in July when Michigan is included, and ends in October in British Columbia, Canada (NASS/USDA, 2003)

In 2002, Michigan had 16,900 acres of highbush blueberries that yielded 64 million lb of fruit. Approximately 66 percent of these berries (42 million lb) were processed and the remainder went to the fresh market (NASS/USDA, 2002). The percentage of Michigan blueberries marketed as fresh fruit is much lower than the

national average because harvest begins in July. July - also known as National Blueberry Month, is the peak harvest season for blueberries with the volume of fruit in the marketplace exceeding demand. Given the drop in price during peak harvest, much of the fruit is sold to the processed market. Allegan, Berrien, Muskegon, Ottawa and Van Buren counties on the western side of Michigan's Lower Peninsula are primary blueberry-growing regions (Hancock et al., 2001). The most popular varieties in Michigan are Jersey, Bluecrop, Elliot, Duke and Rubel. Total Jersey acreage declined from about 55% in 1970 to 40 % in 2000. Rubel, the other historically important variety, has also declined and now represents less than 10% of total acreage. However, the remaining varieties have increased over time with Bluecrop now comprising almost 30% of total production due to its very high and dependable yields. The Elliot variety, almost nonexistent in the 1970's, has become increasingly popular due to its very late harvest and storability, with the Duke variety also increasing significantly after 2000 because of its large, firm fruit, late bloom and early harvest (Hancock et al., 2001). The best-suited varieties for fresh market include Bluecrop, Duke, and Elliot; whereas the Jersey and Rubel varieties are small-fruited and most popular for the processing market (Hancock et al., 2001). Most growers plant a range of varieties to extend the harvest season for both fresh and processed markets.

The time of harvest depends on the blueberry variety, weather, and location. In Michigan, harvesting typically begins in early July with handpicking for the fresh market, and ends in September. Blueberries usually ripen over several weeks, and require two to four pickings to harvest. After handpicking is complete, growers shift to mechanized harvesting for the processed market. Machine-harvested bushes are usually picked when

60 to 70 % of the berries are blue and again 10 to 14 days later (Hanson and Hancock, 1998).

In 2000, Michigan had approximately 575 growers of which 6% had 100 or more acres and accounted for 46% of the total acreage (Michigan Agricultural Statistic Service, 2002). From 1991 to 2000, the trend was for the number of farms to decrease and acreage to increase. Although the numbers of acres planted has increased by almost 10 %, acreage decreased for all size groups except the largest growers (200 or more acres) where acreage increased by 93 %. Approximately half of all growers belong to the Michigan Blueberry Growers Association (MBG) with the remainder market their fruit independently. MBG members market their fresh fruit through Global Berry Farms (MBG's marketing company), and their processed fruits through Peterson Farms, Inc. Some integrated operations also grow, pack and market their own fruit. Most growers pack their own fresh fruit; however, a few growers have their own processing facility (Bertelsen et al., 1995).

A. Processed Market

The market structure for processed blueberries has changed in response to demands by customers for more stringent food safety and quality standards. High costs due to updating facilities, technologies and administration to meet these standards have resulted in fewer individual growers operating their own processing facilities. Most significant was the opening of two new processing cooperatives in 1999 and 2001, in addition to MBG: Northern Pride Processing (NPP) and West Michigan Processing

Cooperative (WMPC). Each processing cooperative has about 24 members (Michigan Blueberry Growers Association, 2001).

The blueberry processing chain begins with mechanical harvesting by large over-row harvesters that shake berries from the bushes onto conveyors. From conveyors, the berries are loaded into 20-lb lugs. The lugs are then stacked on pallets and transported to the processing facility. Upon arrival at the processing facility, the fruits are either processed the same day or stored overnight on pallets until the next day. From lugs, fruits are loaded onto the processing line (conveyer belts) that carries the fruit over a blower to remove leaves, sticks and other debris. A tilt belt is then used to separate soft and cluster berries. Next, the berries are passed through a water tank to remove any green unripened fruit that floats to the surface. This water tank also contains a sanitizer (usually sodium hypochlorite or chlorine dioxide) to decrease the microbial load on the berries. Subsequently, the berries pass through a de-stemmer that consists of a series of rollers. Finally, the fruit is inspected manually and/or automatically (color sorter/ optical sorter) for quality characteristics before being boxed. The color sorter is most popular, but the new optical inspection sorter can distinguish ripened berries from other berries (green, red, multicolored) and also the Japanese beetle more accurately than hand sorting (Fruit Growers News, 2002). Finally, the 30-pound boxes are coded, dated, passed through a metal detector, and stacked on pallets for freezing (U.S. Highbush Blueberry Council, 2002).

B. Processed Blueberry Standards

Blueberry grades and standards have been set by USDA (USDA/AMS, 1995), and MBG. Processors use MBG's grading system because it is more demanding. In grading fruit, considerations include stem counts, detritus, berry color, fruit damage and ripeness. The most important standards for processors are those set by individual customers that may include particular sizes (small and firm or larger), and/or particular characteristics such as sweetness and color. Food safety standards are stringently implemented by processors, who must now have in place a detailed and documented Hazard Analysis Critical Control Point (HACCP) program, metal detection, multiple self-inspection audits, and annual audits by outside parties including an independent, and third-party food safety audit. All of these changes are necessary because of increasing food safety concerns among customers (Gentry, 2002). In addition, some buyers require third-party certification (TPC). A cost-effective solution for meeting the high cost of TPC and changing customer standards was the opening of the new processing co-ops including NPP and WMPC.

Microbial standards are increasing because of customer demands. Particular microbial standards set by customers reflect the need for safety ('zero tolerance' for human pathogens) or a specific use. The industry needs to know how to meet these increasing demands. A scientific understanding of the factors affecting variation in microbial levels, the risk associated with various microbial levels, and the steps needed to attain particular levels is needed in order to set microbial standards. Understanding at which points in production, harvesting, processing and packaging contamination is likely to occur is clearly needed along with improved sanitizing and microbial reduction

strategies. These efforts will eventually lead to a science-based uniform standard for microbial levels that will satisfy buyers and also be effective and affordable for growers and processors.

C. Fresh Market

Investments for fresh market are much lower than those for the processing market. Most fresh blueberries are handpicked using seasonal migrant labor. Fresh blueberries are not sanitized to decrease microbial levels, mainly because immersion in water severely limits product shelf-life. Some large growers run berries through a blower followed by a tilt belt (for soft berries), a color sorter, and an inspection belt before marketing. Smaller growers simply inspect the fruit manually before packaging. In the fresh market, adherence to good agricultural and hygienic practices is the basis for minimizing microbial levels. Third-party certification is also implemented in the fresh sector because of requirements from some major retailers.

2.2 PRODUCE RELATED OUTBREAKS

During the past several decades the number of produce-related outbreaks reported to Centers for Disease Control and Prevention (CDC) produced by foodborne pathogens as greatly increased due to increased consumption of fresh produce, changes in food production and distribution practices, and the emergence of new foodborne pathogens (U.S.G.A.O., 2002). Changes in dietary habits, with a higher per capita consumption of fresh and minimally processed fruits and vegetables, and increasing demand for salad bars and meals eaten outside the home have further amplified the number of foodborne

outbreaks (Altekruse and Swerdlow, 1996). Consumption of fruits and vegetables in the United States has increased substantially, with per capita consumption of produce increasing 24% (from 573 lb in 1970 to 711 pounds in 1997) (Putnam and Allshouse, 1997). The median number of reported produce-associated outbreaks increased from 2 outbreaks per year in the 1970s to 7 per year in the 1980s, and to 16 per year in the 1990s. According to Sumathi et al. (2004), from 190 produce-associated outbreaks reported between 1973 through 1997, nine involved berries - 4 raspberry, 4 strawberry, and 1 blackberry.

2.2.1 SOURCES OF CONTAMINATION

Produce can be contaminated at any point during its growth, harvesting, processing, distribution, and final preparation (Beuchat et al., 2003). Potential sources for pre-harvest contamination of produce can include soil, feces, irrigation water, water used to apply fungicides and insecticides, insects, dust, inadequately composed manure, wild animals, and human handling (Beuchat, 1996). The ability of produce to internalize pathogens, including *Escherichia coli* O157:H7 (Solomon et al., 2002), and *Salmonella* (Guo et al., 2002), from contaminated water was also recently reported. Thus, irrigation wells and other sources for irrigation water should be monitored for human pathogens. Manure should be adequately composted before being used as fertilizer. Domestic and wild animals should also be excluded from contact with produce fields. Post-harvest sources of contamination include feces, human handling, harvesting equipment, transport containers, insects, dust, rinse water, and processing equipment (Burnett and Beuchat, 2001).

Fruits and vegetables are major components of a healthy diet; however, eating fresh uncooked produce is not risk free. Further efforts are needed to better understand the complex interactions between microbes and produce and the mechanisms by which contaminants are spread from field or orchard to the table. Intensive investigations that include traceback to the farm of origin are now being conducted to identify the root cause of these outbreaks (Sumathi et al., 2004).

2.2.2 BERRY-ASSOCIATED OUTBREAKS

Some berry-associated outbreaks have been traced back to single and/or multiple growers that were located both in and outside of the United States. In 1990 one multistate (Montana, Georgia) outbreak of hepatitis A, the source of contamination was traced to frozen strawberries from one processing facility in California with the strawberries coming from their own farm (Niu et al., 1992). Another traceback of a multistate hepatitis A virus outbreak in 1997 led to strawberries from four farms in Mexico that were processed (frozen and packaged) at a single plant in California (Hutin et al., 1999). Several *Cyclospora* outbreaks were also traced to raspberries imported from Guatemala (Herwaldt, 2000).

Regarding blueberries, in 1984 consumption of unwashed strawberries, blueberries or nectarines may have been linked to an outbreak of listeriosis in Connecticut (Ryser, 1999). More recently, blueberries were confirmed as the source of infection in a multi-district outbreak of hepatitis A in New Zealand with domestic blueberries that were traced back to a single orchard. Fourteen tones of blueberries from the orchard had been sold in New Zealand, 14 tones had been exported and 22 tones were

in cold storage (frozen). At the investigation was found a high contamination rate (3/6 samples) among frozen blueberries (Calder et al., 2003). In addition, in 1998 one producer was forced to recall an undetermined quantity of frozen blueberries from California, Illinois and Australia because of contamination with *Listeria monocytogenes* (FDA Enforcement Report, 1998).

2.3 BLUEBERRY MICROBIAL LIMITS

Michigan blueberries have not yet been implicated in any outbreaks of illness. However, since blueberries and other berry types have been linked to outbreaks in the past, microbial safety remains a critical concern for all segments of the blueberry industry. Large buyers now demand microbial testing and processors must meet particular microbial specifications with a third-party certification. These buyers have ‘zero tolerance’ policies for human pathogens such as *L. monocytogenes*, *E. coli* O157:H7, *Staphylococcus*, and *Salmonella*. They also have limits on mesophilic aerobic bacteria (MAB), coliforms, yeasts and molds that vary considerably from buyer to buyer (Table 2.1).

Table 2.1 Microbial limits (CFU/g) for mesophilic aerobic bacteria (MAB), yeasts, molds, coliforms, and human pathogens (*Staphylococcus*, *Listeria* and *E. coli* O157:H7) set by purchasers of blueberries (After MAFMA Research Proposal, 2003).

Test	Company A	Company B	Company C	Company D
M A B	150,000	50,000	50,000	10,000
Yeast	20,000	10,000	5,000	1,000
Mold	5,000	10,000	5,000	1,000
Coliforms	100	100	<1,000	10
<i>E. coli</i>	Absent	<10	Absent	Absent
<i>Staphylococcus</i>	Absent	Absent	Absent	Absent
<i>Listeria</i>	Absent	Absent/25g	Absent/25g	Absent/25g
<i>E. coli</i> O157:H7	Absent	Absent	Absent/25g	Absent

For example, Company A accepts levels of MAB, yeast, mold and coliforms on fruit that are 15, 20, 5, and 10 times higher than Company D. Some limits appear arbitrary, whereas others reflect specific uses. Pie manufacturers who use blueberries with high yeast counts frequently find that their pies do not “set up” because yeast growth results in enzymatic breakdown of starch and other stiffening agents. This can, in turn, lead to considerable financial loss and frequently a change in blueberry suppliers. These microbial standards are difficult to meet since the level of MAB, yeasts and molds and other contaminants can vary widely between fields, seasons, and the time of harvest depending on factors such as moisture, temperature, insect level, plant health, and harvest management practices (Schilder Annemieck – unpublished data).

2.4 HUMAN PATHOGENS

2.4.1 *ESCHERICHIA COLI* O157:H7

A. Health significance of *E. coli* O157:H7

Enterohemorrhagic *E. coli* (EHEC) was first isolated in 1975 and first identified as a human pathogen in 1982 during two outbreaks of hemorrhagic colitis in Oregon and Michigan that were linked to undercooked ground beef hamburgers sold by one fast food restaurant chain (Riley et al., 1983). Individuals who are most susceptible population include children age 2-10 and those over 65 years of age. The oral infective dose is very low (0.3-15 CFU/g of frozen ground beef) as evidenced from the largest multistate outbreak to date. In this outbreak 731 cases were registered, 178 were hospitalized and 4 children died. The incubation period for *E. coli* O157:H7 is 1-5 days after which individuals develop nonbloody diarrhea that can be self-limiting or turn bloody and persist for up to 2 weeks. Hemolytic uremic syndrome (HUS) - the most dangerous complication in children, is characterized by acute renal failure and a high fatality rate. Elderly patients can also develop thrombotic thrombocytopenic purpura (TTP), a rare adult version of HUS that produces clots in the brain due to platelet aggregation with neurological signs. These symptoms result from adherence of the organism to the intestinal tract lining followed by production of one or more verotoxins. All EHEC produce verotoxins (VT's) that are cytotoxic to African green monkey kidney (Vero) cells and similar to Shiga toxin produced by *Shigella* type 1 [Shiga-like toxins (SLT)] (Doyle et al., 1997).

B. *E. coli* O157: H7 outbreaks in produce

Escherichia coli O157: H7 has been traced to outbreaks involving lettuce, apple juice, salad, cantaloupe, and alfalfa sprouts. In 1995, one outbreak was reported in Montana in which 92 people became ill after consuming leaf lettuce consumption. In 1996, another outbreak in Connecticut, Illinois, and New York was traced to mesclun lettuce (Hilborn et al., 1999).

Traceback of lettuce in these outbreaks lead mainly to farms located in the United States. Contamination on farms most likely occurred through contact with contaminated irrigation water, wash water or animal feces (Ackers et al., 1998; Hilborn et al., 1999). Fresh produce can easily become contaminated with fecal pathogens such as *E. coli* O157:H7, which can remain viable in bovine feces for up to 70 days (Wang and Doyle, 1996). When grown adjacent to or downwind from a microbial source of pathogens such as a dairy operation, tree fruits can also become contaminated with *E. coli* (George et al., 2002).

A significant number of outbreaks occurred with fresh apple cider and apple juice. In 1991, 23 people in Massachusetts became ill and 3 developed HUS after consuming unpasteurized cider. In 1996, 12 people in Connecticut were ill, 3 with HUS. In the same year, in multiple states and Canada, another outbreak occurred in which 56 people became ill, 14 developed HUS and 1 died. All of these outbreaks were traced to consumption of unpasteurized apple cider or juice. Practices that increased the risk of producing contaminated juice included the use of dropped fruit or inadequate cleaning of the fruit before juicing (Besser et al., 1993; CDC, 1997; Cody et al., 1999). These outbreaks highlighted the unusual acid tolerance of *E. coli* O157:H7 in acidic foods such as apple

cider that typically has a pH of 3.5-4.0. This increased tolerance to acid is based on the expression of acid shock proteins that provide protection to the organism from normally lethal pH levels (Foster and Hall, 1990; Leyer and Johnson, 1995). *Escherichia coli* O157: H7 can survive up to 7 days in unpasteurized apple cider at room temperature (25°C), but when the cider is refrigerated (8°C) survival is increased to 20 days (Besser et al., 1993).

Internalization has been shown to occur with *Escherichia coli* O157: H7 in sprouts (Itoh et al., 1998) and apples (Buchanan et al., 1999), and with *Salmonella* in tomatoes (Zhuang et al., 1995) and mangoes (Penteado et al., 2004). When warm produce is immersed in cold water, a pressure difference is formed between the produce core and the surrounding water that facilitates entry of bacteria into the core, mainly through the stem end (Bartz and Showalter, 1981).

2.4.2 SALMONELLA

A. Health significance of *Salmonella*

Salmonellosis caused by the bacterium *Salmonella* is one of the most common and widely distributed foodborne diseases. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths (WHO, 2005). The contamination route is mostly fecal to oral with symptoms of infection usually appear 8-72 h after initial exposure of incubation (Ryser, 1998). The clinical manifestations of non-typhoid *Salmonella* infections can range from a self-limited gastroenteritis to septicemia and death. In the mild cases symptoms of gastroenteritis, including nausea and vomiting subside within a few hours. Fever, chills, prostration, myalgia and abdominal

pain that can resemble acute appendicitis with diarrhea can follow in the course of the disease. Diarrhea, the predominant symptom can range from loose stools to bloody and rice-water cholera-like stools in severe cases. The clinical condition can still be self-limiting with remission of signs without intervention within 5 days of onset (D'Aoust, 1997 and Ryser, 1998). In some cases, particularly in the very young and in the elderly, the associated dehydration can become severe and life-threatening. In such cases, as well as in cases where *Salmonella* causes bloodstream infections, effective antibiotics are essential for treatment (WHO, 2005). Of public health concern is shedding of *Salmonella* in infected patient's stools at concentrations 10^6 to 10^9 CFU/g. Administration of antibiotics for gastroenteritis can disturb and/or destroy the normal intestinal microflora that competes with *Salmonella* for nutrients and binding sites, thereby increasing the asymptomatic carrier state (D'Aoust, 1997 and Ryser, 1998).

Another public health concern is the emergence of multi-antibiotic resistant serovars of *Salmonella* that have now become a serious worldwide problem (Glynn et al., 1998). Strains of *Salmonella* subsp. *enterica* serovar Typhimurium Definitive Type 104 isolated in the United States are commonly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines with strains in England showing additional resistance to trimethoprim, fluoroquinolones and ciprofloxacin (Glynn et al. 1998). This resistance results from the use/misuse of antimicrobials in humans and animal husbandry and national/international trade of infected animals is thought to play a major role in the spread (WHO, 2005). A total of 2501 different *Salmonella* serotypes have been identified up to 2004 and all can cause disease in humans with a total cost estimated at 3 billion dollars annually in the United States. *Salmonella* Enteritidis and *Salmonella*

Typhimurium are the two most important serotypes for salmonellosis transmitted from animals to humans (WHO, 2005).

B. *Salmonella* outbreaks in produce

Produce items frequently implicated in *Salmonella* outbreaks include sprouts, melons, apple/orange juice, and tomatoes. Most of the sprout outbreaks involved alfalfa sprouts, affected a high number of persons, and were multistate (Glynn et al., 1998; Mohle-Boetani et al., 2001; Mouzin et al., 1997) or international (Mahon et al., 1997; Van Beneden et al., 1999). These outbreaks were associated with the use of fecally contaminated seeds or contaminated water during sprouting. The melons implicated in outbreaks (including watermelon and cantaloupe) were more likely surface contaminated from the ground where they were grown (CDC, 1991). Between 2000 and 2002, several salmonellosis outbreaks in United States and/or Canada were linked to Mexican-grown cantaloupe. FDA conducted a survey of cantaloupe from Mexico and found that 5 percent of samples were contaminated with *Salmonella*. Possible sources of contamination included irrigation of fields with water containing sewage; processing (cleaning and cooling) produce with *Salmonella*-contaminated water; poor hygienic practices of workers who harvest and process the cantaloupe; pests in packing facilities, and inadequate cleaning and sanitizing of equipment that comes in contact with cantaloupes. FDA now detains whole and precut cantaloupes from Mexico until the producer can demonstrate that the product can be exempt from detention (CDC, 2002).

In 1974 in New Jersey 298 people become ill after consumption of contaminated apples. Twenty-one years later, 62 cases of salmonellosis were linked to consumption of Florida orange juice. In both outbreaks, the unpasteurized juice was prepared from fruit that dropped from the trees (CDC, 1975; Cook et al., 1998). In 2000 another multistate *S. Enteritidis* outbreak was associated with consumption of unpasteurized orange juice prepared from oranges that received a sanitizer treatment before squeezing (Rangel, 2000).

Salmonella is another pathogen that develops tolerance to acidic environments with serovars Hartford and Typhimurim able to survive up to 27 and 60 days in orange juice at pH 3.5 and 4.1, respectively. Hence, consumer of unpasteurized juice can be exposed potentially infectious levels of *Salmonella* (Parish et al., 1997).

2.4.3 *LISTERIA MONOCYTOGENES*

A. Health significance of *Listeria monocytogenes*

Listeria monocytogenes is a pathogenic bacterium that can be found in animal feces. It is a natural inhabitant of soil and has been isolated from a wide variety of fresh produce including bean sprouts, cabbage, chicory, cucumbers, eggplant, lettuce, mushrooms, potatoes, radishes, and salad vegetables (Buck et al., 2003). However, *L. monocytogenes* was not often found on broccoli, carrots, cauliflower and tomatoes, which lead to speculation that less soil contact during growth of these vegetables or the presence of some compounds that inhibits the pathogen (tomatoes and carrots) can be the reason.

The cause of the current “*Listeria* Hysteria” stems from the ubiquitous nature of the pathogen, severity of the disease that has a fatality rate of 20-30%, and increased susceptibility of pregnant women, the elderly, and immunocompromised adults.

Pregnant women may develop flu-like symptoms, but the infection is much more severe for the unborn fetus with spontaneous abortion, fetal death or delivery of a stillborn infant. *Listeria monocytogenes* is a psychrotrophic organism that is also acid and salt tolerant. Although the oral infectious dose is thought to be as low as 1,000 cells, listeriosis remains a rare disease with about 2500 cases and 500 fatalities reported annually in the United States (Mead et al., 1999). However, the gravity of this disease led to the current “0 tolerance policy” for *L. monocytogenes* in ready-to-eat (RTE) foods, and has made this pathogen the leading cause of all microbiologically related Class I recalls.

B. *Listeria monocytogenes* outbreaks in produce

Although *L. monocytogenes* is widely distributed on plant vegetation (Beuchat, 1996), with produce contamination from soil, water, and manure, (Reina et al., 1995), fresh produce has largely remained an uncommon source of foodborne listeriosis. In 1979, raw celery, tomatoes and lettuce were epidemiologically linked as the possible cause of a listeriosis outbreak involving 23 patients from the Boston area (Beuchat, 1996). Two years later, *L. monocytogenes* was first confirmed as a consumption of *Listeria*-contaminated coleslaw was directly linked to 7 adult and 34 perinatal (17 death) cases of listeriosis in the Maritime Provinces of Canada. Cabbage used in manufacturing the coleslaw came from fields that were fertilized with fresh sheep manure from a flock of *Listeria*-infected sheep (Schlech, 1983).

Listeria monocytogenes is known as a “hardy” organism and is able to survive on produce (Zhang and Farber, 1996). Furthermore, as a psychrotroph this pathogen can grow on refrigerated foods (Lou and Yousef, 1999). Beuchat (1996) found that, *L. monocytogenes* grew on endive and lettuce during storage at 10°C. In 1984, consumption

of unwashed strawberries, blueberries or nectarines may have been linked to an outbreak of listeriosis in Connecticut (Ryser, 1999), and in 1998 one producer was forced to recall an undetermined quantity of frozen blueberries from California, Illinois and Australia because of contamination with *Listeria monocytogenes* (FDA Enforcement Report, 1998).

2.5 ECOLOGICAL FACTORS INFLUENCING HUMAN PATHOGENS ON BLUEBERRY

To generate disease, pathogens contaminating any food product need to survive at levels sufficient to cause illness. The minimum infectious dose is lower for *Salmonella* at <100 cells (Blaser and Newman, 1982) and *E. coli* O157:H7 at 2 to 45 cells (Tilden et al., 1996) than for *L.a monocytogenes*. Pathogens that contaminate the surface of fruit are mainly influenced by pH, availability of nutrients, and the natural microflora of the product. Fruits such as blueberries have a pH of 3.5 to 4.0 that is sufficiently low to prevent or retard the growth of bacterial pathogens. However, the pH can be increased by growth of post-harvest fungi that may permit the growth of pathogenic bacteria. In two studies, populations of *L. monocytogenes* and *E. coli* O157:H7 increased on the surface of apples containing *Glomerella cingulata* with this attributed in part to the increase in pH from 4.7 to around 7.0 (Conway et al., 2000; Riordan et al., 2000).

Produce with damaged tissue from spoilage fungi or bacteria may also be more prone to pathogen growth due to the availability of nutrients in the exudates. In a study with healthy and soft-rotted produce, the incidence of *Salmonella* on produce affected by

bacterial soft rot (*Erwinia*, and *Pseudomonas*) was twice that of the control samples (Weissinger and Beuchat, 2000).

Growth of spoilage and non-spoilage microorganisms on fresh fruit and vegetables can result in the formation of biofilms that can protect bacteria from the bactericidal action of sanitizers. On one study, *L. monocytogenes* was unaffected by treatment with 500 ppm free chlorine when the pathogen was present in a multi-species biofilm with *Pseudomonas fragi* and *Staphylococcus xylosus* (Norwood and Gilmour, 2000).

2.6 METHODS TO PREVENT AND ELIMINATE BLUEBERRY AND HUMAN PATHOGENS

Spoilage and illness prevention strategies available to consumers of blueberries are primarily limited to washing. One method to improve the microbial quality of blueberries would involve to improvements in crop and harvest management. One microbial assessment of blueberries from 9 and 12 locations in Michigan during 2002 and 2003, respectively, showed that populations of bacteria, yeasts and filamentous fungi increased considerably from green to ripened fruit and from the 1st to the 2nd harvest. (Schilder Annemieck – unpublished data). Microbial populations varied widely among field locations including those that were irrigated and non-irrigated. Among filamentous fungi were *Colletotrichum* (most common), *Cladosporium*, *Epicoccum*, *Alternaria* and *Phoma* species. Most of these fungi are common in the environment and live on dead and dying plant material. They attack ripe or overripe fruit and can spread from infected to

healthy berries upon contact and can result in serious spoilage and losses in stored blueberries.

Colletotrichum acutatum, the causal fungus of anthracnose fruit rot, is a major cause of pre- and post-harvest fruit rot in most blueberry-growing regions. In Michigan, *C. acutatum* was found in nearly all fields and comprised 95-100% of fungal population (Schilder Annemieck – unpublished data). Cane, twig, and leaf lesions are more sporadic. Fruit rot manifests itself as sunken areas on ripe fruit with gelatinous, orange spore masses. The fungus over-winters in remnants of old fruiting twigs and infected canes. In spring and summer, fruiting bodies release spores that are dispersed by rain to infect flowers, fruit and other tissues. Fruit infections remain latent until the fruit starts to ripen. In Michigan, spore numbers peak around bloom. A second peak occurs when fruits are ripening. Warm humid conditions favor the disease (Michigan blueberry facts, 2005).

Alternaria fruit rot occurs in most blueberry-growing regions. On ripe fruit, sunken areas near the calyx are covered by a dark green, velvety growth. On stored fruit, a grayish-green mold may appear on the stem scar or calyx end and spread over the entire berry. Infected fruit becomes soft and shriveled. The fungus over-winters in old twigs and in plant debris on the ground. Leaf infections occur in the spring during periods of cool, wet weather. Fruit infections occur as the berries start to ripen. Disease development is optimal at 68°F (20°C) (Michigan blueberry facts, 2005).

The fungus *Phomopsis vaccinii* over-winters in infected canes and twigs. *Phomopsis* canker and twig blight occur in most blueberry-growing regions. In the spring, spores are dispersed from fruiting bodies by rain. The fungus is active from bud swell until after harvest. Plants that have been wounded mechanically or damaged by

freezing are more susceptible to infection than undamaged plants. Fruit infection leads to white mold growth and soft fruit that splits when squeezed (Michigan blueberry facts, 2005).

Fungi management involves the use of plant resistant cultivars, pruning to remove old or infected wood, application of fungicides before harvest, harvesting in a timely manner, handling berries dry, and rapidly cooling fruit after harvest (Michigan blueberry facts, 2005).

Some of the common yeasts on blueberry fruit surfaces were identified as *Aureobasidium*, and *Cryptococcus* (most common), *Sporidiobolus*, *Bullera*, *Filobasidium*, and *Rhizosphaera* species. These yeasts are also common on plant surfaces and are not harmful to humans. Bacteria found on blueberries include *Pseudomonas*, *Bacillus*, and *Clavibacter* spp (Schilder Annemieck – unpublished data).

Aureobasidium pullulans (yeast rot) produces a sporadic post-harvest rot characterized by rapid collapse and a wet or slimy appearance of the fruit. Yeast growth may appear as black, shiny bumps with a white or pinkish slime (Michigan blueberry facts, 2005). Representative bacteria, yeast and mold isolates were tested for production of extracellular enzymes including amylase that reduce the viscosity of backed goods as well as pectinase and cellulase that can lead to breakdown of the fruit itself. Most fruit associated microbes were able to break down starch (Schilder Annemieck – unpublished data). According to Janisiewicz and Korsten (2002) several yeast species including *Aureobasidium* and *Cryptococcus* spp. possessed antimicrobial activity against these spoilage organisms, thus suggesting their potential use as biocontrol agents for post-harvest diseases of fruits.

Mechanical harvesting, storage and processing are the most important in obtaining high quality and safe fruit for consumption. According to NeSmith et al. (2002) machine harvesting caused the greatest loss in fruit firmness (20-30%). This was followed by a 10-15% loss in firmness due to grading and sorting. Keeping fruit at ambient temperatures for 24 h after harvest resulted in only a 3-8% loss in firmness as compared to cooling fruit immediately. Removal of harvester padding resulted in a 4-8% loss in fruit firmness. The decay of machine-harvested blueberries during post-harvest holding is perhaps the industry's biggest problem. Mechanical harvesters operate over-the-row and result in loss of berries on the ground with the quality generally inferior to handpicked fruit. However, one over-the-row harvester can cover up to 1 acre/h, replacing over 100 hand pickers. Thus, economics may dictate mechanical harvesting. Blueberry quality changes in response to pre-packing delays and holding temperatures. Jackson et al. (1998) assessed blueberries for the following ten quality attributes during storage: percentage of split berries, bloom, firmness, weight loss, moisture, soluble solids, titratable acids, pH, microbial counts, and percentage of marketable berries at different pre-packing temperatures up to 26°C, delayed processing times up to 45 h, and subsequent storage times up to 21 d at 0°C. Increasing the pre-packing temperature and delaying packing led to a ~1-log increase in microbial populations. Overall, minimizing delays was the best means for maximizing blueberry quality with cooling before packing being beneficial when packing was delayed.

Freezing is one means of prolonging the shelf life of blueberries by preventing microbial growth. The additional killing effect of freezing on microorganisms relates to temperature shock, concentration of extracellular solutes, toxicity of intracellular solutes, and

dehydration and ice formation (Zaritzky, 2000). However, decades ago Schmidt-Lorenz (1963) and Schmidt-Lorenz and Gutschmidt (1969) found that certain bacteria were still able to grow to relatively high numbers at -7.5°C . The authors also found that the growth limit for yeast was -10°C . Microbial growth or metabolic activity also has been reported in permafrost bacteria at -10°C (Gilichinsky et al., 1995) with the temperature limit of bacterial growth in frozen food now generally considered to be -8°C (Geiges, 1996). Rivkina et al. (2000) attempted to quantify metabolic activity at subzero temperatures in the native bacterial population of Siberian permafrost by measuring the incorporation of sodium acetate into lipids at seven temperatures from -5°C to -20°C for times up to 550 days. The minimum doubling times ranged from 1 day (5°C) to 20 days (-10°C) to ca. 160 days (-20°C). Thus, microbiological quality remains an issue until temperatures below -10°C have been reached in the freezing process. Geiges (1996) reviewed the available literature regarding the effect of slow and fast freezing on bacteria and concluded that quick freezing and thawing would result in higher microbial survival rates than those found for slow freezing and thawing. Today, cryogenic substances are routinely used to maintain viability of microorganisms during under long-term storage. Hence, consumers and manufacturers should not take the microbiological safety of cryogenically frozen products for granted. Additional factors that affect the microbiological quality of frozen foods relate to the physical and chemical characteristics of the product, the pre-freezing microbiological quality of the product and the temperature fluctuations during storage that can lead to thawing and refreezing.

Methods to prevent consumption of fresh or frozen blueberries with foodborne pathogens are limited to thorough washing, choosing unblemished product, avoidance of

cross contamination in the kitchen, and eating or refrigerating the berries promptly after purchase in order to prevent microbial growth. If contamination is limited to the surface, various wash treatments have shown to be marginally effective in reducing levels of surface microorganisms. Washing produce with water alone will typically reduce microbial populations by only 1 or 2 logs (Beuchat et al., 2003). Some products such as cantaloupe and raspberries have difficult-to-clean surfaces that support increased adherence of pathogens (Ortega et al., 1997). Moreover, some types of produce such as apples are prone to internal contamination that results from ineffective surface washing treatments (Zhuang et al., 1995). When Crowe et al (2005) commercially washed blueberries in Maine with a sterile spray of distilled water for 30 and 300 sec, populations of bacteria, yeasts and molds decreased $\leq 0.43 \log \text{CFU/g}$.

Another prevention method is to avoid consumption of high risk items such as unpasteurized apple cider by persons at high-risk (young children, elderly). Laboratory and field studies on the behavior of microbes in produce during growing, harvesting, and processing can offer novel and important insights into potential control strategies with an emphasis on bioprevention of pathogen contamination. New control measures that prevent or reduce contamination of fresh produce are needed (Sumathi et al., 2004).

Once fresh produce becomes contaminated with pathogens, the product remains a potential risk until the time of consumption. Washing and rinsing some types of fruit and vegetables will prolong the shelf life by reducing the number of microorganisms on the surfaces. However, with this simple method only some of the pathogens are removed from the surface. To be deemed effective, any antimicrobial treatment (sanitizer) that is

administrated to produce should reduce the populations of pathogens by more than 2 to 3 log CFU/g (Beuchat, 1998).

2.7 SANITIZERS

The use of chemical sanitizers to enhance the microbial safety and shelf-life of fresh fruits and vegetables is of great interest to the food industry. The goal is to obtain a 5-log reduction as set by the Food and Drug Administration (FDA) for selected commodities. Chlorine-based sanitizers are most common for fruits and vegetables including Michigan blueberries; however, other sanitizers including fatty organic acids (OA), aqueous chlorine dioxide and especially chlorine dioxide gas are of increasing interest to blueberry processors. Sanitizers that can be used to wash fruits and vegetables are regulated by the U.S. Food and Drug Administration in accordance with the Federal Food, Drug and Cosmetic Act outlined in the Code of Federal Regulations, Title 21, Ch.1, Section 173.315. The efficacy of a sanitizer is a function of both time and concentration. In general, greater sanitizer efficacy is seen at increasing concentrations. Increasing the contact time and the sanitizer concentration can also increase microbial inactivation. Sodium hypochlorite at 200 ppm was found to be significantly more effective than 100 ppm in inactivating *L. monocytogenes* and *E. coli* O157:H7 on different types of produce after a 5-minute exposure (Rodgers, 2004). There is an optimum sanitizer concentration below which the effectiveness is reduced and above which there is no further improvement. Populations of fecal coliforms on green salad leaves were reduced by 2-logs using 50 ppm chlorine with concentrations up to 200 ppm being no more effective (Mazollier, 1988). Sanitizer antimicrobial action can be influenced by temperature (chlorine compounds) with greater activity at 22°C than at 4°C

(Zhang and Farber, 1996). The pH of the solution can also influence effectiveness of chlorine with a decrease in pH from 9 to 5 increasing the antimicrobial effect of chlorine 4-fold on lettuce (Adams, 1989). Pathogens also vary in their sensitivity to sanitizers with *L. monocytogenes* generally being more resistant to chlorine than *Salmonella* and *E. coli* O157:H7 (Beuchat, 1998).

The general lack of efficacy of sanitizers on raw fruits and vegetables can be attributed, in part, to their inaccessibility to locations within structures and tissues that harbor pathogens. Pathogenic bacteria are able to infiltrate cracks, crevices, and intercellular spaces of seeds and produce. Infiltration is dependent on temperature, time, and pressure, and only occurs when the water pressure on the produce surface overcomes the internal gas pressure and the hydrophobic nature of the surface of the produce (Beuchat, 2002; Burnet and Beuchat, 2001). Infiltration may also be enhanced by the presence of surfactants and when the temperature of the fruit or vegetable is higher than the temperature of the water. The protective mechanism of these sites is not well understood, but the theory that hydrophobicity of microbial cells aids in their protection by inhibiting penetration of the disinfectants has been proposed (Buck, 2003). Major factors limiting sanitizer efficacy include strength and rapidity of microbial attachment, inaccessibility to attachment sites, attachment and growth in cuts and punctures, internalization of microbial contaminants within plant tissues, and biofilm formation (Sapers, 2001).

2.7.1 CLOROX TM/ SODIUM HYPOCHLORITE (NaClO)

A. General characteristics

Chlorine was discovered several hundred years ago and has been since used to noxious odors, and as calcium hypochlorite to sanitize morgues, sewers and hospitals. Today most sanitizers used in the produce industry are chlorine-based (Beuchat, 1998 and Brackett, 1999). Chlorine is used at concentrations of 5 to 200 ppm with contact times of few minutes for raw fruits and vegetables (Beuchat, 1996). Hypochlorites such as calcium hypochlorite (CaCl_2) and sodium hypochlorite (NaCl) are produced when the chlorine compound is dissolved in water. Chlorine is released forming hypochlorous acid (HOCl) and hydrochloric acid. The strong antimicrobial activity of HOCl results from a high oxidation potential (Ong, 1996).

The dissociation rate of HOCl is pH dependent. At $\text{pH} \leq 2$, and ≥ 10 , chlorine is in the elemental form and in the hypochlorite ion form, respectively. As the pH of a solution at 20°C is reduced, the concentration of HOCl increases to 23 and 97% at pH of 8.0 and 6.0 respectively. Toxic chlorine (Cl_2) gas is also formed at pH below 4 (Beuchat, 1996).

Temperature is another factor that influences the reaction with more HOCl produced at lower temperatures and greater loss of chlorine gas at higher temperatures. Chlorine is maximally soluble in aqueous solutions at 4°C (Burnett, 2001). Chlorine also rapidly loses activity when in contact with organic matter, exposed to air, light, metals (copper, cobalt, nickel, other catalysts) or ultraviolet light. The most common method for measurement of “available chlorine content” (strength of hypochlorite solution) is the iodometric method. This method is based on the principal that in the presence of

potassium iodine (KI), free chlorine in water liberates iodine that is titrated with a standardized sodium thiosulfate solution as a quantitative indicator.

B. Antimicrobial performance

Chlorine has good antimicrobial activity in aqueous model systems. However, on the surface of fresh fruits and vegetables, chlorine is less effective in killing microorganisms with microbial reductions typically not exceeding 2 to 3 log CFU/g (Beuchat, 1998) due mainly to its inactivation by other organic materials that are present on the product.. According to Park and Beuchat (1999), treating cantaloupe with 2000 ppm chlorine reduced *E. coli* O157:H7 populations less than 1 log due to interference from organic matter. Treatment of blueberries with 100 ppm for 5 min reduced the population of bacteria, yeast, and mold by 0.83, 0.77, and 0.61 log CFU/g, respectively (Crowe et al., 2005). Another factor that makes the effectiveness of chlorine compounds unpredictable is insufficient wetting of the hydrophobic surface (waxy cuticle) of fruits and vegetables (Adams, 1989). Although chlorine-based sanitizers are relatively inexpensive, some concerns have been raised regarding corrosivity, instability and the production of residual chlorine by-products such as chloroform, trihalomethane (THM), bromodichloromethane, and MX [3-chloro-4-(dichloromethyl)-5-hydroxyl-2(5H)-furanone] (Richardson, 1998). These organochloride compounds have been demonstrated to cause cancer in laboratory animals (Wei, 1985). Because the organochloride compounds can enter the environment through wastewater and gain access to drinking water, the US Environmental Protection Agency established a maximal THM limit in drinking water of 100 µg/L (Richardson, 1998). Because of these limitations in efficacy

along with recent environmental and public health concerns, the produce industry is stimulated to find alternative treatments.

2.7.2 CHLORINE DIOXIDE

A. General characteristics

Chlorine dioxide is a yellow-green gas generated through oxidation when concentrated hydrochloric acid is added to sodium chlorite. When done in water, the end result is an aqueous chlorine dioxide solution. Chlorine dioxide is receiving increased attention as a produce sanitizer due to the following advantages over chlorine: greater effectiveness over a wider pH range, not affected by high levels of organic matter, no rapid dissociation in water, an oxidation capacity 3 times greater than chlorine (White, 1972), does not react with organic compounds to produce carcinogenic by-products, formation of by-products that are less toxic and 3-5 times lower compared with chlorine (Richardson et al., 1998), and a wide germicidal activity including spores (Richardson et al., 1994), viruses (Sobsey, 1988) and protozoa including *Cryptosporidium* and *Giardia* oocysts (Finch et al., 1997) that are more resistant to chlorine. Because of these qualities chlorine dioxide is used today in the disinfection of water, air, and was the principal agent used in the decontamination of buildings in the US after the 2001 anthrax attacks.

The food and Drug Administration (FDA 2005) amended the food additive regulation to allow the safe use of chlorine dioxide as an antimicrobial agent in water used to wash fruits and vegetables that are not raw agricultural commodities in an amount not to exceed 3 ppm residual chlorine dioxide. The treatment of the fruits and vegetables with chlorine dioxide shall be followed by a potable water rinse or by

blanching, cooking, or canning. The U.S. Environmental Protection Agency (EPA) approved use of chlorine dioxide as a disinfectant for potable water treatment limiting the residual to 1 ppm (U.S. Federal Register, 2000). There is a lot of effort underway to use chlorine dioxide gas for raw agricultural commodities such as tomato, cantaloupe, onion, flower bulb and vegetable seed trials (ICA TriNova, LLC Forest Park, GA) but nothing is commercial yet.

The germicidal action of chlorine dioxide is based on a loss of membrane permeability that results from oxidative damage to the outer cell membrane followed by destruction of the trans-membrane ionic gradient and suspension of protein synthesis (Berg et al., 1986).

B. Antimicrobial performance

The efficacy of chlorine dioxide is high when studied in model aqueous systems with a > 5 log reduction for *E. coli* O157:H7 and *L. monocytogenes* following a 5-min exposure to 3 and 5 ppm chlorine dioxide (Rodgers et al., 2004). On produce, the efficacy of chlorine dioxide against pathogens is far lower with reductions ranging from 1 log on shredded lettuce and cabbage (Zhang and Farber, 1996) to ~ 4 logs on whole apples (Wisniewsky et al., 2000). The surface seems to play a more important role for the efficacy of sanitizers. A 3 mg/L aqueous ClO₂ treatment achieved reductions of 3.7- and 0.4-log for *L. monocytogenes* on uninjured and injured surfaces of green pepper, respectively, while populations decreased >6 and ~3.5 logs/ 5g on the same surfaces using similar concentrations of chlorine dioxide gas (Han et al., 2001). A ClO₂ gas treatment was the most effective in reducing *L. monocytogenes* on both uninjured and

injured green pepper surfaces, when compared with aqueous ClO₂. The difference in log reductions for uninjured and injured green pepper surfaces and results from confocal laser scanning microscopy (CLSM) analysis suggested that injured surfaces protected more bacteria from sanitation treatments than did uninjured surfaces or that the injured surfaces could also support growth of the pathogen. Fruit treatment with aqueous chemical solutions can promote yeast and mold growth. Growth of molds can increase the pH of blueberries from ~ 3.35 (Jackson et al., 1998) to over 4 and thus enhance the growth of bacteria including foodborne pathogens and increasing safety risk.

Thus, alternatives to aqueous sanitizers such as gaseous chlorine dioxide have been assessed for microbial reductions on fresh produce. Studies have shown gaseous chlorine dioxide to be effective in microbial reductions including enteric pathogens on apple (Du et al., 2002; Du et al., 2003), green peppers (Han et al., 2000; Han et al., 2001), lettuce (Lee et al., 2004), tomato, cabbage, carrots, peaches (Sy et al., 2005a) strawberries (Han et al., 2004; Sy et al., 2005b), raspberries and blueberries (Sy et al., 2005b) resulting an increase in the popularity of using chlorine dioxide gas as a sanitizer. Chlorine dioxide gas proved also to be effective as a sanitizer in food processing plants by reducing the > 4 log populations of yeast and molds on stainless steel surface of the tanks used for aseptic juice storage below detectable limits (Han et al., 1999) and in libraries by effectively controlling the spread of molds (Weaver-Meyers et al., 1998). Treatment of surface-injured green peppers with 1.2 mg/L chlorine dioxide gas resulted also in a 6.4 log reduction in *E. coli* O157:H7 per spotted site (Han et al., 2000). Treatment with 18 mg/L for 10 min reduced numbers of *E. coli* O157:H7 on calyx, stem, and skin surfaces of apples by 3.8, 3.8, and >7 logs CFU per inoculated spot (Du et al., 2003). Similarly, a

nonpathogenic strain of *E. coli* was reduced by 4.5 log CFU/g on whole apples using 0.3 mg/L chlorine dioxide gas (Sapers et al., 2003). Gaseous chlorine dioxide was also tested for reducing populations of *Salmonella*, yeasts and molds on blueberries surface (Sy et al., 2005). Treatment with 4.1 to 8 mg/L chlorine dioxide gas released in 30 to 120 min, and 75 to 90% relative humidity (RH), reduced the populations of *Salmonella*, yeasts and molds on spot-inoculated blueberries (skin, calyx and stem scar) by 1.9 to 3.7, and 1.4 to 2.5 log CFU/g, respectively. The reductions achieved using increased concentrations of chlorine dioxide gas were similar, with higher reductions on skin than the stem scar and calyx. Sensory attributes including appearance, color, aroma, and overall quality after treatment with 4.1 mg/L chlorine dioxide gas were not significantly different compared with the ungassed control after 0 and 3 days of storage. However, after 7 and 10 days, the treated samples were ranked significantly higher for overall quality and aroma, respectively.

CHAPTER 3

MICROBIAL CONTAMINATION IN Highbush Blueberries Before, During, and After Processing

ABSTRACT

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 (Y) and molds (M). Thereafter, blueberries from these same fields were harvested and similarly assessed at different points during processing (after-harvest, blower exit, after washing, and before packaging for freezing) at six facilities along with environmental samples (blower and filler conveyor belts, chlorinated wash water). Duplicate blueberry (100g), wash water (50 ml) and environmental swab samples (~10 x 10 cm) were analyzed for MAB, coliforms, *E. coli*, Y and M by plating on tryptic soy agar containing 0.6% yeast extract and cyclohexamide, Petrifilm™ *E. coli*/coliform plates, and potato dextrose agar containing streptomycin and ampicillin, respectively. Average MAB, Y and M 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.

3.1 INTRODUCTION

The United States is the world's leading blueberry producer with 55% of total production and approximately one-third of the total U.S. highbush blueberry crop coming from Michigan. In 2002, Michigan had 16,900 acres of highbush blueberries that yielded 64 million lb of fruit. Approximately 33 percent of these berries (42 million lb) were marketed as fresh berries with the remainder processed and frozen for later use in jams and baked goods (NASS/USDA, 2002).

Food safety concerns surrounding blueberries include a possible link to a 1984 outbreak of listeriosis in Connecticut (Ryser, 1999), one confirmed outbreak of hepatitis A in New Zealand (Calder et al., 2003), and a 1998 recall involving an undetermined quantity of frozen blueberries from California, Illinois and Australia that was contaminated with *Listeria monocytogenes* (FDA Enforcement Report, 1998). Michigan blueberries have not yet been implicated in any outbreaks of illness. However, since blueberries and other berry types have been linked to outbreaks in the past, microbial safety remains a critical concern for all segments of the blueberry industry.

Besides 'zero tolerance' policies for *L. monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* in blueberries, buyers of frozen berries now demand microbial testing for levels of spoilage bacteria, yeasts and molds. Different purchasers have now developed varying microbial standards for frozen blueberries with these standards

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reflecting different uses. Pie manufacturers need a low level of amylase in their blueberries in order to produce baked goods with a low viscosity blueberry filling. Because most bacteria, yeasts and molds isolated from blueberries produce the enzyme amylase (Schilder Annemieck – unpublished data), the microbial standards for frozen berries are now becoming increasingly stringent. A better understanding of factors contributing to high microbial levels in blueberries along with improved microbial reduction strategies will allow blueberry growers and processors to better meet these increasingly strict microbial standards.

The specific objective of this study was to assess the levels of microbial contamination at various points during blueberry harvesting, handling, cleaning, and packaging. These findings will eventually help to establish a science-based uniform standard for microbial levels in blueberries that will satisfy the needs of buyers as well as growers and processors.

3.2 MATERIAL AND METHODS

Blueberry samples

During the 2003 and 2004 harvest seasons, 18 500-g blueberry samples were obtained by hand-picking specific rows or sections at 12 and 6 fields locations in Michigan, respectively. These samples were then assessed for microbial levels at five points during processing at six and two facilities in 2003 and 2004, respectively. Fruit samples (500 g) were collected in 1- pint plastic clam shell containers at pre-harvest (prior to mechanical harvesting), post-harvest (when loaded onto the conveyer belt 4 to 18 h after harvesting), blower-exit, water tank-exit, and pre-packaging for freezing

(Figure 2.1). All clam shells containing blueberries were placed in individual plastic bags, stored on ice and analyzed within 24 h of collection. Portions of these samples (450g) from the 2003 harvest season were placed in sterile 20 x 10 cm polyethylene bags (Whirl-PackTM, Fisher Scientific, Pittsburgh, N.J.) and analyzed after 3 and 6 months of storage at -20°C. Storage time between pre-harvest and post-harvest varied between 4 to 18 h.

Environment samples

Environment samples were collected during processing (Figure 3.1) from 10 x 10 cm areas of the conveyer belt entering the blower and pre-packaging areas using Enviro-sponges (Fisher Scientific, Pittsburgh, N.J.) that were hydrated in 30 ml of neutralizing buffer (Difco, Becton Dickinson, Sparks, MD). Water tank samples were collected in 50-ml sterile centrifuge tubes containing 1 ml of neutralizing buffer (Difco). All samples were transported to the laboratory on ice and analyzed within 24 h of collection.

Microbial data and additional information obtained at the time of sampling included the field location, fruit variety, date and time of harvest, date and time of processing, visual cleanliness of the fruit, and type of sanitizer (Appendix D).

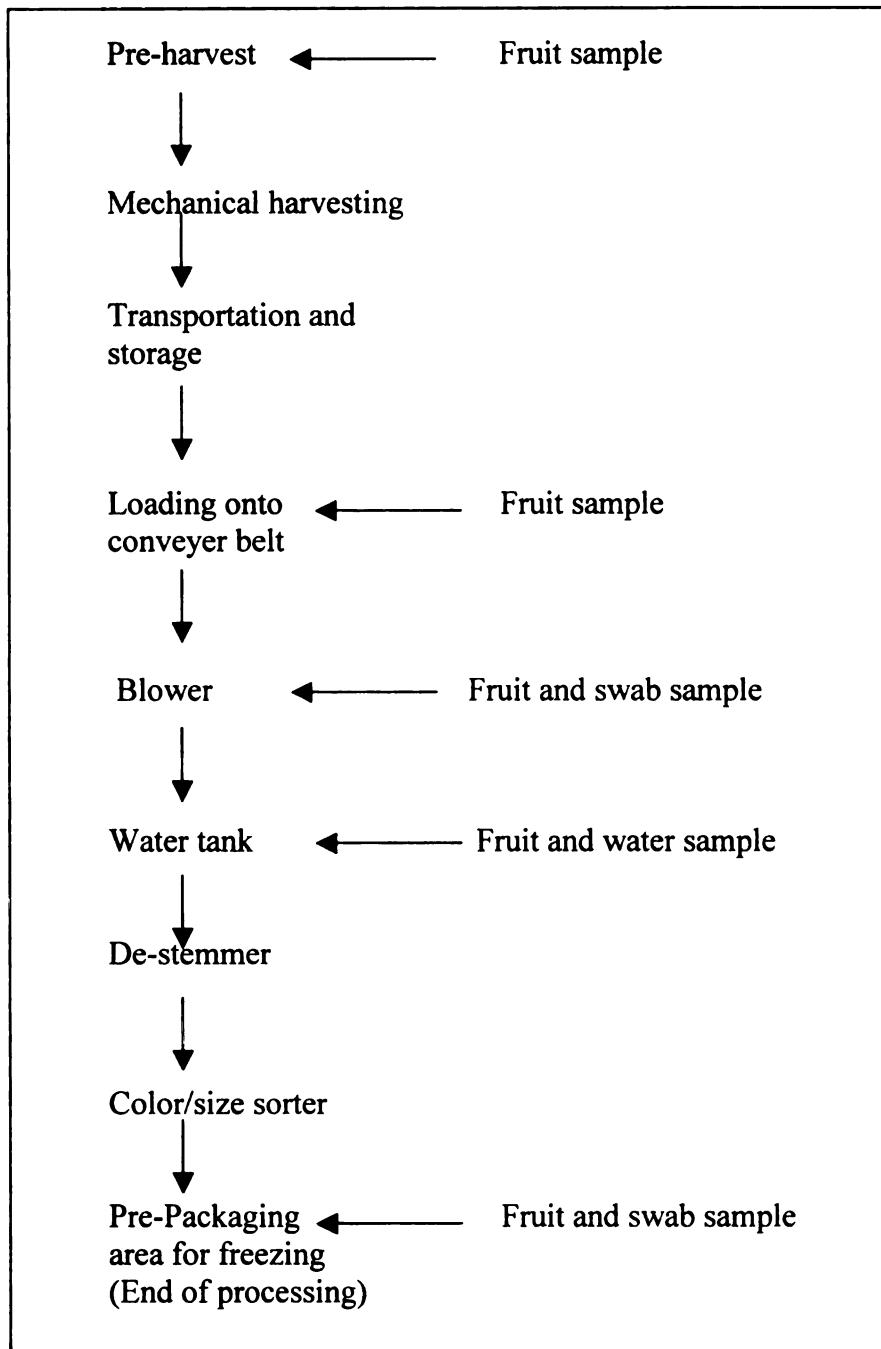


Figure 3.1 Sample collection points for highbush blueberries. Swab and water samples were taken before the start of processing and after the berries were processed.

Microbial Analysis

Blueberry samples (25 g) were placed in sterile 20 x 10 cm polyethylene bags (Whirl-Pack™) containing 100 ml of neutralizing buffer (Difco). The sealed bags were shaken horizontally at 100 rpm for 20 min on a G2 Gyrotory Shaker platform (New Brunswick Scientific Co., Inc., Edison, N.J.) and then pulsed for 1 min using a Pulsifier, (Filataflex Ltd., Ontario, Canada). Thereafter, 1-ml samples were serially diluted in sterile phosphate buffer solution (PBS) (Sigma-Aldrich Co., St. Louis, MO) and spiral-plated (Autoplate 4000 –Spiral Biotech, Exotech, Inc, Gaithersburg, MD) in duplicate on tryptic soy agar (Difco) containing 0.6% yeast extract (Difco) and 100 ppm cyclohexamide (Sigma) (TSAYE-C) for enumeration of mesophilic aerobic bacteria (MAB), and Potato Dextrose Agar (Difco) containing 20 ppm streptomycin (Sigma) and 50 ppm ampicillin (Sigma) (PDA-SA) for enumeration of yeast and mold. Additional 1-ml aliquots were plated on *E. coli*/Coliform Petrifilm™ plates (3M Corp., St. Paul, MN) for quantification of coliforms and *E. coli*. TSAYE-C and *E. coli* /Coliform plates were counted after 48 h of incubation at 37°C. PDA-SA plates were counted after 72 to 96 h of incubation at room temperature (22°C). Environmental sponge and water samples were serially diluted in PBS and similarly examined for the same microorganisms.

Statistical analysis

Analysis of Variance (ANOVA) was done on all microbial count data obtained from fresh / frozen fruit samples, environmental sponge, and water samples using the

Statistical Analysis System (SAS, Version 8, SAS[®] Institute Inc., Cary, NC) to assess microbial changes during processing and frozen storage. Data in the graphs and tables (Appendix B) are means from replicates and significance between means were determined using least significant difference (LSD) test at the 95% confidence level ($P=0.05$).

3.3 RESULTS

Fruit samples

Numbers of MAB, yeasts, molds, coliforms and *E. coli* at pre-harvest varied from 1 to 3.41 logs for the different field locations with highest populations seen near the end of the harvest season (Appendix B, Table 3.1). Microbial populations increased 0.6 to 1.46 logs (Appendix B, Table 3.1) from pre-harvest to post-harvest with this difference statistically significant ($P < 0.05$) (Figure 3.2).

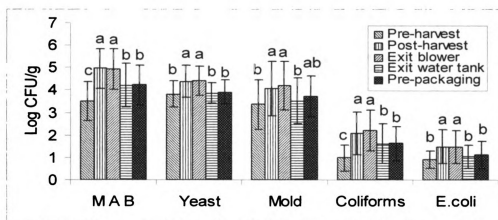


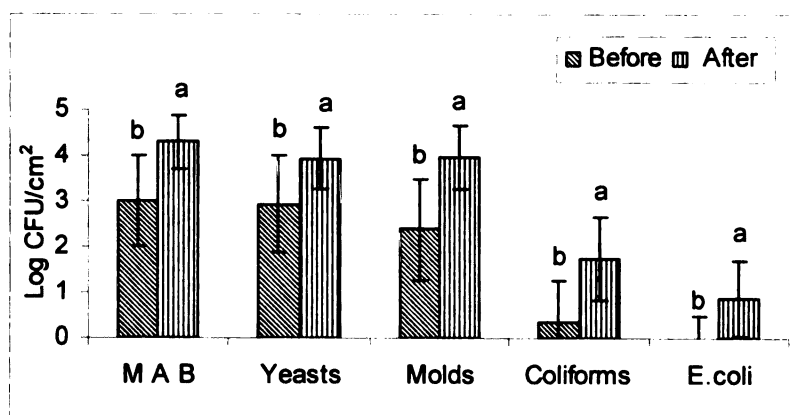
Figure 3.2 Populations of MAB, yeasts, molds, coliforms and *E. coli* (mean $\pm$ std. dev., $n=18$) on blueberries sampled at pre-harvest, post-harvest, blower exit, water tank exit, and pre-packaging. Values with different letters within the same microbial category are significantly different ($P < 0.05$).

Only washing of the fruit significantly reduced ($P < 0.05$) the numbers of microorganisms 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 (Figure 3.2).

Environmental samples

Populations of MAB, yeasts, molds, coliforms and *E. coli* in time from before to after processing the fruits, increased on conveyer belts surface entering the blower area by 1.29, 1.00, 1.58, 1.39 and 0.97 log CFU/cm², respectively (Fig. 3.3 A), and on conveyer belts surface entering the pre-packaging area by 1.17, 1.15, 1.12, 1.06 and 0.66 log CFU/cm² (Fig. 3.3 B), respectively (Appendix B, Table 3.2), with all of these increases being statistically significant ($P < 0.05$).

(A)



(B)

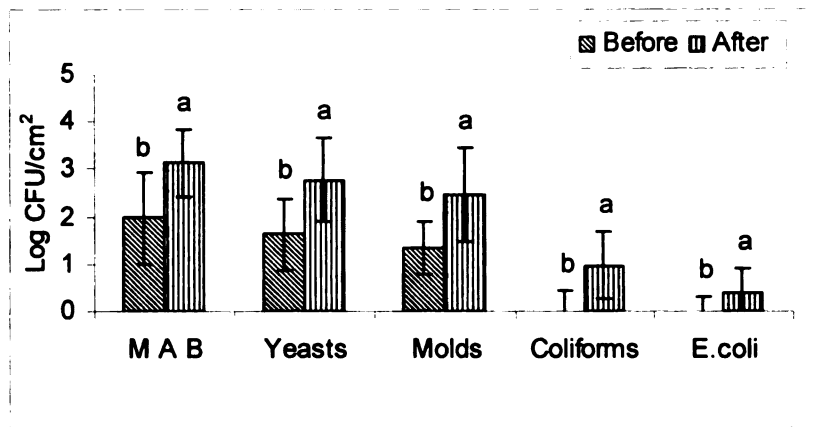


Figure 3.3 Populations of MAB, yeasts, molds, coliforms and *E. coli* (mean $\pm$ std. dev., $n=18$) in time from before to after processing the fruits, increased on conveyer belts surface entering the blower (A) and pre-packaging areas (B). Values with different letters within the same microbial category are significantly different ($P < 0.05$).

Water tank samples

During fruit processing, populations of MAB, yeasts, molds, coliforms and *E. coli* in water tanks increased in time from before to after processing the fruits by 0.56, 0.74, 0.86, 0.41 and 0.32 log CFU/ml (Table 3.3), respectively, with all these increases being statistically significant ($P < 0.05$) (Figure 3.4).

Frozen fruit storage studies

After 3 months at -20°C populations of yeasts, molds, and *E. coli* on blueberries decreased by 0.44, 0.46, and 0.68 log CFU/g (Appendix B Table 3.4), respectively, with all of these reductions being statistically significant ($P < 0.05$) No change was seen for

MAB or coliforms with no reduction in numbers of MAB, yeasts, molds, and *E. coli* evident after 6 months of frozen storage (Figure 3.5).

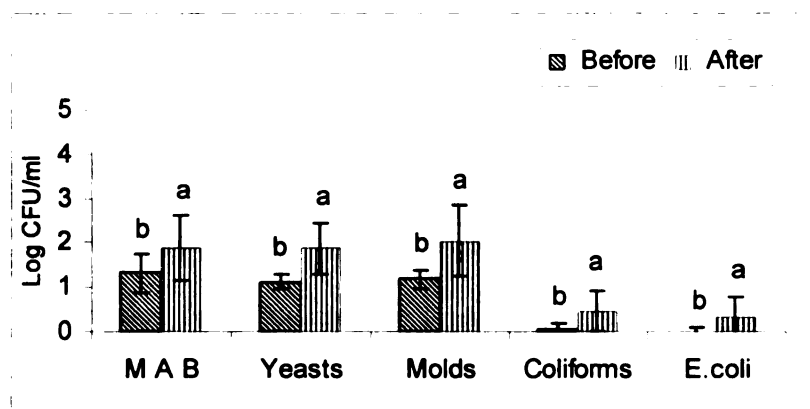


Figure 3.4 Populations of MAB, yeasts, molds, coliforms and *E. coli* (mean $\pm$ std. dev., $n=18$) in water samples (log CFU/ml) collected at the time before and after fruit washing. Values with different letters within the same microbial category are significantly different ($P < 0.05$).

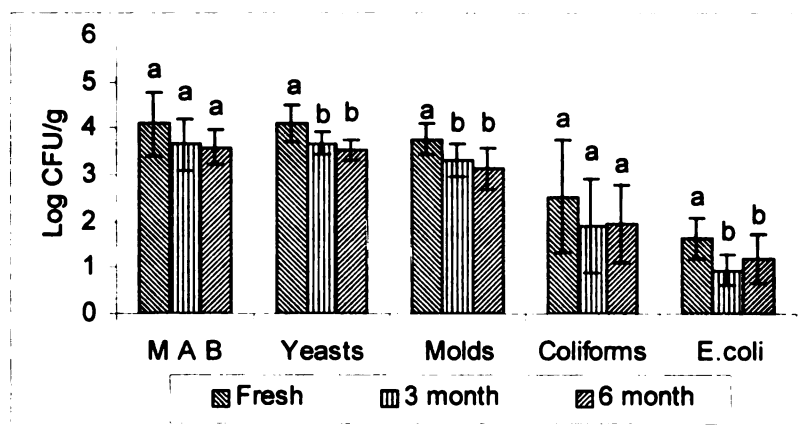


Figure 3.5 Populations of MAB, yeasts, molds, coliforms and *E. coli* (mean $\pm$ std. dev., $n=18$) on blueberries before and after 3 and 6 months of storage at -20°C . Values with different letters within the same microbial category are significantly different ($P < 0.05$).

3.4 DISCUSSIONS

Results showed some consistent trends. Populations of MAB, yeasts, molds, coliforms and *E. coli* at pre-harvest varied widely among field location, increased over the harvest season for these same fields, and increased 1 log or more from pre-harvest to post-harvest. These observations are similar to another microbial survey of Michigan blueberries collected from irrigated and non-irrigated fields during 2002 and 2003 (Schilder Annemieck – unpublished data) with populations of bacteria, yeasts and filamentous fungi increasing 1 to 2 logs between unripened green fruit and the 2nd harvest.

Variations in microbial load between fields and increases within the same field over time suggest multiple pre-harvest sources of contamination including soil, feces, irrigation water, water used to apply fungicides and insecticides, insects, dust, inadequately composted manure, wild animals, and human handlers (Beuchat, 1996). The ability of produce to internalize pathogens, including *Escherichia coli* O157:H7 (Solomon et al., 2002), and *Salmonella* (Guo et al., 2002), from contaminated water was also recently reported. Thus, sources for irrigation water, including wells, should be monitored for microbial levels. Manure needs to be adequately composted before being used as fertilizer. Domestic and wild animals also should be discouraged from entering blueberry fields. Calder et al. (2003) confirmed a multi-district outbreak of hepatitis A (HAV) associated with consumption of fresh blueberries in New –Zealand with the contaminant likely coming from infected field workers or polluted groundwater.

During handpicking, blueberries can become contaminated with bacterial pathogens that can readily survive on the non-acidic blueberry surface.

After handpicking is complete, growers shift to mechanized harvesting for the processed market. The increase in bacteria, yeast and mold during the harvest season is due mainly to microbial spread and multiplication facilitated by mechanical harvesting. According to NeSmith et al. (2002) machine harvesting caused the greatest loss in blueberry firmness (20-30%). Such damaged blueberries are more susceptible to attack from spoilage fungi that are easily spread to non-infected by harvest equipment that can result in serious spoilage and major financial losses during extended storage (Schilder Annemieck – unpublished data).

Pathogens that contaminate the surface of blueberries are mainly influenced by pH, availability of nutrients, and the natural microflora. Blueberries have an internal pH of 3.5 to 4.0 that is sufficiently low to prevent or retard the growth of bacterial pathogens. However, pH can increase from growth of spoilage fungi that could lead to the growth of bacterial pathogens. In two studies, growth of *L. monocytogenes* and *E. coli* O157:H7 on the surface of apples containing *Glomerella cingulata* was attributed to an increase in pH from 4.7 to 7.0 (Conway et al., 2000; Riordan et al., 2000). In a study with healthy and soft-rotted produce, the incidence of *Salmonella* on produce affected by bacterial soft rot (*Erwinia* and *Pseudomonas*) was twice that of the control samples (Weissinger and Beuchat, 2000).

In addition to contamination during mechanical harvesting, the higher microbial load on blueberries at post-harvest also likely resulted from a delay in processing. Upon arrival at the processing facility, most blueberries were held for 12 hours or more before processing began. According to Jackson et al. (1998), microbial populations on fresh blueberries were ~1 log higher after 45 h of storage at temperatures up to 26°C.

Microbial populations on blueberries decreased less than 1 log after exposure to ~ 10 to 200 ppm chlorine in the water tank. The limited efficacy of chlorinated water may be due to short contact times, which were typically less than 1 minute. Increasing amounts of organic matter in the wash water also decreased the effectiveness of chlorine as evidenced by an increase in the levels of microorganisms in the water tank over time with additional contaminants transferred from the conveyor belt to the berries after washing. The fact that blueberries generally contain higher microbial levels after processing than before harvest demonstrate the lack of efficacy in reducing microbial loads in industry settings.

Microbial populations on blueberries decreased less than 0.7 logs after either 3 or 6 months of storage at -20°C , demonstrating that maintaining freezing and maintaining berries in the freezer for extended storage periods is not an effective microbial reduction strategy. Decades ago, Schmidt-Lorenz (1963) and Schmidt-Lorenz and Gutschmidt (1969) found that certain bacteria were still able to grow to relatively high numbers at -7.5°C . The authors also found that the growth limit for yeast was -10°C . Microbial growth or metabolic activity also has been reported in permafrost bacteria at -10°C (Gilichinsky et al., 1995) with the temperature limit for bacterial growth in frozen food now generally considered to be -8°C (Geiges, 1996). In a bacterial population study of Siberian permafrost, Rivkina et al. (2000) reported doubling times of 1 (5°C) to 20 days (-10°C) and 160 days (-20°C). Thus, microbiological quality of blueberries remains an issue during frozen storage.

CHAPTER 4

EFFICACY OF CHLORINE DIOXIDE GAS SACHETS FOR ENHANCING THE MICROBIOLOGICAL QUALITY AND SAFETY OF BLUEBERRIES

ABSTRACT

In response to increasingly stringent microbial specifications being imposed by purchasers of blueberries, chlorine dioxide (ClO₂) gas generated by a dry chemical sachet was 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 ~10⁶ CFU/g and exposed to ClO₂ (4 mg/L, 0.16 mg/g) for 12 h in a sealed 20 liter container (99.9% RH) at ~22°C (3 replicates). After gassing, blueberries (25 g) were diluted 1:5 in neutralizing buffer, pulsed 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 ClO₂ (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, ClO₂ sachets may provide a simple, economical and effective means of enhancing the microbial shelf-life and safety of fresh blueberries.

4.1 INTRODUCTION

Microbial safety and quality are critical concerns to all segments of the blueberry marketing chain. A single widely publicized outbreak of a blueberry-related illness would negatively impact the entire industry. Although the blueberry industry in the U.S. has not yet experienced such event, one recent multi-district outbreak of hepatitis A (HAV) associated with the consumption of fresh blueberries was reported in New-Zealand with these berries likely contaminated from infected food handlers or fecally polluted groundwater (Calder et al., 2003).

Most Michigan blueberries are processed and frozen rather than fresh marketed for economic reasons (NASS/USDA, 2002). Some buyers of frozen blueberries now demand microbial testing in order to ensure that the product does not exceed their specifications for mesophilic aerobic bacteria (MAB), coliforms, yeasts and molds. These buyers also have ‘zero tolerance’ policies for human pathogens such as *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella*. Microbial specifications vary considerably from buyer to buyer with many appearing arbitrary, whereas others reflect specific uses. Pie manufacturers who use blueberries with high yeast populations frequently find that their pies do not “set up” because yeast growth results in enzymatic breakdown of starch and other stiffening agents. In one Michigan study, most bacteria, yeasts and molds isolated from fresh blueberries were found to produce amylase which

facilitates starch breakdown with some isolates also producing pectinase and cellulase that can breakdown the fruit cell walls (Schilder Annemieck – unpublished data). These enzymatic concerns can lead to considerable financial loss and frequently a change in blueberry suppliers.

For blueberry industry, these microbial standards are difficult to meet since the level of MAB, yeasts and molds and other contaminants can vary widely between fields, seasons, and the time of harvest depending on factors such as moisture, temperature, insect level, plant health, and harvest management practices with microbial populations peaking at the end of the harvest season (Schilder Annemieck – unpublished data).

Given the relative ineffectiveness of chlorinated water in reducing microbial levels on blueberries during processing, new microbial reduction strategies are needed in order to meet the new microbial standards.

Chlorine dioxide is a yellow-green gas generated through oxidation when concentrated hydrochloric acid is added to sodium chlorite. When done in water, the end result is an aqueous chlorine dioxide solution. Chlorine dioxide is increasing in popularity due to the numerous advantages over chlorine: greater effectiveness over a wider pH range, not affected by high levels of organic matter, no rapid dissociation in water, an oxidation capacity 3 times greater than chlorine (White, 1972), does not react with organic compounds to produce carcinogenic by-products, formation of by-products that are less toxic and 3-5 times lower compared with chlorine (Richardson et al., 1998), and a broader germicidal activity including spores (Richardson et al., 1994), viruses (Sobsey, 1988) and protozoa as *Cryptosporidium* and *Giardia* oocysts (Finch et al., 1997) that are more resistant to chlorine. Thus, chlorine dioxide is used today in the

disinfection of water, air, and was the principal agent used in the decontamination of buildings in the US after the 2001 anthrax attacks.

The Food and Drug Administration (FDA 2005) amended the food additive regulation “to allow the safe use of chlorine dioxide as an antimicrobial agent to wash fruits and vegetables that are not raw agricultural commodities in an amount not to exceed 3 ppm residual chlorine dioxide that shall be followed by a potable water rinse or by blanching, cooking, or canning.” The U.S. Environmental Protection Agency (EPA) approved use of chlorine dioxide as a disinfectant for potable water treatment limiting the residual to 1 ppm (U.S. Federal Register, 2000).

Studies have shown gaseous chlorine dioxide to be effective in microbial reductions including enteric pathogens on apple (Du et al., 2002; Du et al., 2003), green peppers (Han et al., 2000; Han et al., 2001), lettuce (Lee et al., 2004), tomato, cabbage, carrots, peaches (Sy et al., 2005a) strawberries (Han et al., 2004; Sy et al., 2005b), raspberries and blueberries (Sy et al., 2005b) in laboratory conditions. Chlorine dioxide gas proved also to be effective as a sanitizer in food processing plants by reducing the > 4 log populations of yeast and molds on stainless steel surface of the tanks used for aseptic juice storage below detectable limits (Han et al., 1999) and in libraries by effectively controlling the spread of molds (Weaver-Meyers et al., 1998).

This study assessed the efficacy of gaseous chlorine dioxide for inactivating *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, mesophilic aerobic bacteria, coliforms, *E. coli*, yeasts and molds on the surface of blueberries in industrial conditions before processing.

4.2 MATERIAL AND METHODS

EXPERIMENTAL DESIGN

Two different studies were conducted to assess the efficacy of ClO₂ gas for microbial reductions on blueberries - (a) a pilot study in which fresh blueberries were inoculated to contain various foodborne pathogens, spoilage yeasts or molds and then exposed to ClO₂ gas for 12 h in sealed 20 L buckets and -(b) a pallet study in which 30 lugs of blueberries (~272 kg) were placed on a pallet and exposed to ClO₂ gas for 12 h under a tarp.

A. PILOT STUDY

Blueberries

Blueberries (*Vaccinium corymbosum*) variety 'Bluecrop' were obtained from a local retailer and stored at 4°C for a maximum 2 days before inoculation. Before inoculation the fruit was tempered for 1 to 2 h at room temperature (22±1°C).

Bacterial Strains

Three strains of *Escherichia coli* O157:H7 (AR, AD305, AD317), and *Listeria monocytogenes* (CWD 95, CWD102, CWD184) were previously obtained from Catherine W. Donnelly (Dept. of Nutrition and Food Sciences, University of Vermont, Burlington, VT). Three additional *Salmonella* strains (*S. Typhimurium* H 3380, *S. Heidelberg* F5038 BG1, and *S. Enteritidis* H3502) were obtained from V.K. Juneja (USDA-ARS-ERRC, Wyndmoor, PA). All strains were maintained at -80°C in trypticase soy broth (TSB) (Difco, Becton Dickinson, Sparks, MD) containing 10% (v/v) glycerol. Individual strains

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were separately activated by transferring a loop of frozen stock culture into 9 ml of sterile TSB containing 0.6 % (w/v) yeast extract (TSBYE) (Difco) followed by 18-24 h of incubation at 35°C and then subjected to an identical transfer in 20 ml of TSBYE before use.

Yeasts and Molds

Five spoilage molds (*Colletotrichum* sp., *Epicoccum* sp., *Cladosporium* sp., *Phoma* sp., and *Alternaria* sp.) and yeasts (*Aureobasidium* sp., *Bullera* sp., *Cryptococcus* sp., *Sporidiobolus* sp., and *Filobasidium* sp.) originally isolated from blueberry fields in Michigan were obtained from A.C. Schilder (Dept. of Plant Pathology, Michigan State University, E. Lansing, MI). All yeasts and molds were maintained at -80°C in TSB containing 10% (v/v) glycerol. Individual strains were separately activated by transferring a loop of frozen stock culture onto duplicate plates of Potato Dextrose Agar (PDA) (Difco) containing 20 ppm streptomycin (Sigma-Aldrich Co., St. Louis, MO) and 50 ppm ampicillin (Sigma) (PDA-SA) with the yeasts and molds incubated 3 - 4 and 10 - 12 days at 26°C, respectively, before use.

Preparation of the inoculum

The cultures of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* were harvested by centrifugation (Sorvall Super T21, Newtown, CT) at 7,000 rpm for 10 min at 4°C and re-suspended in equal volumes of sterile phosphate buffer solution (PBS). Suspensions of each strain containing approximately equal populations (10^9 CFU/ml) were combined to generate three separate 3-strain cocktails (~60 ml each) of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella*. Populations in these cocktails were

determined by plating appropriate serial dilutions in PBS on Sorbitol McConkey Agar (SMAC) (Difco), Modified Oxford Agar (MOX) (Difco), and McConkey Agar (MAC) (Difco), respectively. Two separate 5-strain 100-ml cocktails of yeasts and molds containing approximately equal populations (10^8 CFU/ml) were obtained by washing the previous PDA-SA plates with 20 ml of PBS. Yeast and mold populations in these cocktails were determined by surface plating appropriate serial dilutions on PDA-SA followed by 3 - 4 and 10 - 12 days of incubation at 26°C, respectively.

Inoculation of blueberries

Five 125-g blueberry samples were placed in separate 25 x 20 cm sterile polyethylene bags (Whirl-Pack™, Fisher Scientific, Pittsburgh, N.J.) containing 100 ml of each of the five cocktails and then gently swirl-agitated at 100 rpm for 20 min on a G2 Gyrotory Shaker platform (New Brunswick Scientific Co., Inc., Edison, N.J.). These inoculated samples containing $\sim 10^6$ CFU/g of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, yeasts or molds were then air-dried under laminar flow in a biosafety cabinet for 2 h, stored overnight at 4°C and finally re-dried under laminar flow for 2 h before use.

Chlorine Dioxide Exposure

Five 100-g samples of inoculated blueberries were placed in separate half pint plastic clamshell containers and in a 20 L bucket. The berries were exposed to ClO₂ gas (4 mg/L, 0.16mg/g fruit) in the sealed 20L bucket (Figure 4.1) for 12 h at $\sim 22^\circ\text{C}/99.9\%$ RH. Chlorine dioxide gas was generated inside the bucket using a 20-g commercial ClO₂ sachet (ICA TriNova, LLC Forest Park, GA). The ends of the sachet were pulled to

remove the spine clip; granules from the two compartments of the sachet were fully mixed and pooled at one end, after which the sachet was placed in the bottom of the bucket. High humidity was maintained by placing a Petri dish containing 20 ml of SDW on the bottom of the bucket next to the inoculated fruit and ClO_2 sachet. Chlorine dioxide gas was circulated inside the sealed bucket using a brushless 12 VDC cooling fan (5 by 5 by 1 cm) (Model D50SM, RadioShack, Fort Worth, TX) that was attached to the underside of the bucket lid. Temperature (22°C) and R.H. were continuously monitored using a Thermo-Hygrometer (Model Traceable[®], Fischer Scientific) that was sealed in the lid of the container.

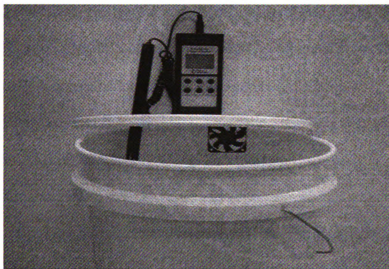


Figure 4.1 Chlorine dioxide gas system used to treat inoculated blueberries in Pilot study. This system consisted of a 20 L bucket, a Petri dish containing SDW (inside), a lid with a fan and an attached Thermo-Hygrometer. Inoculated fruit was placed in the container and exposed to ClO_2 gas (4 mg/L, 0.16mg/g fruit) for 12 h at $22^\circ\text{C}/99.9\%$ RH.

Microbial Analysis

After treatment, fruit samples (25 g) were placed in sterile 20 x 10 cm polyethylene bags (Whirl-Pack™) containing 100 ml of neutralizing buffer (Difco). The sealed bags were shaken horizontally at 100 rpm for 20 min on a G2 Gyrotory Shaker platform and then pulsed for 1 min using a Pulsifier (Filtaflex Ltd., Almonte, Ontario, Canada). Samples of neutralizing buffer (1 ml) were serially diluted in PBS and spiral-plated (Autoplate 4000–Spiral Biotech, Exotech, Inc, Gaithersburg, MD) in duplicate on SMAC, MOX, MAC, and PDA-SA to enumerate *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, and yeasts and molds, respectively. SMAC, MOX, and MAC plates were counted after 48 h of incubation at 37°C, whereas PDA-SA plates were counted after 72 to 96 h of incubation at room temperature (22°C).

B. PALLET STUDY

Blueberries

Fresh mechanically harvested blueberries from different field locations at the same grower were obtained through the Michigan Blueberry Growers Association (Grand Junction, MI). Thirty lugs of blueberries (~ 9.1 kg/lug) were stacked on 4 x 4 ft wooden pallets (~ 272 kg fruit/pallet) at a blueberry processing facility with 5 lugs/level and 6 levels/pallet (Figure 4.2)



Figure 4.2 Pallet study with gassed (tarpred) and un-gassed pallets (control).

Chlorine Dioxide Exposure

Each of the six replicated experiments included one gassed (tarpred) and one un-gassed pallet (control) that were held for 12 h at $\sim 12\text{--}14^\circ\text{C}$. Chlorine dioxide gas was generated using three 3-kg sachet containers (ICA TriNova) according to the manufacturer's instructions: The sachets were removed from their containers and opened after which their content were poured back into the container and thoroughly mixed by shaking. Two containers per pallet were then placed on the top lugs with their lids opened to allow ClO_2 gas to escape. Two 10-cm diameter flexible hoses ~ 2 meters in length containing ventilating fans were run along two sides of each pallet from bottom to top to circulate ClO_2 through the pallet (Figure 4.2). The pallets were tarpred with a plastic sheet, sealed, and exposed to ClO_2 gas for 12 h. According to the manufacturer 1 g

of this mixture generated 4 mg of ClO₂ over a 12 h period with the three 3-kg sachets generating a total of 36,000 mg ClO₂ gas in each 2000 L pallet, giving a final ClO₂ estimated concentration of 18 mg/L or 0.13mg/g fruit.

Pallet samples

Before and after 12 h of gassing, blueberry samples (500-g) were collected from the gassed and ungassed pallets. Each pallet contained 30 lugs (5 lugs x 6 levels) (Figure 4.3). Samples were taken from 25 surface and bottom collection points (5 surface and 5 bottom collection points/lug) and then composited to obtain one surface and one bottom sample for each of the six levels. All samples were placed in individually bagged clamshell containers, transported to the laboratory on ice and analyzed within 24 h of collection.

Microbial analysis

All blueberry samples (100 g each) were placed in sterile 25 x 20 cm Whirl-Pack™ bags containing 200 ml of neutralizing buffer, shaken and pulsified as previously described. Appropriate dilutions in PBS were spiral-plated in duplicate on trypticase soy agar (Difco) containing 0.6% (w/w) yeast extract and 100 ppm cyclohexamide (Sigma-Aldrich Co., St. Louis, MO) (TSAYE-C) for enumeration of mesophilic aerobic bacteria (MAB), and PDA-SA for enumeration of yeasts and molds. Additional 1-ml aliquots were plated on *E. coli* /coliform count plates (Petrifilm™, 3M Corp., St.Paul, MN) for quantification of coliforms and *E. coli*. TSAYE-C and *E. coli* / coliform count plates

were counted after 48 h of incubation at 37°C, whereas PDA-SA plates were counted after 72 to 96 h of incubation at room temperature (22°C).

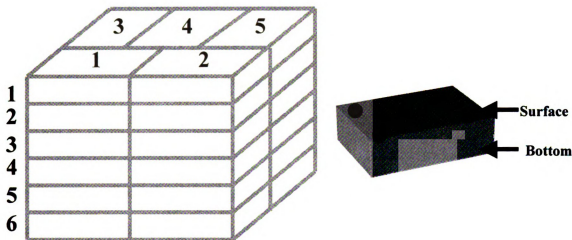


Figure 4.3 Blueberry pallet (left) and lug (right). Every lug has 5 sampling points from the surface (shown) and 5 sampling points at the bottom (not shown). The pallet has 6 levels with 5 lugs per level. Surface and bottom samples were collected from each lug at each level (25 surface and 25 bottom samples per level) and then composited to obtain 1 surface and 1 bottom sample for each of the 6 levels.

Statistical analysis

Analysis of Variance (ANOVA) was done on all microbial count data obtained from the pilot and pallet studies using the Statistical Analysis System (SAS, Version 8, SAS[®] Institute Inc., Cary, NC). Data in the graphs and tables (Appendix B) are means from replicates and significance between means were determined using least significant difference (LSD) test at the 95% confidence level ($P=0.05$).

Sensory Analysis

Uninoculated blueberries from Grand Junction, MI, variety 'Bluecrop' were exposed to 0.19 mg ClO₂ gas /g fruit under the same conditions as for inoculated fruit (12 h at 22°C/99.9% R.H.). Gassed and un-gassed samples (control) were few seconds rinsed in tap water, air-dried for 1 h, dispensed into individual sample cups with lids (15- 20 berries each) and stored aerobically overnight at 4°C before being evaluated by 110 panelists for various sensory attributes. Panelists were presented with a legal consent form (Appendix C), individual trays containing coded samples, and a set of instructions regarding evaluation. The samples were analyzed using a Hedonic scale with "like extremely" (9) to "dislike extremely (1) for the following sensory attributes: appearance, aroma, texture, flavor, and overall acceptability. The data were analyzed using SIMS 2000 computer software program at a significance level of $P < 0.05$.

4.3 RESULTS AND DISCUSSION

Blueberries were inoculated by dipping rather than spotting to more closely mimic contamination during irrigation, mechanical harvesting, and processing including direct contact with belts, blowers and water tanks. Surface washing was chosen as the method to remove bacteria, yeasts and molds from the surface of blueberries rather than homogenizing. Homogenizing blueberries in a diluent would decrease the pH to about 4.0, thereby hampering recovery of any injured cells. When Sy et al. (2005) compared washing and stomaching for recovery of *Salmonella* from inoculated blueberries, populations were 10-fold lower after stomaching. Han and Linton (2004) also reported that stomaching was less effective than washing for recovery of *L. monocytogenes* from

inoculated strawberries. Although acid tolerant, *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* are unable to survive in foods at pH < 4.0 (D'Aoust, 2000). Pulsification by ultrasound after surface washing further improved release and subsequent recovery of microorganisms from the surface without rupturing the fruit and decreasing the pH as occurred after stomaching.

A. PILOT STUDY

Initial populations of *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, yeasts and molds on the inoculated berries before treatment were 6.46, 6.38, 6.35, 5.99, and 6.22 logs CFU/g, respectively. Significant ($P < 0.05$) microbial reductions of 3.94, 3.62, 4.25, 3.10, and 3.17 log CFU/g, respectively, were achieved after exposing the inoculated blueberries to 4 mg/L, 0.16mg/g fruit ClO₂ gas in a sealed bucket for 12 h at 22°C/99.9% RH (Appendix B, Table 4.1). High relative humidity (99.9 %) was maintained to enhance the lethality of chlorine dioxide gas against bacteria, yeasts and molds, with Han et al. (1999) also reporting increased efficacy at higher relative humidity. In later work by Han et al (2001, 2000) *L. monocytogenes* and *E. coli* O157:H7 populations decreased 3.5 and 6.4 logs on the surface of injured green peppers after exposure to 3 and 1.2 mg/L chlorine dioxide gas for 30 min, respectively. When the skin, calyx and stem scar areas of apples were surface-inoculated with *L. monocytogenes* and exposed to 4 mg/L chlorine dioxide gas for 10 min at 21°C/90% RH, Du et al. (2002) obtained higher reductions of 5.5, 3.2, and 3.6-logs, respectively. Exposing *Salmonella*-inoculated blueberries to 4.1 to 8 mg/L chlorine dioxide gas / 75-95% RH for 30 to 120 min reduced *Salmonella* up to 3.7 logs

with populations of inherent yeasts and molds decreasing as much as 2.5-logs (Sy et al., 2005).

Differences in the surface structure and microbial attachment sites on green peppers, apples and blueberries are likely responsible for the different biocidal efficacies seen in these studies. Waxes on the surface of apples contain alcohols, morpholine and surfactants that may enhance the penetration of sanitizers resulting in higher microbial reductions (Kenney and Beuchat, 2002). The wax on the surface of blueberries is composed mainly of β -diketone that produces a dense network of interlocking branched rodlets or closed tube-like structures (Freeman et al., 1979). The hydrophobicity of β -diketone also hinders penetration of aqueous-based sanitizers leading to decreased efficacy.

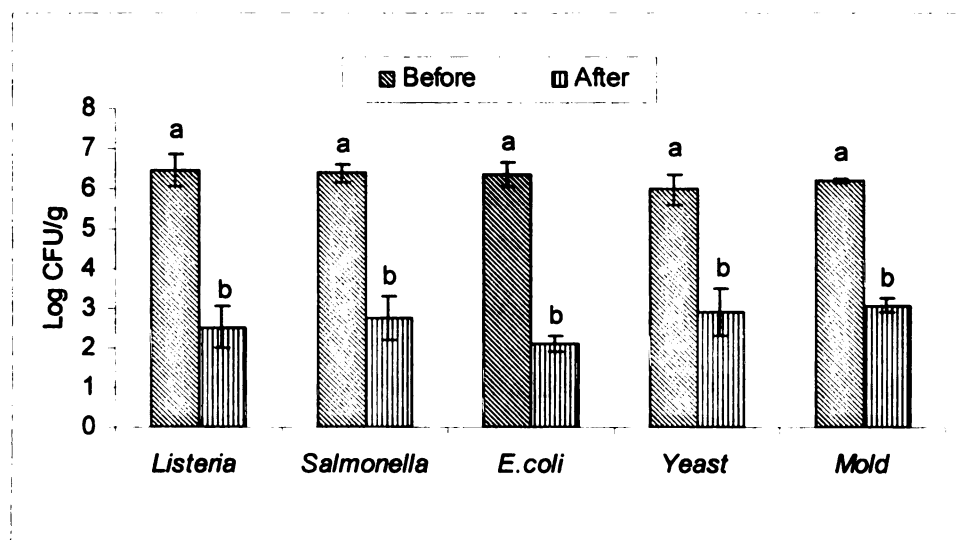


Figure 4.4 Populations of *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, yeasts and molds (mean $\pm$ std. dev., n=3) on inoculated blueberries before and after exposure to 4 mg/L, 0.13mg/g ClO₂ gas in a sealed 20 L bucket for 12 h at 22°C/99.9% RH. Values with different letters within the same microbial category are significantly different ($P < 0.05$).

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B. PALLET STUDY

Initial populations of MAB, yeasts, molds, coliforms, and *E. coli* on naturally contaminated untreated blueberries were 4.03, 4.32, 4.57, 1.12, and 0.51 logs CFU/g, respectively (Appendix B, Table 4.2). After 12 h of storage, significant ($P < 0.05$) microbial growth was observed in the un-gassed pallets with populations of MAB, yeasts, molds, coliforms, and *E. coli* increasing 0.69, 0.36, 0.11, 1.00 and 0.50 logs, respectively. However, after 12 h of storage under the same conditions, significant ($P < 0.05$) reductions of 2.33, 1.63, 0.48, 1.47, and 0.52 log CFU/g, respectively, were seen between the gassed and un-gassed pallets. Overall, reductions for bacteria, yeasts and molds were 1 to almost 3 logs higher on inoculated as compared to uninoculated blueberries. One reason for this decrease in efficacy is likely related to the lower chlorine dioxide concentration in the pilot (0.16 mg/g) as opposed to the pallet study (0.13 mg/g). Decreased efficacy of chlorine dioxide gas in the pallet size study could also be related to the use of uninoculated blueberries. Compared to bacteria, populations of yeasts and molds naturally present on blueberries were less susceptible to chlorine dioxide gas. These findings are in agreement with Rodgers et al. (2004) who reported reductions of up to 5 log CFU/g for *L. monocytogenes* and *E. coli* O157:H7 compared to 1.5 log CFU/g for yeasts and molds when apples were treated with aqueous solutions of 3 and 5 ppm chlorine dioxide. The lower reductions for coliforms and *E. coli* on gassed uninoculated blueberries can be explained by the relatively low initial populations with numbers decreasing below the limit of detection (0.48 log CFU/g) after gassing.

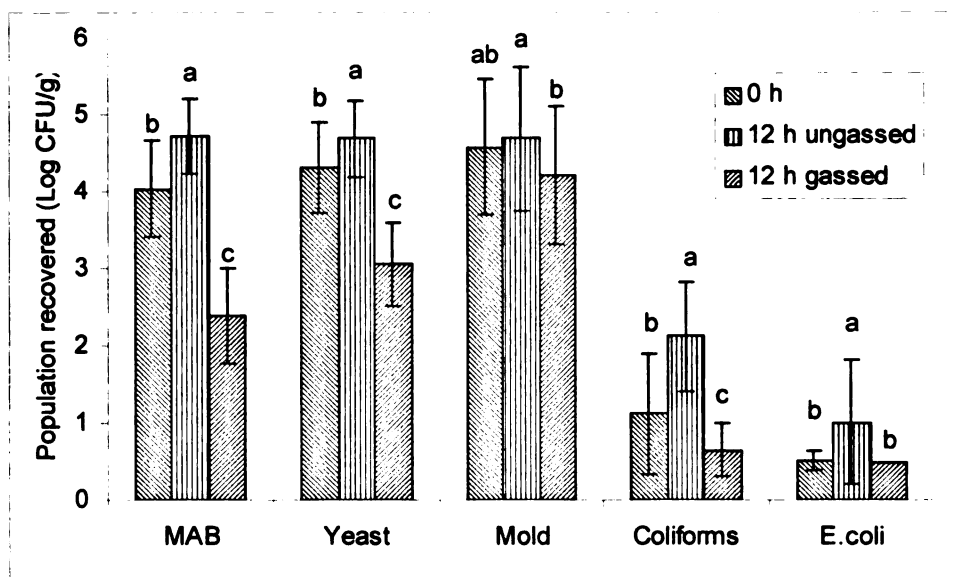
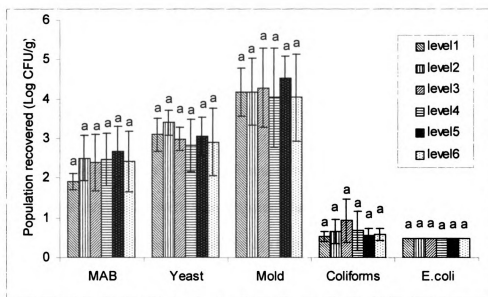


Figure 4.5 Populations of MAB, yeasts, molds, coliforms, and *E. coli* (mean $\pm$ std. dev., $n=6$) recovered from blueberries initially (0 h) and from gassed and un-gassed fruit after 12 h of storage. Blueberry pallets containing fruit harvested from the same grower were stored under the same conditions for 12 h at 12 to 14°C. Gassed pallets were exposed to 18 mg/L (0.13mg/g) ClO_2 gas. The limits of detection were 1.78 (MAB, yeast, and mold) and 0.48 log CFU/g (coliforms, and *E. coli*). Values with different letters within the same microbial category are significantly different ($P < 0.05$).

Uniformity in the reduction of MAB, yeasts, molds, coliforms, and *E. coli* on palletized blueberries at different pallet levels (Figure 4.6 A) and sample locations within the lugs (Figure 4.6 B) using ClO_2 gas was also evaluated. After gassing, no significant differences ($P > 0.05$) in microbial counts were seen between pallet levels 1 to 6 and, except for MAB, no significant differences were evident between the bottom and top surface of the lugs. Based on the analysis of variance, regarding gassed pallets levels (1 to 6) and positions (surface and bottom) no interactive effect resulted on the fruit microbial load (Appendix B, Table 4.3). These findings demonstrate uniform dispersion and penetration of chlorine dioxide gas throughout the pallets. Regarding quality of the

gassed blueberry pallets, no visible changes in the fruit were evident; however, some bleaching of the blueberry leaves was observed.

(A)



(B)

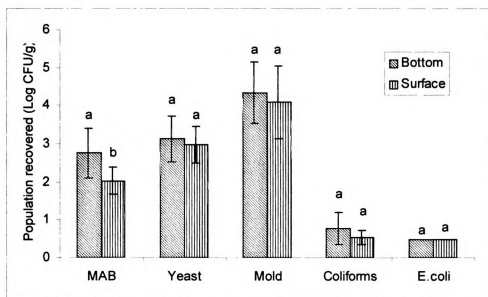


Figure 4.6 Populations of MAB, yeasts, molds, coliforms, and *E. coli* (mean $\pm$ std. dev., n=6) recovered from gassed blueberry pallets at different pallet levels (A) and between the bottom and top surface of lugs from the same pallet level (B). Blueberry pallets were stored under the same conditions at 12 to 14°C, exposed to 18 mg/L (0.13mg/g) ClO₂ gas, tarped and sealed for 12 h. The limits of detection were 1.78 (MAB, yeast, mold) and 0.48 (coliforms, *E. coli*) logs CFU/g. Values with different letters within the same microbial category are significantly different ($P < 0.05$).

Sensory Analysis

No significant ($P < 0.05$) differences regarding appearance, aroma, texture, flavor, or overall acceptability were seen between blueberries exposed to 0.19 mg/g ClO₂ gas for 12 h and ungassed (control) blueberries (Figure 4.6). Similarly, Sy et al., 2005 reported no significant changes in sensory attributes including appearance, color, aroma, and overall quality between blueberries exposed to 4.1 mg/L chlorine dioxide gas and ungassed blueberries after 0 and 3 days of storage. However, in the same study the treated samples were ranked significantly higher for overall quality and aroma after 7 and 10 days of storage, respectively.

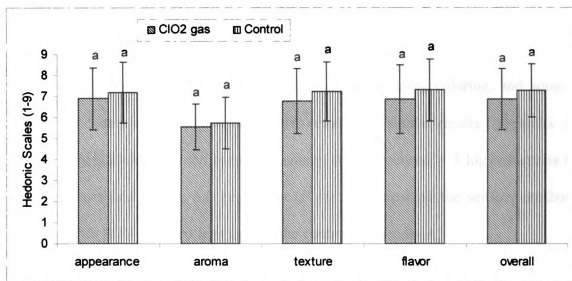


Figure 4.7 Average consumer (n=110) ratings for appearance, aroma, texture, flavor, and overall acceptability of treated (0.19 mg/g ClO_2 /12 h) and untreated (control) blueberries.

4.4 CONCLUSIONS

The results of these studies indicate that chlorine dioxide gas can be used by the blueberry industry to reduce microbial populations before processing. This pre-processing microbial reduction strategy will help blueberry processors to better meet current the microbial standards that are being imposed by blueberry purchasers, without changes in current processing technology. Gassing of fruit can be done when the fruit is stored at the processing facility (up to 18 h). In most blueberry processing operations, the sole microbial reduction step involves brief passage of the berries through a tank of sanitizer solution. The water tanks were originally employed to separate buoyant green unripened from mature blueberries but sanitizers are also used to decrease the microbial load on the berries. In most processing facilities the fruit is typically exposed to a chlorine-based sanitizer for less than one minute. These wash treatments are only marginally effective in reducing the number of microbial contaminants on the surface of

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blueberries. Treatment of blueberries with 100 ppm chlorine for 5 min reportedly reduced the population of bacteria, yeast and mold by 0.83, 0.77, and 0.61 log CFU/g, respectively (Crowe et al., 2005). Our preliminary work using chlorine, and aqueous chlorine for 5 min on inoculated blueberries resulted in similar results (Appendix A). Organic fatty acids used in the same preliminary work produced > 3 log reductions for bacteria, yeasts and molds, but negatively ($P < 0.05$) impacted the sensory attributes compared with SDW-washed fruit used as the control (Appendix A).

To answer to the actual needs of the industry, this study evaluated gaseous chlorine dioxide for its effectiveness in inactivation of the human pathogens *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*, as well as mesophilic aerobic bacteria, coliforms, *E. coli*, yeasts and molds known for fruit spoilage. Small-scale experiments done on inoculated blueberries yielded reductions of > 3 logs for known human pathogens and spoilage yeasts and molds.

Pallet size studies using ~ 272 kg of uninoculated blueberries/pallet (industrial scale) were done to determine the effectiveness of gaseous chlorine dioxide (18 mg/L, 0.13mg/g) on naturally occurring MAB, yeasts, molds, coliforms, and *E. coli* with significant ($P < 0.05$) reductions achieved for all microbial categories compared to the ungassed control pallets.

Overall, reductions of bacteria, yeasts and molds were higher on inoculated (Pilot study) than on uninoculated blueberries (Pallet study). One reason would be the use of a lower gas concentration in the pallet (0.13 mg/g) as opposed to the pilot study (0.16 mg/g).

Sensory analysis of blueberries exposed to chlorine dioxide (0.19 mg/g fruit) revealed no significant ($P < 0.05$) differences compared to ungassed (control) blueberries.

These results are in agreement with those of Sy et al. (2005) and suggest that chlorine dioxide gas can be used as to both enhance the microbial quality of blueberries and meet the present microbial standards being imposed by purchasers.

5. CONCLUSIONS

Microbial contamination on highbush blueberries before, during, and after processing from the 2003-2004 survey demonstrated that populations of MAB, yeasts, molds, coliforms and *E. coli* varied widely among field location without a consistent correlation to field condition or handling procedure and increased over the harvest season for the same fields, and from pre-harvest to post-harvest due mainly to microbial spread and multiplication facilitated by mechanical harvesting, and from a 12-h or more delay in processing, respectively. Microbial populations on the blueberries were not efficiently reduced after exiting the chlorinated water tank (~ 10 to 200 ppm chlorine). Reduced efficacy of chlorinated water is due to the short contact times that were typically less than 1 minute. Increasing amounts of organic matter in the wash water also decreased the effectiveness of chlorine as evidenced by an increase in the levels of microorganisms in the water tank over time with additional contaminants transferred from the conveyor belt to the berries after washing. The fact that blueberries generally contained higher microbial levels after processing than before harvest demonstrates the lack of efficacy in reducing microbial loads in industry settings. Microbial reductions of less than 0.7 logs after either 3 or 6 months of storage at -20°C confirms that freezing is also not an effective microbial reduction strategy for blueberries.

Treating blueberries with 100 ppm chlorine for 5 min reportedly reduced the population of bacteria, yeast and mold by less than 1 log CFU/g (Crowe et al., 2005). Our preliminary work using chlorine and aqueous chlorine dioxide for 5 min on inoculated blueberries resulted in similar reductions (Appendix A). Organic fatty acids used in the same preliminary work produced > 3 log reductions for bacteria, yeasts and molds, but negatively ($P < 0.05$) impacted the sensory attributes compared with SDW-washed fruit used as the control (Appendix A).

To answer to the actual needs of the industry, gaseous chlorine dioxide was evaluated for its effectiveness in inactivation of the foodborne pathogens *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*, as well as mesophilic aerobic bacteria, coliforms, *E. coli*, yeasts and molds known for fruit spoilage. Small-scale experiments on inoculated blueberries (0.16 mg/g ClO₂ gas) yielded reductions of > 3 logs for known human pathogens and spoilage yeasts and molds. When 600 lb pallets of uninoculated blueberries were exposed to 0.13 mg/g ClO₂ gas for 12 h, populations of naturally occurring MAB, yeasts, molds, coliforms, and *E. coli* decreased significantly ($P < 0.05$) compared to the ungassed control pallets. Sensory analysis of blueberries exposed to higher chlorine dioxide gas concentrations (0.19 mg/g fruit) revealed no significant ($P < 0.05$) differences compared to ungassed (control) blueberries. Thus, chlorine dioxide shows considerable promise to enhance the microbial quality of blueberries before processing and perhaps of fresh fruit, and may help producers and processors meet the present microbial standards demanded by purchasers.

Future research goals focusing on the microbial spoilage and safety of blueberry would include the following:

1. Optimize chlorine dioxide gas treatment for sanitizing blueberries without changing sensory attributes.

2. Scale up the gaseous chlorine dioxide treatment for industrial processing of blueberries.

3. Assess the ability of this gaseous chlorine dioxide treatment followed by washing of berries in water containing 200 ppm chlorine to meet the current microbial standards demanded by buyers of frozen berries.

4. Assess the ability of gaseous chlorine dioxide to extend the shelf life of fresh blueberries.

APPENDIX A

MICROBIAL REDUCTIONS ON BLUEBERRIES USING SODIUM HYPOCHLORITE, CHLORINE DIOXIDE AND ORGANIC ACIDS

OBJECTIVE

The objective of this preliminary work study was to better characterize the efficacy of chlorine, aqueous chlorine dioxide, organic fatty acid A and B sanitizers at concentrations and exposure times deemed to appropriate for blueberries processors. These sanitizers were tested at a contact time of 5 min in an aqueous model system and on inoculated fruits against 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.).

MATERIAL AND METHODS

All bacterial strains, yeasts and molds for this study were grown as described in Chapter 4.

Sanitizers

The following sanitizer solutions were investigated for microbial reductions: chlorine (100, 200 and 400 ppm), aqueous chlorine dioxide (ClO₂) (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), with sterile distilled water (SDW) used as the control.

Sodium hypochlorite solutions containing 100, 200 and 400 ppm chlorine were prepared by diluting CloroxTM (Clorox Company, Oakland, CA) with SDW and then confirming the concentration with a chlorine test kit (La Motte Chemical Products Co., Inc., Chestertown, MD). Aqueous chlorine dioxide solutions (3 and 5 ppm ClO₂) were prepared from commercial 20-g chlorine dioxide sachet (ICA TriNova, LLC Forest Park, GA) by placing the mixed contents of the sachet in a sealed bottle containing 1150 ml of SDW for 12 h. This stock solution containing 800 ppm ClO₂ was transferred in smaller sterile bottles and stored at 4°C with the ClO₂ concentration remaining stable up to 30 days. Working solutions containing 3 and 5 ppm ClO₂ were obtained by diluting the stock solution with SDW and then confirming the ClO₂ concentration with a chlorine test kit (La Motte Chemical Products Co.). The organic fatty acid sanitizers were obtained from Bob Coleman and are not yet commercially available. These fatty acid sanitizer solutions were freshly prepared just before use by adding 1.5 ml OB, and 3, 6, or 9 ml OA stock solution to 1 L SDW to obtain 0.15% OB, and 0.3, 0.6, or 0.9% OA work solutions, respectively.

Aqueous Model System Study

Sterile centrifuge tubes containing 9-ml aliquots of each sanitizer solution at the above concentrations were inoculated with 1ml of a 19-strain cocktail containing three strains each of the mesophilic bacterial pathogens *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* as well as five yeasts (*Aureobasidium* sp., *Bullera* sp., *Cryptococcus* sp.,

Sporidiobolus sp., and *Filobasidium* sp.) and five molds (*Colletotrichum* sp., *Epicoccum* sp., *Cladosporium* sp., *Phoma* sp., and *Alternaria* sp.), with SDW used as the control. Over a period of 5 minutes, 1 ml aliquots were removed and diluted 1:10 in neutralizing buffer (Difco) for sodium hypochlorite, chlorine dioxide and double strength neutralizing buffer (Guthery, 2001) for organic fatty acids A and B, followed by serial dilution in sterile phosphate buffer solution (PBS). Double strength neutralizing buffer solution (Guthery, 2001) was prepared by adding 10 g peptone (Difco), 2 g sodium thiosulfate (JT Baker, Phillipsburg, NJ), 0.5 g mono-potassium phosphate (JT Baker), 0.5 g catalase (Sigma), 60 g Tween 80 (Sigma), and 10 g lecithin (Sigma) to 1 L of Letheen broth (Difco). Appropriate dilutions in PBS were spiral-plated in duplicate on trypticase soy agar (Difco) containing 0.6% yeast extract and 100 ppm cyclohexamide (Sigma-Aldrich Co., St. Louis, MO) (TSAYE-C) for enumeration of mesophilic aerobic bacteria (MAB), and potato dextrose agar (Difco) containing 20 ppm streptomycin (Sigma) and 50 ppm ampicillin (Sigma) (PDA-SA) for enumeration of yeasts and molds. TSAYE-C plates were counted after 48 h of incubation at 37°C. PDA-SA plates were counted after 72 to 96 h of incubation at room temperature (22°C).

Fruits

Fresh blueberries of uniform size and shape were obtained from the local market and stored at 4°C. Before inoculation the fruits were held at room temperature to dry.

Inoculation

Blueberries (275g) were inoculated by immersion in a 25 x 20 cm sterile polyethylene bag (Whirl-Pack™, Fisher Scientific, Pittsburgh, N.J.) containing ~370 ml of the 19-strain cocktail of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, yeasts and

molds and gently swirl-agitated at 100 rpm for 20 min on a G2 Gyrotory Shaker platform, (New Brunswick Scientific Co., Inc., Edison, N.J.). The fruits were air dried under laminar flow in a biosafety cabinet for 2 h, stored overnight at 4°C and then re-dried under a laminar flow hood for 2 h before use.

Sanitizer Exposure

Fruit samples (25g) placed in sterile 20x 10 cm polyethylene bags (Whirl-Pack™) containing 100 ml of the various sanitizers were horizontally shaken at 100 rpm for 5 minutes on a G2 Gyrotory Shaker platform (New Brunswick Scientific Co.).

Microbial Analysis

After treatment, the sanitizer was discarded and replaced by 100 ml of neutralizing buffer (Difco) for sodium hypochlorite, chlorine dioxide, and 100 ml of double strength neutralizing buffer (Guthery, 2001) for organic fatty acids A, and B. The sealed bags were horizontally shaken at 100 rpm for 20 min on a G2 Gyrotory Shaker platform (New Brunswick Scientific Co.) and then pulsed for 1 min using a Pulsifier, (Filataflex Ltd., Ontario, Canada). Samples of neutralizing buffer (1ml) were serially diluted in sterile phosphate buffer solution (PBS) (9ml) and appropriate dilutions were spiral-plated (Autoplate 4000 –Spiral Biotech, Exotech, Inc, Gaithersburg, MD) in duplicate on TSAYE-C for enumeration of mesophilic aerobic bacteria (MAB), and PDA-SA for enumeration of yeasts and molds. TSAYE-C plates were counted after 48 h incubation at 37°C. PDA-SA plates were counted after 72 to 96 h incubation at room temperature (22°C).

Statistical analysis

Analysis of Variance (ANOVA) was done on all microbial reduction data for each sanitizer concentrations using the Statistical Analysis System (SAS, Version 8, SAS[®] Institute Inc., Cary, NC). Data in the graphs and tables (Appendix B) are means from replicates and significance between means were determined using least significant difference (LSD) test at the 95% confidence level ($P=0.05$).

Sensory Analysis

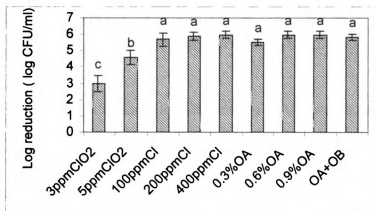
Uninoculated fruit samples were exposed to each of the following sanitizers for 5 min : 200 ppm chlorine, 5 ppm ClO_2 , 0.9% OA, , 0.3% OA + 0.15% OB. After treatment, the samples were water rinsed for 1-2 sec., air dried for 1 h, dispensed into individual sample cups with lids (15- 20 berries each) and stored aerobically overnight at 4°C before being given to 110 panelists comprising students, staff and faculty at Michigan State University. The panelists were presented with a legal consent form (Appendix C), individual trays containing the coded samples, and a set of computer instructions regarding sample evaluation. The samples were analyzed using a Hedonic scale with “like extremely” (9) to “dislike extremely” (1) for the following sensory attributes: appearance, aroma, texture, flavor, and overall acceptability. The data were analyzed using SIMS 2000 computer software program at a significance level of $P<0.05$.

RESULTS

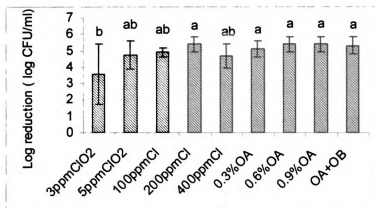
Aqueous Model System Studies

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 (Appendix B, Table A.1), respectively, with these reductions only statistically significant ($P < 0.05$) for MAB, and molds. Chlorine and 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 (Figure A.1).

MAB



Yeast



Mold

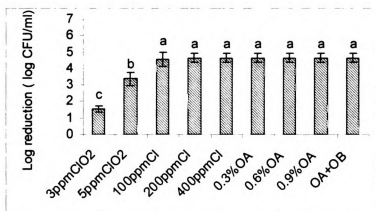
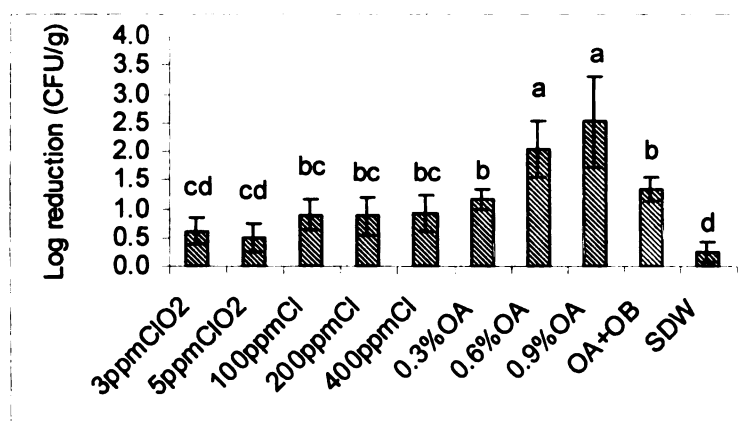


Figure A 1 Reductions (log CFU/ml) of MAB, yeasts, and molds (mean $\pm$ std. dev., $n=3$) after a 5-minute exposure to various sanitizers in an aqueous model system: 3 ppm chlorine dioxide (3 ppm ClO₂), 5 ppm ClO₂, 100 ppm sodium hypochlorite (100 ppm Cl), 200 ppm Cl, 400 ppm Cl, 0.3% organic fatty acid A (0.3% OA), 0.6% OA, 0.9% OA, and 0.3% OA + 0.15% OB (OA+OB). Microbial reductions with different letters are significantly different ($P<0.05$).

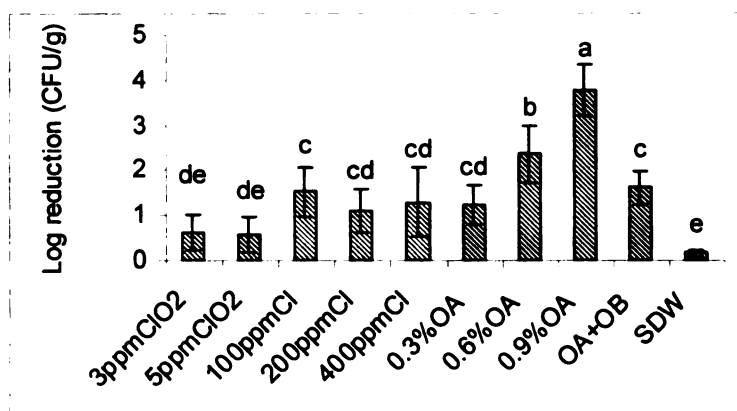
Blueberries Inoculation Studies

The two organic fatty acids were the most effective sanitizers. 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 (Appendix B, Table A.2). Increasing the OA concentration from 0.3 to 0.9% resulted in significantly ($P < 0.05$) greater reductions for MAB and yeast. 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 SDW for MAB and yeast (Figure A.2). Treatment of inoculated blueberries with SDW for 5 minutes reduced populations of MAB, yeast and mold by 0.24, 0.15 and, 0.49 log CFU/g, respectively.

MAB



Yeast



Mold

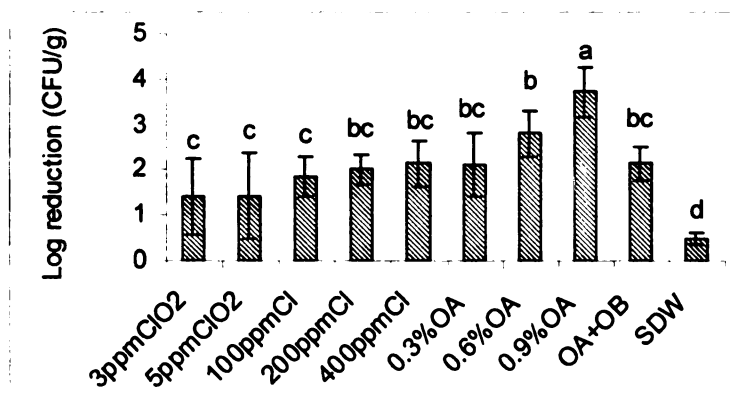


Figure A 2 Reductions (log CFU/g) of MAB, yeasts, and molds (mean $\pm$ std. dev., $n=3$) after a 5-minute exposure of inoculated fruit to various sanitizers: 3 ppm chlorine dioxide (3 ppm ClO₂), 5 ppm ClO₂, 100 ppm sodium hypochlorite (100 ppm Cl), 200 ppm Cl, 400 ppm Cl, 0.3% organic fatty acid A (0.3% OA), 0.6% OA, 0.9% OA, and 0.3% OA + 0.15% OB (OA+OB) and SDW. Microbial reductions with different letters are significantly different ($P<0.05$).

Sensory Analysis

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 (Figure A 3). OA 0.9% compared with OA+OB treated fruit was also significantly ($P < 0.05$) lower in terms of texture, flavor, and overall acceptability.

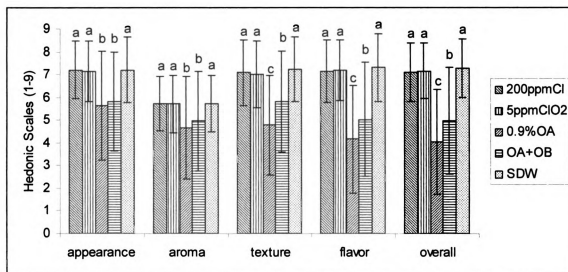


Figure A 3 Average consumer acceptability for fresh blueberries subjected for 5 min to wash treatment with 200 ppm sodium hypochlorite (200 ppm Cl), 5 ppm chlorine dioxide (5 ppm ClO₂), 0.9% organic fatty acid A (0.9% OA), 0.3% OA + 0.15% OB (OA + OB), and sterile distilled water (SDW) as control.

CONCLUSION

The most effective sanitizers were organic fatty acids A and B followed by chlorine, and chlorine dioxide. On fruits organic fatty acid A and B negatively impacted all sensory attributes ($P < 0.05$) compared to the control and other sanitizers. Aqueous solutions of chlorine dioxide chlorine produced similar microbial reductions and sensory

attributes ($P > 0.05$) for fruit. SDW reduced microbial populations by < 0.5 logs indicating that just simply washing of fruit is not a solution for enhancing the safety and quality of blueberries.

Appendix B

Table 3.1 Populations of MAB, yeasts, molds, coliforms and *E.coli* (mean $\pm$ std. dev., n=18) and range on blueberries (Log CFU/g) sampled at pre-harvest, post-harvest, blower exit, water tank exit, and pre-packaging.

Fruit Samples	MAB		Yeasts		Molds		Coliforms		<i>E.coli</i>	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Pre-H	3.49 $\pm$ 0.86	2.00-5.41	3.81 $\pm$ 0.58	2.69-5.04	3.35 $\pm$ 1.09	1.69-5.08	0.98 $\pm$ 0.58	0.69-2.44	0.89 $\pm$ 0.39	0.69-1.69
Post-H	4.95 $\pm$ 0.87	2.60-5.90	4.37 $\pm$ 0.71	3.07-5.73	4.06 $\pm$ 1.02	2.00-5.90	2.09 $\pm$ 0.95	0.69-3.99	1.49 $\pm$ 0.75	0.69-3.23
Blower E	4.93 $\pm$ 0.90	2.30-6.23	4.40 $\pm$ 0.65	2.69-5.77	4.21 $\pm$ 1.08	2.30-5.90	2.20 $\pm$ 0.92	0.69-3.53	1.48 $\pm$ 0.74	0.69-3.20
W Tank E	4.21 $\pm$ 0.98	2.30-5.94	3.86 $\pm$ 0.45	3.11-4.87	3.52 $\pm$ 1.01	1.69-5.00	1.62 $\pm$ 0.90	0.69-3.55	1.05 $\pm$ 0.52	0.69-2.17
Pre-P	4.22 $\pm$ 0.89	2.47-5.63	3.90 $\pm$ 0.53	2.74-4.89	3.72 $\pm$ 0.92	2.30-4.96	1.63 $\pm$ 0.76	0.69-3.27	1.12 $\pm$ 0.59	0.69-2.54

Table 3.2 Populations of MAB, yeasts, molds, coliforms and *E.coli* (mean $\pm$ std. dev., n=18) on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab Samples	MAB		Yeasts		Molds		Coliforms		<i>E.coli</i>	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
BA before	3.00 $\pm$ 1.01	1.08-4.69	2.93 $\pm$ 1.05	0.78-4.18	2.37 $\pm$ 1.09	0.78-4.34	0.35 $\pm$ 0.90	-0.52-2.69	0.10 $\pm$ 0.49	-0.52-1.14
BA after	4.29 $\pm$ 0.58	2.67-4.95	3.93 $\pm$ 0.68	1.90-4.89	3.95 $\pm$ 0.71	2.41-4.58	1.74 $\pm$ 0.92	-0.52-2.47	0.87 $\pm$ 0.83	-0.52-2.73
PA before	1.97 $\pm$ 0.98	0.30-3.30	1.63 $\pm$ 0.76	0.47-2.49	1.33 $\pm$ 0.57	0.47-2.23	0.10 $\pm$ 0.45	-0.52-1.08	0.26 $\pm$ 0.32	-0.52-0.47
PA after	3.14 $\pm$ 0.71	2.23-4.57	2.78 $\pm$ 0.89	1.65-5.08	2.45 $\pm$ 1.00	1.07-4.53	0.96 $\pm$ 0.71	0.00-2.17	0.40 $\pm$ 0.50	-0.52-1.47

Table 3.3 Populations of MAB, yeasts, molds, coliforms and *E.coli* (mean $\pm$ std. dev., n=18) in water samples (log CFU/ml) taken before and after fruit washing.

Water samples	MAB		Yeasts		Molds		Coliforms		<i>E.coli</i>	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Before	1.31 $\pm$ 0.45	1.00-2.65	1.12 $\pm$ 0.15	1.00-1.30	1.17 $\pm$ 0.19	1.00-1.54	0.04 $\pm$ 0.15	0.00-0.60	0.02 $\pm$ 0.01	0.00-0.30
After	1.87 $\pm$ 0.73	1.00-3.54	1.86 $\pm$ 0.58	1.00-3.46	2.03 $\pm$ 0.80	1.30-3.77	0.45 $\pm$ 0.40	0.00-1.00	0.33 $\pm$ 0.30	0.00-1.00

Table 3.4 Populations of MAB, yeasts, molds, coliforms and *E.coli* (mean $\pm$ std. dev., n=18) on blueberry samples (log CFU/g) before (fresh) and after 3 and 6 months of storage at -20°C.

Fruit Sample	MAB		Yeasts		Molds		Coliforms		<i>E.coli</i>	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Fresh	4.09 $\pm$ 0.69	2.00-5.87	4.11 $\pm$ 0.39	2.69-5.17	3.77 $\pm$ 0.32	1.69-5.57	2.53 $\pm$ 1.20	0.69-3.99	1.62 $\pm$ 0.44	0.69-3.23
3 months	3.65 $\pm$ 0.55	1.70-5.67	3.67 $\pm$ 0.25	2.58-4.48	3.31 $\pm$ 0.34	1.70-4.76	1.90 $\pm$ 1.03	0.69-2.66	0.94 $\pm$ 0.33	0.69-1.70
6 months	3.59 $\pm$ 0.39	1.30-5.49	3.51 $\pm$ 0.22	2.18-4.40	3.15 $\pm$ 0.44	1.70-4.66	1.94 $\pm$ 0.85	0.69-3.34	1.18 $\pm$ 0.53	0.69-2.63

Table 4.1 Populations of *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, yeasts and molds (mean $\pm$ std. dev., n=3) on inoculated blueberries before, after, and reductions (log CFU/g) from exposure to 4 mg/L, 0.13mg/g ClO₂ gas in a sealed bucket for 12 h at 22°C/99.9% RH.

Microbial category	Before	After	Reduction
<i>L. monocytogenes</i>	6.46 $\pm$ 0.39	2.52 $\pm$ 0.54	3.94 $\pm$ 0.15
<i>Salmonella</i>	6.38 $\pm$ 0.21	2.76 $\pm$ 0.56	3.62 $\pm$ 0.42
<i>E. coli</i> O157:H7	6.35 $\pm$ 0.32	2.10 $\pm$ 0.20	4.25 $\pm$ 0.50
Yeasts	5.99 $\pm$ 0.38	2.89 $\pm$ 0.61	3.10 $\pm$ 0.50
Molds	6.22 $\pm$ 0.05	3.05 $\pm$ 0.18	3.17 $\pm$ 0.15

Table 4.2 Populations of MAB, yeasts, molds, coliforms, and *E. coli* (mean $\pm$ std. dev., n=6) recovered from blueberries before (0 h) and from gassed and un-gassed fruit after 12 h of storage at 12 to 14°C. Gassed pallets were exposed to 18 mg/L, 0.13mg/g ClO₂ gas.

Microbial category	Before (0h)	After 12 h un-gassed	After 12 h gassed
M A B	4.03 $\pm$ 0.63	4.72 $\pm$ 0.49	2.39 $\pm$ 0.62
Yeasts	4.32 $\pm$ 0.59	4.68 $\pm$ 0.49	3.05 $\pm$ 0.53
Molds	4.57 $\pm$ 0.89	4.68 $\pm$ 0.94	4.20 $\pm$ 0.89
Coliforms	1.12 $\pm$ 0.79	2.12 $\pm$ 0.71	0.65 $\pm$ 0.35
<i>E. coli</i>	0.51 $\pm$ 0.12	1.01 $\pm$ 0.81	0.48 $\pm$ 0.00

Table 4.3 Analysis of variance on the effect of levels and positions on microbial load on fruit
(P-values for independent variables and interactions)

Source of variance			
	level	position	Interaction (level * position)
MAB	0.5865	0.0035**	0.7000
Yeast	0.7327	0.1884	0.9329
Mold	0.9239	0.2396	0.9223
Coliforms	0.2278	0.0556	0.1215
<i>E. coli</i>	ND	ND	ND

* Significant at $P < 0.05$; ** significant at $P < 0.01$

MAB: mesophilic aerobic bacteria

ND: not determined because the numbers were below detection limits

Table A.1 Reductions (mean $\pm$ std. dev., n=3) of MAB, yeasts, and molds (log CFU/ml) after a 5-minute exposure to various sanitizers in an aqueous model system.

Treatment	Concen- tration	MAB			Molds			Yeasts		
		Mean	Range		Mean	Range		Mean	Range	
Sodium hypochlorite	100 ppm	5.67 $\pm$ 0.41	5.41-6.15		4.52 $\pm$ 0.44	4.02-4.85		4.88 $\pm$ 0.29	4.60-5.18	
Sodium hypochlorite	200 ppm	5.86 $\pm$ 0.24	5.71-6.15		4.62 $\pm$ 0.27	4.32-4.85		5.38 $\pm$ 0.43	5.08-5.88	
Sodium hypochlorite	400 ppm	5.96 $\pm$ 0.22	5.71-6.15		4.62 $\pm$ 0.27	4.32-4.85		4.67 $\pm$ 0.75	3.80-5.13	
Chlorine dioxide	3 ppm	2.95 $\pm$ 0.48	2.43-3.04		1.51 $\pm$ 0.17	1.37-1.71		3.55 $\pm$ 1.84	1.46-4.97	
Chlorine dioxide	5 ppm	4.56 $\pm$ 0.43	4.2-5.04		3.34 $\pm$ 0.37	2.96-3.71		4.71 $\pm$ 0.86	3.74-5.40	
Organic acid A	0.3%	5.53 $\pm$ 0.18	5.4-5.74		4.62 $\pm$ 0.27	4.32-4.85		5.11 $\pm$ 0.48	4.63-5.60	
Organic acids A+B	0.3+0.15%	5.80 $\pm$ 0.20	5.67-6.04		4.62 $\pm$ 0.27	4.32-4.85		5.29 $\pm$ 0.53	4.82-5.88	
Organic acid A	0.6%	5.96 $\pm$ 0.22	5.71-6.15		4.62 $\pm$ 0.27	4.32-4.85		5.38 $\pm$ 0.43	5.08-5.88	
Organic acid A	0.9%	5.96 $\pm$ 0.22	5.71-6.15		4.62 $\pm$ 0.27	4.32-4.85		5.38 $\pm$ 0.43	5.08-5.88	

Table A.2 Reductions (mean $\pm$ std. dev., n=3) and ranges of MAB, yeasts, and molds (log CFU/g) after a 5-minute exposure of inoculated fruit to various sanitizers and SDW (washed fruit).

Treatment	Concen- tration	MAB			Mold			Yeast		
		Mean	Range		Mean	Range		Mean	Range	
Sodium hypochlorite	100 ppm	0.88 $\pm$ 0.26	0.6-1.16		1.85 $\pm$ 0.43	1.21-2.2		1.53 $\pm$ 0.54	0.95-2.1	
Sodium hypochlorite	200 ppm	0.86 $\pm$ 0.34	0.39-1.15		2.01 $\pm$ 0.33	1.56-2.37		1.08 $\pm$ 0.48	0.6-1.66	
Sodium hypochlorite	400 ppm	0.91 $\pm$ 0.31	0.47-1.21		2.14 $\pm$ 0.50	1.65-2.85		1.28 $\pm$ 0.77	0.58-2.3	
Chlorine dioxide	3 ppm	0.61 $\pm$ 0.22	0.33-0.84		1.41 $\pm$ 0.82	0.73-2.55		0.62 $\pm$ 0.39	0.18-1.12	
Chlorine dioxide	5 ppm	0.50 $\pm$ 0.23	0.3-0.84		1.42 $\pm$ 0.95	0.57-2.68		0.56 $\pm$ 0.40	0.18-1.51	
Organic acid A	0.3%	1.16 $\pm$ 0.18	0.96-1.34		2.10 $\pm$ 0.70	1.26-2.85		1.23 $\pm$ 0.42	0.78-1.81	
Organic acids A+B	0.3+0.15%	1.35 $\pm$ 0.21	1.04-1.51		2.13 $\pm$ 0.36	1.61-2.45		1.61 $\pm$ 0.36	1.28-1.98	
Organic acid A	0.6%	2.02 $\pm$ 0.49	1.39-2.52		2.79 $\pm$ 0.52	2.26-3.26		2.35 $\pm$ 0.64	1.4-2.84	
Organic acid A	0.9%	2.52 $\pm$ 0.79	1.39-3.25		3.72 $\pm$ 0.55	3.15-4.21		3.77 $\pm$ 0.56	3.14-4.48	
Washed fruits	-	0.24 $\pm$ 0.18	0.11-0.51		0.49 $\pm$ 0.13	0.36-0.65		0.15 $\pm$ 0.06	0.06-0.2	

Table E.1 Growth of MAB, yeasts, molds, coliforms, and *E.coli* (mean $\pm$ std. dev., n=3) populations on naturally contaminated blueberries (log CFU/g) initially (0 h) and during 24, 48, and 96 h of storage at room temperature ($21\pm1^{\circ}\text{C}$) and 99% R.H.

Microbial category	0 h	24 h	48 h	96 h
M A B	4.72 $\pm$ 0.10	5.81 $\pm$ 0.10	6.30 $\pm$ 0.10	6.19 $\pm$ 0.11
Yeasts	4.83 $\pm$ 0.10	4.87 $\pm$ 0.10	5.65 $\pm$ 0.10	6.19 $\pm$ 0.10
Molds	5.41 $\pm$ 0.10	5.37 $\pm$ 0.10	5.79 $\pm$ 0.15	6.13 $\pm$ 0.10
Coliforms	0.85 $\pm$ 0.27	1.41 $\pm$ 0.39	2.51 $\pm$ 0.10	3.58 $\pm$ 0.10
<i>E.coli</i>	0.69 $\pm$ 0.10	1.05 $\pm$ 0.39	2.03 $\pm$ 0.10	3.31 $\pm$ 0.15

Table E.2 Reductions of MAB, yeasts, molds, coliform, and *E.coli* (mean $\pm$ std. dev., n=4) on inoculated and uninoculated blueberries (log CFU/g) after 12 h exposure to 4 mg/L, 0.16 mg/g ClO₂ gas in a sealed bucket at 22°C and 99.9% RH.

Sample	M A B	Yeasts	Molds	Coliform	<i>E.coli</i>
Inoculated	3.71±0.47	2.78±0.10	2.52±0.24	3.39±0.08	3.44±0.07
Uninoculated	2.66±0.08	2.26±0.13	1.87±0.07	3.34±0.08	3.18±0.13

APPENDIX C

Consumer Consent Form

“Microbial Reduction Strategies for Highbush Blueberries ”

Department of Food Science and Human Nutrition, Michigan State University

Sample:

Before you decide to sign this consent form and continue to participate in our study, please read carefully and thoroughly the reverse side of this form for the sample ingredients and preparation information, purpose and procedure of this study, potential risks and benefits from your participation, our assurance of your privacy, your rights as a human subject in our study, etc. If you have any question during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator. Feel free to contact Dr. Elliot Ryser, the principle investigator of this study, via phone at 517-355-7713 ext.185 (Department of Food Science and Human Nutrition, 2108 S. Anthony, Michigan State University, East Lansing, MI 48823) You can also reach me via email at ryser@msu.edu for any inquiry you might have due to your participation in our study. In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Peter Vasilenko, Ph.D., Chair of University Committee on Research Involving Human Subject (UCRIHS), 202 Olds Hall, Michigan State University, East Lansing, MI 48824-1046, PHONE (517) 355-2180 FAX (517) 432-4503, E-Mail - UCRIHS@msu.edu, WEB SITE -<http://www.humanresearch.msu.edu>

If you have read all the information given to you in this consent form and decide to participate in this study and provide your valuable response to our questionnaire, you can go ahead and sign this form now. Otherwise, you can stop here and feel free to discontinue participation in our study without any penalty.

PLEASE NOTE UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN THIS STUDY. YOUR SIGNATURE INDICATES YOU HAVE READ THE INFORMATION PROVIDED ABOVE AND THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS UPON YOUR REQUEST.

SIGNED _____ DATE _____

You indicate your voluntary agreement to participate by signing above.

Consent Form

“Microbial Reduction Strategies for Highbush Blueberries ”

Department of Food Science and Human Nutrition, Michigan State University

INVITATION TO PARTICIPATE

You are invited to participate in this study that assesses the quality attributes of blueberries.

PURPOSE OF THIS STUDY

This study is intended to study the quality of blueberries that have been washed in either water or one or more FDA-approved sanitizer solutions that are widely used commercially. Texture, appearance and flavor characteristics of blueberries will be evaluated.

PROCEDURE OF THIS STUDY

Each participant will be presented with a series of three blueberry samples. They will be asked to **evaluate visually and after tasting**, score the attributes as presented on the score sheet for each sample. Samples will be presented using three digit random codes. Consumer marketing questions will also be asked.

SAMPLE PREPARATION

All treatments given to our blueberries are FDA approved.

POTENTIAL RISKS

Because all ingredients we use in our study are food grade and FDA approved for food applications, these samples pose no adverse health risk, provided the subject has not been identified as being susceptible to an allergic reaction to the previously listed sample ingredients. If you believe there is a potential of an **allergic reaction upon sniffing and tasting**, notify the on-site sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this study, Michigan State University will provide emergency medical care, if necessary, but this and any other medical expense must be paid from your own health insurance program.

POTENTIAL BENEFITS

There are no benefits gained directly from your participation in this study. However, your participation and response will provide us valuable data, which can be used to identify optimum microbial reduction strategies for blueberries that will help keep the Michigan blueberry industry strong and profitable.

ASSURANCE OF CONFIDENTIALTY

Any information obtained in connection with this study that could be identified with you will be kept confidential by ensuring that all consent forms are securely stored. All data collected and analyzed will be reported in an aggregate format that will not permit associating subjects with specific responses or findings. **Your privacy will be protected to the maximum extent allowable by law.**

WITHDRAWAL FROM THIS STUDY

Participation in this study is voluntary. Your decision to refuse participation or discontinue participation during this study will be honored promptly and unconditionally.

Questionnaire

Product: Fresh blueberry

Instructions:

You will be provided with 6 fruit samples and a questionnaire. Please look carefully at the sample number and find the corresponding blueberry cup with that number. Then answer the following questions:

1. How do you like the APPEARANCE of the blueberries?

- 9 – Like extremely
- 8 – Like very much
- 7 – Like moderately
- 6 – Like slightly
- 5 – Neither like nor dislike
- 4 – Dislike slightly
- 3 – Dislike moderately
- 2 – Dislike very much
- 1 – Dislike extremely

Instruction: Please sniff the blueberries and answer to the following question.

2. How do you like the AROMA of the blueberries?

- 9 – Like extremely
- 8 – Like very much
- 7 – Like moderately
- 6 – Like slightly
- 5 – Neither like nor dislike
- 4 – Dislike slightly
- 3 – Dislike moderately
- 2 – Dislike very much
- 1 – Dislike extremely

Instruction:

Please taste the blueberries and answer the following questions.

3. How do you like the TEXTURE of the blueberries?

- 9 – Like extremely
- 8 – Like very much
- 7 – Like moderately
- 6 – Like slightly
- 5 – Neither like nor dislike
- 4 – Dislike slightly
- 3 – Dislike moderately
- 2 – Dislike very much
- 1 – Dislike extremely

4. How do you like the FLAVOR of the blueberries?

- 9 – Like extremely
- 8 – Like very much
- 7 – Like moderately
- 6 – Like slightly
- 5 – Neither like nor dislike
- 4 – Dislike slightly
- 3 – Dislike moderately
- 2 – Dislike very much
- 1 – Dislike extremely

5. How do you like the OVERALL ACCEPTABILITY of the blueberries?

- 9 – Like extremely
- 8 – Like very much
- 7 – Like moderately
- 6 – Like slightly
- 5 – Neither like nor dislike
- 4 – Dislike slightly
- 3 – Dislike moderately
- 2 – Dislike very much
- 1 – Dislike extremely

Instruction:

Please answer the following marketing questions.

6. Please check all that apply: Which of these factors are important to you as reasons why you purchase blueberries.

- ☐ Nutritious/healthy food
- ☐ Good value
- ☐ Taste

- ☐ Little/no waste
- ☐ Safe
- ☐ Do not purchase blueberries

7. Thinking back over these past two weeks, did you purchase blueberries and if yes, how did you purchase them?

- 5 – Did not purchase
- 4 – Other forms
- 3 – Fresh blueberries
- 2 – Frozen blueberries
- 1 – Canned blueberries

8. I prefer to eat fresh blueberries rather than eating frozen blueberries.

- 5 – Strongly agree
- 4 – Agree
- 3 – Neither agree nor disagree
- 2 – Disagree
- 1 – Strongly disagree

9. Are you Male or Female?

- 2 – Female
- 1 – Male

10. Of the following, which category best represents your 2004 household income before taxes?

- 4 – Less than \$20,000
- 3 – \$20,000 to \$39,999
- 2 – \$40,000 to \$59,999
- 1 – Greater than \$60,000

11. How many people are in your household?

12. How many people in your household are under 18?

THANK YOU FOR YOUR PARTICIPATION!

APPENDIX D

Frozen Sampling Log 2005

Fruit samples of processed fruits before freezing were taken from Hartman on 8/25/2005 and microbial analysis within 4 h. Fruit were packed the same day in two-30 lb boxes.

Room temperature 66°F. Product temperature 60°F. Both boxes (A and B) were located in the middle of the pallet. Product was frozen to 32 °F 8/27/05 6:45 AM @ freezer

according to RFID Tag. Last read of RFID Tag 9/19/05 @ 1:15 PM with 0° F recorded.

Fruit samples after freezing taken on 9/20/2005 and microbial analysis done within 4 h.

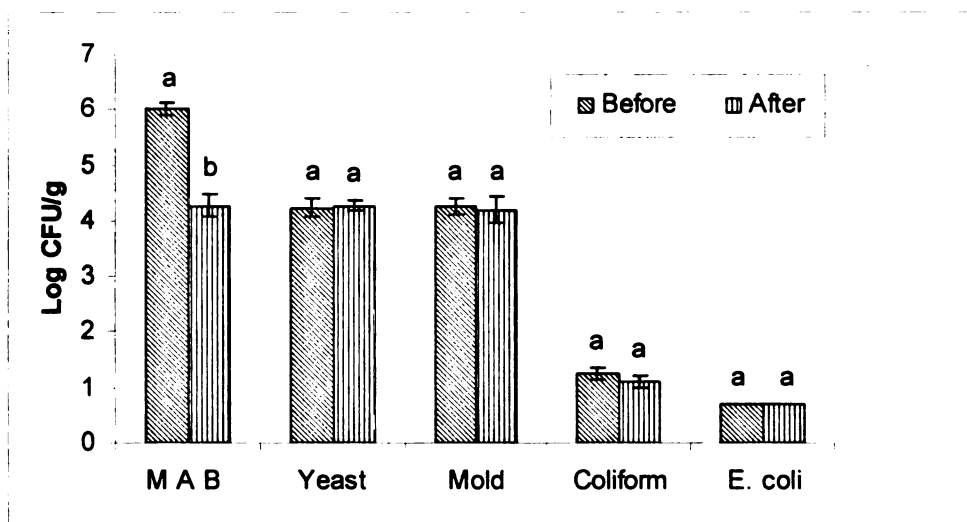


Figure D.1 Populations of MAB, yeasts, molds, coliforms and *E. coli* (mean $\pm$ std. dev., $n=6$) on blueberry samples before and after frozen storage in industrial conditions. Values with different letters within the same microbial category are significantly different ($P<0.05$).

Microbiological Survey of Blueberries 2003-2004

Sampling Log - 2004

Hartman 1 'Rancocas' Type of blueberry.

Pre-harvest sample collection: 13 July 04 at 08:30 by Steven and Iuliano.

Weather Conditions: ~75°F, cloudy, calm, humidity 85 – 90%.

Machine-harvested sample collection: 11:00.

Weather Conditions: ~80°F, cloudy, calm, humidity 85 – 90%.

Processing began at 13:00 – 13:45 on the same day.

Wash water for processing: 'Chlorox' at 10 ppm Cl

HA 1- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	4.78	4.9	4.3	1.6	0.7
Pre-harvest ₂	5	5.04	4.9	0.7	0.7
Post-harvest ₁	5.77	5.04	5.71	1.3	1.18
Post-harvest ₂	5.9	5.73	5.9	1.74	1.4
Blower exit ₁	4.85	5	5.62	3.18	2.23
Blower exit ₂	5.96	5.18	5.23	3.18	2.48
Water tank exit ₁	4.3	4.3	4.9	2.35	0.7
Water tank exit ₂	5.04	4.7	4.7	1.48	0.7
Pre-packaging ₁	5.23	4.6	4.7	1.81	0.7
Pre-packaging ₂	4.6	4.6	4.7	2.08	0.7

HA 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	1.08	1.48	0.78	0	0
PA before	1.48	1.08	0.78	0	0
BA after	4.63	3.38	3.92	2.32	1.48
PA after	4.38	5.08	4.53	0	0

HA 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.3	1.3	1.3	0	0
After	1.3	1.3	2.53	0	0

HA 2 'Rubel' Type of blueberry.

Pre-harvest sample collection: harvested 19 July by Eric.

Weather conditions: unknown on 19 July, and sunny, still, ~78°F on July 20.

Processing began at 08:05- 08:15 on 20 July 04.

Wash water for processing: 'Chlorox'. During processing time the free chlorine and the pH of the water tank were: Water drops -10ppm and 8.51; Water tank 0 min-48 ppm and 8.90; 10 min (end) 44 ppm and 8.18.

HA 2- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	3.04	3.34	2.9	0.7	0.7
Pre-harvest ₂	3	3.62	2.3	0.7	0.7
Post-harvest ₁	5.59	5.48	5.6	2.11	1
Post-harvest ₂	5.75	5.36	5.58	1.95	2
Blower exit ₁	5.79	5.77	5.54	2.78	1.7
Blower exit ₂	5.91	5.67	5.9	2.18	1.7
Water tank exit ₁	5.54	4.65	4.58	0.7	0.7
Water tank exit ₂	5.94	4.57	5	2.3	1
Pre-packaging ₁	5.58	4.48	4.96	2	1
Pre-packaging ₂	5.63	4.48	4.59	2.54	0.7

HA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	1.86	0.78	0.78	0	0
PA before	3.08	2.59	1.26	1.08	0
BA after	4.11	4.08	4.57	0.85	0.3
PA after	3.61	2.89	3.36	0.3	0

HA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.3	1.3	1.3	0	0
After	1.3	1.9	1.3	0.3	0

HA 3 'Rancocas' Type of blueberry.

Pre-harvest sample collection: 20 July 04 from 09:30 – 09:40 by Steven and Iuliano.

Weather Conditions: sunny, still, ~78°F.

Harvested (handpicked) for processing on 21 July from 12:20 – 13:20.

Weather Conditions: partly cloudy, still, ~83°F.

Processing began at 14:00, finished at 14:20.

Wash water for processing: 'Chlorox'.

During processing time the free chlorine and the pH of the water tank were: 0 min-2 ppm and 7.64; 10 min 20 ppm and 6.84; 20 min-18 ppm and 7.88.

HA 3- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	5.41	4	5.08	0.7	0.7
Pre-harvest ₂	4.9	4	4.7	0.7	0.7
Post-harvest ₁	5.45	4.85	5.45	1	0.7
Post-harvest ₂	5.2	4.9	5.52	1.85	1.4
Blower exit ₁	5.08	4.78	5.38	2.65	1.7
Blower exit ₂	5.53	4.7	5.36	2.48	1.7
Water tank exit ₁	3.3	3.6	4	1.4	0.7
Water tank exit ₂	3	3.6	4.34	1.7	0.7
Pre-packaging ₁	3	3.7	4.52	1.18	0.7
Pre-packaging ₂	3.48	4.11	4.58	1.95	0.7

HA 3-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.63	3.28	2.26	0	0
PA before	2.88	1.89	0.78	0	0
BA after	4.15	4.15	4.58	0.48	1.65
PA after	2.96	2.11	2.36	0	0

HA 3-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.3	1.3	1.3	0	0
After	1.3	1.3	1.3	0	0

HA 4

Pre-harvest sample collection: July 26 taken by Eric and Steven from 12:00 – 12:15.

Weather Conditions: sunny, slight breeze, ~73F.

Harvested for processing on 26 July from 11:00 - ~13:00.

Processing began at 08:15 on 27 July.

Weather Conditions: cloudy, still, rain in the area, ~70F.

Wash water for processing: 'Chlorox'.

During processing time the free chlorine and the pH of the water tank were: Water drops

-32 ppm and 8.62; 0 min-250 ppm and 9.08;5 min- 108ppm and 8.29;10 min(end)

208ppm and 8.68, respectively.

HA 4- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries (Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	2.48	3.67	3.4	0.7	0.7
Pre-harvest ₂	2.85	3.23	3.28	0.7	0.7
Post-harvest ₁	5.72	4.58	4.85	2	0.7
Post-harvest ₂	5.18	4.81	4.08	2.81	0.7
Blower exit ₁	6.23	4.48	4.58	1	0.7
Blower exit ₂	5.83	4.52	4.56	2.18	1.18
Water tank exit ₁	3.59	3.52	2.95	0.7	0.7
Water tank exit ₂	3.46	3.6	2.78	1.4	1.4
Pre-packaging ₁	4.15	3.3	3.94	1.7	0.7
Pre-packaging ₂	4	3	3.96	0.7	0.7

HA 4-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.63	3.6	2.68	0	0
PA before	1.38	0.78	0.78	0	0
BA after	4.54	4.23	4.51	1.98	0.78
PA after	2.23	2.04	1.92	1.26	0.48

HA 4-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.3	1.3	1.3	0	0
After	1.3	1.3	1.3	0	0

KA 1 'Bluecrop' Type of blueberry.

Preharvest sample taken by Eric on 2 Aug 04.

Weather conditions: ~67F, sunny/foggy, still.

Harvested for processing on 2 Aug from 13:00 – 17:00.

Processing began next day at 11:45-12:00, finished at 12:30-12:45.

During processing time the free chlorine and the pH of the water tank were: 0min -12 ppm and 6.84; 5 min-18 ppm and 6.98; 15 min- 10ppm and 6.94; 20 min- 8ppm and 6.91; rinse water-2ppm and 7.01.

KA 1- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	2.7	3.78	2.3	0.7	0.7
Pre-harvest ₂	3.2	4.66	2.48	0.7	0.7
Post-harvest ₁	4.32	3.95	2	0.7	0.7
Post-harvest ₂	4.08	3.48	2	0.7	0.7
Blower exit ₁	4.61	4.23	2.3	0.7	0.7
Blower exit ₂	4.26	3.78	2.3	0.7	0.7
Water tank exit ₁	4.67	4.15	3.7	0.7	0.7
Water tank exit ₂	4.36	4.11	3	0.7	0.7
Pre-packaging ₁	4.28	4.2	3.6	0.7	0.7
Pre-packaging ₂	3.95	4.4	3.9	1.4	0

KA1-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.46	4	3.91	0.48	0
PA before	3.3	2.62	1.82	0	0
BA after	3.86	3.38	4.15	1.78	0
PA after	2.38	2.08	2.08	0.9	0

KA 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.3	1.3	1.3	0	0
After	1.3	1.3	1.3	0	0

KA 2 'Bluecrop' Type of blueberry.

Preharvest sample taken on 3 Aug 04 from 13:25 – 13:35 by Steven and Iuliano.

Harvested for processing on 3 Aug from 13:00 – 17:00.

Weather conditions: raining, cloudy, cool, ~65F.

Processing began at 10:45, finished at 11:15 on 4 Aug.

During processing time the free chlorine and the pH of the water tank were: 0 min -6 ppm and 6.35; 5 min -16 ppm and 6.90; 20 min -22 ppm and 7.00; rinse water -3 ppm and 7.08.

KA 2- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	3.85	3.7	2.7	0.7	0.7
Pre-harvest ₂	4.26	3.6	2	0.7	0.7
Post-harvest ₁	4.93	3.96	2.78	0.7	0.7
Post-harvest ₂	4.79	3.99	3	0.7	0.7
Blower exit ₁	5.04	4.61	3.7	2	0.7
Blower exit ₂	4.99	4.65	4.34	2.3	0.7
Water tank exit ₁	4.49	3.75	3.28	0.7	0.7
Water tank exit ₂	4.08	3.18	2.3	1.48	0.7
Pre-packaging ₁	3.51	4.08	2.6	1.3	0.7
Pre-packaging ₂	3.61	4	2.3	0.7	0.7

KA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	4.69	4.18	4.34	0.6	0.3
PA before	3.28	2.46	2.23	0	0
BA after	4.92	4.63	4.26	1.26	0
PA after	3.26	2.92	1.78	0	0

KA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.78	1.3	1.3	0	0
After	1.3	1.3	1.3	0	0

Sampling Log - 2003

EL 1 'Earliblue' fruit first picking.

Preharvest sample taken on 7 July 03 by Hanson just NW of packing shed. Good shape, little field rot. About 70 F.

Processing samples taken on 8 July by Hanson and Berkheimer from Ellis processing line in Grand Junction. First fruit run of the season for them. Fruit had sat in shed @ 60-70F all night. Ran first thing in morning. Fruit collected over 2 hr period. Older shed and equipment generally clean, but not an MBG-Marketing approved processor.

Some question about whether chlorine injector was working when pre-run water samples were taken.

No swab samples taken – all stainless steel mesh belts. Line included one blower, water tank with bleach injector, sorting belt, and then back into lugs.

EL 1- Populations of MAB, yeasts, and molds on blueberries (Log CFU/g)

Fruit sample	MAB	Yeast	Mold
Pre-harvest ₁	2.17	3.47	1.69
Pre-harvest ₂	3.86	4.72	2.69
Post-harvest ₁	3.41	3.79	2.39
Post-harvest ₂	2.87	3.79	3.07
Blower exit ₁	3.68	4.04	2.54
Blower exit ₂	3.79	4.41	3
Water tank exit ₁	3.32	3.17	<1.69
Water tank exit ₂	2.30	3.11	1.69
Pre-packaging ₁			
Pre-packaging ₂			

HA1 'Bluecrop' Type of blueberry.

Preharvest sample take by Berkheimer on 28 July 03.

Weather conditions: sunny, still and warm; ~80F.

First mechanical harvest of 'Bluecrop' on July 28. Field was hand-picked once.

Berries had sat in shed over night at 60-65F. Berries with 1-4% green plus some over-ripe and rot. Fruit collected over 1 hr period.

Processing samples taken by Hanson and Montecino on July 29.

Line set up with two blowers, de-stemmer, custom water tank continuously flushed with bleach water (try to maintain 10-20 ppm), rinse spray as they leave water tank (2-4 ppm), color sorter, hand sorting belts, and boxer. Berries in water for just short time, just to treat, not to sort out the green.

Water chlorinated with Chlorox.

HA 1- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	4.11	4.23	3.90	<0.69	<0.69
Pre-harvest ₂	4.41	4.11	4.25	<0.69	<0.69
Post-harvest ₁	4.75	3.59	3.30	1.30	0.69
Post-harvest ₂	4.81	3.07	2.95	1.54	1
Blower exit ₁	4.65	2.69	4.60	1.39	0.69
Blower exit ₂	4.59	4.04	3.77	1.17	0.69
Water tank exit ₁	4.27	3.65	3.04	1	<0.69
Water tank exit ₂	4.54	3.23	3.49	0.69	<0.69
Pre-packaging ₁	4.17	2.74	3.11	<0.69	<0.69
Pre-packaging ₂	3.74	2.81	2.90	<0.69	<0.69

HA 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.27	3.25	2.55	<-0.52	-0.52
PA before	1.77	1.07	0.47	<-0.52	<-0.52
BA after	3.94	3.74	3.77	2.05	1.25
PA after	2.66	2.50	1.07	1.50	0.90

HA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	<1	<1	<1	<0	<0
After	2.62	1.60	1.77	0.77	0.30

EL 2 'Bluecrop' Type of blueberry.

Preharvest sample collected by Hanson and Montecino.

Third picking of fruit behind (west) of pond. Some fruit over-ripe and rot.

EL 2 - Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries (Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	2.30	2.92	1.69	<0.69	<0.69
Pre-harvest ₂	2	2.87	1.69	<0.69	<0.69
Post-harvest ₁	2.60	3.30	2.96	<0.69	<0.69
Post-harvest ₂	3.04	3.27	3.33	<0.69	<0.69
Blower exit ₁	2.30	3.38	3.38	<0.69	<0.69
Blower exit ₂	2.87	3.14	3.14	<0.69	<0.69
Water tank exit ₁	3.62	3.66	3.66	<0.69	<0.69
Water tank exit ₂	2.83	3.79	3.79	0.69	<0.69
Pre-packaging ₁	2.47	3.60	3.60	<0.69	<0.69
Pre-packaging ₂	3.74	3.66	3.66	<0.69	<0.69

EL 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	2.75	2.47	1.79	<-0.52	<-0.52
PA before					
BA after	2.67	1.91	2.41	<-0.52	-0.52
PA after					

EL 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	<1	<1	<1	<0	<0
After	3.54	3.46	2.23	0.60	0.30

HA 2 'Bluecrop' Type of blueberry.

Pre-harvest sample collected on 4 Aug 03 by Hanson.

Harvest same day-3rd picking. Field placed on CR 380. Had rained 2 inches day before.

Fruits quite ripe. Bushes 15+ years-old.

HA 2- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	3.30	3.47	3	<1.69	<1.69
Pre-harvest ₂	3.30	3.69	3.92	<1.69	<1.69
Post-harvest ₁	5.44	5.17	3.60	3.20	3.04
Post-harvest ₂	5.41	5.14	4.53	3.17	3.04
Blower exit ₁	5.34	4.87	4.81	3.39	3
Blower exit ₂	5.30	4.11	4.83	3.46	2.65
Water tank exit ₁	4.88	4.14	3.65	2.69	2.17
Water tank exit ₂	4.77	4.04	3	2.74	2.17
Pre-packaging ₁	4.76	3.81	3	2.60	2
Pre-packaging ₂	5.11	4.34	3.95	2.65	2.54

HA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.17	3.41	<1.47	<0.47	<0.47
PA before	0.77	1.77	<1.47	<0.47	<0.47
BA after	4.95	4.89	4.23	3.20	2.73
PA after	4.57	3.71	2.47	1.95	1.47

HA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	2.65	1	<1	<0	0.30
After	<2	<2	3.38	<0	<0

KA1 'Bluecrop' Type of blueberry.

Pre-harvest sample collected by Hanson.

First picking of Bluecrop field at Riley x 152nd. Field had overhead irrigation. Bushes 10-15 years old. Well pruned, heavy crop. Soil dry – no recent rain.

Processing samples collected by Berkheimer, Montecino and Thornbush.

KA 1- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	2.39	3.46	2.39	<0.69	<0.69
Pre-harvest ₂	2.81	3.64	2.60	0.69	<0.69
Post-harvest ₁	4.67	3.77	3	2.65	2.36
Post-harvest ₂	4.47	4.11	3.30	2.30	2.17
Blower exit ₁	3.77	3.81	3.17	1.25	1.11
Blower exit ₂	4.14	4.07	3.47	1	1
Water tank exit ₁	3.17	3.65	<2.69	<0.69	<0.69
Water tank exit ₂	3.77	3.6	<2.69	<0.69	<0.69
Pre-packaging ₁	3.60	3.65	3.17	<0.69	0.90
Pre-packaging ₂	3.74	3.87	3	1.25	1.39

KA 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.92	3.90	2.65	1.55	1.14
PA before	3.04	2.30	0.95	<-0.52	<-0.52
BA after	4.95	3.90	2.62	2.46	1.17
PA after	2.55	1.87	1.17	<0.47	<0.47

KA 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.47	<1	1.47	<0	<0
After	<2	<2	<2	<1	<1

TH 1

Pre-harvest samples from this field collected a few days earlier by Siva and Iuliano.

Processing samples taken on 10 Aug 03 at Northern Pride facility in Hartford, by

Hanson and Berkheimer. Ran first thing in morning. Berries delivered previous night, left outside over-night. Sampled 2 pallets of fruit over a 10 min period.

Line set up with conveyor to single large blower, into water filled flume to water tank (all chlorinated), water tank quite large and retained fruit for unknown length of time on bottom, Conveyed to color sorter, over de-stemmer, and to boxer.

TH 1- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries (Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	3.99	4.34	3.23	0.69	0.69
Pre-harvest ₂	3.99	4.34	3.28	0.69	0.69
Post-harvest ₁	5.41	3.69	4.65	2.25	2.36
Post-harvest ₂	5.50	5.20	4.39	2.47	2
Blower exit ₁	6.04	4.65	5.04	3.25	3.20
Blower exit ₂	5.75	4.30	4.54	3.04	3
Water tank exit ₁	5.71	4	4.30	1.90	0.69
Water tank exit ₂	5.59	3.36	4.59	1.65	<0.69
Pre-packaging ₁	5.20	3.63	4.04	2.17	1.77
Pre-packaging ₂	5.20	3.47	4.25	1.87	1.69

TH 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	2.07	2.23	1.32	-0.52	-0.52
PA before	1.23	0.47	<0.47	-0.30	<-0.52
BA after	4.67	3.61	4.81	2.47	2.14
PA after	3.67	1.65	3.25	1.23	1.17

TH 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1	<1	<1	<0	<0
After	2	<2	2.17	<1	<1

KA 2 'Bluecrop' Type of blueberry.

Pre-harvest sample collected on 11 Aug 03 by Hanson. First picking of 4-6 year-old Bluecrop bushes on Brewer farm west of 148th, just north of Kamphuis processing facility. Fruit very nice, average crop load. Little rot.

Processing samples taken on 12 Aug by Berkheimer and Montecino (Denny Brewer).

Cloudy and warm. Run took 45 minutes.

Kamphuis 2- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries (Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	3.11	4.04	2.44	<0.69	<0.69
Pre-harvest ₂	3.74	4.44	2.69	<0.69	1.17
Post-harvest ₁	5.30	4.61	3	2.44	1.11
Post-harvest ₂	5.61	4.53	2.39	2.59	1
Blower exit ₁	5.14	4.44	3.11	2.07	1.17
Blower exit ₂	5.17	4.46	3.30	1.94	0.90
Water tank exit ₁	3.68	4.07	2.39	1.96	0.69
Water tank exit ₂	4.30	3.90	3.86	2.64	<0.69
Pre-packaging ₁	4.17	3.72	<2.69	0.69	<0.69
Pre-packaging ₂	3.83	3.77	2.87	1	<0.69

KA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.90	3.74	3.30	2.69	0.47
PA before	1.64	1.43	1.65	0.30	<-0.52
BA after	4.55	4.43	3.43	2.41	0.47
PA after	3.25	1.95	1.17	0.90	<0.47

KA 2-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	<1	<1	<1	<0	<0
After	<2	<2	2	<1	<1

HA 3 'Jersey' Type of blueberry.

Pre-harvest sample taken on 19 Aug 03 by Berkheimer and Montecino.

Harvest same day. First picking (CPPU trial site). Fruit over-ripe with considerable rot.

30-50 year-old bushes. Weather conditions: sunny and hot. Processing samples taken on 20 Aug.

HA 3- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	3.63	<2.69	4.79	<0.69	<0.69
Pre-harvest ₂	3.94	3.11	4.79	0.69	<0.69
Post-harvest ₁	5.87	4.60	5.43	3.99	3.23
Post-harvest ₂	5.85	4.72	5.57	2.51	1.30
Blower exit ₁	5.36	4	4.89	1.81	0.69
Blower exit ₂	5.43	4.25	4.72	2.55	1
Water tank exit ₁	5	3.69	4.57	<0.69	0.69
Water tank exit ₂	4.63	3.69	4.86	<0.69	<0.69
Pre-packaging ₁	5.34	4.74	4.36	1.25	<0.69
Pre-packaging ₂	5.27	4.17	4.69	1.68	<0.69

HA 3-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.92	3.79	3.25	0.30	<-0.52
PA before	1.92	2.49	0.47	<-0.52	<-0.52
BA after	4.61	4.60	4.11	2.25	1.36
PA after	3.50	3.38	3.72	1.90	<-0.52

HA 3-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1	<1	<1	<0	<0
After	2	2	3.77	<1	<1

KL 1 'Elliott' Type of blueberry.

Pre-harvest sample collected on 23 Aug 03 by Berkheimer and Montecino.

Weather conditions: dry day, little soil moisture, sunny and clear, ~80F.

Harvest with smaller harvester. First picking of fruit.

Processing samples collected on 24 Aug by Hanson and Montecino, Hartmann processing facility. Fruit delivered previous evening, held in shed over night. Ran very fast first thing in morning.

KL 1- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	2.94	3.46	2.39	<0.69	<0.69
Pre-harvest ₂	3.04	3.41	2.11	<0.69	<0.69
Post-harvest ₁	4.49	3.54	4.11	<1.69	<1.69
Post-harvest ₂	4.74	3.47	2.87	<1.69	<1.69
Blower exit ₁	4.11	3.51	2.69	1.69	<1.69
Blower exit ₂	4.39	4.04	<2.69	<1.69	<1.69
Water tank exit ₁	2.69	3.57	<2.69	<1.69	<1.69
Water tank exit ₂	2.69	3.63	<2.69	<1.69	<1.69
Pre-packaging ₁	<2.69	3.81	2.69	<1.69	<1.69
Pre-packaging ₂	<2.69	3.84	<2.69	<1.69	<1.69

KL 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	<1.47	<1.47	<1.47	<-0.52	<-0.52
PA before	<1.47	<1.47	<1.47	<-0.52	<-0.52
BA after	3.53	3.62	2.89	0.77	<0.47
PA after	2.36	2.77	1.47	<0.47	<0.47

HA 3-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	<1	<1	<1	<0	<0
After	<2	<2	<2	<1	<1

HA 4 'Rubel' and 'Bluecrop' Type of blueberry.

Pre-harvest sample collected on 24 Aug 03 by Hanson and Montecino. Third picking of mixed field (very little left), 8th St west (N of Hartmann's), cross RR tracks, first 2-track on the left. Bushes variable in size, 15-30 years-old.

Processing samples collected on 25 Aug by Hanson. Question about whether initial fill and sample from water tank was chlorinated. Run at 1:00 and took 20 min. Fruit sat in shed overnight and through morning.

HA 4- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	<2.69	4.17	3.30	<0.69	<0.69
Pre-harvest ₂	<2.69	3.57	4.47	<0.69	<0.69
Post-harvest ₁	5.43	5.07	4.76	3.79	2.17
Post-harvest ₂	5.61	5.07	4.68	3.62	<1.69
Blower exit ₁	5.56	5.14	4.99	3.53	<1.69
Blower exit ₂	5.53	4.97	4.84	3.50	<1.69
Water tank exit ₁	4.59	3.92	4.23	3.55	<1.69
Water tank exit ₂	4.64	3.63	4.41	3.51	<1.69
Pre-packaging ₁	4.34	3.47	4.47	2.57	<1.69
Pre-packaging ₂	4.30	3.63	4.14	3.27	<1.69

HA 4-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	2.96	1.85	1.25	1.23	<-0.52
PA before	0.95	0.69	<0.47	<-0.52	<-0.52
BA after	4.34	4.04	4.20	2.30	<0.47
PA after	3.46	3.72	2.77	2.17	<0.47

HA 4-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	<1	<1	<1	<0	<0
After	3.38	2.17	2	<1	<1

KA 3 First picking of older Burlington bushes.

Pre-harvest sample taken on Aug 27 by Hanson. Off North Holland Road, N of 160th.

Overhead irrigated, nice quality, average crop load.

Processing samples collected on 28 Aug by Hanson. Fruit sat in shed overnight at 65F.

Sampled over 25 min run. Line set up with blower, de-stemmer, water bath, rinse (3-5 ppm chlorine) blower-drier, color sorter, hand sorting belt, and boxer.

KA 3- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	4.44	3.36	4.82	2.44	<1.69
Pre-harvest ₂	4.39	3.25	4.69	2.17	<1.69
Post-harvest ₁	5.53	4.77	5.20	2.96	1.69
Post-harvest ₂	5.57	4.57	5.34	3.07	<1.69
Blower exit ₁	5.69	5.07	5.50	2.94	1.69
Blower exit ₂	5.60	4.99	5.38	3.14	<1.69
Water tank exit ₁	5.77	4.87	4.89	3	1.69
Water tank exit ₂	5.68	4.81	4.11	3.04	<1.69
Pre-packaging ₁	5.23	4.63	4.57	2.74	2.09
Pre-packaging ₂	5.55	4.89	4.96	2.89	<1.69

KA 3-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	1.81	2.59	3.04	<-0.52	<-0.52
PA before	0.30	0.69	1.07	<-0.52	<-0.52
BA after	4.36	4.44	4.47	2.04	0.47
PA after	2.50	2.89	3.14	1.23	<0.47

KA 3-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	<1	<1	1	<0	<0
After	<1	1	1.30	<0	<0

BO 1 'Jersey' Type of blueberry.

Pre-harvest samples collected on 4 September by Hanson and Montecino. First thing in morning From field off Tyler west of US31. 2nd pick from old field, light yield, nice quality.

Weather conditions: dry, 65F and cloudy.

Processing samples run same morning at West Central Processing Facility. About 25 lugs of 30 lb each, run in 15 min. Slowed down to accommodate us. Crew had cleaned the line well before run, emptied and re-filled water tank. Tank maintained at 2 ppm with chlorine dioxide generator. Setup blower, water tank, clean water rinse, de-stemmer, color sorter, and then boxer.

BO 1- Populations of MAB, yeasts, molds, coliforms and *E. coli* on blueberries
(Log CFU/g)

Fruit sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Pre-harvest ₁	3.83	4.39	4.97	2.57	<1.69
Pre-harvest ₂	3.18	4.34	4.26	2.11	<1.69
Post-harvest ₁	4.59	4.11	4.45	2.36	<1.69
Post-harvest ₂	4.30	4.26	4.23	2.30	<1.69
Blower exit ₁	4.55	4.18	4.32	2.58	<1.69
Blower exit ₂	4.49	4.28	4.34	2.63	<1.69
Water tank exit ₁	3.25	3.89	4.11	<1.69	<1.69
Water tank exit ₂	3.94	3.94	3.68	<1.69	<1.69
Pre-packaging ₁	3.60	3.76	4.49	1.87	<1.69
Pre-packaging ₂	3.60	3.65	4.28	1.87	<1.69

BO 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* on conveyer belts (Log CFU/cm²) entering the blower (BA) and pre-packaging areas (PA), before and after fruit processing.

Swab samples	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
BA before	3.41	3.77	3.39	1.25	-0.52
PA before	2.97	2.27	1.91	0	<-0.52
BA after	4.14	3.74	4.11	1.47	0.47
PA after	2.77	2.84	2.79	0.95	<0.47

BO 1-Populations of MAB, yeasts, molds, coliforms and *E. coli* in water tank samples (log CFU/ml) taken before and after fruit washing.

Water sample	MAB	Yeast	Mold	Coliforms	<i>E. coli</i>
Before	1.92	1.30	1.54	0.60	<0
After	1.47	1.77	2.02	<0	<0

APPENDIX E

EFFICACY COMPARISON OF CHLORINE DIOXIDE GAS SACHETS FOR THE MICROBIOLOGICAL QUALITY ON SURFACE-INNOCULATED AND – UNINOCULATED BLUEBERRIES

OBJECTIVE

The objective of this work study was to better characterize and compare the efficacy of chlorine dioxide gas generated by a commercial sachet in reducing the populations of MAB, coliforms, *E. coli*, yeasts and molds on inoculated and uninoculated blueberries. The sanitizer was tested at concentrations and exposure times similar to pilot study (Chapter 4) that are appropriate for blueberries processors.

MATERIAL AND METHODS

Blueberries

During the 2005 harvest season, blueberry samples (500 g) were collected in 1-pint plastic clamshell containers from a field in Grand Junction, MI, placed in individual plastic bags, stored on ice and analyzed within 4 h of collection.

Bacteria, yeasts and molds

For the inoculated fruit study, MAB, yeasts, molds, coliforms and *E. coli* strains were isolated from these same blueberry field fruits. Fruit samples (25 g) were placed in sterile 20 x 10 cm polyethylene bags (Whirl-Pack™) containing 100 ml of neutralizing buffer (Difco), shaken, pulsed, diluted, plated on TSAYE-C, *E. coli* / coliform count plates and incubated as previously described for the isolation of MAB, *E. coli* and

coliforms, respectively. Five isolates each of MAB, *E. coli* and coliforms were transferred from plates into 9 ml of TSBYE (Difco) followed by 18-24 h of incubation at 35°C and then subjected to an identical transfer in 30 ml of TSBYE before use. Five representative yeasts and molds were isolated on PDA-SA from the same fruit 3 weeks before using previously described procedure. Individual strains of yeasts and molds were separately activated by transferring a loop culture from isolation plates onto culture PDA-SA plates, surface-plated, and grown for 3 - 4 and 10 - 12 days at 26°C, respectively, before use.

For the uninoculated fruit study, MAB, yeast, molds, coliforms and *E. coli* were obtained by fruit storage in ventilated buckets at 22°C and 99.9 RH for 96 h. Fruit sample (25g) were taken initially, at 24, 48, and 96 h and monitored for the microbial growth of MAB, yeast, molds, coliforms and *E. coli* using similar procedure as described above.

Preparation of the inoculum

The five culture each of MAB, *E. coli* and coliforms were harvested by centrifugation (Sorvall Super T21) at 7,000 rpm for 10 min at 4°C and re-suspended in equal volumes (30ml) of sterile PBS. The five culture each of yeast and mold were harvested by washing the previous yeast and molds culture plates with 30 ml of sterile PBS. Suspensions (~30ml) of each bacterial strain containing approximately equal populations (10^9 CFU/ml) and each yeast and mold strain containing approximately equal populations (10^8 CFU/ml) were combined to generate a single inoculum cocktail (~ 750 ml). Populations of MAB, yeast and molds, and *E. coli*, coliforms from inoculum cocktail

were determined by plating appropriate serial dilutions in PBS on TSAYE-C, PDA-SA, and *E. coli* / coliform count plates, respectively.

Inoculation of blueberries

Blueberries (500g) were inoculated by immersion in a 25 x 20 cm sterile polyethylene bag (Whirl-Pack™) containing ~750 ml of inoculum cocktail and gently swirl-agitated at 100 rpm for 20 min on a G2 Gyrotory Shaker platform (New Brunswick Scientific Co.). Inoculated fruit samples were then air-dried under laminar flow in a biosafety cabinet for 2 h, stored overnight at 4°C and finally re-dried under laminar flow for 2 h before use.

Sanitizer Exposure

Inoculated and uninoculated fruit samples (500 g) were placed in separate 1-pint plastic clam shell containers that were then placed in sealed 20 L buckets and were either gassed (4 mg/L, 0.16mg/g fruit) or un-gassed (control) for 12 h at ~ 22°C/99.9 RH. Chlorine dioxide gas was generated in the sealed bucket using a 20-g ClO₂ sachet (ICA TriNova). After 12 h fruit samples (25g) were placed in sterile 20 x 10 cm polyethylene bags (Whirl-Pack™) containing 100 ml of neutralizing buffer (Difco) and assessed for populations of MAB, yeasts, molds, *E. coli* and coliforms as previously described

Statistical analysis

Analysis of Variance (ANOVA) was done on all microbial count data obtained from microbial growth, inoculated and un-inoculated fruit studies using the Statistical Analysis System (Proc Anova, SAS, Version 8, SAS[®] Institute Inc., Cary, NC). Data in the graphs and tables (Appendix B) are means from replicates and significance between means were

determined using least significant difference (LSD) test at the 95% confidence level ($P=0.05$).

RESULTS AND DISCUSSIONS

Initial populations of MAB, yeasts, molds, coliforms, and *E. coli* on naturally contaminated berries were 4.72, 4.83, 5.41, 0.85, and 0.69 logs CFU/g, respectively (Appendix B, Table E.1). After 96 h of storage at $21\pm1^{\circ}\text{C}$ / 99% RH in a sealed and ventilated bucket, significant ($P < 0.05$) microbial growth was observed on the blueberries with populations of MAB, yeast, mold, coliforms, and *E. coli* increasing 1.47, 1.36, 0.72, 2.73, and 2.62 logs, respectively.

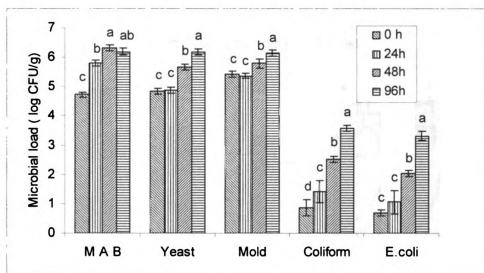


Figure E 1. Growth (log CFU/g) of MAB, yeasts, molds, coliforms, and *E. coli* (mean $\pm$ std. dev., $n=3$) on naturally contaminated blueberries during storage at $21\pm1^{\circ}\text{C}$ /99 % RH. Values with different letters within the same microbial category are significantly different ($P < 0.05$).

Populations of MAB, yeasts, molds, coliforms, and *E. coli* on inoculated blueberries decreased 3.71, 2.78, 2.52, 3.39, and 3.44 logs CFU/g, respectively, after a 12-h exposure

to 4 mg/L (0.16 mg/g) ClO_2 gas in a sealed bucket at 22°C 99.9% RH (Appendix B, Table E.2). Significantly ($P < 0.05$) greater microbial survival was seen after similarly exposing naturally contaminated blueberries to chlorine dioxide. Numbers of MAB, yeasts, molds, and *E. coli* decreased 1.05, 0.52, 0.65, and 0.26 logs CFU/g, respectively; whereas the 0.05 log CFU/g reduction for coliforms was not significant ($P > 0.05$) lower. Surface-inoculated microorganisms were likely attached to the waxy blueberry surface and thus more susceptible to sanitizers than naturally occurring microbial contaminants that were more likely embedded in the hydrophobic wax layer and thereby protected from the action of chlorine dioxide (Freeman et al., 1979).

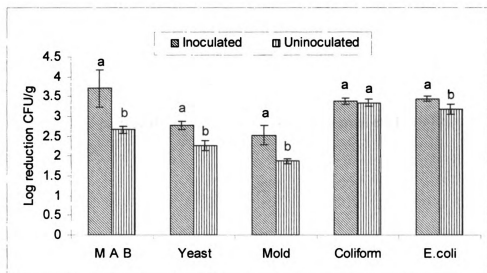


Figure E 2. Reduction (log CFU/g) of MAB, yeasts, molds, coliforms, and *E. coli* (mean $\pm$ std.dev., $n=4$) on inoculated and uninoculated blueberries after a 12-h exposure to 4 mg/L (0.16 mg/g) ClO_2 gas in a sealed 20 L bucket 22°C/ 99.9% RH. Values with different letters within the same microbial category are significantly different ($P < 0.05$).

Higher efficacy of the sanitizer on inoculated as opposed to uninoculated blueberries is also likely due to less internalization of microorganisms on the surface-inoculated fruit

which results in greater exposure to chlorine dioxide gas as opposed to naturally contaminated fruit with microorganisms embedded in the wax layer. Yeasts and molds naturally present on blueberries were less susceptible to chlorine dioxide gas than bacteria with these findings in agreement with those of Rodgers et al. (2004) and Sy et al. (2005).

Work with inoculated vs. uninoculated blueberries revealed significantly ($P < 0.05$) lower microbial reductions after similar exposure of naturally contaminated blueberries (uninoculated) to chlorine dioxide for MAB, yeasts, molds, and *E. coli* as well as significant ($P > 0.05$) differences for coliforms. Surface inoculation of the berries likely led to microorganisms that were attached to the blueberry wax. These surface-inoculated organisms were likely more exposed to chlorine dioxide gas than those on naturally contaminated fruit which would tend to be embedded in the hydrophobic wax structure and thus more protected from chlorine dioxide (Freeman et al., 1979).

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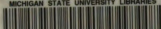
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