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Effects of Post-harvest Treatments on the Microbiological Quality and Pesticide Residues of Lowbush Blueberries

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EFFECTS OF POST-HARVEST TREATMENTS ON THE MICROBIOLOGICAL QUALITY AND PESTICIDE RESIDUES OF LOWBUSH BLUEBERRIES

By

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An Abstract of the Thesis Presented
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Lowbush blueberries of the species *Vaccinium angustifolium* are native to the Northeast United States. In the state of Maine, lowbush blueberries are commercially managed and harvested each year to be marketed as either IQF (individually quick frozen) or fresh pack. Since IQF berries are processed at the field level, post-harvest treatments must be effective in ensuring crop quality and safety. Furthermore, with the increase in concern for microbiological and chemical food safety, post-harvest treatment of lowbush blueberries must be effective in reducing microbial populations and pesticide residues. Currently, the Maine Wild Blueberry Industry incorporates a 50-100ppm chlorinated water spray into the processing of lowbush blueberries. However, the possibility of future regulatory constraints on the use of chlorine as a sanitizer calls for investigation of alternative treatments that would be equally effective in improving microbial quality and safety. This thesis examined the influence of post-harvest

treatments namely chlorine, hydrogen peroxide, citric acid, and distilled water on the microbiological quality and residual phosmet levels of lowbush blueberries. This research also assessed the degradation of phosmet on commercial blueberries in Maine.

Field samples were collected from one of Maine's commercial blueberry fields immediately after treatment with aerial sprays of Imidan 70-W (phosmet). Sampling continued for a total of five weeks during the summer of 2001. Berries were subjected to 500mL sprays of 100ppm chlorine, 0.5% hydrogen peroxide, 0.5% citric acid, or distilled water. Microbiological analysis of total aerobes, yeast, and mold were conducted using FDA Standard Methods. Residual phosmet was measured by GC/MSD.

Microbial reductions of up to 1.5 log were observed on individual washed samples. Significant differences ($p<0.05$) in antimicrobial effectiveness existed among treatments allowed the same contact time. Overall, samples treated with 100ppm chlorine had the lowest mean counts of total aerobes, yeast, and mold. No significant differences ($p<0.05$) were observed in treatment effectiveness after holding for 30 seconds versus 300 seconds. During the five weeks pre-harvest, results showed that as the microbial load increased, residual phosmet levels decreased. Residual phosmet levels of treated samples were significantly lower ($p<0.05$) than residues on unwashed berries. The extent to which treatments removed residual phosmet was influenced by the week of sampling. The greatest reductions in residual levels were observed on blueberries treated during the first three weeks when residual levels on unwashed controls were highest. Although treatments were less effective during the final weeks of the study, the residual phosmet levels on the control samples fell below the EPA tolerance level of 10,000ppb.

Furthermore, 100% of all blueberries sampled during the final three weeks of the study contained residual phosmet at levels below the EPA tolerance.

Results of this study demonstrate the efficacy of treating lowbush blueberries with 100ppm chlorine sprays to improve the microbiological quality and safety of the crop; however, additional research on alternative sanitizers and their effective concentrations should be conducted to aid the industry should the use of chlorine be restricted.

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WILD BLUEBERRY CULTURE

Crop Production

Lowbush blueberries of the *Vaccinium* genus are native to the Northeast United States and Canada. These wild blueberries account for 50% of the total blueberry production in North America with Maine producing 25% annually. The remaining 25% of the wild blueberry crop is produced in the Canadian provinces of Nova Scotia, Quebec, New Brunswick, Prince Edward Island, and Labrador.

The state of Maine commercially manages 60,000 acres of wild blueberry fields. However, only half of the fields are harvested each year due to pruning methods used to stimulate higher yields (Yarborough, 1996). Fields require minimal management because the berries are indigenous to Maine and are adapted to the naturally acidic, low fertility soil. The fruits ripen throughout the summer months and harvest begins in late July or early August and usually continues until the first Monday in September.

Over the past five years, management intensity and production has increased with each harvest. 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 marketed as either IQF (individually quick frozen) or canned berries with only 1% of the crop sold as fresh pack. Berries are frozen or canned at one of seven processing facilities in the state of Maine.

Botanical Classification

From the genus *Vaccinium*, blueberries belong to the Ericaceae plant family. Several species of wild blueberries are found in Maine but the most common species are

Vaccinium angustifolium and *Vaccinium myrtilloides.* Maine's most abundant blueberry variety is *Vaccinium angustifolium* also known as the low sweet blueberry. Plants of this species have smooth stems that vary in color from tan to red and range in height from 4 to 15 inches. Leaves are dark green with slightly toothed edges. The blossoms are bell-shaped and white or pinkish-white in color. The fruit is usually dark blue or powder blue in color depending on the presence of a waxy coating that gives the fruit a lighter appearance. Another variety of the low sweet blueberry, nigrum, is black in color due to the absence of this waxy coating or bloom.

The second most common variety of lowbush blueberries is *Vaccinium myritilloides* or the sour top blueberry. These berries are more prevalent in the mountains or hills of Maine and range in height from 6 to 24 inches. The stems and leaves are covered with tiny hairs and are more branched than the low sweet blueberries. The fruits are bright blue in color and are covered with a waxy coating. In contrast to the low sweet variety, the bell-shaped blossoms of the sour top blueberry are greenish-white in color with smaller, generally less sweet fruit.

Growth Characteristics

Wild blueberry plants are indigenous to the forests and mountains of Maine. These plants are composed of underground stems called rhizomes, which grow near the soil surface. As the plant matures, roots develop on the rhizomes. Each plant or clone possesses different genetic characteristics than neighboring clones. The growing area of each clone depends on its age with younger clones covering less area.

Only half of Maine's 60,000 fruit bearing acres are harvested each year. Immediately after harvest, blueberry fields are pruned to the ground either by mechanical mowing or by fire. The growing season immediately following harvest is set aside for vegetative and formative growth thus setting up a two-year growth cycle. The naturally acidic soil of Maine allows for optimum growth of wild blueberries. The optimum acidity of the soil should remain near pH 4.5. Soil testing is necessary to determine if fields need to be treated with sulfur to lower the pH or with lime in order to increase the pH making the soil less acidic.

Blueberry plants require insect pollination to increase the fruit set and seed numbers resulting in higher yields. Honeybees are the insects of choice for the pollination of blueberries. Current recommendations call for 2 to 4 hives per acre.

Harvesting the berries takes place in late July and early August when the majority of the berries are ripe. Ninety percent of the berries are raked by hand although some companies prefer mechanical harvesting techniques. The typical raking season lasts through the first Monday in September.

SAFETY OF ORGANOPHOSPHOROUS PESTICIDES FOR WILD BLUEBERRIES

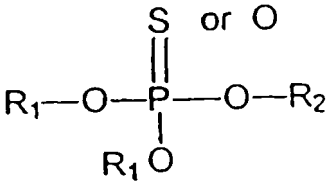
Organophosphates

The safety of our food supply is important to agribusiness as well as to consumers. Today, there is a high demand for our food supply to be free of potentially toxic substances including those used in the production of raw products. During the production and processing of agricultural commodities, various chemicals and foreign substances may enter food commodities. These chemicals are considered indirect or non-intentional food additives. Pesticides used in the production of raw products are among the most common indirect food additives. Most pesticides are acutely toxic and can produce adverse health effects in humans and animals when ingested at low levels over long periods of time (Smith, 2000). When pesticides are used according to their prescribed applications, very few foods produced in the United States contain toxic pesticide levels; however, most foods contain some residual levels depending on the food product. Acceptable residual levels are established for all foods by the Environmental Protection Agency (EPA) based on the use and toxicity of the pesticide. Title 40 of the Code of Federal Regulations (parts 150 to 189) provides an alphabetical listing of approved chemicals, specific uses, and acceptable residual levels as approved by the EPA.

Pesticide classifications are based on the specific organism to be eliminated or controlled. Organophosphates (OP's) are classified as insecticides although they are commonly used as herbicides, fungicides, and acaricides. First synthesized in the 1800's, the first organophosphates were highly neurotoxic to mammals but were later modified to be less toxic in order to facilitate use. Organophosphates can be divided into 15

subgroups depending on the type of organic group bound to the core phosphate atom (Smith, 2000). In most organophosphates, the core phosphate atom is double-bonded with sulfur because the presence of sulfur enhances the absorption of the organophosphate through the cuticle layer of the insect's exoskeleton (Figure 1). Upon absorption, the organophosphate is converted to the active oxon form which generally possesses the same toxicity as the parent compound. The toxicity of organophosphates is attributed to their ability to irreversibly inhibit acetylcholinesterase.

Figure 1: General Formula of Organophosphate Pesticides



Phosmet: Chemical Characterization and Nomenclature

Phosmet is a broad-spectrum organophosphate insecticide/acaricide applied to terrestrial food areas to control a variety of pests including alfalfa weevil, boll weevil, codling moth, leafrollers, and blueberry maggot (EPA, 2001). Other applications of phosmet include nursery and ornamental plants as well as bovine livestock, poultry, and dogs (Purdey, 1998; Smith, 2000; EPA, 2001).

According to the EPA's risk assessment for phosmet, over 95% of its usage is for insect control on commercial tree and vine fruit (EPA, 2001). Approximately 80% of this usage is applied to apples throughout the northeastern and western states, while the

remaining 15% is applied to pears, pecans, peaches, cherries, almonds, plums, prunes, blueberries, and grapes. On average, approximately 1 million pounds of phosmet are applied annually to approximately 1.1 million acres across the United States. In Maine, phosmet is used on commercial blueberry fields to control a wide variety of pests including the blueberry maggot, flea beetles, and the sawfly and spanworm larvae.

The chemical name for phosmet is N-(mercaptomethyl)-phthalimide-S-(O,O-dimethyl phosphorodithioate) and the trade name is Imidan (Gowan Chemicals, Arizona). Phosmet is an off-white crystalline solid that is sparingly soluble in water. Agricultural applications include delayed dormant spray or foliar application performed using aerial or handheld equipment while application to livestock and dogs is performed via backpack sprays or dipping.

Phosmet: Degradation

Factors which influence the persistence of organophosphorous insecticides include pH, temperature, vapor pressure, sunlight and other radiations, and the presence of microorganisms (Chapman and Cole, 1982; Garcia-Repetto et al., 1994).

Phosmet is relatively stable in acidic conditions but is rapidly hydrolyzed in neutral and alkaline media. At 20°C, the insecticide is stable for 13 days at pH 4.5, < 12 hours at pH 7, and < 4 hours at pH 8.3 (Pesticide Manual, 1997). Therefore, the pH of the media plays an important role in the stability of phosmet. Furthermore, the chemical structure of phosmet makes it susceptible to hydrolysis in aqueous environments (Figure 2) (Garcia-Repetto et al., 1994; Sharom et al., 1980). Thus,

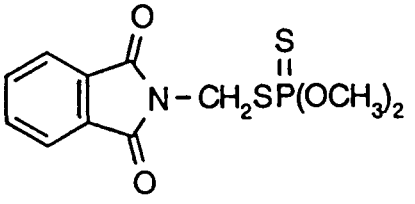
hydrolysis of phosmet under neutral and alkaline conditions is a major dissipation pathway that contributes to its low environmental persistence (EPA, 2001).

In addition to chemical degradation, phosmet also undergoes microbial degradation within the soil. Enhanced microbial degradation occurs when a population of soil microorganisms, which has adapted to exposure of a pesticide, rapidly degrades a subsequent application of the pesticide (Racke and Coats, 1988). This type of mineralization by soil microorganisms contributes to the decreased persistence of phosmet. After hydrolysis under neutral or alkaline conditions, microbial degradation under aerobic conditions appears to be the most important degradation pathway of phosmet (EPA, 2001). In environmental fate assessment studies conducted by the EPA, phosmet was found to degrade rapidly under aerobic conditions in the soil (half-life, $t_{1/2}$ = 3 days, pH 7.4), and more slowly under anaerobic conditions ($t_{1/2}$ = 15 days, pH 7.1). Fields evaluated 150 days after initial phosmet application showed residual phosmet levels less than 0.01ppm under aerobic soil conditions. In soil mobility studies, phosmet was largely detected in the upper 7 inches of the soil with no detection below the 10.5-inch soil layer (EPA, 2001).

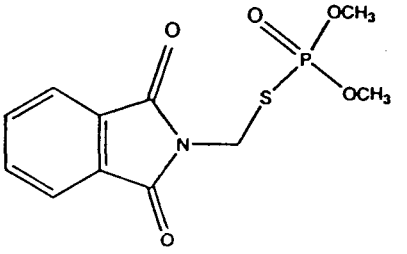
The primary hydrolysis products identified by the EPA during fate assessment studies include phosmet oxon, phthalamic acid, phthalic acid, and phthalimide. These degradates were qualitatively the same in both aerobic and anaerobic soils but differed in the amounts formed. This is due in part to the varying rates of formation among degradates. Of the known degradates identified by the EPA, phosmet oxon (0,0-dimethyl-S-phthalimido-methylphosphorothioate) is the only degradate identified for toxicological concern (Figure 2). In soil mobility studies, phosmet oxon appears to be

limited to upper soil layers (0- to 3-inch soil layer) while the parent compound has been detected as low as the 10.5-inch soil layer (EPA, 2001). Additional research is needed to formulate a full fate assessment of the oxygen analog as well as to determine the persistence of other degradates relative to the parent compound.

Figure 2: Structural Formula of Phosmet and Phosmet Oxon



Phosmet



Phosmet Oxon

Phosmet: Bioaccumulation and Toxicity

Due to the short half-life of phosmet under both aerobic and anaerobic conditions, the likelihood for bioaccumulation should be decreased. However, there are no studies to confirm this theory and the bioaccumulation of phosmet remains unclear. Even though phosmet is not considered to be persistent ($t_{1/2}$ = 3 days under aerobic conditions), the application rates and frequency of applications can influence residues that may trigger chronic toxicity concerns. Data published by the EPA for the Phosmet Reregistration Eligibility Decision in 1998 suggest that on certain crops where there is high application rate and frequency of application, expected environmental concentrations can lead to acute and chronic risk to both terrestrial and aquatic species.

Toxicity concerns for phosmet center on its ability to irreversibly inhibit acetylcholinesterase. This enzyme plays an important role in the central nervous system by hydrolyzing acetylcholine at the pre-synaptic terminal to prevent re-firing from the synapse. The hydrolysis products include acetic acid and choline of which the choline may be taken up by the pre-synaptic terminal or returned to the bloodstream for future use. Symptoms of cholinesterase inhibition include salivation, sweating, headache, nausea, muscle twitching, tremors, incoordination, blurred vision, tears, abdominal cramps, diarrhea, and chest discomfort. Severe cases of cholinesterase inhibition may lead to convulsions, pulmonary edema, respiratory failure, and death.

As with any pesticide, phosmet falls under the regulation of the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the US Department of Agriculture – Food Safety and Inspections Services (USDA-FSIS). Tolerances for residual phosmet on blueberries are determined by the EPA and published in Title 40 of the Code of Federal Regulations. According to the EPA, tolerance is defined as the maximum amount of a specific pesticide and/or its by-products that is permitted to remain in or on foods. As published in Title 40 CFR180.261, the tolerance for residual phosmet on blueberries is 10ppm. According to the EPA, phosmet is classified as a class II non-systemic insecticide and acaricide with predominantly contact action. Table 1 details the potential toxicity risks associated with phosmet exposure. Results from these tests on surrogate species are considered applicable to other member species within their class.

Table 1: Ecological Effects Hazard Assessment of Phosmet

SPECIES	TOXICITY	TOXICITY LEVEL
Avian (acute)	moderate to non-toxic	840->5,000ppm
Avian (chronic)	possible reproductive concern	60-150ppm
Mammalian (acute)	moderate	113mg/kg
Mammalian (chronic)	possible reproductive concern	*LOEC = 20ppm
Honey bee (chronic)	very highly toxic	1.06µg/bee
Fish, freshwater (acute)	very high to moderate	0.07-11.0ppm
Fish, marine/estuarine (acute)	highly toxic	0.17ppm
Invertebrates - freshwater (acute)	very highly toxic	0.002-0.008ppm
- freshwater (chronic)	possible growth effects	0.0011ppm
- marine/estuarine (acute)	very highly toxic	0.016-0.017ppm
- marine/estuarine (chronic)	1st and 2nd generation survival	0.0005ppm

*LOEC – Level of Estimated Concern
Results obtained from the EPA Environmental Risk Assessment for Phosmet (2001).

Phosmet: Application and Use

Imidan 70-W (EPA registration number 10163-169) is aerially applied to Maine's wild blueberry fields when indicated by insect infestations. According to the 2001 Insect Control Guide for Wild Blueberries published by the University of Maine Cooperative Extension, pesticides are applied to blueberries for insect management of blueberry spanworm, blueberry flea beetle, thrips, and blueberry maggot. The use rate is 0.67 to 1 1/3 pounds of Imidan 70-W in a minimum of 2 gallons of water per acre of field or row crops. The rate required for thorough, uniform coverage varies with plant growth at the time of application. Its use is restricted to no more than 2.67 pounds per acre per season and cannot be applied < 7 days prior to harvest. This product is not to be applied while bees are actively pollinating the treatment area or in residential areas. Because phosmet has a pH value of 3.0 and is rapidly hydrolyzed in alkaline media, its insecticidal activity may be reduced when the pH of the spray solution is $\geq$ 7. The pH of the solution must be corrected by the addition of a suitable buffering or acidifying agent for optimal insecticidal activity. Phosmet is also incompatible with alkaline agricultural materials such as spray lime, lime sulfur, and Bordeaux mixtures.

During the summer of 2001 when this research began, the restricted entry interval for treated fields was 24 hours. However, as of November 5, 2001, the EPA announced new restrictions on the use of phosmet including a 3-day restricted entry interval. Furthermore, blueberries were listed as one of nine crops with authorized use of phosmet for five years under specific terms. Under an agreement with the registrant, Gowan Company, three uses of phosmet were voluntary cancelled including application on domestic pets, household ornamentals, and household fruit trees.

SAFETY AND QUALITY ISSUES ASSOCIATED WITH FRUITS AND VEGETABLES

Food Safety and the Produce Industry

In recent years, the consumption of fresh fruits and vegetables in the United States has dramatically increased due to consumer perception that fresh fruits and vegetables are more nutritious (Garg et al., 1990). During this time, the incidence of food-borne illness due to food pathogens, chemicals, and wastewater has also increased (Xu, 1999; Tauxe et al., 1997). In the United States, food-borne diseases have been estimated to cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year (Mead et al., 1999). Since such statistics are drawing significant public and government attention, an increasing amount of research is being directed to investigate the safety of fresh fruits and vegetables and in particular processing methods to reduce and eliminate human pathogens. For example, in 1997 the President of the United States along with Congress issued the President's Food Safety Initiative to improve the nation's food safety system and its influence on the environment. One of the goals of this initiative was to identify an alternative sanitizer to replace the more traditional sanitizing agents like chlorine. At present, chlorine is the most widely used commercial sanitizing agent for fresh produce; however, important health and environmental concerns have arisen over its continued use (Xu, 1999).

Shelf-life and safety are important aspects of fresh produce that dictate processing steps used in industry. The shelf-life of produce is largely dependent on the microbiological quality while safety is a factor of both the microbiological quality as well as the presence of chemical residues. It is estimated that losses due to microbiological spoilage of fresh produce between the time of harvest and consumption are as high as

30% (Beuchat, 1991). For this reason, large amounts of pesticides are applied annually to fruit and vegetable crops in order to delay spoilage and control pests. At the time of harvest, residual levels remain on these products usually in concentrations below tolerance levels set by the EPA for each product. Unfortunately, current technologies cannot totally remove residual pesticides and by-products from the surface of fruits and vegetables; therefore, residues are ingested by consumers on a daily basis. The extent to which these residues affect public health is not completely understood.

Microbiological Concerns Associated with Fresh Produce

As global trade increases, once seasonal produce is now available to consumers year round. Thus, produce reflects variations in microflora due to varying conditions of cultivation. The microflora of fruits and vegetables ultimately reflects the soil in which it is cultivated, the climate, and cultivation practices of the growing region. Furthermore, the extent to which the commodity is processed will also affect the post-harvest microflora. According to a preliminary report by the National Advisory Committee on Microbiological Criteria for Foods, the potential for microbial contamination of produce exists at every level of processing and production (Tauxe et al., 1997). Contamination may occur pre-harvest from the use of a contaminated water source as well as the use of improperly composted and treated manure as fertilizer. The use of manure can introduce food-borne pathogens such as *Salmonella* spp. and *E. coli* O157:H7. For example, it has been shown that *E. coli* O157:H7 can survive in bovine feces for 70 days, which means that regulations requiring aging of bovine manure for 60 days before using it as fertilizer may be inadequate (Wang et al., 1996). In addition, the quality of the water or ice used in

processing is a special concern because of the potential for amplification associated with the use of contaminated water or improperly maintained wash water.

In general, the quality of fresh produce is dependent on the growth and survival of microorganisms. According to Tauxe et al. (1997), microbial quality is determined by the interaction of four broad factors: (1) the characteristics of the microorganism present, (2) the physiologic state of the plant tissue and its resistance to microbial metabolic processes, (3) the characteristics of the environment surrounding the plant tissue (e.g., pH, water activity, atmospheric composition), and (4) the effect of food processing on microbial populations and plant metabolism.

The general composition of fruit is 85% water and 13% carbohydrate along with various vitamins and organic compounds. The nutrient content of most fruits would support the growth of bacteria, yeast, and mold, but yeasts and molds predominate as spoilage organisms due to their ability to thrive at low pH levels (Jay, 2000). A variety of yeast genera commonly bring about the spoilage of fruits especially in the field. Compared to molds, yeasts typically initiate the spoilage of fruit due to their faster growth rate. Initially, yeasts ferment fruit sugars producing alcohol and carbon dioxide. Next, molds utilize this alcohol as energy and then proceed to destroy the fruit by attacking the rinds and structural polysaccharides.

Of the spoilage bacteria associated with fruits and vegetables, gram-negative mesophilic rods predominate with species of *Pseudomonas, Enterobacter,* and *Erwinia* among the most numerous. Psychrotrophic bacteria, lactic acid bacteria, molds, and fungi are also capable of bringing about spoilage of fruits and vegetables.

Produce: A Vehicle for Food-borne Pathogens

Due to the apparent increase in the number of food-borne disease outbreaks, the National Advisory Committee on Microbiological Criteria for Foods investigated the association of fresh produce with food-borne diseases and microbial pathogens (Tauxe et al., 1997). All reports were preliminary since new and emerging pathogens continuously appear in various food vehicles as a result of the globalization of food production and consumption.

According to the report, many different bacteria, viruses, and protozoa have caused outbreaks that were linked epidemiologically with fresh produce consumption. However, most outbreaks were bacterial in origin and among these outbreaks, *Salmonella* spp. were the most commonly reported. Table 2 lists the bacterial pathogens most commonly associated with fresh or minimally processed produce outbreaks and the food items that have been linked epidemiologically to outbreaks caused by these pathogens.

Table 2: Bacterial Pathogens Commonly Associated with Produce

Bacterial Pathogen	Fresh Produce	References
Bacillus cereus	Sprouts	Portney et al., 1976
Clostridium botulinum	Chopped garlic	St. Louis et al., 1988
Enterotoxigenic *E. coli*	Carrots	CDC, 1994
Escherichia coli 0157:H7	Apple cider Lettuce	Besser et al., 1993 Ackers et al., 1996
Listeria monocytogenes	Cabbage	Schlech et al., 1993
Salmonella spp.	Tomatoes Watermelon Cantaloupe Orange juice	CDC, 1993 Blostein, 1991 Ries et al., 1990 Cook et al., 1996
Shigella spp.	Lettuce Scallions	Davis et al., 1988 Cook et al., 1995
Toxigenic *Vibrio cholerae* O1	Coconut milk, froz.	Taylor et al., 1993

To reduce the microbial hazards associated with fresh produce, growers and producers must minimize environmental contamination during cultivation, harvesting, and processing. Proper temperatures should be maintained during processing, transportation, and storage of fresh and minimally processed produce. In addition, produce should be exposed to a critical control step during processing that would eliminate any contaminating organisms. Such a step may be achieved by the addition of adequate sanitizing agents to the processing water or by lowering the pH of the processing water. Traditionally, the produce industry utilizes water washes with and without chemical sanitizers to bring about microbial reductions. However, the efficacy of common chemical sanitizers to bring about significant microbial reductions on the surface of fruits and vegetables may be limited and unpredictable (Nguyen-the and Carlin, 1994). Most post-harvest washes are applied by processing companies to reduce the microbial load in order to increase the shelf-life and suppress the growth of a wide range of microflora. Very few, if any, chemical sanitizers are used for the purpose of removing pesticide residues.

POST-HARVEST TREATMENTS ASSOCIATED WITH FRESH PRODUCE

Citric Acid

Although chlorine is the most widely used industrial sanitizing agent, research has shown that alternative treatments are also effective. As an organic approach to chemical sanitizers, citric acid has been evaluated for its effectiveness in reducing microbial populations on agricultural commodities by lowering the pH to a level that would not sustain microbial growth (Shapiro and Holder, 1960). In this study, salad mixes were dipped in solutions of 500ppm citric acid (buffered at pH 5.0), acidified tap water (pH 5.0), and regular tap water (pH 7.0). The samples were cultured on successive days for one week and examined for variations in total plate counts. A treatment was considered effective if it decreased the original count and suppressed growth of the residual population beyond 48 hours. A 48-hour delay in microbial activity was considered significant since the shelf-life of salad greens is typically 7 days or less. Table 3 compares the effectiveness of dipping treatments on total bacterial counts.

Table 3: Effect of Citric Acid Dips on Total Bacterial Numbers

Treatment	Viable Bacteria / g Salad Mix		
	0 hr	**24 hr**	**72 hr**
Untreated	7.30		
Tap water, pH 7.0	7.20	7.27	7.94
Acidified tap water, pH 5.0	6.68	7.59	7.85
Citric acid, pH 5.0, 500ppm	**6.40**	**6.42**	**7.35**

Note: For a complete summary, see Shapiro and Holder, 1960.

Results indicated that both the acidified water and the citric acid treatments substantially reduced the initial plate counts by lowering the pH below which most microorganisms can survive. In addition, the citric acid treatment continued to suppress the microbial growth for up to 72 hours at which point there was increased proliferation. Those samples exposed to tap water (pH 7.0) had increased microbial counts at 24 and 72 hours. It is evident that the introduction of untreated water supplies available moisture for growth and increases the opportunity for proliferation of microorganisms. However, exposure of fruits and vegetables to tap water did result in a small reduction in counts. To verify the holding action of citric acid and to determine the critical concentration at which the inhibition of bacterial growth occurs, a group of salad greens were exposed to citric acid treatments of 150, 500, and 1500ppm. Treatments of 1500ppm citric acid significantly reduced the initial microbial population; however, treatments of 150ppm and 500ppm continued to suppress growth for up to 96 hours. Altogether, results indicated 150ppm citric acid treatments to be the most effective in reducing initial microbial populations and suppressing microbial growth on the surface of salad greens.

Hydrogen Peroxide

Hydrogen peroxide is generally recognized as safe (GRAS) for use in food products as a bleaching agent, oxidizing and reducing agent, and antimicrobial agent. As an antimicrobial agent, hydrogen peroxide is approved for use in the preparation of modified whey and thermophile-free starch and as a treatment for milk used in the production of cheese (CFR, 1994). For each food application, the FDA specifies use

levels and requires that residual hydrogen peroxide fall below tolerance levels after processing.

In recent years, hydrogen peroxide has received much attention as an antimicrobial agent with the potential to prolong the shelf-life of fruits and vegetables. Preparations such as Sanosil-25 in which hydrogen peroxide is the active compound are currently approved for use in Europe and Israel for use in drinking water and in the food industry at concentrations of 0.005-0.03% and as a surface disinfectant at concentrations up to 3% (Fallik et al., 1994). Sanosil-25 is highly effective oxidant which contains 48% hydrogen peroxide and 0.05% silver ion as a stabilizing agent. As a disinfectant, it is shown to be effective against pathogenic bacteria, fungi, algae, viruses, and amoebae. In work by Fallik et al. (1994), dips of 0.1 and 0.5% Sanosil-25 significantly inhibited decay of commercially harvested eggplants during 14 days storage at 12°C. Less than 4% decay was observed on eggplant treated with 0.5% Sanosil-25 after 14 days compared to 11% decay observed on the untreated control. Furthermore, sweet red peppers treated with 0.5% Sanosil-25 showed 80% less decay when compared to the control after storage for 14 days at 8°C.

Juven and Pierson (1996) explained the bactericidal effects of hydrogen peroxide in biological systems such as the growth inhibition of one bacterial species by another and killing of invading microorganisms by activated phagocytic cells. Most aerobic bacteria generate hydrogen peroxide; however, it is usually detected in catalase-negative aerobes. In aerobic environments, some microorganisms use oxygen (O_2) as an alternative electron acceptor and reduce it to hydrogen peroxide and water. If hydrogen peroxide is present in quantities in excess of the organism's capacity to degrade it,

cytotoxic effects are seen. As an intermediate in oxygen reduction, hydrogen peroxide is also a powerful oxidizing agent due to its metabolic ability to form radicals such as the hydroxyl radical (OH·). Hydrogen peroxide may be converted to hydroxyl radicals by transition metal ions (e.g., iron and copper) and by UV radiation among other ways. Once produced, this radical can initiate oxidation and cause damage to nucleic acids and other cellular structures. However, some bacterial cells possess oxidative defense proteins which might repair DNA damage caused by hydrogen peroxide and the hydroxyl radical (Imlay et al., 1988).

Factors affecting inhibitory and bactericidal properties of hydrogen peroxide include the concentration of the solution, bacterial strains present, catalase activity of competing bacterial strains, and environmental factors such as pH and temperature. Bacteria with high catalase activity can break down hydrogen peroxide as a defense mechanism to prevent peroxide toxicity. For this reason, catalase has been proposed as a potential virulence factor among bacterial pathogens because their ability to detoxify peroxide may contribute to their survival (Beaman and Beaman, 1984). Therefore, more research is needed to determine the sensitivity of human pathogens to hydrogen peroxide treatment. Temperature is another contributing factor to the bactericidal and sporicidal activity of hydrogen peroxide. Toledo et al. (1973) found that hydrogen peroxide is only a weak sporicide at room temperature but is very potent at higher temperatures. Significant sporicidal activity was observed at 76°C versus 24°C when spores of *Bacillus subtilis* var. *globigii* were exposed to concentrations of 25.8% hydrogen peroxide. At higher temperatures, sporicidal activity was less dependent on length of exposure whereas the sporicidal activity of hydrogen peroxide was more dependent on length of

exposure at lower temperatures (<76°C). In contrast, other research suggests that cells exhibiting enhanced tolerance to high temperatures such as *Salmonella enteritidis* and *S. typhimurium* appear better able to survive in the presence of hydrogen peroxide (Morgan et al., 1986; Humphrey et al., 1995). Although bacterial resistance has been seen in species that are able to utilize and reduce O_2 while coping with the toxic, reactive oxygen by-products such as the hydroxyl radical and hydrogen peroxide, research indicates that hydrogen peroxide vapor and wash treatments are capable of reducing total microbial populations in mushrooms, salad vegetables, berries, and fresh-cut melons (Sapers et al., 1994; Sapers et al., 1995).

Sapers et al. (1994) tested the efficacy of washing mushrooms in a 5% hydrogen peroxide solution for 30 seconds to suppress the growth of spoilage bacteria primarily *Pseudomonas tolaasii*. Following the hydrogen peroxide dip, mushrooms were held for 5 minutes to allow unreacted peroxide to be decomposed by endogenous catalase and then dipped in a browning inhibitor. Results indicate that this combination treatment was effective in suppressing bacterial blotch development and extending the shelf-life of mushrooms inoculated with *P. tolaasii* for 7 days at 4°C. It was also noted that soil was removed from the mushroom surfaces by the oxygen bubbles produced from the catalase-hydrogen peroxide reaction. This mechanical action could be beneficial in removing loosely attached microorganisms located on the surface of produce. Furthermore, residual hydrogen peroxide on the mushroom surface was undetectable by two hydrogen peroxide testing measures with sensitivity as low as 0.1 ppm. The low residual levels reflect the rapid decomposition of residual hydrogen peroxide by endogenous catalase and the reduction of hydrogen peroxide by erythorbate in the browning inhibitor dip.

Having established the antimicrobial activity of hydrogen peroxide, Sapers et al. (1995) conducted a follow-up study to determine the residual hydrogen peroxide levels of fresh-cut produce immersed in solutions of 5-10% hydrogen peroxide. Gas evolution was used as an indicator of endogenous catalase activity with little or no catalase activity possibly indicating a hydrogen peroxide residue problem. Apple and pear wedges, sweet cherries, raspberries, strawberries, broccoli, cauliflower, and whole cherry tomatoes showed little or no gas evolution due to low catalase activity. Thus, the residual levels of hydrogen peroxide on these products were higher. In contrast, vigorous gas evolution was observed in shredded cabbage, carrot sticks, celery sticks, diced green bell pepper, shredded lettuce, peeled potato, and sliced zucchini. The higher catalase activity of these vegetables contributed to their lower residual hydrogen peroxide levels. Overall, results indicated that the endogenous catalase activity of the produce contributed to the level of residual hydrogen peroxide after treatment. In an attempt to prevent high concentrations of residual hydrogen peroxide, the study was repeated with samples receiving a 5% hydrogen peroxide wash treatment followed by a dip in either a 1% erythorbate solution or water. These samples were evaluated against those receiving only the 5% hydrogen peroxide wash. Results of this study indicated that residual hydrogen peroxide could be reduced to levels below FDA regulations when rinsed in water or dipped in 1% erythorbate solutions. However, for those fruits and vegetables that were not washed following treatment with hydrogen peroxide, residual levels were dependent on the endogenous catalase activity of the individual fruit or vegetable.

Studies have shown that hydrogen peroxide not only initiates microbial degradation but also assists photocatalytic degradation of some organophosphate

insecticides. Doong and Chang (1998) found hydrogen peroxide to contribute to the degradation of organophosphorous insecticides in the presence of UV radiation. Although organophosphorous pesticides are known to degrade in the presence of UV radiation, the oxidation efficiency of UV radiation can be enhanced with the addition of hydrogen peroxide to this reaction. Furthermore, the presence of high-valent iron, such as Fe^{2+} and Fe^{3+}, can be used in combination with hydrogen peroxide to generate more OH· radicals capable of attacking organic compounds. Results of the experiment showed the degradation efficiency of organophosphorous pesticides in the UV/Fe/H_2O_2 system were more effective than that of organophosphorous pesticides in the UV/ H_2O_2 system.

Chlorine

Chlorine, in the form of sodium, potassium, or calcium hypochlorite, is the most widely used sanitizing agent in the fruit and vegetable product industry (Sapers and Simmons, 1998). This relatively inexpensive product is easily incorporated into water to improve the microbiological quality and control pathogens by reacting with microbial cells present on the surface of fruits and vegetables. Much data exist to support the potent oxidizing potential of chlorine and its ability to reduce initial microbial populations.

In general, the effectiveness of chlorine depends on the solution concentration including the amount of free chlorine, pH of the solution, length of exposure, and surface characteristics of the commodity receiving treatment. As free chlorine in the solution reacts with organic materials, the antimicrobial activity of chlorine decreases upon contact. Thus, the antimicrobial effectiveness of chlorine is also dependent on the

amount of organic material surrounding the microbial cell. For example, natural openings in the exterior covering of produce expose organic matter which would be expected to weaken the effectiveness of chlorine. The length of time in which chlorine is allowed to react with microbial cells on the surface varies between products and is also dependent on the surface characteristics of the product. For example, cells positioned within protective hydrophobic pockets of some commodities are less accessible to chlorinated water and are not as easily removed as cells positioned on exposed areas.

Park and Lee (1995) evaluated the efficacy of various chlorine concentrations in reducing the initial microbial load of cut water cress. Chlorine concentrations ranged from 50 to 1,000ppm. All samples were dipped into chlorine solutions for 1 minute. Quality evaluations of ascorbic acid retention and surface color were done on all samples in addition to total microbial counts. Based on the microbial and quality results of the study, 100ppm was the maximum chlorine concentration at which microbial reductions were seen without significant deterioration in the quality of cut water cress. At this concentration, the initial microbial population was reduced from $10^{7.5}$ to $10^{6.7}$ cfu/g. When stored at 5°C for 7 days, the total microbial population of the control exceeded 10^{8}cfu/g by day 7. The microbial population of chlorine-treated samples also exceeded 10^{8}cfu/g by day 7. Thus, the antimicrobial effectiveness of chlorine was limited to short-term storage. Additional research studies have supported the short-lived effectiveness of chlorine to increase shelf-life. According to Sapers and Simmons (1998), the maximum reduction that can be expected at permitted chlorine concentrations is a 1- to 2-log population reduction. More recently, Soylemez et al. (2001) concluded that chlorine concentrations of 200ppm were effective in reducing the initial microbial populations on

treated alfalfa seeds; however, during the sprouting process, no significant differences ($p < 0.05$) in aerobic plate counts (APC) existed between the chlorine-treated sprouts and the control.

In addition to improving the microbiological quality of fruits and vegetables, chlorine treatments are also used to reduce the risks of food-borne pathogens such as *Listeria, Escherichia*, and *Salmonella*. Zhang and Farber (1996) tested the efficacy of chlorine to bring about reductions in viable cells of *Listeria monocytogenes* on lettuce and cabbage. The maximum observed log reduction of *L. monocytogenes* was seen in lettuce and cabbage samples exposed to concentrations of 200ppm chlorine at 22°C for 10 minutes. Reductions of up to 1.7 log for lettuce and 1.2 log for cabbage were recorded. Overall, chlorine solutions were more effective against *L. monocytogenes* on lettuce as compared to cabbage. This data supports the theory that the effectiveness of chlorine is dependent on the type of vegetable and its surface characteristics. The effectiveness of chlorine against *L. monocytogenes* was also influenced by the temperature of the solution as evidenced by the increased bactericidal effect of chlorine on lettuce samples washed in solutions at 22°C versus 4°C. The influence of temperature on the listericidal efficacy on lettuce was similar to that obtained by El-Kest and Marth (1988). This evidence suggests that the rate of diffusion of chlorine into the microbial cell is increased at higher temperatures. Therefore, not only does the solution concentration affect the antimicrobial activity of chlorine but also the temperature of the solution may play an important part in chlorine's effectiveness against bacterial cells.

Numerous outbreaks of *E. coli* 0157:H7 have been associated with the consumption of raw fruit and vegetables. Recent outbreaks have implicated improperly

treated sewage sludge, irrigation water, and improperly composted manure as probable sources of contamination. With increased transmission of this pathogen to fruits and vegetables, the effectiveness of commercial sanitizers such as chlorine has been challenged. Beuchat (1999) investigated the effectiveness of chlorinated water sprays in the treatment of lettuce inoculated with *E. coli* 0157:H7 from bovine feces. Lettuce samples were inoculated at four levels ranging from 10^0 to 10^5 CFU of *E. coli* 0157:H7 per gram of lettuce. Spray treatments of 200ppm chlorine and deionized water (control) were applied to lettuce samples prior to determination of populations of *E. coli* 0157:H7. Samples were held for 1 minute and 5 minutes following treatment and then rinsed with water to conclude each contact time. Spray treatments of lettuce with 200ppm chlorine solution and deionized water were equally effective in killing or removing *E. coli* 0157:H7 from lettuce samples receiving the lowest inoculum. However, populations on lettuce samples receiving the two highest inocula were significantly lower ($p \leq 0.05$) after treatment with 200ppm chlorine compared to the control. Holding the lettuce samples for 5 minutes after spray treatment was not more effective in reducing populations than holding for 1 minute before rinsing with water. In further studies evaluating the effectiveness of chlorine against pathogenic bacteria, Beuchat et al. (2001) inoculated alfalfa seeds with *Salmonella*. The inoculated seeds were washed in a 200ppm chlorine solution for 30 minutes. The 200ppm chlorine treatment resulted in a 1.9 log reduction of *Salmonella* cells. Both investigations confirm that chlorine has the potential to reduce pathogenic populations from the surface of fruits and vegetables.

MATERIALS AND METHODS

Experimental Design

Field samples were hand-raked from a commercial blueberry field in Deblois, Maine, in the summer of 2001. Five designated plots around the field served as markers for sampling. Blueberries from each plot were collected into separate ½ gallon bags. Sampling began 24 hours after initial aerial application of phosmet and continued each week thru harvest for a total of 5 weeks. Samples for microbial and pesticide analysis were collected at the beginning of each week. In addition, the five designated plots around the field were sampled every three days to assess phosmet degradation over time. Once collected, blueberry sample bags were transported on ice to the Department of Food Science and Human Nutrition where five treatment solutions (100ppm chlorine, 0.5% hydrogen peroxide, 0.5% citric acid, distilled water, and a commercial produce wash (Appendix A)) were evaluated for their effectiveness in reducing surface microbial populations as well as reducing residual phosmet.

Solution Preparation and Treatment

One liter stock solutions of 100ppm chlorine, 0.5% hydrogen peroxide, 0.5% citric acid, and distilled water were prepared each day before treatment began. Chlorine solutions were made according to common industry practices (Camire, 1994) using household bleach (6% sodium hypochlorite). Spray treatments were applied using Home and Garden Sprayers (RL Flomaster, Root-Lowell Manufacturing Co., Lowell, Michigan) modified with Whirljet (1/4 B ss 3) nozzles (Spraying Systems Co., Wheaton, Illinois). Treatment volumes of 500mL 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 350g were spread on a sterile wire screen and subjected to 500mL sprays of 100 ppm chlorine, 0.5% hydrogen peroxide, 0.5% citric acid, or distilled water. After spraying, berries were held for 30 or 300 seconds. To initiate the end of each contact time, blueberries were transferred on wire screens to the blast freezer where they were held for 3 minutes at -30°C. Unwashed berries served as the control for this study. All treatments were applied over two days with samples allowed a 30 second contact time treated the day of sampling and samples allowed a 300 second contact time treated the following day. Blueberry samples allowed a 300 second contact time were held under refrigerated storage until treatment.

Microbiological Analysis

Triplicate samples of 50g were taken immediately after freezing for microbial analysis of total aerobes, yeast, mold, and coliforms. 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 10% tartaric acid. Coliform enumeration was performed according to the Most Probable Number (MPN) method using Lauryl Tryptose Broth, Brilliant Green Bile (2%), and EC broth (Difco Laboratories, Detroit, Michigan).

Phosmet Extraction and Analysis

A phosmet analytical standard (99% purity) was obtained from the EPA Repository (Fort Meade, Maryland) and used to prepare a standard stock solution. The stock solution of phosmet was prepared in methanol and serially diluted in ethyl acetate. From this stock solution, a 7-point standard curve (196ng/ml to 9821ng/ml) was prepared within linear range of the GC-MSD operated in the pulsed splitless mode.

Extraction of residual phosmet from blueberry samples was performed according to a method used for the determination of organophosphate insecticides in fruits and vegetables (Bushway and Fan, 1998). Extraction of phosmet was accomplished using methanol followed by a solid phase extraction (SPE) clean-up step. All solvents used including methanol, ethyl acetate, and water were ACS HPLC-grade and pesticide-grade (Fisher Scientific Co., Fair Lawn, New Jersey). Extraction of phosmet from blueberry samples was performed using 5g sub-samples of each treated sample as well as the control. A 5g quantity of each sample was weighed into a 50ml conical polypropylene centrifuge tube followed by the addition of 20ml of ACS-grade methanol. The sample was processed with a Polytron homogenizer (Kinematica, CH-6010 Kriens, Lucerne, Switzerland) at medium speed for 3 minutes and then centrifuged for 10 minutes at 5,000 x g using a Beckman TJ-6 centrifuge (Beckman, Palo Alto, California). A 10ml aliquot was removed and added to 90ml of HPLC-grade water before passing the entire 100ml solution through an activated 500mg Strata SDB-L cartridge (Phenomenex, Torrence, California). The activation step for the SPE cartridge included 5ml of methanol followed by 5ml of HPLC-grade water. The Sep-Pak cartridge was dried under light vacuum for 1

hour before it was eluted with ethyl acetate. The first 1ml of eluate was collected for analysis using GC-MSD.

GC-MSD analysis was performed with an Agilent Model 6890 gas chromatograph interfaced with an Agilent Model 5973 mass spectrophotometric detector (Agilent, Wilmington, Delaware). A Hewlett-Packard (HP) 5MS capillary column (5% phenylmethyl-siloxane: 30cm x 0.25mm x 0.25um film thickness) was employed for the separation (HP, Andover, Massachusetts). Data was analyzed by Chemstation Environmental Data Analysis (Agilent, Wilmington, Delaware). Helium, at a total flow rate of 54.5ml/minute and a pressure of 11.3psi, was the carrier gas for the system which was operated in the pulsed splitless mode. The initial oven temperature was held at 80°C for 1 minute and increased to 250°C at a rate of 20°C per minute during separation and detection of phosmet. The final temperature of 250°C was held for an additional 10 minutes to elute highly retained analytes. Sample volumes of 2 µl were injected into the system with a solvent delay of 3.5min. Phosmet was plotted using Selected Ion Monitoring (SIM) in which three ions characteristic of phosmet were monitored.

Statistical Analysis

Microbial analyses were conducted using three replicates per treatment and control and residual phosmet analyses were conducted using two replicates per treatment and control. All data were subjected to one-way analysis of variance and Tukey's HSD Multiple Comparison to determine if significant differences ($p<0.05$) existed in mean values of microbial populations and residual phosmet levels among treatments. A multi-way analysis of variance was also conducted using the variables of time, treatment, and

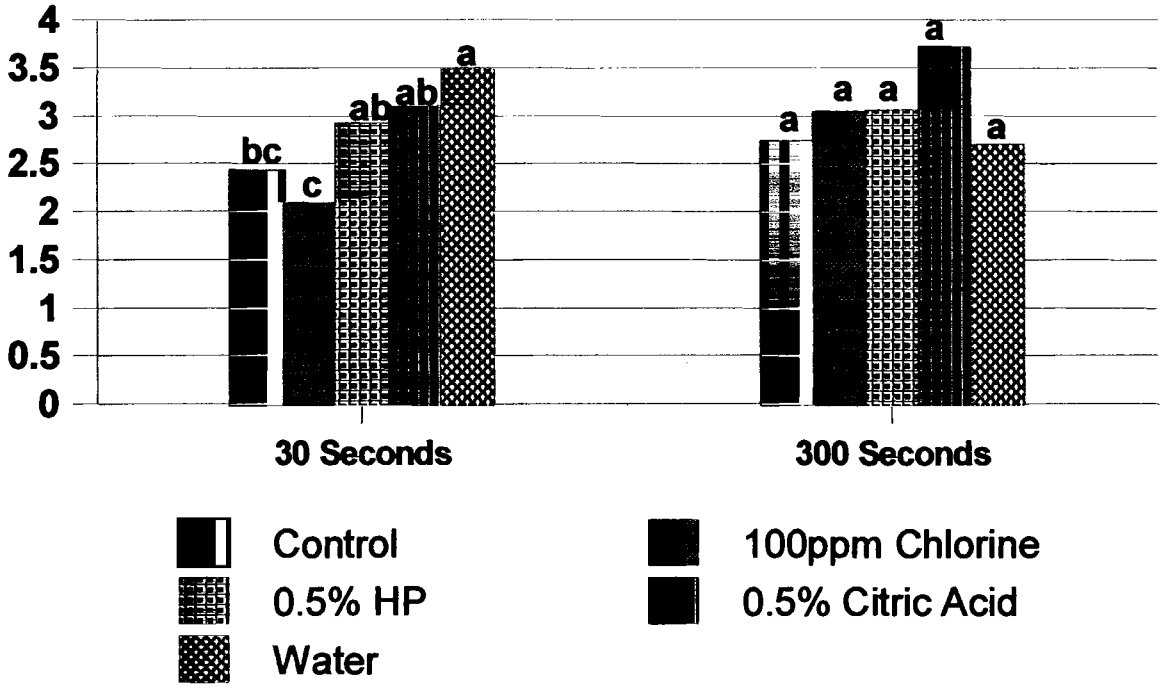
week to determine if these variables significantly influenced ($p<0.05$) microbial populations and residual phosmet levels of lowbush blueberries. Statistical analysis was performed using Systat Analytical Software, version 9.0 (Chicago, Illinois).

RESULTS AND DISCUSSION

Microbiological Analyses

Results of the influence of contact time, treatment, and week of sampling on microbial populations of lowbush blueberries are presented in Figures 3 – 17. Log reductions in microbial populations are calculated from the unwashed control samples within each contact time. Additionally, Tables B1-B3 present microbial log reductions in lowbush blueberries as influenced by treatment across the 5 weeks.

Figure 3: Week One - Aerobic Plate Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

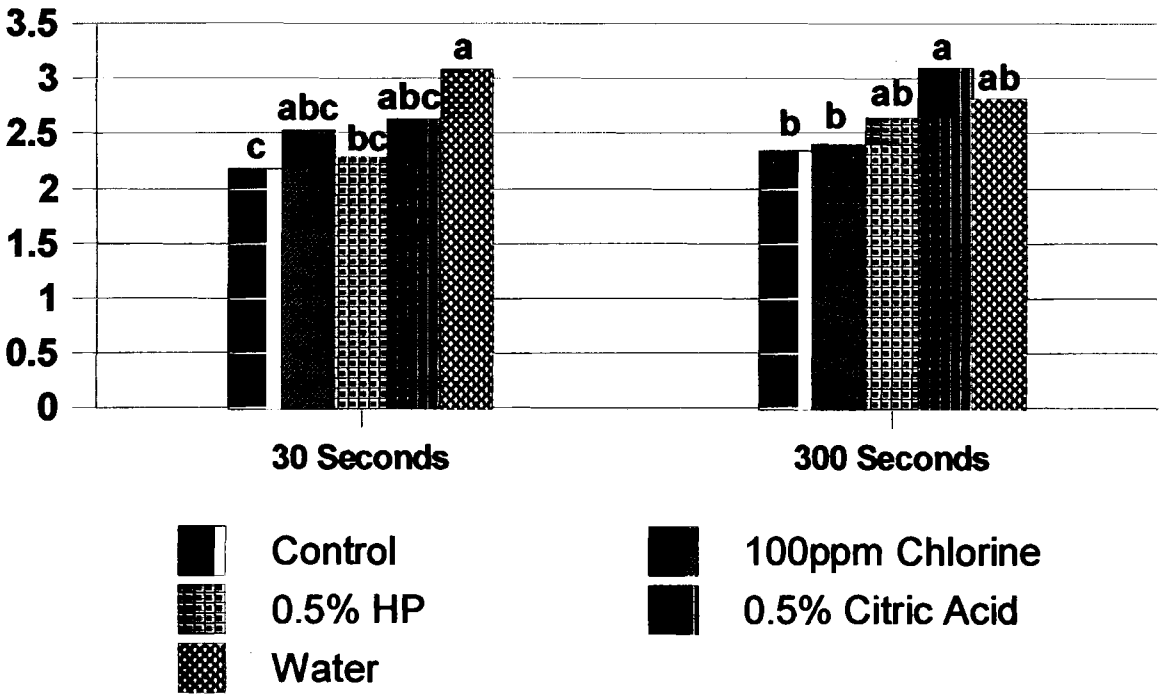


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The APC

of the control sample for the 30 second treatment was 2.44 ± 0.15 log. Treatment with 100ppm chlorine yielded reductions in aerobic plate counts; however, results were not significantly different ($p<0.05$) than the control. An increase in aerobic plate counts was observed on samples treated with 0.5% hydrogen peroxide, 0.5% citric acid, and distilled water.

The APC of the control sample for the 300 second treatment was 2.75 ± 0.30 log. Although significant differences ($p<0.05$) did not exist among treatments, samples washed with distilled water showed reductions in aerobic plate counts. An increase in aerobic plate counts was observed on samples treated with 100ppm chlorine, 0.5% hydrogen peroxide, and 0.5% citric acid.

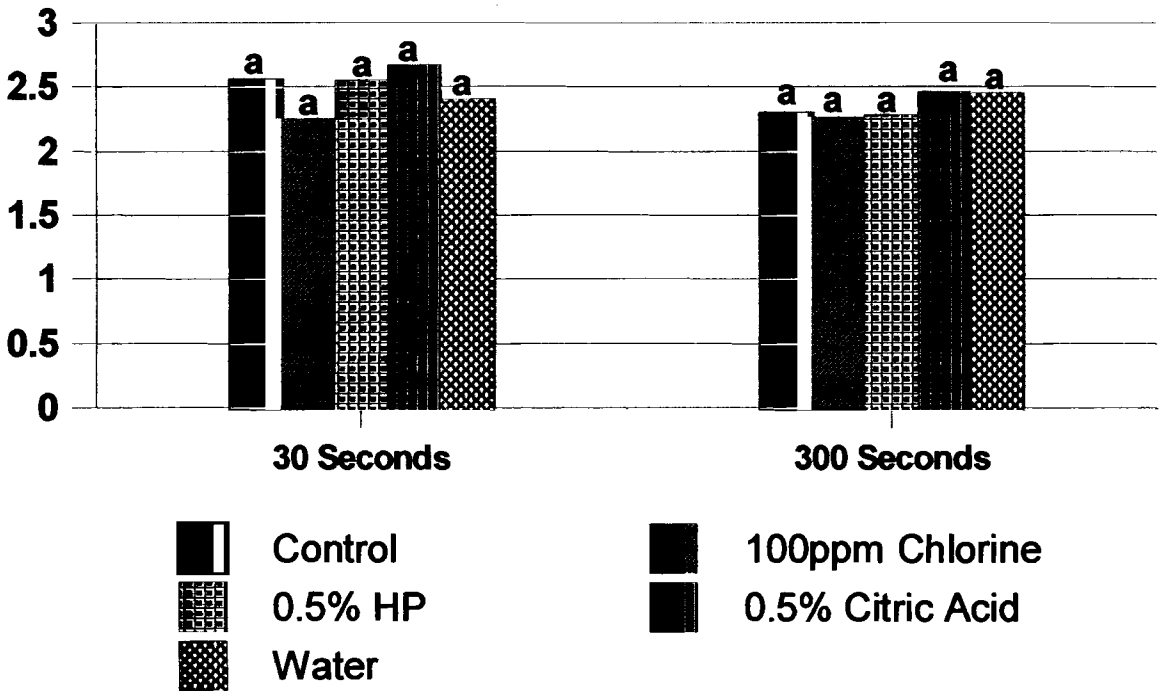
Figure 4: Week One - Yeast Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The yeast count of the control sample for the 30 second treatment was 2.18 ± 0.11 log. Higher yeast counts were observed in all treated samples with significantly higher counts ($p<0.05$) observed on samples treated with distilled water.

The yeast count of the control sample for the 300 second treatment was 2.34 ± 0.20 log. Higher yeast counts were observed on all treated samples with significantly higher counts ($p<0.05$) observed on samples treated with citric acid for 300 seconds.

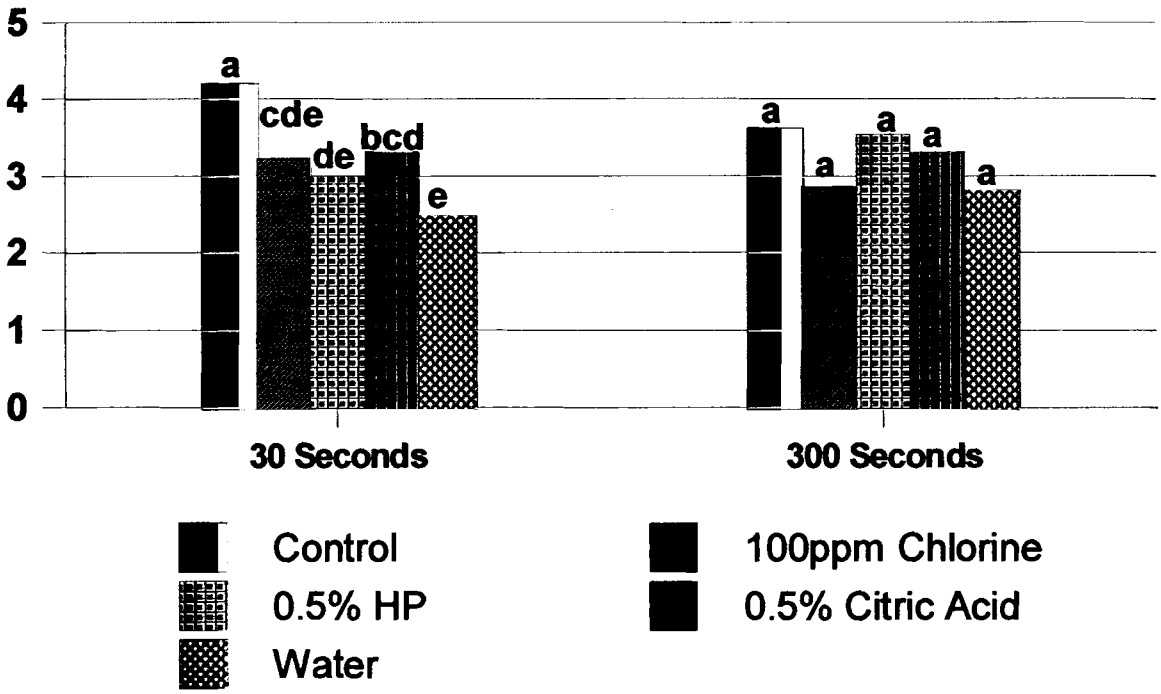
Figure 5: Week One – Mold Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The mold count of the control sample for the 30 second treatment was 2.56 ± 0.10 log. All treatments resulted in reductions in initial mold counts although significant reductions ($p<0.05$) were not seen among treatments when compared to the control. The greatest reduction (0.30 log) in mold counts was observed on samples treated with 100ppm chlorine.

The mold count of the control sample for the 300 second treatment was 2.31 ± 0.15 log. Samples washed with 0.5% citric acid and distilled water showed an increase in mold counts when compared to the control. Similar to results observed at the 30 second contact time, significant differences ($p<0.05$) in mold counts were not observed among treated samples and the control.

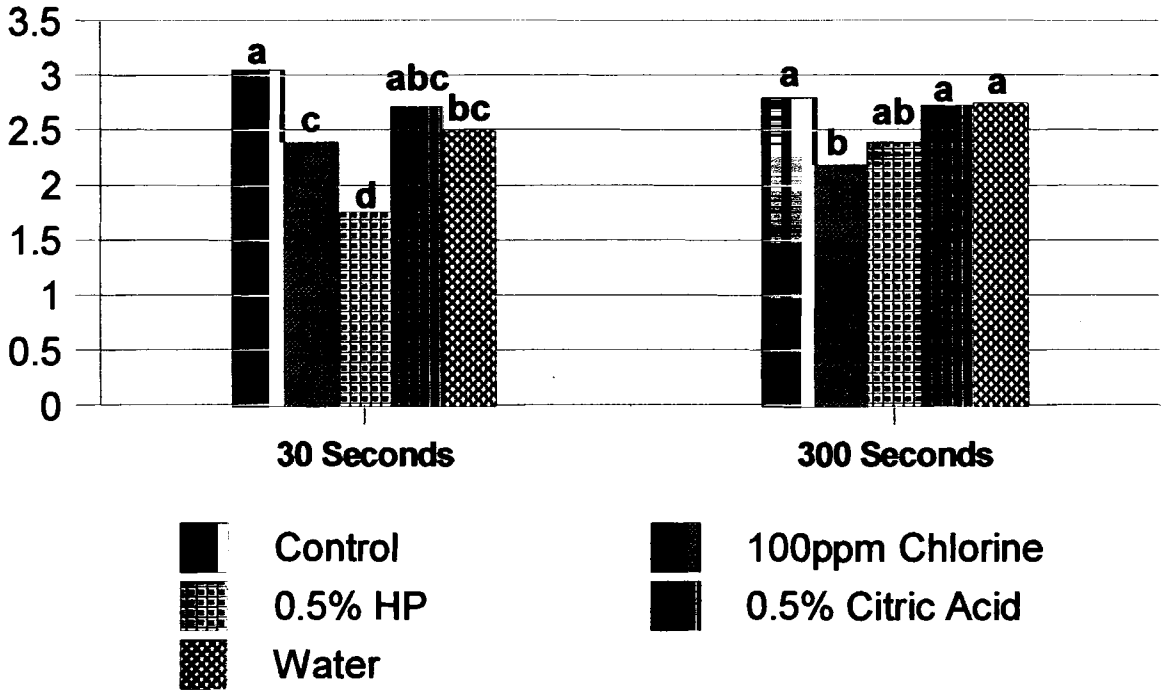
Figure 6: Week Two – Aerobic Plate Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The APC of the control sample for the 30 second treatment was 4.21 ± 0.17 log. All treatments resulted in significant reductions ($p<0.05$) in aerobic plate counts when compared to the control. Chlorine 100ppm, 0.5% hydrogen peroxide, and 0.5% citric acid treatments were not significantly different ($p<0.05$) in their antimicrobial effectiveness. Overall, the greatest reduction (1.72 log) in aerobic plate counts was observed on samples treated with distilled water; however, these counts were not significantly different ($p<0.05$) than counts observed on samples treated with 0.5% hydrogen peroxide.

The APC of the control sample for the 300 second treatment was 3.62 ± 0.19 log. In contrast to results observed at the 300 second contact time, significant differences ($p<0.05$) did not exist among treatments and the control; however, reductions in aerobic plate counts were observed in all treated samples. Chlorine treatments and distilled water treatments were similar in antimicrobial effectiveness resulting in reductions of 0.76 log and 0.80 log, respectively.

Figure 7: Week Two - Yeast Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

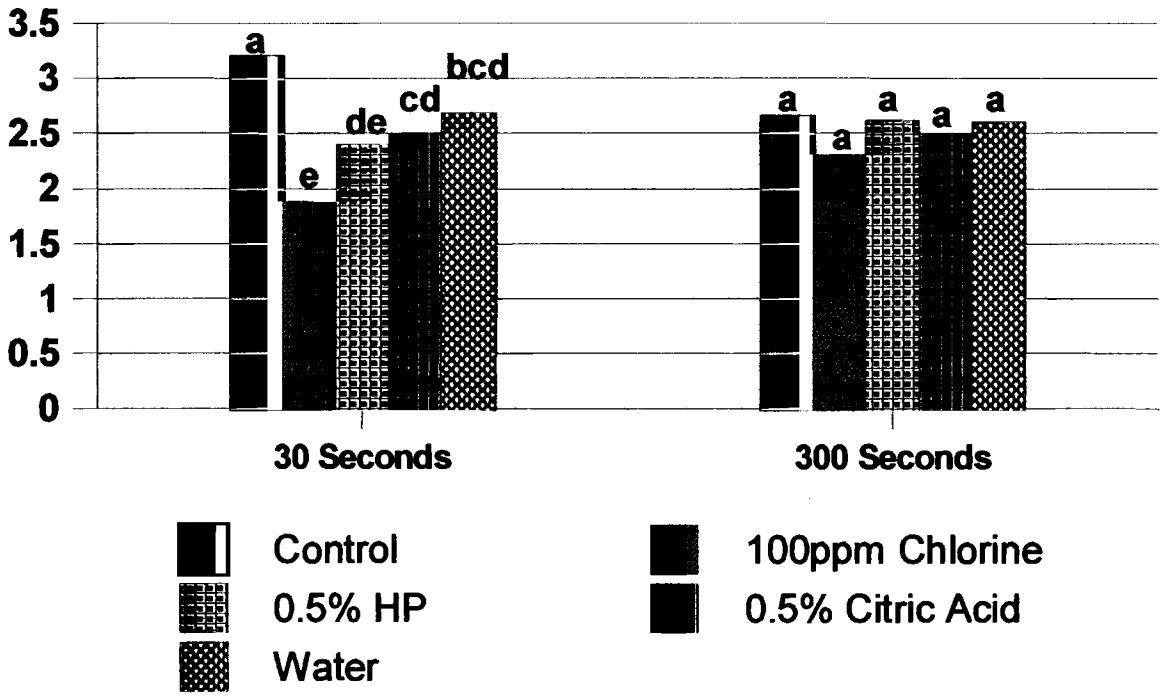


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The yeast count of the control sample for the 30 second treatment was 3.05 ± 0.09 log. Chlorine

100ppm, 0.5% hydrogen peroxide, and distilled water treatments significantly reduced ($p<0.05$) yeast counts compared to the control. Chlorine 100ppm, 0.5% citric acid, and distilled water treatments were similar in their effect on population reduction; however, only samples treated with 100ppm chlorine, 0.5% hydrogen peroxide, and distilled water were significantly different ($p<0.05$) from the control. Overall, the greatest reduction (1.30 log) was seen in samples receiving 0.5% hydrogen peroxide treatment.

The yeast count of the control for the 300 second treatment was 2.80 ± 0.10 log. All treatments reduced the initial yeast population of blueberry samples; however, only samples treated with 100ppm chlorine were significantly different ($p<0.05$) from the control resulting in a 0.62 log reduction.

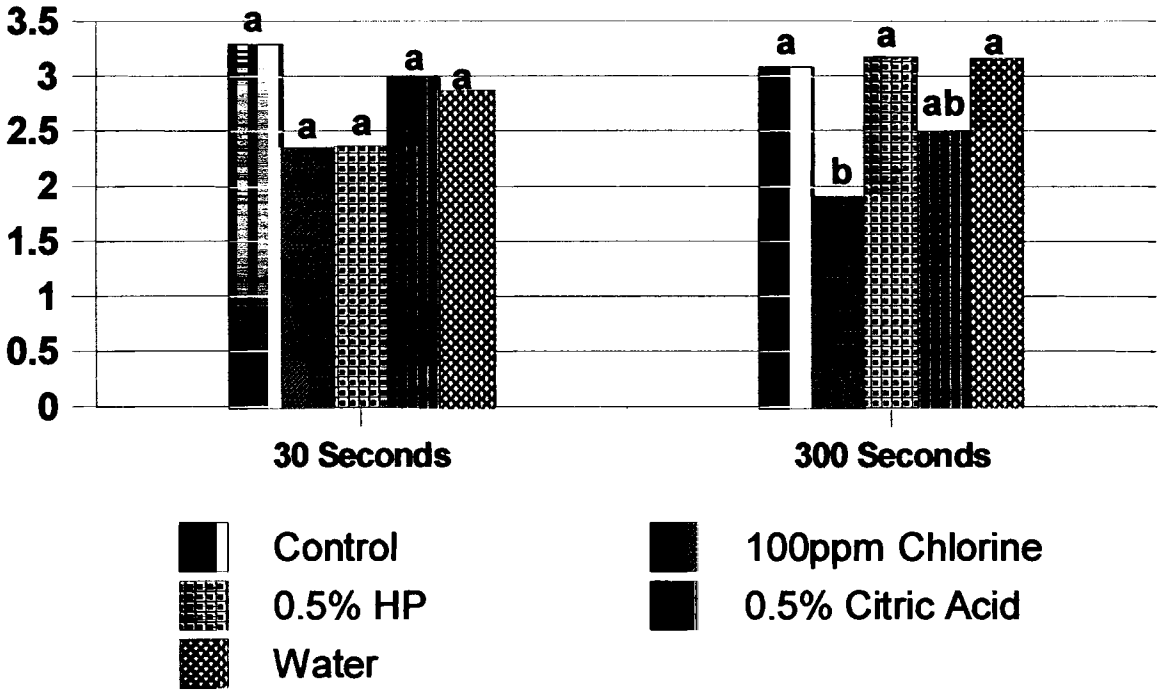
Figure 8: Week Two - Mold Count (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The mold count of the control sample for the 30 second treatment was 3.21 ± 0.10 log. Significant reductions ($p<0.05$) in mold counts were observed in all treated samples when compared to the control. Treatments of 0.5% hydrogen peroxide, 0.5% citric acid, and distilled water were similar in their antimicrobial effectiveness. Chlorine-treated samples showed the greatest reduction (1.33 log) in mold counts although counts were not significantly lower ($p<0.05$) than those observed in hydrogen peroxide-treated samples.

The mold count of the control sample for the 300 second treatment was 2.67 ± 0.13 log. Although mold count reductions were observed in all treated samples, significant differences ($p<0.05$) did not exist between treatments and the control. The greatest reduction in mold counts (0.36 log) was observed in samples treated with 100ppm chlorine.

Figure 9: Week Three – Aerobic Plate Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

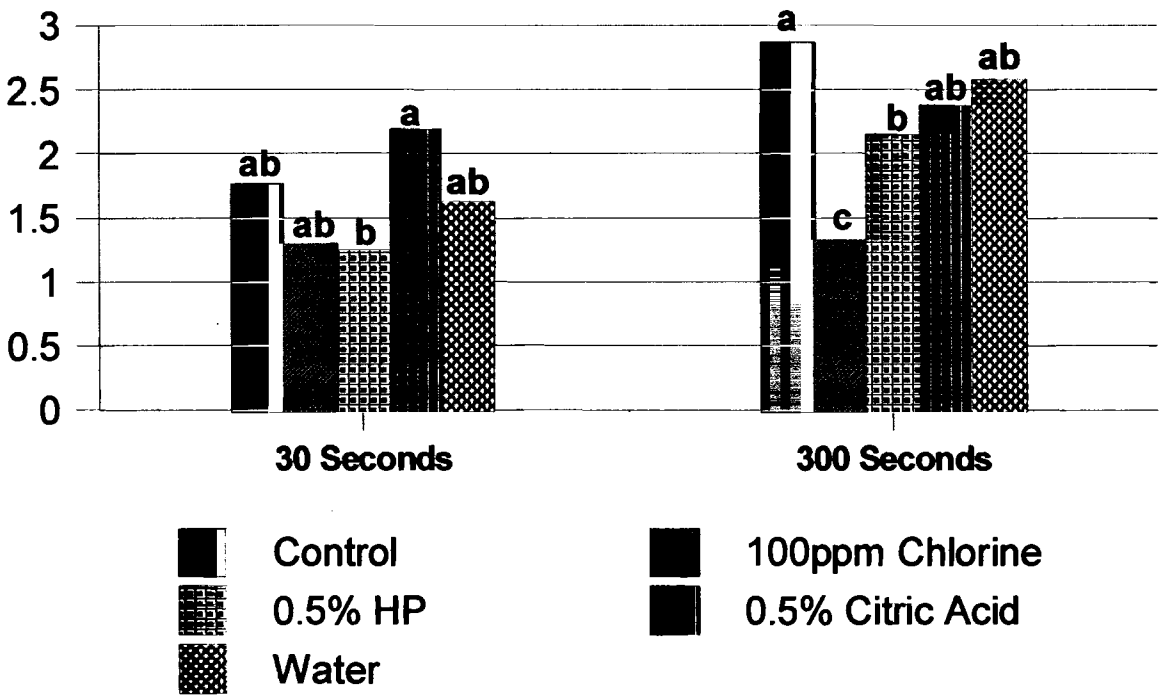


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The APC of the control sample for the 30 second was 3.29 ± 0.22 log. Significant differences ($p<0.05$) did not exist among treatments and the control; however, all treatments resulted in aerobic plate counts reductions with the greatest reductions seen in samples treated with 100ppm chlorine (0.94 log) and 0.5% hydrogen peroxide (0.93 log).

The APC of the control sample for the 300 second treatment was 3.08 ± 0.15 log. An increase in aerobic plate counts was observed on samples treated with 0.5% hydrogen peroxide and distilled water. Although aerobic population reductions were observed on

samples treated with 100ppm chlorine and 0.5% citric acid, only chlorine-treated samples showed significant reductions ($p<0.05$) (1.18 log) in aerobic populations when compared with the control.

Figure 10: Week Three - Yeast Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

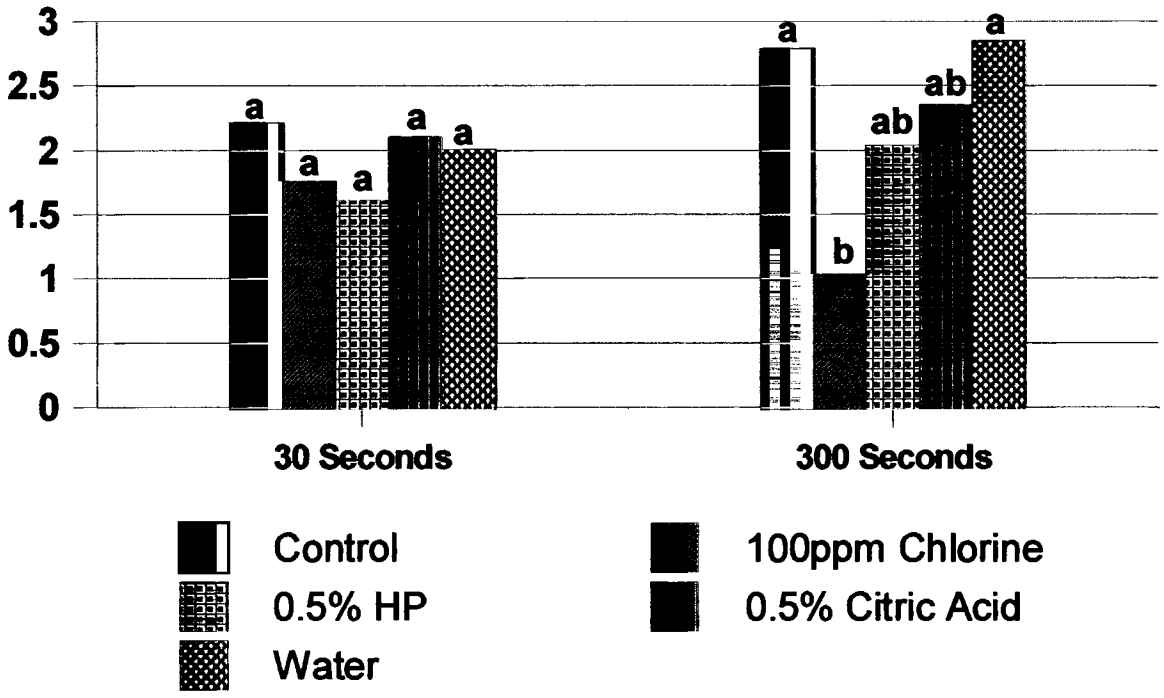


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The yeast count of the control sample for the 30 second treatment was 1.76 ± 0.19 log. Yeast count reductions were observed on samples treated with 100ppm chlorine, 0.5% hydrogen peroxide, and distilled water; however, results were not significantly different ($p<0.05$) when compared to the control. The greatest reduction (0.51 log) in yeast counts was

observed on samples treated with 0.5% hydrogen peroxide while an increase in yeast counts was observed in samples treated with 0.5% citric acid.

The yeast count of the control sample for the 300 second treatment was 2.87 $\pm$ 0.10 log. Significant reductions ($p<0.05$) in yeast counts were observed on samples treated with 100ppm chlorine and 0.5% hydrogen peroxide. Although significant reductions ($p<0.05$) were observed on hydrogen peroxide-treated samples, the treatment was similar to 0.5% citric acid and distilled water treatments in antimicrobial effectiveness. Overall, samples treated with 100ppm chlorine resulted in the greatest reduction (1.54 log) in yeast counts.

Figure 11: Week Three - Mold Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

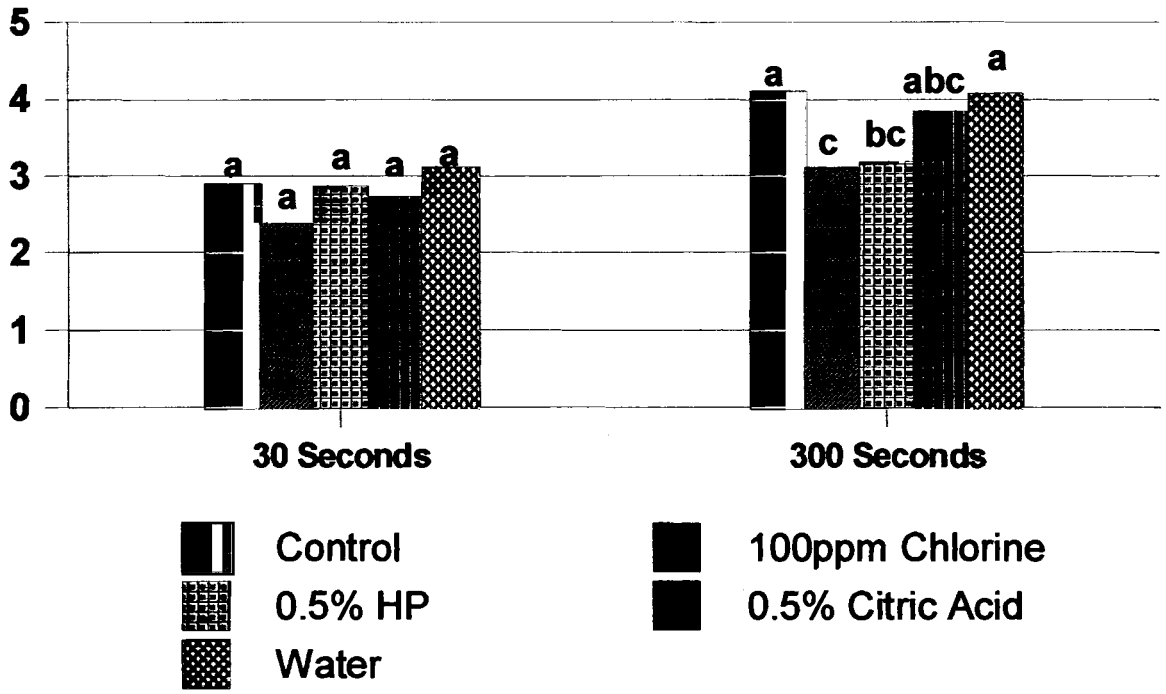


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend.

Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The mold count of the control sample for the 30 second treatment was 2.22 ± 0.18 log. Although mold count reductions were observed in all treated samples, significant differences ($p<0.05$) did not exist among treatments and the control. Samples treated with 0.5% hydrogen peroxide showed the greatest reduction (0.61 log) in mold counts.

The mold count of the control sample for the 300 second treatment was 2.79 ± 0.30 log. Mold count reductions were observed on samples treated with 100ppm chlorine, 0.5% hydrogen peroxide, and 0.5% citric acid; however, only chlorine-treated samples showed significant reductions ($p<0.05$) (1.76 log) when compared to the control. An increase in mold counts was observed on samples treated with distilled water.

Figure 12: Week Four – Aerobic Plate Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

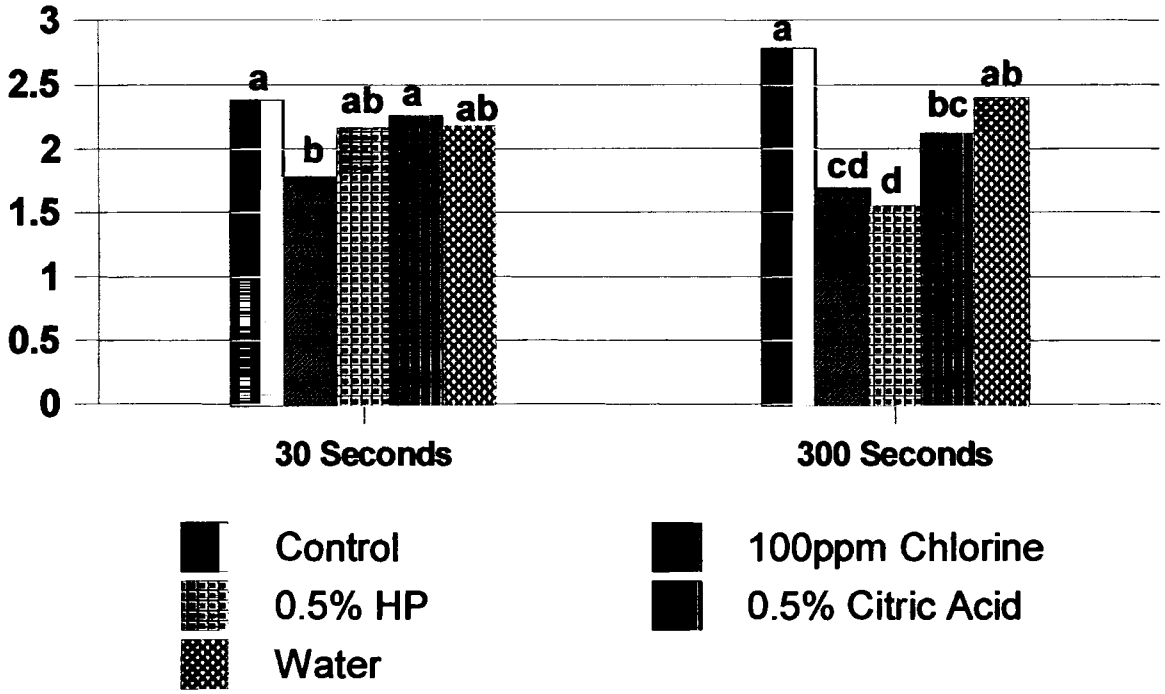


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The APC of the control sample for the 30 second treatment was 2.90 ± 0.18 log. Samples treated with 100ppm chlorine, 0.5% hydrogen peroxide, and 0.5% citric acid showed reductions in aerobic plate counts; however, significant reductions ($p<0.05$) did not exist among treatments and the control. The greatest reduction (0.51 log) in aerobic plate counts was observed on samples treated with 100ppm chlorine. A population increase of 0.21 log was observed on samples treated with distilled water.

The APC of the control sample for the 300 second treatment was 4.10 ± 0.16 log. Although similar in their antimicrobial effectiveness, both 100ppm chlorine and 0.5%

hydrogen peroxide treatments produced significant reductions ($p<0.05$) (0.98 log and 0.92 log, respectively) in the aerobic populations of blueberry samples. Population reductions were seen in samples treated with 0.5% citric acid and distilled water; however, significant differences ($p<0.05$) did not exist between these treatments and the control.

Figure 13: Week Four - Yeast Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

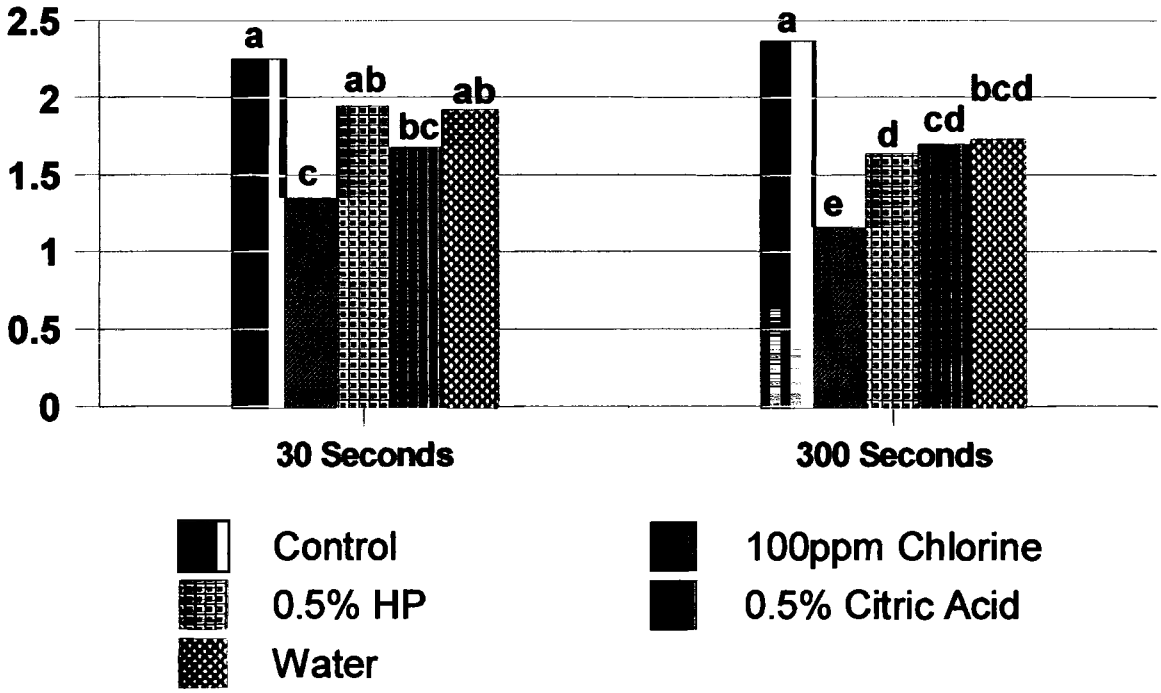


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The yeast count of the control sample for the 30 second treatment was 2.38 ± 0.09 log. Only samples treated with 100ppm chlorine showed significant reductions ($p<0.05$) (0.60 log)

in yeast counts when compared to the control; however, hydrogen peroxide and distilled water treatments were similar to chlorine in antimicrobial effectiveness.

The yeast count of the control sample for the 300 second treatment was 2.78 $\pm$ 0.12 log. Reductions in yeast counts were observed on all treated samples; however, only samples treated with 100ppm chlorine, 0.5% hydrogen peroxide, and 0.5% citric acid showed significant reductions ($p<0.05$) in yeast counts when compared to the control. Overall, the greatest reduction (1.23 log) was observed on samples treated with 0.5% hydrogen peroxide.

Figure 14: Week Four - Mold Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

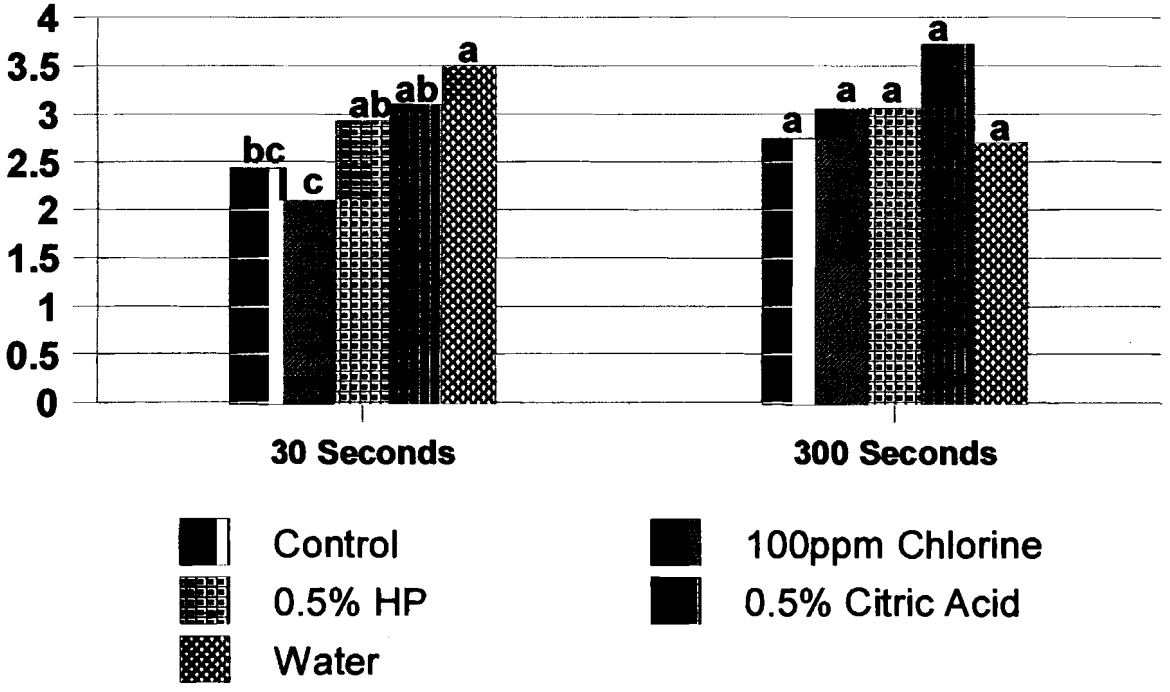


Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different

($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The mold count of the control sample for the 30 second treatment was 2.25 ± 0.10 log. Mold count reductions were observed in all treated samples. Significant reductions ($p<0.05$) were observed on samples treated with 100ppm chlorine and 0.5% citric acid. The greatest reduction (0.90 log) was observed on samples treated with 100ppm chlorine.

The mold count of the control sample for the 300 second treatment was 2.37 ± 0.09 log. Significant reductions ($p<0.05$) in mold counts were observed on all treated samples when compared to the control. Furthermore, samples treated with 100ppm chlorine showed significant reductions ($p<0.05$) (1.21 log) in mold counts when compared with other treated samples. Hydrogen peroxide, citric acid, and distilled water treatments were similar in their antimicrobial effectiveness.

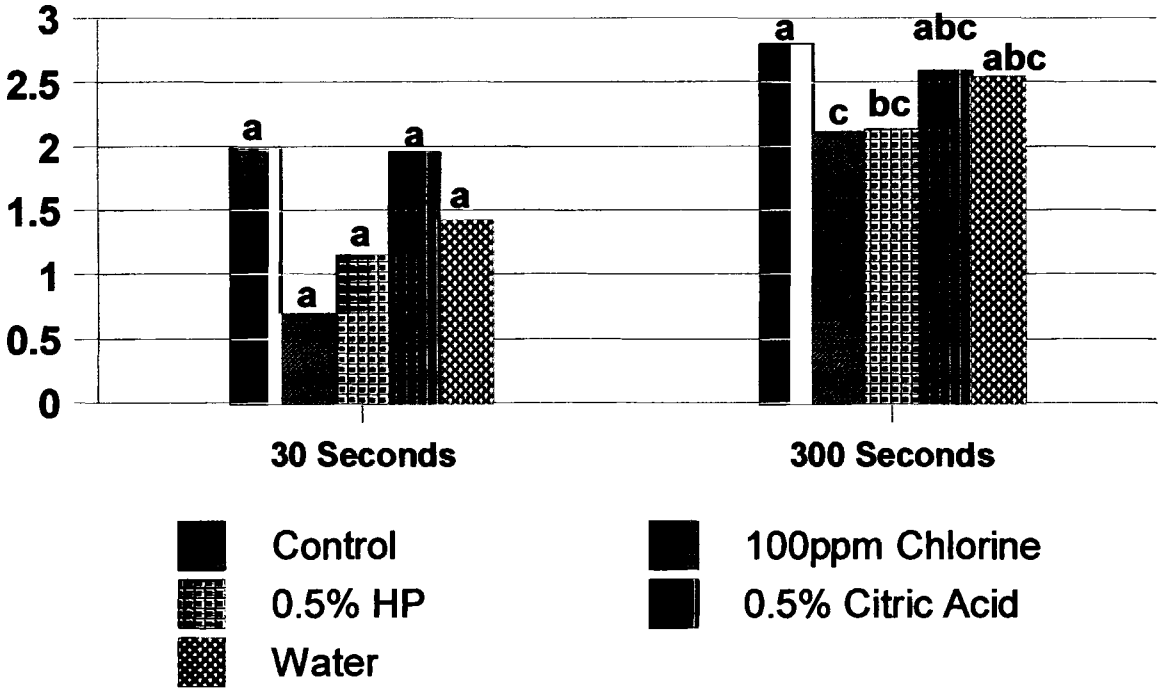
Figure 15: Week Five – Aerobic Plate Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The APC of the control sample for the 30 second treatment was 3.27 ± 0.13 log. Treatments were similar in their antimicrobial effectiveness; however, significant differences ($p<0.05$) existed between all treatments and the control. Overall, the greatest reduction (1.57 log) in aerobic plate counts was observed on samples treated with 0.5% hydrogen peroxide.

The APC of the control sample for the 300 second treatment was 3.99 ± 0.18 log. All treatments except distilled water produced significant reductions ($p<0.05$) in aerobic plate counts when compared to the control. Although 100ppm chlorine, 0.5% hydrogen peroxide, and 0.5% citric acid treatments were similar in antimicrobial effectiveness, the greatest reduction (1.50 log) in aerobic plate counts was observed on samples treated with 100ppm chlorine.

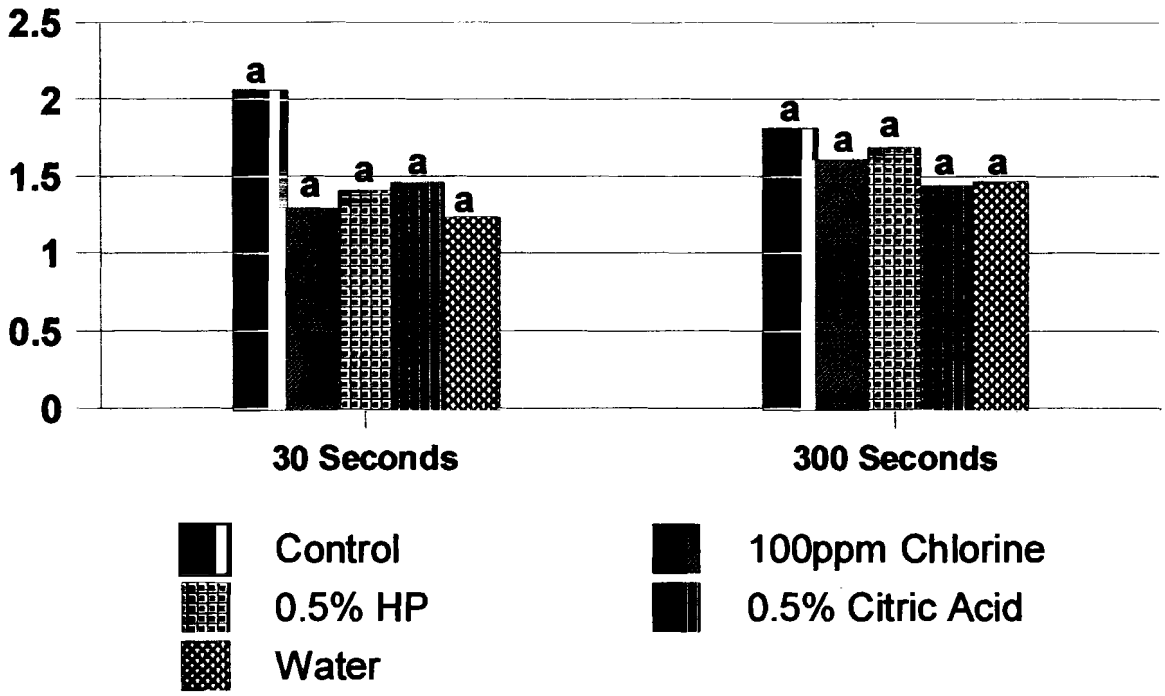
Figure 16: Week Five - Yeast Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The yeast count of the control sample for the 30 second treatment was 2.00 ± 0.32 log. All treatments resulted in yeast count reductions although significant differences ($p<0.05$) did not exist between treatments and the control. Overall, the greatest reduction (1.30 log) in yeast counts was observed on samples treated with 100ppm chlorine.

The yeast count of the control sample for the 300 second treatment was 2.80 ± 0.12 log. All treatments resulted in yeast count reductions; however, only samples treated with 100ppm chlorine and 0.5% hydrogen peroxide resulted in significant reductions ($p<0.05$) (0.68 log and 0.66 log, respectively) when compared to the control.

Figure 17: Week Five - Mold Counts (log CFU/g) of Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Triplicate samples were taken after each treatment and plated in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The mold count of the control sample for the 30 second treatment was 2.06 ± 0.18 log. Although mold count reductions were observed in all treated samples, significant differences ($p<0.05$) did not exist between treatments and the control. Overall, samples treated with distilled water resulted in the greatest reduction (0.83 log) in mold counts.

The mold count of the control sample for the 300 second treatment was 1.81 ± 0.14 log. Although mold count reductions were observed in all treated samples, significant differences ($p<0.05$) did not exist between treatments and the control.

Samples treated with 0.5% citric acid and distilled water showed the greatest reductions in mold counts, 0.37 and 0.34 log, respectively.

Upon further microbial analysis, coliforms were detected in the control samples during the 1st and 2nd week of the study; however, coliforms were not detected in any of the treated samples throughout the study. Results of testing reveal a MPN of 23CFU/ml detected in the control sample during the 1st week of the study and only 9.1CFU/ml of coliforms detected in control sample during the 2nd week of the study. Upon further testing, all samples failed to produce acid or gas in EC broth. Although these samples tested positive for coliforms, it is not likely that the samples contained fecal coliforms since all failed to produce acid or gas when transferred to EC broth.

During the 2nd week of the study, the microbial population of blueberry samples markedly increased as evidenced by higher log values for APC, yeast, and mold. This noticeable increase coincides with an increase in irrigation. During June 2001, the field was irrigated once a week. This frequency was maintained until the second week of the study at which time the irrigation frequency was increased to 2 times per week until harvest. This increase in available water could have contributed to the microbial proliferation, especially bacterial proliferation, seen at week 2 and the subsequent weeks.

Prior to administering treatment, it was hypothesized that the antimicrobial effectiveness of a treatment would be improved with an increase in contact time. However, results show that contact time did not significantly influence ($p<0.05$) a treatment's effectiveness at reducing populations of total aerobes, yeast, and mold. Thus, significant differences did not exist between the effectiveness of a treatment allowed a 30 second contact time versus a 300 second contact time. For example, at week 2, an APC

reduction of 0.98 log was seen in samples treated with 100ppm 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 100ppm 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), Beuchat (1999), and Adams et al. (1989).

Although microbial populations were not significantly influenced by the interaction of contact time and treatment, contact time, independent of treatment, had a significant effect ($p<0.05$) on total aerobes and yeast populations. Samples allowed a 30 second contact time after treatment reported significantly lower log values ($p<0.05$) for each of these populations as compared with samples allowed a 300 second contact time. In contrast, mold counts were not significantly ($p<0.05$) affected by the length of contact time after treatment. Log reductions in mold populations were similar at each contact time. The extent to which contact time significantly influenced ($p<0.05$) microbial populations varied across the weeks. For example, log reductions in total aerobes were greater at the 30 second contact time at weeks 1, 4, and 5 than at weeks 2 and 3. Furthermore, results indicate that a treatment's effectiveness at reducing populations of total aerobes, yeast, and mold also varied across the weeks. The weekly differences in microbial reductions in response to treatment and contact time are likely influenced by berry maturity. As berries mature and ripen, structural pectins are degraded by pectinases

resulting in a softer fruit texture and weakening of cellular structures. The weakening of cell walls leaves intracellular nutrients more accessible to microorganisms for growth and proliferation. For this reason, berry maturity is usually accompanied by microbial proliferation resulting in a greater possibility of interaction between antimicrobial treatments and this larger population. For example, at week 1, the mean initial APC for unwashed berries (control) was 2.44 $\pm$ 0.15 log. Samples treated with 100ppm chlorine for 30 seconds produced a mean reduction of 0.35 log. However, at week 5 when the mean initial APC for unwashed berries was higher, 3.27 $\pm$ 0.13 log, the same treatment produced a mean reduction of 1.47 log.

Significant differences ($p<0.05$) in antimicrobial effectiveness among treatments allowed the same contact time were observed as evidenced by total aerobe, yeast, and mold counts presented in Figures 3-16. Table 4 and 5 summarize the mean log reductions of total aerobe, yeast, and mold populations in response to treatments allowed a 30 second contact time versus a 300 second contact time.

Table 4: Mean[a] Log (CFU/g) Reduction in Microbial Populations of Treated Blueberries Allowed a 30 Second Contact Time

	APC	Yeast	Mold
100ppm Chlorine	0.85	0.53	0.75
0.5% Hydrogen Peroxide	0.66	0.55	0.47
0.5% Citric Acid	0.43	-0.08	0.37
Distilled Water	0.43	0.11	0.40

[a]Mean value of 30 samples across 5 weeks.

Table 5: Mean[a] Log (CFU/g) Reduction in Microbial Populations of Treated Blueberries Allowed a 300 Second Contact Time

	APC	Yeast	Mold
100ppm Chlorine	0.83	0.77	0.61
0.5% Hydrogen Peroxide	0.36	0.54	0.34
0.5% Citric Acid	0.24	0.14	0.30
Distilled Water	0.22	0.10	0.17

[a]Mean value of 30 samples across 5 weeks.

Results indicate that samples treated with 100ppm chlorine had lower mean values of total aerobes, yeast, and mold when compared to samples treated with 0.5% hydrogen peroxide, 0.5% citric acid, or distilled water. 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 individual washed samples, cumulative results report a mean population reduction of less than 1 log for all chlorine-treated samples. 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 organic materials, like plant tissue components, 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 value of the chlorinated wash water may also have influenced the effectiveness of chlorine. Since the antimicrobial effectiveness of chlorine is dependent on the concentration of hypochlorous acid in solution, the pH of the solution should remain at or below pH 7-8 at which point hypochlorous acid remains undissociated (Adams et al, 1989). Consequently, since the chlorine solutions used in this study had a mean pH of 9.1, the effectiveness of these treatments may have been reduced. This fluctuation in pH is commonly observed in industry and is compensated for by readjusting the pH of the chlorine solution to 8.0.

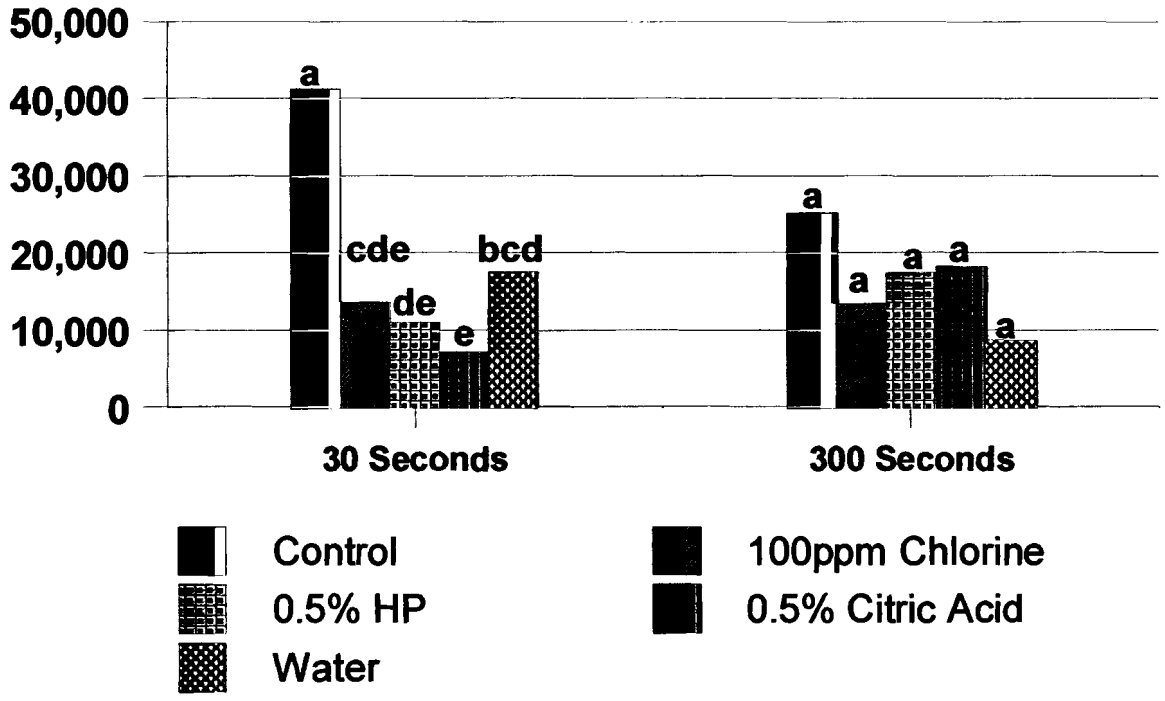
In comparison with other treatments, 0.5% hydrogen peroxide treatments were second to chlorine in effectiveness at bringing about population reductions. Total aerobe, yeast, and mold populations responded similarly to treatment with 0.5% hydrogen peroxide. Microbial populations of samples treated with 0.5% citric acid and distilled water were least affected by treatment. Total aerobe and mold populations responded similarly to treatment with citric acid and distilled water; however, both populations showed greater reductions in response to citric acid and distilled water than did yeast.

Differences in effectiveness of treatments throughout the 5 weeks may have been influenced by microbial differences within the 5 sampling plots. Differences in berry maturity, clone, irrigation, and amount of organic material present on the surface of blueberries would be expected to vary across the field and such differences would affect the microbial population of the representative samples. For example, if at week 2, more berries were collected from plots 1-3 than from plots 4 and 5, the microbial population of the individual samples that week would more closely resemble that of plots 1-3. Thus,

the antimicrobial effect of a treatment may vary due to the microbial characteristics of the population.

Residual Phosmet Analyses

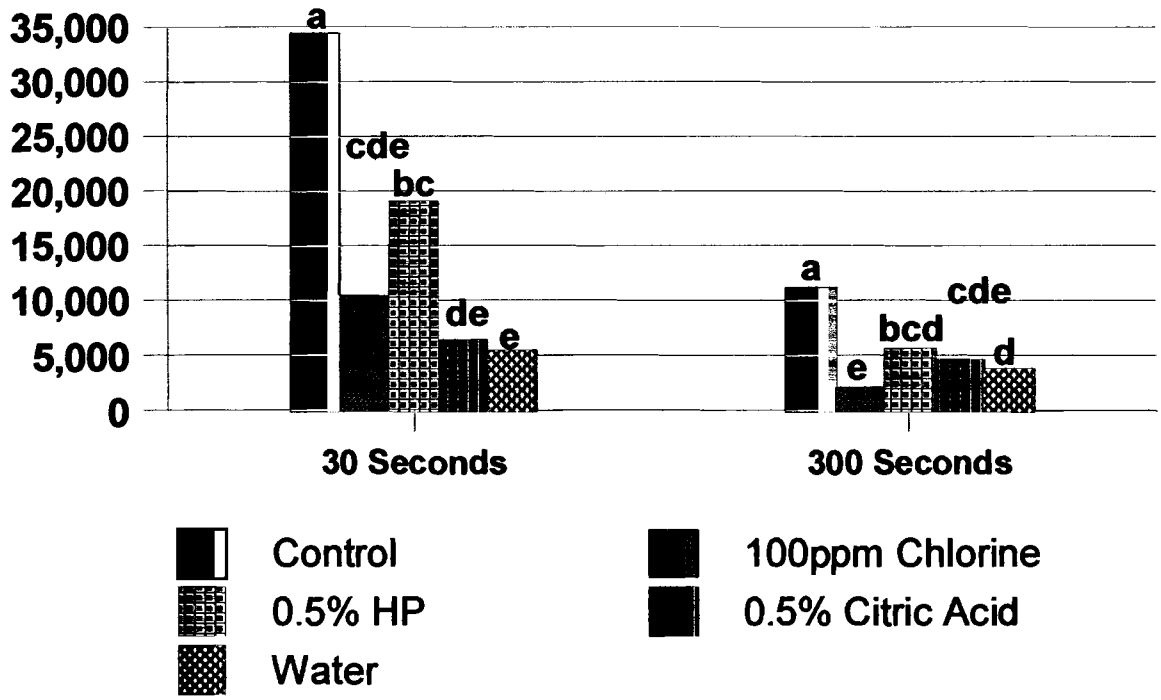
Figure 18: Week One – Residual Phosmet Levels (ppb) on Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Extraction of phosmet from all samples was performed in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The residual phosmet level of the control sample for the 30 second treatment was 41,249ppb. All treatments significantly reduced ($p<0.05$) residual phosmet levels compared to the control. Samples treated with 0.5% citric acid showed the greatest reductions with residual levels falling below the EPA tolerance of 10,000ppb.

The residual phosmet level of the control sample for the 300 second treatment was 25,253ppb. All treatments significantly reduced ($p<0.05$) residual phosmet levels compared to the control; however, significant differences ($p<0.05$) did not exist among treatments. Samples treated with distilled water resulted in residual phosmet levels below the EPA tolerance of 10,000ppb.

Figure 19: Week Two – Residual Phosmet Levels (ppb) on Lowbush Blueberries Following Treatment for 30 and 300 Seconds

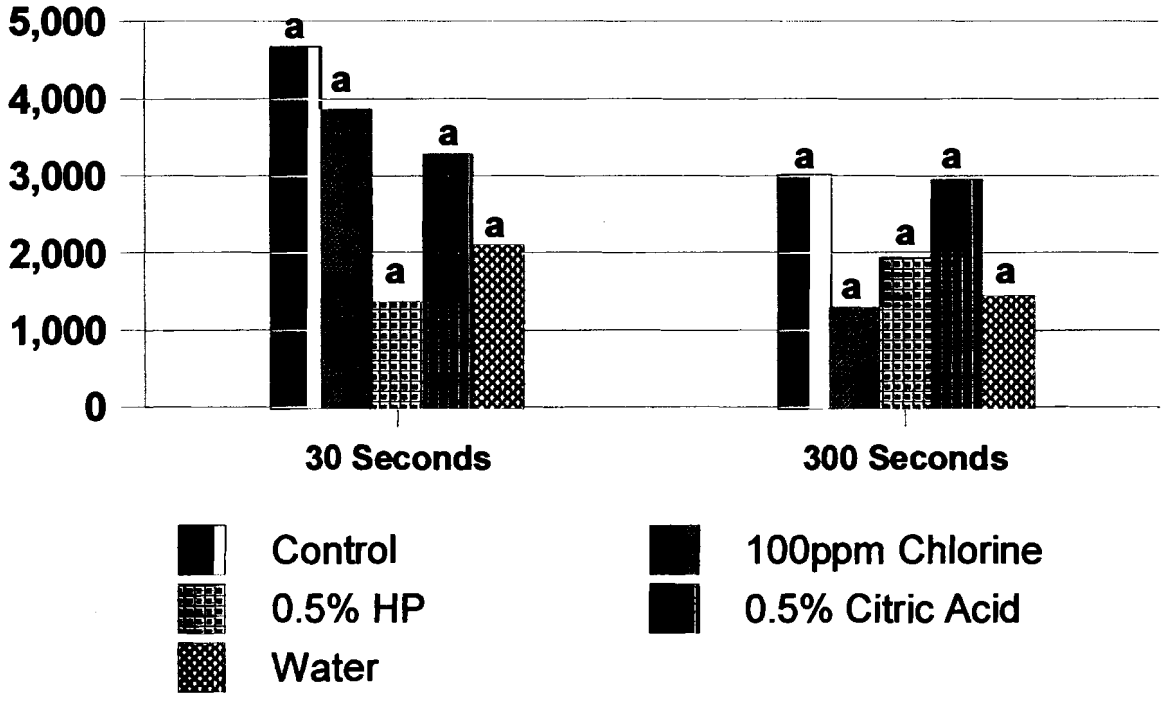


Extraction of phosmet from all samples was performed in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The residual phosmet level of the control sample for the 30 second treatment was 34,319ppb. All treatments significantly reduced ($p<0.05$) residual phosmet levels compared to the

control. Samples treated with 100ppm chlorine, 0.5% citric acid, and distilled water retained residues in amounts within the EPA tolerance level of $\leq$ 10,000ppb.

The residual phosmet level of the control sample for the 300 second treatment was 11,143ppb. All treatments significantly reduced ($p<0.05$) residual phosmet levels below the EPA tolerance of 10,000ppb. Overall, treatment with 100ppm chlorine resulted in the greatest residual reduction. Significant differences ($p<0.05$) did not exist between the effectiveness of 0.5% hydrogen peroxide, 0.5% citric acid, and distilled water treatments of wild blueberries.

Figure 20: Week Three – Residual Phosmet (ppb) on Lowbush Blueberries Following Treatment for 30 and 300 Seconds

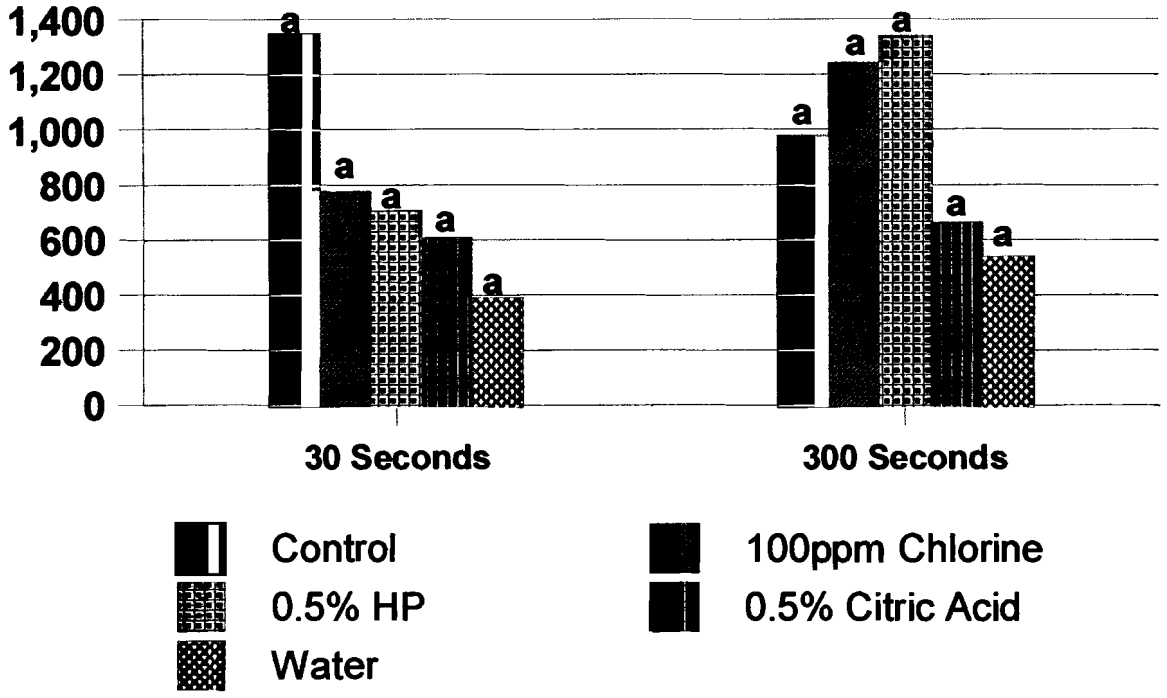


Extraction of phosmet from all samples was performed in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$)

using a one-way ANOVA and Tukey's HSD Multiple Comparison. The residual phosmet level of the control sample for the 30 second treatment was 4,668ppb. Significant differences ($p<0.05$) did not exist among treatments and the control; however, residual phosmet levels of all samples including the control contained residual phosmet in amounts below the EPA tolerance of 10ppm or 10,000ppb. Among treated samples, those samples treated with 100ppm chlorine sprays showed the greatest reduction in phosmet residues.

The residual phosmet level of the control sample for the 300 second treatment was 3,019ppb. Significant differences ($p<0.05$) did not exist among treatments and the control; however, all samples including the control contained residual phosmet in amounts below the EPA tolerance of 10,000ppb. Samples treated with 100ppm chlorine and distilled water showed the greatest reduction in residual phosmet.

Figure 21: Week Four – Residual Phosmet Levels (ppb) on Lowbush Blueberries Following Treatment for 30 and 300 Seconds

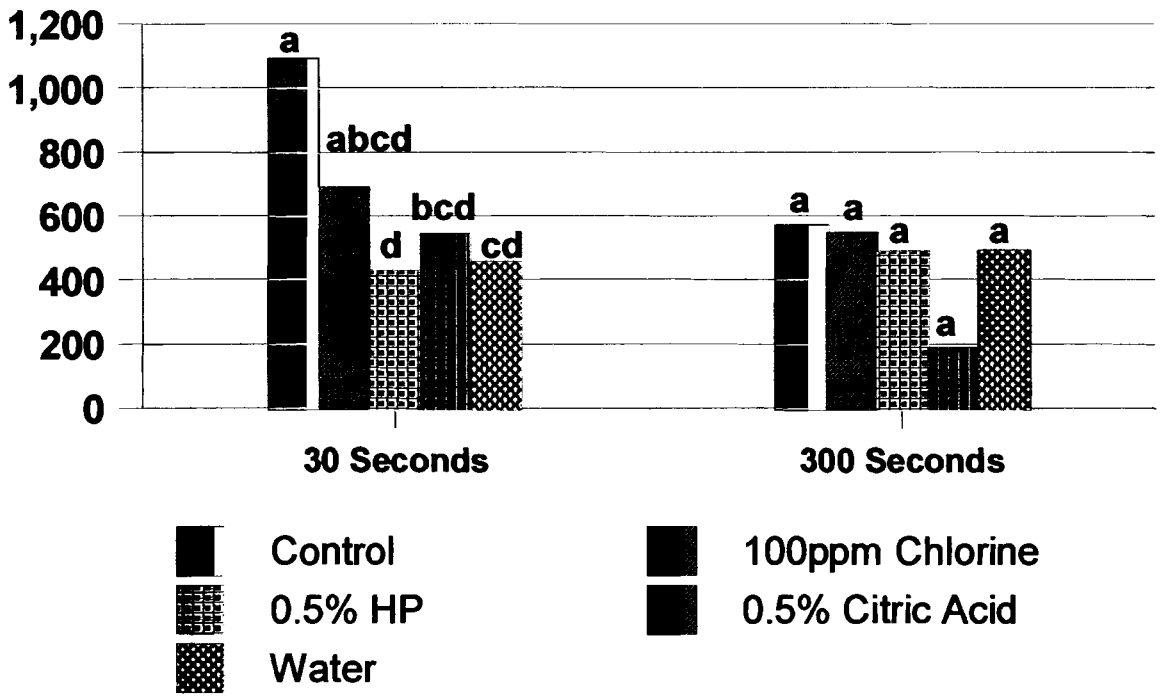


Extraction of phosmet from all samples was performed in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The residual phosmet level of the control sample for the 30 second treatment was 1,348ppb. Significant differences ($p<0.05$) did not exist among treatments and the control; however, residual phosmet levels of all samples including the control fell below the EPA tolerance of 10,000ppb. Samples treated with distilled water contained the lowest levels of residual phosmet compared to other treated samples.

The residual phosmet level of the control sample for the 300 second treatment was 978ppb. Significant differences ($p<0.05$) did not exist among treatments and the control; however, all samples including the control contained residual phosmet in

amounts below the EPA tolerance of 10,000ppb. The increased residual levels observed on samples treated with 100ppm chlorine and 0.5% hydrogen peroxide could be the result of variations in sampling. Since a representative sample was obtained by raking 5 separate plots around the field, any variations in sampling may be reflected in the residual phosmet levels of individual samples.

Figure 22: Week Five – Residual Phosmet Levels (ppb) on Lowbush Blueberries Following Treatment for 30 and 300 Seconds



Extraction of phosmet from all samples was performed in duplicate. Samples treated with 0.5% hydrogen peroxide are represented by 0.5% HP on the legend. Values not followed by the same letter were determined to be significantly different ($p<0.05$) using a one-way ANOVA and Tukey's HSD Multiple Comparison. The residual phosmet level of the control sample for the 30 second treatment was 1,094ppb. All samples including the control contained residual phosmet in amounts below the EPA

tolerance of 10,000ppb. Although significant differences ($p<0.05$) did not exist among treatments, only samples treated with 0.5% hydrogen peroxide, 0.5% citric acid, and distilled water showed significant reductions ($p<0.05$) in residual phosmet levels when compared to the control.

The residual phosmet level of the control sample for the 300 second treatment was 575ppb. All samples including the control contained residual phosmet in amounts below the EPA tolerance of 10,000ppb; however, only samples treated with 0.5% citric acid showed significant reductions ($p<0.05$) in residual phosmet when compared to the control.

Overall, results indicate that all treatments significantly reduced ($p<0.05$) residual phosmet levels of lowbush blueberries. Berries treated with distilled water, 0.5% citric acid, and 100ppm chlorine showed greater reductions in residual phosmet than did samples treated with 0.5% hydrogen peroxide. Distilled water, citric acid, and chlorine treatments were similar in their ability to lower residual phosmet levels; however, the greatest residual reduction in phosmet was observed on samples treated with distilled water. The effectiveness of distilled water washing is also reflected in the pattern of phosmet degradation seen after intensive irrigation of the field by an overhead irrigation system. For example, lowbush blueberries showed substantial decreases in residual phosmet levels during the 2nd week of the study at which time irrigation frequency increased. For the remainder of the study, the field was irrigated 2 times per week while residual levels steadily decreased. Although additional factors like temperature, vapor pressure, sunlight, and other radiations may have contributed to phosmet degradation, water treatment methods including sprays and irrigation resulted in significant reductions

($p<0.05$) in residual phosmet levels on lowbush blueberries. The influence of water on phosmet degradation is most likely the combined effect of hydrolysis and pH on the pesticide. The chemical structure of organophosphorous pesticides contributes to their susceptibility to hydrolysis in aqueous environments (Garcia-Repetto et al., 1994). Furthermore, since phosmet is rapidly hydrolyzed in neutral and alkaline media, water, at pH 7.0, may also contribute to the degradation kinetics of phosmet.

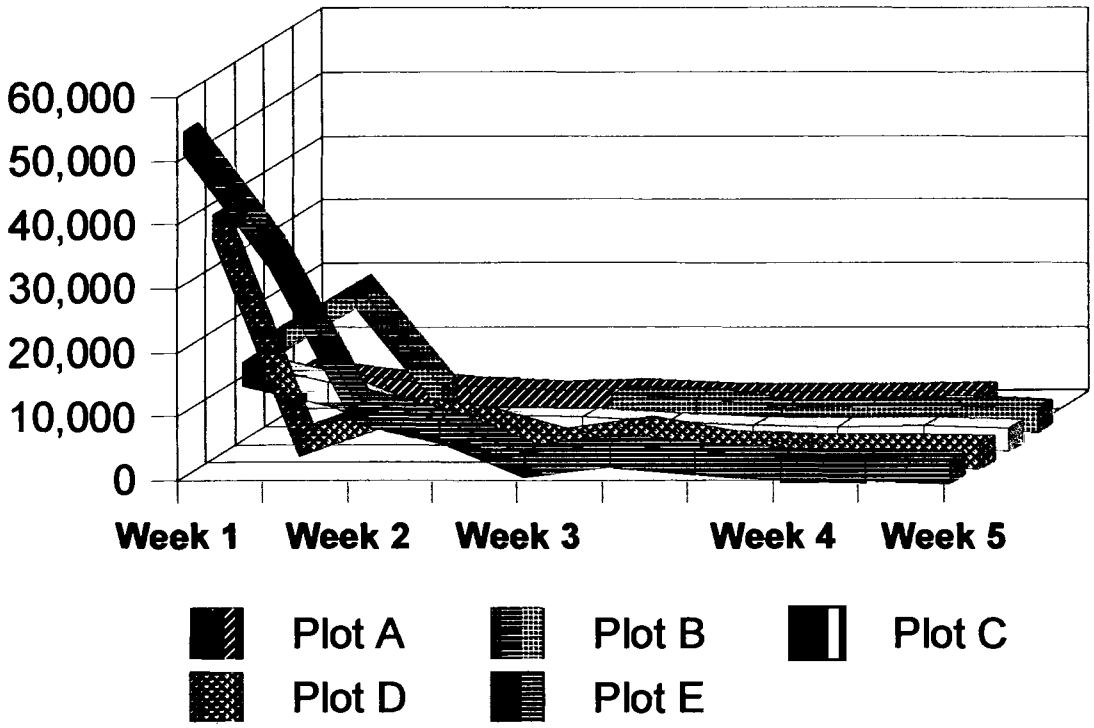
Significant differences ($p<0.05$) in residual phosmet levels of lowbush blueberries were observed throughout the 5 weeks of sampling (Figure 23). For example, after the initial application of phosmet, residual levels showed substantial decreases over the next 5 weeks as evidenced by residual levels of unwashed control samples. Furthermore, the week of sampling also significantly influenced ($p<0.05$) the effectiveness of treatments at removing phosmet residues. For example, at week 1 and 2, when residual phosmet levels were elevated, greater residual reductions were observed in treated samples. However, at weeks 3-5 when residual levels were lower, treatments showed decreased effectiveness in removing residues from blueberry samples.

Results of phosmet analyses reveal residual reductions to be significantly greater ($p<0.05$) in samples held for 30 seconds following treatment versus 300 seconds. Less residual reduction at the 300 second contact time could be attributed to lower control values for these samples and to results implicating contact time to be less influential in bringing about residual phosmet reductions when initial levels were decreased, for example, at weeks 3-5. Therefore, when initial residual levels are lower, contact time and treatment, independent of each other, have less of an influence on residual phosmet levels of lowbush blueberries. Although microbial populations were not significantly

influenced ($p<0.05$) by the interaction of contact time and treatment, results indicate that contact time did significantly influence ($p<0.05$) a treatment's effectiveness at removing residual phosmet from the surface of lowbush blueberries. For example, when treatments were allowed a 30 second contact time, samples treated with 0.5% citric acid showed the greatest reductions in residual phosmet, but when allowed a 300 second contact time, samples treated with distilled water retained the lowest residual levels.

Results on the assessment of phosmet degradation around the field are summarized in Figure 23. By the 2nd week of the study, residual phosmet levels of samples from 4 of the 5 plots fell below the EPA tolerance of 10,000ppb. Furthermore, this data reflects the pattern of degradation seen in unwashed control samples throughout the 5 weeks of the study. Differences in initial residual levels of the 5 plots may be influenced by their respective position around the field. For example, aerial application of phosmet may not be applied as uniformly to the perimeter of the field, Plot A, as it would to the center of the field, Plot D.

Figure 23: Degradation of Phosmet (ppb) from Initial Application until Harvest



In addition to temperature, vapor pressure, sunlight and other radiations, microbial degradation under aerobic conditions also principally influences the persistence of phosmet (Chapman and Cole, 1982; Garcia-Repetto et. al, 1994; EPA, 2001). For example, in Fate Assessment Studies conducted by the EPA, phosmet degraded with a calculated half-life of approximately 3 days in moist loam soil at pH 7.4. Although mineralization by microorganisms has only been evaluated under aerobic soil conditions, the possibility exists that surface microflora of lowbush blueberries may also be capable of degrading phosmet under aerobic conditions. Furthermore, microbial degradation may be enhanced by an increase in water activity via irrigation or rain since microbial proliferation is accelerated at higher water activity levels. Although a decrease in residual phosmet was observed in conjunction with an increase in irrigation and microbial proliferation, more research is needed to support this theory.

CONCLUSIONS

Factors such as contact time, post-harvest treatment, and time of sampling influence the individual effectiveness of post-harvest treatments to improve the microbiological quality and safety of fruits and vegetables. Although previous observations have suggested that chlorine is limited in its effectiveness when used at permitted concentrations, microbial results reveal chlorine to be the most effective of the treatments tested at improving the microbiological quality of lowbush blueberries. All treatments were found to be more successful when allowed a 30 second contact time versus a 300 second contact time. Overall, results indicate that treatment of blueberries with 100ppm chlorine solutions for 30 seconds results in a marked decrease in microbial populations of total aerobes, yeast, and mold. The results of this study are in support of the Maine Wild Blueberry Industry standard of treating blueberries with 100ppm chlorine sprays to improve microbial quality prior to freezing. In addition to improving the microbial quality, chlorine treatments also contributed to the removal of residual phosmet on lowbush blueberries. Although not administered for this purpose, the secondary effect of chlorine is beneficial in improving the safety of IQF blueberries.

In addition to chlorine, results of the study reveal that distilled water treatments also significantly contributed ($p<0.05$) to the degradation or removal of phosmet on the surface of lowbush blueberries. Furthermore, samples showed a marked decrease in residual levels in response to irrigation. The application of this data is important to consumers who may only rinse blueberries prior to consumption. However, the applicability of this data to other fruits and vegetables would be dependent on the type of pesticide and surface characteristics of the produce. Independent of treatment,

degradation contributed to decreased residual levels of phosmet seen on lowbush blueberries at the time of harvest. Therefore, according to the degradation pattern of phosmet, it appears that washing to decrease residual levels may not be a necessary commercial application.

Overall, results of the study support the use of 100ppm chlorine treatments to improve the microbiological quality and safety of lowbush blueberries. However, since chlorine is known to form potentially hazardous by-products, future regulatory constraints on the use of chlorine as a sanitation agent may be eminent. Therefore, additional research is needed to identify alternative treatments and their effective concentrations.

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APPENDIX A

Evaluation of a Liquid Produce Wash on Improving the Microbiological Quality And Reducing Pesticide Residues of Lowbush Blueberries

The effectiveness of commercial treatments in reducing initial microbial populations of fresh fruits and vegetables has been extensively researched. Numerous chemical sanitizers have been evaluated as alternatives to chlorine, the most common sanitizer used in the fruit and vegetable product industry (Xu, 1999). However, little research has been conducted on the effectiveness of commercial liquid prototype produce washes at improving the microbial quality of produce. If proven efficacious, such washes would be beneficial for consumers handling fresh produce in the home. The following research was conducted to evaluate the effectiveness of Fit® (Procter & Gamble, Cincinnati, Ohio), a commercial produce wash, at improving the microbial quality of lowbush blueberries. Similar research has been conducted to compare chlorine and Fit® for effectiveness in killing *Salmonella* and *E. coli* 0157:H7 on alfalfa seeds. Results by Beuchat et al. (2001) show similar and significant reductions in populations of *Salmonella* and *E. coli* 0157:H7 on alfalfa seeds after treatment with either 20,000ppm chlorine or Fit® compared with treatment with 200ppm chlorine. Thus, Fit would be an effective and safe alternative to high concentrations of chlorine in killing pathogenic bacteria on alfalfa seeds.

Fit® is an alkaline solution consisting of generally recognized as safe ingredients including water, oleic acid, glycerol, ethanol, potassium hydroxide, sodium bicarbonate, citric acid, and distilled grapefruit oil. Wash treatments were prepared according to product directions using distilled water. A 350g sample of blueberries was immersed in

the wash solution for 120 seconds and then rinsed with distilled water at the end of the contact time. Unwashed berries served as the control for this study. Microbial and pesticide analysis was conducted on washed berries according to the same methods previously described for other treated samples. Microbial and residual phosmet results from experiments using Fit® washes are summarized in Tables A1-A3.

Table A1: Aerobic Plate Counts – Mean Log (CFU/g) and Log Reduction[a] of Lowbush Blueberries After Treatment with Fit®

	Control	Fit® Treatment	Log Reduction
Week 1	2.44 ± 0.06	2.40 ± 0.21	0.04
Week 2	4.20 ± 0.16	3.60 ± 0.28	0.60
Week 3	3.29 ± 0.18	2.20 ± 0.17	1.09
Week 4	2.90 ± 0.31	3.69 ± 0.50	-0.79[b]
Week 5	3.27 ± 0.24	2.09 ± 0.36	1.18

[a]Log reduction is the difference between aerobic plate counts before and after treatment with Fit® wash.
[b]Negative log reductions indicate an increase in aerobic plate counts after treatment.

Triplicate samples were taken after treatment with Fit® and plated in duplicate. With the exception of week 4, log reductions in microbial populations of total aerobes were observed in all treated samples. As the microbial population of blueberries increased, greater population reductions were observed. This data is similar to results from previous studies by the author.

Table A2: Yeast – Mean Log (CFU/g) and Log Reduction[a] of Lowbush Blueberries After Treatment with Fit®

	Control	Fit® Treatment	Log Reduction
Week 1	2.18 ± 0.09	2.57 ± 0.16	-0.39[b]
Week 2	3.05 ± 0.06	2.44 ± 0.13	0.61
Week 3	1.76 ± 0.36	1.27 ± 0.38	0.49
Week 4	2.37 ± 0.19	2.46 ± 0.42	-0.09
Week 5	2.00 ± 0.30	1.29 ± 0.26	0.71

[a]Log reduction is the difference between yeast counts before and after treatment with Fit® wash.
[b]Negative log reductions indicate an increase in yeast counts after treatment.

Triplicate samples were taken after treatment with Fit® and plated in duplicate. Log reductions in yeast counts were observed in some samples; however, increases in yeast populations were also seen in samples after treatment with Fit®. The antimicrobial effectiveness of Fit® did not appear to follow any predictable pattern as evidenced by yeast log reductions.

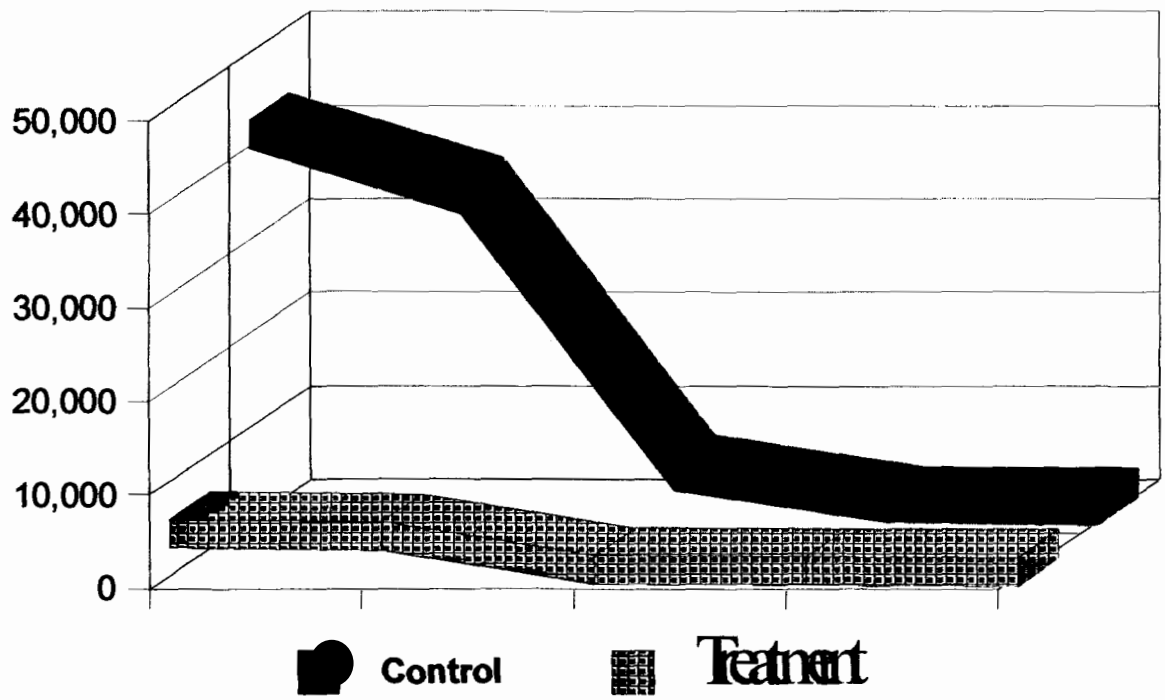
Table A3: Mold – Mean Log (CFU/g) and Log Reduction[a] of Lowbush Blueberries After Treatment with Fit®

	Control	Fit® Treatment	Log Reduction
Week 1	2.56 ± 0.16	2.10 ± 0.06	0.46
Week 2	3.21 ± 0.11	2.17 ± 0.06	1.04
Week 3	2.22 ± 0.22	1.09 ± 0.12	1.13
Week 4	2.24 ± 0.16	1.90 ± 0.58	0.34
Week 5	2.06 ± 0.32	1.18 ± 0.00	0.88

[a]Log reduction is the difference between mold counts before and after treatment with Fit® wash.

Triplicate samples were taken after treatment with Fit® and plated in duplicate. Mold count reductions were observed in all samples after treatment. Greater mean reductions in mold counts were observed in samples treated with Fit® for 120 seconds than in samples treated with 100ppm chlorine, 0.5% hydrogen peroxide, 0.5% citric acid, or distilled water for either 30 or 300 seconds.

Figure A1: Phosmet Residues (ppb) on Lowbush Blueberries Before and After Treatment with Fit®



Blueberries treated with Fit® showed significant reductions ($p<0.05$) in residual phosmet levels. Each week, treated samples retained phosmet in levels below the EPA tolerance of 10,000ppb despite relatively high residues on unwashed controls. Therefore, results of the study indicate that treatment of lowbush blueberries with Fit® produce wash is effective in reducing residual phosmet although its effectiveness in improving microbial quality is limited.

APPENDIX B

Log Reductions in Microbial Populations of Lowbush Blueberries Following Treatment

Table B1 - Log (log CFU/g) Reductions in Total Aerobic Populations of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

	Week 1	Week 2	Week 3	Week 4	Week 5
Control – 30 Seconds	2.44 ± 0.15	4.21 ± 0.17	3.29 ± 0.22	2.90 ± 0.18	3.27 ± 0.13
100ppm Chlorine	0.35	0.98	0.94	0.51	1.47
0.5% Hydrogen Peroxide	-0.49[a]	1.22	0.93	0.03	1.57
0.5% Citric Acid	-0.66	0.89	0.29	0.17	1.47
Distilled Water	-1.06	1.72	0.42	-0.21	1.27
Control – 300 Seconds	2.75 ± 0.30	3.62 ± 0.19	3.08 ± 0.15	4.10 ± 0.16	3.99 ± 0.18
100ppm Chlorine	-0.30	0.76	1.18	0.98	1.50
0.5% Hydrogen Peroxide	-0.31	0.08	-0.10	0.92	1.19
0.5% Citric Acid	-0.98	0.30	0.57	0.25	1.06
Distilled Water	0.04	0.80	-0.08	0.03	0.33

[a] Log reduction is the difference between aerobic plate counts before and after treatment.
[b] Negative reductions indicate an increase in aerobic plate counts after treatment.

Table B2 - Log (log CFU/g) Reductions[a] in Yeast Populations of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

	Week 1	Week 2	Week 3	Week 4	Week 5
Control – 30 Seconds	2.18 ± 0.11	3.05 ± 0.09	1.76 ± 0.19	2.38 ± 0.09	2.00 ± 0.32
100ppm Chlorine	-0.35[b]	0.66	0.46	0.60	1.30
0.5% Hydrogen Peroxide	-0.10	1.30	0.51	0.22	0.85
0.5% Citric Acid	-0.45	0.34	-0.43	0.12	0.04
Distilled Water	-0.90	0.54	0.13	0.20	0.58
Control – 300 Seconds	2.34 ± 0.20	2.80 ± 0.10	2.87 ± 0.10	2.78 ± 0.12	2.80 ± 0.12
100ppm Chlorine	-0.06	0.62	1.54	1.09	0.68
0.5% Hydrogen Peroxide	-0.31	0.41	0.72	1.23	0.66
0.5% Citric Acid	-0.76	0.08	0.49	0.66	0.21
Distilled Water	-0.48	0.05	0.29	0.38	0.26

[a] Log reduction is the difference between yeast counts before and after treatment.
[b] Negative reductions indicate an increase in yeast counts after treatment.

Table B3 - Log (log CFU/g) Reductions[a] in Mold Populations of Lowbush Blueberries Following Treatment for 30 and 300 Seconds

	Week 1	Week 2	Week 3	Week 4	Week 5
Control – 30 Seconds	2.56 ± 0.10	3.21 ± 0.10	2.22 ± 0.18	2.25 ± 0.10	2.06 ± 0.18
100ppm Chlorine	0.30	1.33	0.46	0.90	0.77
0.5% Hydrogen Peroxide	0.01	0.81	0.61	0.30	0.65
0.5% Citric Acid	-0.11[b]	0.70	0.11	0.57	0.60
Distilled Water	0.16	0.52	0.21	0.33	0.83
Control – 300 Seconds	2.31 ± 0.15	2.67 ± 0.13	2.79 ± 0.30	2.37 ± 0.09	1.81 ± 0.14
100ppm Chlorine	0.04	0.36	1.76	0.90	0.20
0.5% Hydrogen Peroxide	0.02	0.05	0.75	0.30	0.12
0.5% Citric Acid	-0.15	0.17	0.44	0.57	0.37
Distilled Water	-0.14	0.06	-0.06	0.33	0.34

[a] Log reduction is the difference between mold counts before and after treatment.
[b] Negative reductions indicate an increase in mold counts after treatment.

BIOGRAPHY OF THE AUTHOR

Kristi Michele Crowe was born in Baton Rouge, Louisiana on February 17, 1977. She was raised in Baton Rouge, Louisiana and graduated from Central Private School in 1995. In May of 2000, she graduated from Samford University with a Bachelor of Science in Nutrition and Diet Therapy. As an undergraduate, she worked for Weight Watcher's Magazine as an editorial foods intern and for Oxmoor House, Inc. as a test kitchen assistant and sensory analyst collaborating on projects such as *Christmas with Southern Living* and *Cooking Light Cookbooks.* In the spring of 2001, she entered the graduate program in Food Science at The University of Maine.

While at The University of Maine, she has become a member of the Institute of Food Technologists, Phi Tau Sigma Honorary Society, and the Food Science Club where she served one term as vice-president. In the summer of 2002, she won 1st place in the Institute of Food Technologists' Fruit and Vegetable Products Division Poster Competition with a poster presenting the results of this thesis. Kristi is a candidate for the Master of Science degree in Food Science and Human Nutrition from The University of Maine in December, 2002.