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Effects of Alternative Postharvest Treatments on the Microbiological Quality of Lowbush Blueberries

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ABSTRACT. The applicability of commonly used postharvest treatments to control produce decay is often limited by toxicity regulations and residual by-product accumulation. Chlorine is the most widely used sanitizer in the produce industry for improving microbial quality and extending shelf-life of most minimally processed fruits and vegetables. Although effective, recent environmental and public health concerns associated with the use of chlorine have stimulated the produce industry to identify alternative treatments equivalent to chlorine in antimicrobial effectiveness. This study compared the effectiveness of alternative postharvest treatments, hydrogen peroxide and citric acid, to the current industry standard, chlorine, in improving the microbiological quality of lowbush blueberries. Significant differences ($p < 0.05$) in antimicrobial effectiveness existed among treatments allowed the same contact time. Samples treated with 100 ppm chlorine resulted in the greatest microbial

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reduction in total aerobes, yeast, and mold counts compared to samples treated with 0.5% hydrogen peroxide, 0.5% citric acid, or distilled water; however, chlorine was second in antimicrobial effectiveness when a 1% hydrogen peroxide treatment was administered. Overall, samples treated with 1% hydrogen peroxide for 120 seconds resulted in population reductions two times greater than reductions observed on samples treated with 100 ppm chlorine. Results indicate postharvest treatments of 1% hydrogen peroxide are an effective alternative to chlorine for improving the microbiological quality and safety of lowbush blueberries during processing without compromising blueberry color.

KEYWORDS. Microbiological quality, blueberries, postharvest treatments, processing

INTRODUCTION

Lowbush blueberries account for 50% of the total blueberry crop in North America. Each year, the state of Maine harvests 25% of this wild blueberry crop from its 60,000 acres of commercially managed fields. Since 1996, Maine wild blueberry production has increased at an average of 7.6% annually (University of Maine Cooperative Extension, 2002). The majority of this crop is processed for marketing as either IQF (individually quick frozen) or canned berries with only 1% of the crop sold as fresh pack.

Currently, the Maine wild blueberry industry incorporates a 50-100 ppm chlorinated water spray into the processing of lowbush blueberries to bring about microbial reductions prior to freezing or canning. However, research has indicated that the efficacy of common chemical sanitizers to bring about significant microbial reductions on the surface of fruits and vegetables may be limited and unpredictable (Cena, 1998; Nguyen-the and Carlin, 1994). For example, when chlorine is used at permitted concentrations, a 1- to 2-log population reduction is the most that can be expected (Sapers, 1998). Furthermore, in recent years, important health and environmental concerns have arisen over chlorine's continued use due to its reactivity with some food constituents to form toxic by-products (Cena, 1998; Graham, 1997). Thus with the possibility of future

regulatory constraints on the use of chlorine as a sanitizer, alternative treatments are being sought to improve the microbiological quality and safety of agricultural commodities. Numerous studies have been conducted supporting the use of hydrogen peroxide (Sapers and Sites, 2003; Sapers and Simmons, 1998), ozone (Kim et al., 1999), and citric acid (Brennan et al., 2000; Shapiro and Holder, 1960) as effective postharvest treatments capable of extending the shelf-life of agricultural commodities. For this study, citric acid and hydrogen peroxide were investigated based on the adaptability of these treatments to current blueberry processing facilities. The objective of this study was to compare the effectiveness of alternative postharvest treatments to the current industry standard, chlorine, for improving the microbiological quality of lowbush blueberries.

MATERIALS AND METHODS

Field samples were obtained from a commercial blueberry field in Deblois, Maine, for two consecutive years (2001-2002). Designated plots around the field served as markers for sample collection. Sampling began after initial aerial pesticide application and continued each week through harvest. Samples for microbial analysis were collected at the beginning of each week and transported on ice to the Department of Food Science and Human Nutrition for treatment and analysis. In 2001, four treatments were evaluated including 100 ppm chlorine, 0.5% hydrogen peroxide, 0.5% citric acid, and distilled water sprays with each treatment allowed a 30 or 300 second contact time. Microbial results from the 2001 crop year served as the basis for treatment and contact time selection for the 2002 crop year. Second year treatments included 100 ppm chlorine, 1% hydrogen peroxide, and distilled water sprays allowed a contact time of 60 or 120 seconds.

In order to replicate industrial processes, spray treatments were applied using Home and Garden Sprayers (RL Flomaster, Root-Lowell Manufacturing Co., Lowell, Michigan) modified with Whirljet nozzles (1/4 B ss 3) (Spraying Systems Co., Wheaton, Illinois). Treatment volumes of 500 mL were applied using this spray system. Before each treatment, sprayers were pumped 100 times to provide the necessary pressure to apply the solution. Blueberry sub-samples of 350 g were spread on a sterile wire screen and subjected to 500 mL sprays of each treatment. After spraying, berries were held for the specified contact time before microbial analysis. To initiate the end of each contact time,

berries were blast frozen at −30°C for 3 minutes. Unwashed berries served as the control for this study.

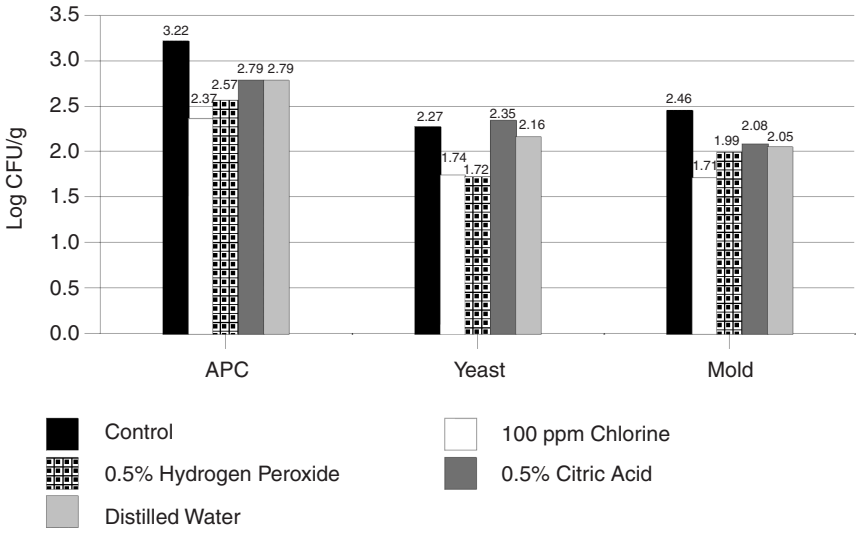
Triplicate samples of 50 g were taken immediately after freezing for microbial analysis of total aerobes, yeast, and mold populations. Microbial analysis was performed according to FDA Standard Methods (FDA, 1998). Appropriate serial dilutions were prepared in sterile 0.1% Bacto-Peptone (Difco Laboratories, Detroit, Michigan) and plated in duplicate. Total aerobic plate counts (APC) were performed using Plate Count Agar (Difco Laboratories, Detroit, Michigan). Yeast and mold were enumerated using Potato Dextrose Agar (Difco Laboratories, Detroit, Michigan) acidified to a final pH of 3.5 with tartaric acid.

Microbial data were analyzed using Systat Analytical Software, version 9.0 (Chicago, Illinois). One-way analysis of variance and Tukey's HSD Multiple Comparison was performed to determine if significant differences ($p < 0.05$) existed in mean values of microbial populations among treatments. A multi-way analysis of variance was also conducted using the variables of time, treatment, and week to determine if these variables significantly influenced ($p < 0.05$) microbial populations.

RESULTS AND DISCUSSION

Overall results from the 2001 crop year indicate that samples treated with 100 ppm chlorine showed significantly greater log reductions ($p < 0.05$) in total aerobes, yeast, and mold populations compared to samples treated with 0.5% hydrogen peroxide, 0.5% citric acid, or distilled water. Figures 1 and 2 summarize the mean microbial counts of lowbush blueberries in response to treatments at both contact times. The effectiveness of chlorine at bringing about microbial reductions was similar to results obtained by Hazen (2001) and Sapers (1998). According to Sapers, a 1- to 2-log population reduction is the most that can be expected when chlorine is used at permitted concentrations. Although microbial reductions of up to 1.5 log were observed on individually-treated samples, cumulative results had a mean population reduction of less than 1 log for all chlorine-treated samples (Table 1). The reduced effectiveness of chlorine at inactivating surface microorganisms may be a result of organic matter surrounding the target cells (Beuchat et al., 2001). As fruits mature, internal cellular structures are degraded by pectinases. Furthermore, fruit degradation is accompanied by softening of cell walls and increased fragility of the fruit skin which can lead to the release of plant tissues onto the surface of the berries. If

FIGURE 1. Mean[z] log CFU/g[y] aerobic mesophillic bacteria (APC), yeast, and mold counts following treatment for 30 seconds–crop year 2001.

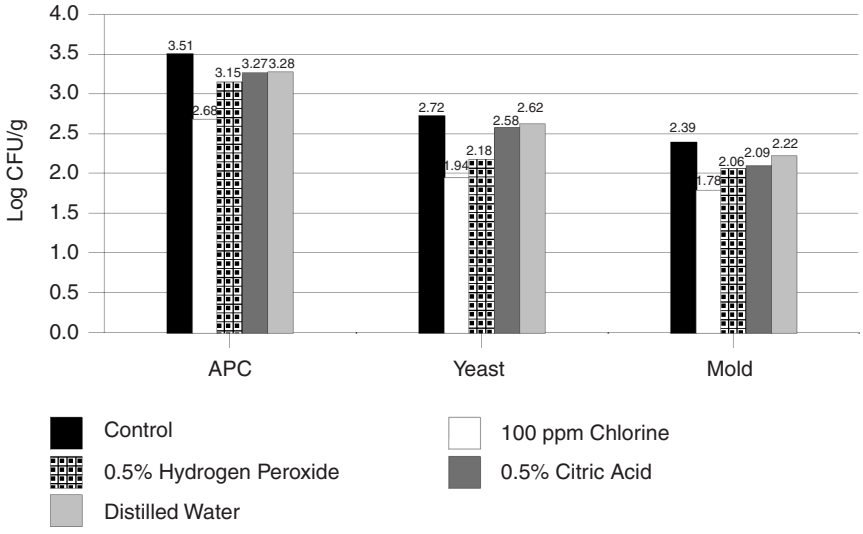


[z] Values represented are the mean of 30 samples across 5 weeks.
[y] All values obtained from analysis were converted to CFU/g blueberries.

organic materials, like plant tissues, interact with chlorine before it makes contact with target cells, the free chlorine in solution becomes neutralized on contact. Thus, the effectiveness of chlorine is limited by the presence of organic materials including any organic impurities present in water. In addition, the pH of the chlorinated wash water may have also influenced the effectiveness of chlorine. The antimicrobial activity of chlorine is dependent upon the concentration of hypochlorous acid (HOCl) in solution; therefore, the pH of the solution should remain at or below pH 7 at which point hypochlorous acid remains undissociated. Consequently, the effectiveness of chlorine treatments in this study may have been reduced since solutions had a mean pH of 9.1 and did not undergo pH adjustment before use. This fluctuation in pH is commonly observed in industry and is compensated for by adjusting the pH of chlorine treatments with hydrochloric acid (HCl).

Among treatments administered during the 2001 crop year, individual treatment effectiveness was not significantly influenced ($p < 0.05$) by the duration of contact time. For example, at week 2, an APC reduc-

FIGURE 2. Mean[z] log CFU/g[y] aerobic mesophillic bacteria (APC), yeast, and mold counts following treatment for 300 seconds–crop year 2001.



[z] Values represented are the mean of 30 samples across 5 weeks.
[y] All values obtained from analysis were converted to CFU/g blueberries.

TABLE 1. Log CFU/g[z] (mean ± SD) and log reduction in microbial populations of treated blueberries–crop year 2001.

	APC	Yeast	Mold
Control–30 Seconds	3.22 ± 0.65	2.27 ± 0.49	2.46 ± 0.46
100 ppm Cl_2	0.85	0.53	0.75
0.5% H_2O_2	0.66	0.55	0.47
0.5% Citric Acid	0.43	−0.08y	0.37
Distilled Water	0.43	0.11	0.40
Control–300 Seconds	3.51 ± 0.58	2.72 ± 0.21	2.39 ± 0.38
100 ppm Cl_2	0.83	0.77	0.61
0.5% H_2O_2	0.36	0.54	0.34
0.5% Citric Acid	0.24	0.14	0.30
Distilled Water	0.22	0.10	0.17

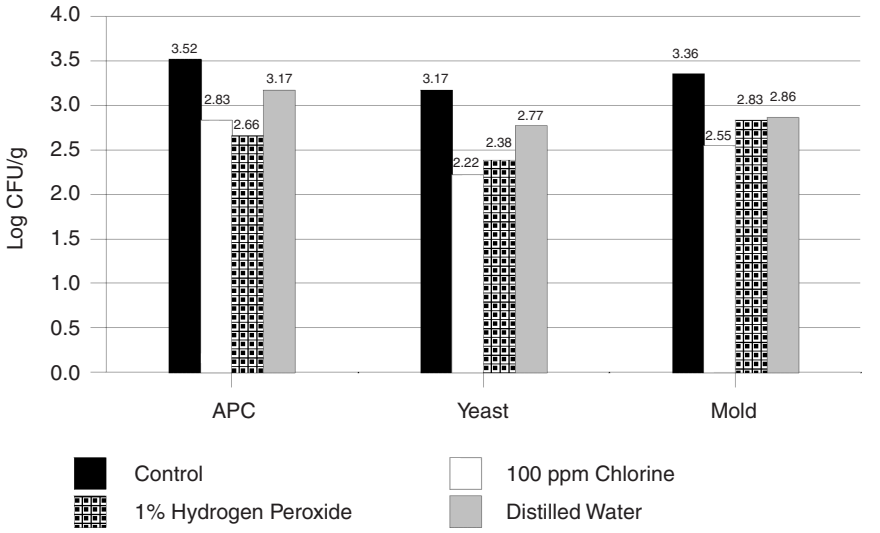
[z] All values obtained from analysis were converted to CFU/g blueberries.
[y] Negative reductions indicate an increase in aerobic late counts after treatment.

tion of 0.98 log was seen in samples treated with 100 ppm chlorine for 30 seconds versus a mean reduction of 0.76 log for chlorine-treated samples allowed a 300 second contact time. By week 5, the interaction of contact time and treatment influenced the population differently. Samples treated with 100 ppm chlorine for 30 seconds resulted in a mean APC reduction of 1.47 log versus a 1.50 log reduction seen in chlorine-treated samples allowed a 300 second contact time. Therefore, holding treated samples for an additional 270 seconds did not result in a marked decrease in microbial populations. The influence of contact time on the effectiveness of chlorine as a sanitizer was similar to results by Hazen (2001) and Beuchat (1999). Although contact time did not significantly ($p < 0.05$) influence a treatment's effectiveness, greater population reductions were observed on treated samples that were allowed a 30 second contact time (Table 1).

Among alternative sanitizers investigated, 0.5% hydrogen peroxide sprays were second to 100 ppm chlorinated water sprays in antimicrobial activity with total aerobe, yeast, and mold populations responding similarly to hydrogen peroxide treatments (Table 1). Microbial results indicate that 0.5% citric acid and distilled water treatments were comparable in their antimicrobial effectiveness with yeast populations influenced the least by either treatment. The reduced effectiveness of 0.5% hydrogen peroxide and 0.5% citric acid treatments may be attributable to the relatively low concentration of these sanitizers in solution. According to Shapiro and Holder (1960), solutions of 150 ppm and 500 ppm citric acid were capable of suppressing bacterial growth of salad greens for up to 96 hours. Furthermore, recent studies have proven hydrogen peroxide treatments to be more effective than chlorine in reducing the microbial load and extending the shelf-life of fresh-cut fruits and vegetables when used in concentrations ranging from 1-10% (Sapers and Sites, 2003; Sapers and Simmons, 1998). Although treatments containing higher concentrations of citric acid and hydrogen peroxide may be beneficial in improving the microbial quality and extending the shelf-life of agricultural commodities, the use of these treatments in blueberry processing may be limited due to excessive surface oxidation resulting in product discoloration. Based on this data and emerging research into the use of hydrogen peroxide as an alternative postharvest sanitizer, 1% hydrogen peroxide treatments were evaluated during the second year of this study and compared to the current industry standard of 100 ppm chlorinated water sprays. Contact times of 60 and 120 seconds were selected based on actual contact times achieved during blueberry line processing.

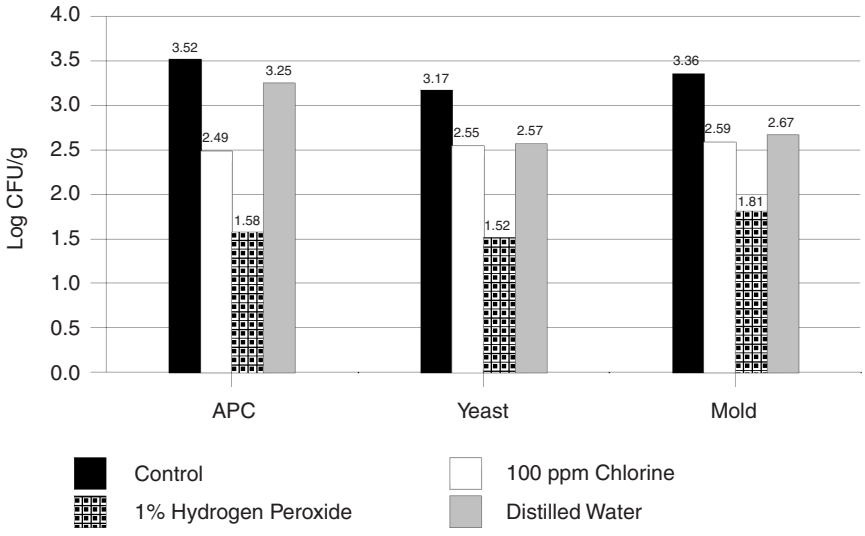
Significant reductions ($p < 0.05$) in microbial populations were observed in blueberry samples treated with 1% hydrogen peroxide for 60 and 120 seconds. Figures 3 and 4 summarize the mean microbial counts of lowbush blueberries in response to treatments administered during the 2002 crop year. Among treatments evaluated, the interaction of 1% hydrogen peroxide treatments and contact time significantly influenced ($p < 0.05$) total aerobe, yeast, and mold populations with the greatest log reductions achieved following a 120 second contact time. For example, hydrogen peroxide-treated samples held for 120 seconds resulted in log reductions up to two times greater than reductions observed on samples treated with 100 ppm chlorine or 1% hydrogen peroxide for only 60 seconds. Population reductions approaching 2 logs were obtained on all samples treated with 1% hydrogen peroxide for 120 seconds without compromising blueberry color (data not shown) as evidenced by color analysis using the Hunter Labscan (Hunter Associates Laboratory, Reston, VA). Overall, samples treated with 1% hydrogen peroxide sprays

FIGURE 3. Mean[z] log CFU/g[y] aerobic mesophillic bacteria (APC), yeast, and mold counts following treatment for 60 seconds–crop year 2002.



[z] Values represented are the mean of 18 samples across 3 weeks.
[y] All values obtained from analysis were converted to CFU/g blueberries.

FIGURE 4. Mean[z] log CFU/g[y] aerobic mesophillic bacteria (APC), yeast, and mold counts following treatment for 120 seconds–crop year 2002.



[z] Values represented are the mean of 18 samples across 3 weeks.
[y] All values obtained from analysis were converted to CFU/g blueberries.

for 120 seconds reported the greatest mean log reductions in total aerobe, yeast, and mold populations (Table 2).

GROWER BENEFITS

Overall results indicate that 1% hydrogen peroxide sprays applied to lowbush blueberries for a contact time of 120 seconds result in a marked decrease in surface microbial populations of total aerobes, yeast, and mold. Because a contact time of 120 seconds is attainable within current processing parameters, 1% hydrogen peroxide sprays may be considered an effective alternative to chlorine for use in lowbush blueberry processing. Due to the similarity in treatment application, incorporation of this technology into Maine's blueberry processing facilities should take place without any necessary additions in current processing. Furthermore, since potentially hazardous residual by-products are not known

TABLE 2. Log CFU/g[z] (mean ± SD) and log reduction in microbial populations of treated blueberries–crop year 2002.

	APC	Yeast	Mold
Control–60 Seconds	3.52 ± 0.40	3.17 ± 0.13	3.35 ± 0.16
100 ppm Cl_2	0.69	0.95	0.81
1% H_2O_2	0.87	0.79	0.53
Distilled Water	0.36	0.40	0.50
Control–120 Seconds	3.52 ± 0.40	3.17 ± 0.13	3.35 ± 0.16
100 ppm Cl_2	1.03	0.62	0.76
1% H_2O_2	1.94	1.66	1.55
Distilled Water	0.27	0.60	0.69

[z] All values obtained from analysis were converted to CFU/g blueberries.

to form in treatment waste water following its use, hydrogen peroxide sprays also represent a less environmentally-demanding method of improving the microbial quality and safety of blueberry products should the use of chlorine be restricted.

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