AN ABSTRACT OF THE THESIS OF

Lisa D. Weller for the degree of Master of Science in Food Science and Technology presented on November 14, 2012.

Title: Efficacy of Water, Sodium Hypochlorite, Peroxyacetic Acid, and Acidified Sodium Chlorite for Reducing Microorganisms on In-Shell Hazelnuts

Abstract approved:

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Hazelnuts are commonly consumed raw and are valued for their numerous health benefits and antioxidant properties. Increased foodborne illness outbreaks associated with *Salmonella* and *Escherichia coli* O157:H7 contamination of tree nuts and peanuts generate a need for improving agricultural sanitation procedures. Food-safe chemical sanitizers have shown promise for reducing pathogenic organisms on fresh produce, but minimal research has been conducted for in-shell nuts. The purpose of this study was to determine the effects of water and three food-safe sanitizers on a) the natural microbial load of postharvest in-shell hazelnuts and b) populations of pathogenic *Salmonella* (*S. enterica* subsp. *enterica* ser. Panama) inoculated and dried onto the surfaces of in-shell hazelnuts.

The first phase of the study investigated the effectiveness of water, sodium hypochlorite (NaOCl; 25 ppm, 50 ppm), peroxyacetic acid (PAA; 80 ppm, 120 ppm), and acidified sodium chlorite (ASC; 990 ppm) as sanitizers for use on postharvest in-shell hazelnuts.

Treatments were applied to two groups of freshly harvested hazelnut samples to examine their effects on total aerobic microorganism populations during different times of harvest (Group 1 = early season, dry weather; Group 2 = late season, rainy weather). Treatments within each group included hazelnuts that underwent a tap water rinse, a tap water rinse followed by a water spray, and a tap water rinse followed by a chemical spray. Due to excess soil attached to shell surface, hazelnuts harvested later in the season (Group 2) had an initial population mean 2.24 log CFU/hazelnut greater than hazelnuts harvested earlier in the season (Group 1). All treatments, including water, resulted in significant population reductions compared to untreated controls ($P \leq 0.05$). Rinsing with tap water produced reductions of 0.38 log units in both groups, and additional water spraying resulted in reductions of 0.83 and 0.73 log units in Group 1 and Group 2, respectively. None of the chemical treatments were significantly more effective than the water spray treatment in Group 1; however, several chemical treatments in Group 2 were significantly more effective than water spraying. Tight adherence to shell surfaces during dry weather may have increased the chemical resistance of microorganisms on hazelnuts. Treatment with ASC produced the greatest reduction in Group 1 and Group 2 compared to the control (1.22 and 2.08 log units, respectively) and water spray treatments (0.39 and 1.39 log units, respectively), but the efficacies varied between treatment groups. Wide variation between Group 1 and Group 2 treatment results made determination of chemical efficacy difficult.

The second phase of the study analyzed the effectiveness of water, sodium hypochlorite (NaOCl; 25 ppm, 50 ppm), peroxyacetic acid (PAA; 80 ppm, 120 ppm), and acidified sodium chlorite (ASC; 450 ppm, 830 ppm, 1013 ppm) as sanitizers for reducing *Salmonella* on in-shell hazelnuts. Hazelnut samples were soaked in pure cultures of *S.* Panama for 24 h, air dried for 66 h, and then sprayed with water and chemical treatments. Surviving *S.* Panama populations were evaluated using a non-selective medium (tryptic soy agar), followed by a selective overlay (xylose lysine deoxycholate agar) after a 3 h incubation period. Tight adhesion prevented significant population decreases from

physical removal by water, which allowed for clear demonstration of chemical effectiveness. All of the chemical treatments significantly reduced the *S*. Panama population ($P \leq 0.05$) compare to untreated and water-sprayed samples. The most effective concentrations of ASC, PAA, and NaOCl treatments resulted in mean microbial population reductions of 2.65, 1.46, and 0.66 log units, respectively.

Overall, physical removal of excess dirt appeared to have the greatest effect on the microbial population reductions of postharvest in-shell hazelnuts, and adherence to shells during dry weather appeared to increase the chemical resistance of microorganisms. Future sanitation experiments should consider weather and levels of excess soil on hazelnuts as factors in the apparent efficacy of chemical sanitizers. Testing chemical sanitizers against tightly-adhered S*almonella* cells provided consistent results with clear demonstration of chemical efficacies. Acidified sodium chlorite at 1013 ppm was significantly more effective at reducing *Salmonella* populations than other treatments and shows the greatest potential for use as a postharvest sanitation treatment. Thorough rinsing of hazelnuts in clean tap water, followed by spraying with high concentrations of acidified sodium chlorite could help increase the efficacy of current hazelnut processing procedures.

Efficacy of Water, Sodium Hypochlorite, Peroxyacetic Acid, and Acidified Sodium Chlorite for Reducing Microorganisms on In-Shell Hazelnuts

By

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Lisa D. Weller, Author

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CONTRIBUTION OF AUTHORS

Dr. Mark Daeschel of Oregon State University provided guidance on the project design, chemical use, and microbial growth and enumeration techniques. Dr. Cathy Durham of Oregon State University provided the statistics software, statistics training, and aided in the data analysis.

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1. INTRODUCTION

Hazelnuts are a type of tree nut commonly eaten raw, roasted, or incorporated into snack foods worldwide. Regular consumption of hazelnuts is known to positively impact cholesterol levels (Alasalvar and others 2003), and research shows that consumption of raw hazelnuts may offer more health benefits than roasted or processed nuts (Schmitzer and others 2011). Oregon is renowned for providing uniquely large in-shell hazelnuts, and more than 75 percent of Oregon hazelnuts are sold in-shell. Unfortunately, recent foodborne illness outbreaks have been associated with in-shell tree nuts contaminated with *Salmonella* and *Escherichia coli* O157:H7. The peanut and many tree nut industries are taking proactive approaches to food safety by investigating new methods of postharvest sanitization.

Cultivation of hazelnuts inevitably allows microorganisms to attach to shell surfaces. Thermal processing procedures (e.g. roasting and blanching) have been validated for reduction of pathogens on tree nuts, but these processes often result in alteration of physical and sensory properties. Chemical sanitizers have been investigated for use on various fruits and vegetables, but little research has been conducted for sanitation of in-shell nuts. In addition, no research has been published that determines the total microbial populations of postharvest in-shell hazelnuts.

Sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite are food-safe chemicals approved for use on many agricultural commodities and organically processed foods. Testing these chemicals on freshly harvested hazelnuts will help determine their potential for use as postharvest processing aids. Investigation of the natural microbial loads of shell surfaces will also provide more insight to the sanitation needs of the hazelnut industry. This research will also include inoculating in-shell hazelnuts with high

concentrations of *Salmonella*, then exposing them to chemical treatments. Results will determine the efficacy of sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite for eliminating pathogens on hazelnut shell surfaces. Overall, this project will lay the groundwork for improving current hazelnut sanitation procedures, and provide a valuable reference for other nut industries interested in non-thermal processing methods.

2. LITERATURE REVIEW

2.1 HAZELNUTS

2.1.1 General Overview

Hazelnuts, also known as filberts, are a type of tree nut that has been consumed by humans for more than 5,000 years (Huntrods 2012). Appreciation and consumption of hazelnuts stem from awareness of their vitamin and antioxidant contents, as well as their complex "sweet" and "bitter" taste profiles (Alasalvar and others 2012). Hazelnuts are commonly eaten raw; roasted; or incorporated into value-added products such as nut butters, ice creams, baked goods, and other snack foods (Huntrods 2012).

Hazelnuts belong to the *Betulaceae* (birch) family and the genus *Corylus* (Nesom 2007). The *Corylus* genus consists of seven species, but world production is based on *C. avellana*, the most commercial species. Hazelnuts are native to the Mediterranean region of Europe and optimal growth occurs in temperate climates. Main producers include Turkey, the European Union (EU-27), Azerbaijan, and the United States. Hazelnut harvests take place between August and October, with most marketing years beginning annually in July[1]. The 2011/2012 global hazelnut production consisted of 66.5 percent from Turkey, 22.4 percent from the EU-27 (mainly Italy and Spain), 5.7 percent from Azerbaijan, and 5.4 percent from the U.S. (FAS 2011; FAS 2012). As of Oct 26, 2012, the 2012/2013 global production consisted of 78 percent from Turkey, 13 percent from the EU-27, 4 percent from Azerbaijan, and 4 percent from the U.S.(FAS 2012).

Oregon, which proudly names the hazelnut as its official state nut, produces over 99 percent of the U.S. hazelnut crop (Mehlenbacher and Olsen 1997). The 2011 U.S. hazelnut crop was valued at 89.7 million dollars and continued to make Oregon one of the top global producers of hazelnuts (NASS 2012b). The main varietals include Barcelona, Ennis, and Lewis, with Barcelona making up the majority of current farm acreage. However, popularity of Barcelona trees has decreased since the early 1990s, and

[1] Turkey's marketing year begins annually in August

only about 1 percent of new plantings are from this varietal (NASS 2012a). From 2008-2012, Jefferson, a cultivar developed by Dr. Shawn Mehlenbacher and David Smith of Oregon State University (McCluskey and others 2011), became the most commonly planted varietal in Oregon and made up 56 percent of newly planted acreage (NASS 2012a). According to Jeff Olsen, a professor of horticulture at Oregon State University, the Jefferson varietal has gained popularity due to its resistance to eastern filbert blight (Olsen 2012a). Eastern filbert blight is a fungal disease that causes dramatic branch dieback, and loss of susceptible trees has been devastating to many hazelnut farmers. However, genetic research conducted on hazelnut trees has acted as a valuable resource for development of resistant cultivars (Lunde and others 2000; Mehlenbacher 1995)

2.1.2 Growth, Harvest, and Processing Procedures

Hazelnut trees, unlike most Oregon plants, pollinate during the winter and develop nuts in the spring. Hazelnuts grow as green clusters during the spring and summer, then ripen during early fall. Harvest takes place after the nuts ripen, turn hazel colored, and fall to the ground separated from their husks (HMB 2012b).

Harvest generally takes place between late August and October and lasts for about a month. After hazelnuts fall to the ground, sweepers align the nuts in long, narrow piles between rows of trees in the orchard (Olsen 2012b). Harvesting machines pick up the hazelnuts, separate the nuts from other plant debris, and deposit the nuts in trailers or large totes (Olsen 2012b). Nuts are quickly transported from orchards to processing facilities throughout the region.

Postharvest hazelnuts go through a variety of processing procedures to ensure that only clean, quality nuts are distributed to consumers. General processing steps include washing and drying, but the washing methods vary by processing company. Washing hazelnuts removes the excess dirt and debris from hazelnut shells and involves rinsing or spraying with water or diluted food-safe sanitizers. Hazelnuts must contain less than 0.02

of one percent (w/w) of foreign material after processing (CFR 2008b). Clean hazelnuts are immediately dried to reduce the moisture to less than 6 percent (CFR 2008b); Drying preserves the nut meats and prevents development of off flavors. Drying can take place in warm, dry locations over several weeks or in food dryers for several days. After drying, hazelnuts can be distributed as in-shell nuts or go through additional processing steps. Shelling machines are used to crack hazelnut shells and separate the kernels from the shells. Kernels are either packaged raw or processed further via roasting. Roasting usually takes place on conveyor belts or in drums and the level of roasting varies depending on the intended use. Most products utilize lightly roasted hazelnuts with subtle flavor enhancements. According to The Hazelnut Council (THC), darker roasted hazelnuts tend to have intense flavors which are favored by the ice cream industry.

2.1.3 Health Benefits

Hazelnuts offer many health benefits when eaten in moderation. According to the Nutrient Data Laboratory (NDL), one serving (1.5 oz or 31 whole kernels) of hazelnuts contains 267 calories, 6.36 g of protein, 25.83 g of fat, 7.1 g of carbohydrates, 4.1 g of fiber, and 1.85 g of sugar. The Code of Federal Regulations (CFR) declares one serving of hazelnuts as a "good source" of protein, fiber, iron, magnesium, phosphorous, thiamin, and folate, and an "excellent source" of vitamin E (CFR 2012b). Hazelnuts contain no cholesterol and are low in saturated fats (NDL). Numerous research studies show that regular consumption of hazelnuts can improve cholesterol profiles and decrease the risk of heart disease and cardiovascular disease. Tey and others (2003) and Mercanghil and others (2007) both found that addition of hazelnuts into the diets of hypercholesterolemic people lowered total cholesterol and LDL cholesterol levels and raised HDL cholesterol levels. In 2003, the U.S. Food and Drug Administration (FDA) approved a qualified health claim stating:

"Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts [such as name of specific nut] as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease. [See nutrition information for fat content.]" (Taylor 2003).

Additionally, hazelnuts act as a rich source of phytochemicals (NDL ; Shahidi and others 2007; Alasalvar and others 2003; Alasalvar 2003), specifically phenolic acid compounds (Alasalvar and others 2009; Del Rio and others 2011). Many phytochemicals found in hazelnuts have strong antioxidant and antiradical properties (Alasalvar and others 2003; Alasalvar and others 2009; Shahidi and others 2007), which can lower the risk of diseases related to oxidative stress (Shahidi and others 2007) (e.g. some cancers (Valko and others 2006), osteoporosis (Sánchez-Rodríguez and others 2007)) and even increase overall life expectancy (Finkel and Holbrook 2000).

2.1.4 Safety and Health Concerns

Despite validation of numerous health benefits of hazelnuts, investigations of the safety of minimally processed tree nut and peanut products are ongoing. Exposure to microorganisms from soil is inevitable during the cultivation and harvest of hazelnuts. Contact with soil allows for attachment of natural yeast, mold, viruses, and bacteria, and can act as a pathway for pathogens. S*almonella* spp. have been known to live ubiquitously in the environment (Thomason and others 1977) and investigations of almond orchard soils have shown that some pathogenic strains may persist long-term in contaminated orchards (Uesugi and others 2007; Danyluk and others 2008). *Salmonella* is the leading cause of bacterial foodborne illness in the United States (Scallan and others 2011; CDC 2011a), as well as the leading pathogen for fatal foodborne illness. The ease of pathogenic contamination of hazelnuts prompts the need for improvement of postharvest sanitation procedures.

2.1.5 Outbreaks and Recalls Associated with Minimally Processed Nuts

Recent outbreaks of foodborne illness have been associated with consumption of numerous nut products, including in-shell hazelnuts. In 2001, in-shell peanuts were associated with an international outbreak of salmonellosis that caused 109 infections across Australia, Canada, England, and Scotland (Kirk and others 2004). In 2000-2001 (Isaacs and others 2005) and 2003-2004 (CDC 2004), raw almonds were also linked to

outbreaks of salmonellosis in North America that sickened 168 and 29 people, respectively. In 2009, detection of *Salmonella* in a processing facility resulted in a recall of 114,350 lbs of hazelnut kernels, but no illnesses were reported (FDA 2009c). In-shell pistachios were also recalled in 2009 due to *Salmonella* contamination (CDC 2009b), but only one illness was reported in the final update from the CDC. In 2010, a second hazelnut recall took place after eight cases of *Escherichia coli* O157:H7 illnesses were reported in Michigan, Minnesota, and Wisconsin (CDC 2011b). Traceback methods determined that the sources of the illnesses were in-shell hazelnuts packed by an Oregon company and distributed by a California company (Miller and others 2012). Numerous recalls were associated with *Salmonella* contaminated nut products prior to this illness outbreak, but this was the first outbreak of *E. coli* O157:H7 documented for nuts. The following year, raw walnuts were also associated with 14 cases of *E. coli* O157:H7 infections in Canada (CFIA 2011b; CFIA 2011a). More recently, a nation-wide outbreak of salmonellosis caused 35 illnesses (at the time of reporting) and resulted in recalls of numerous peanut products including nut butters, ice creams, nutrition bars, in-shell peanuts, and more (FDA 2012).

The previous paragraph summarizes notable recalls and outbreaks pertaining to raw and in-shell nut products. Minimally processed nuts pose the greatest risk for causing foodborne illnesses because the majority do not undergo processing procedures designed for pathogen elimination. For reference, Palumbo and others (2009) provide an updated table that lists all U.S. recalls and illnesses pertaining to tree nuts and peanuts.

2.2 PATHOGENS ASSOCIATED WITH TREE NUTS & PEANUTS

2.2.1 *Salmonella*

Salmonella is a gram negative, non-spore forming, bacilli bacterium (FSIS 2011). Daniel Salmon and Theobald Smith first isolated *Salmonella* in1885 when they described an organism causing disease in hogs (ASM 2012). There has been debate about the classification of *Salmonella* based on factors such as serologic antigens or clinical roles

(Brenner and others 2000). Nomenclature currently used by the Centers for Disease Control and Prevention (CDC) classifies *Salmonella* into two species: *S. enterica* and *S. bongori*. *S. enterica* includes subspecies *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* ; alternatively referred to as subspecies I, II, IIIa, IIIb, IV, and VI, respectively (Brenner and others 2000; Su and Chiu 2007). *S. enterica* subspecies are classified by genomic relatedness and biochemical differences (Brenner and others 2000). In 2004, Shelobolina and others (2004) described a new *Salmonella* species, *S. subterranean*, which was validated in 2005 and may be integrated into nomenclature in the future (Su and Chiu 2007).

Salmonella strains are divided into more than 2,500 serovars, with about 60 percent belonging to subspecies I, *S. enterica* subsp. *enterica* (further referenced as *S. enterica* or subsp. I) (CDC 2011c). In addition, 99 percent of salmonellosis illnesses are caused by subsp. I (CDC 2011c; FSIS 2011). Nontyphoidal salmonellosis is a common foodborne illness that can be caused by consumption of less than 10 viable cells (Hammack 2012). Symptoms generally include vomiting and diarrhea, which can cause fatal dehydration and electrolyte imbalances. Nontyphoidal salmonellosis can also cause septicemia (bacteria in blood, generally followed by a severe inflammatory response) or bacteremia (infection of blood) (Hammack 2012). Typhoid fever, caused by *S.* Typhi and *S.* Paratyphi, is less common and causes a rash, high fever, diarrhea, and can be fatal if untreated (Hammack 2012). Typhoid fever can also result in septicemia or septic arthritis, which can be difficult to treat.

Salmonella causes more hospitalized foodborne illness cases and foodborne illness deaths than any other foodborne pathogen in the U.S. (CDC 2011a). In 2011, studies estimated that nontyphoidal *Salmonella* causes 1 million foodborne illnesses, 19,336 foodborne illnesses requiring hospitalization, and 378 foodborne illnesses resulting in death, annually (CDC 2011a; Scallan and others 2011). Additionally, *S. enterica* serovars Enteritidis and Typhimurium cause more than half of all foodborne disease (FSIS 2011).

Typhoid fever accounts for an estimated 1,821 additional cases of *Salmonella* infections annually (CDC 2011a).

2.2.2 *Escherichia coli* O157:H7

Escherichia coli is a gram negative, non-spore forming, bacillus bacterium that is a natural inhabitant of human and animal gastrointestinal tracts. Theodor Escherich first described *E. coli* in 1885 as a natural gut microorganism of humans (ASM 1999). Interestingly, pathogenic *E. coli* strains were not declared as food pathogens until 1971 when 400 people became ill from contaminated cheeses (Jay and others 2005). Despite recent recognition, historical evidence suggests that *E. coli* spp. were associated with infant diarrhea as early as the 1700s, and that they caused several foodborne illness outbreaks in the mid-1900s (Jay and others 2005).

E. coli is in the Enterobacteriaceae family, which also includes pathogens such as *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., and *Citrobacter* spp. Similar to *Salmonella*, *E. coli* is classified serologically and more than 200 serotypes have been identified (Jay and others 2005). Pathogenic *E. coli* is grouped into six categories based upon virulence and disease factors. The groups include enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroaggressive (EAEC or EAggEC), and diffusely adherent (DAEC) (Feng 2012). ETEC infections are the leading cause of traveler's diarrhea, and large amounts of watery diarrhea result from enterotoxin production in the small intestine. ETEC strains have relatively high infective doses, and generally require ingestion of 10^8-10^{10} viable cells in order to cause symptoms (Jay and others 2005; Feng 2012). EIEC foodborne illnesses are rarer in the U.S., but require as few as 10 viable cells to cause illness. EIEC strains are generally not lethal but can cause bloody or non-bloody diarrhea (Feng 2012). EPEC strains do not generally produce enterotoxins and do not result in bloody diarrhea (Jay and others 2005). Adhesion properties appear to be the key virulence factor for EPEC, and as many as 10^{10} viable cells are needed to cause symptoms in adults. EHEC is the

most common cause of *E. coli* foodborne illnesses and is characterized by frequent, voluminous amounts of bloody diarrhea. EHEC O157:H7 (*E. coli* O157:H7) cause more than 75 percent of EHEC infections and can require as few as 10 cells to cause infection (Jay and others 2005; Feng 2012).

E. coli O157:H7 is ranked as the fifth most common pathogen for causing foodborne illnesses requiring hospitalization. In 2011, studies estimated that *E. coli* O157:H7 causes 63,153 foodborne illnesses, 2,138 foodborne illnesses requiring hospitalization, and 20 foodborne illnesses resulting in death, annually (Scallan and others 2011; CDC 2011a).

2.3 PATHOGEN CONTAMINATION AND PERSISTENCE IN TREE NUTS AND PEANUTS

2.3.1 Contamination

Microbial contamination of tree nuts and peanuts can happen during several phases of nut production. Ground cultivation naturally results in microbial presence on hazelnuts, and immediate postharvest microorganism populations are likely to reflect the microbiota of orchard soils. Therefore, introduction of pathogens into orchard soil can easily result in the presence of pathogens on postharvest nuts. Potential sources of pathogen contamination in soil include contaminated irrigation water, contaminated groundwater, poor worker hygiene, and animal defecation in orchards (Beuchat 2002; Suslow and others 2003).

E. coli O157:H7 has been isolated from contaminated water systems (Olsen and others 2002), as well as from contaminated pasture runoff (Gelting and others 2011). Wild animals, farm animals, and humans are all known reservoirs for *E. coli* O157:H7 and studies have shown that many carriers may be asymptomatic (Deng and others 1998; Callaway and others 2003). *E. coli* O157:H7 is more commonly isolated from ruminant animals than poultry and the population of infected cattle and dairy animals is thought to

vary based upon diet, season, and herd (Callaway and others 2003). Research suggests that as many as 80 percent of feedlot cattle may be infected with *E. coli* O157:H7 during the summer, and as few as 10 percent may be infected during the winter (Callaway and others 2003). *E. coli* infections in farm animals can result in foodborne infections from raw produce via shared contaminated groundwater. In 2006, more than 200 people became ill from *E. coli* O157:H7 infections when fresh spinach was contaminated by waste water runoff from a nearby cattle farm (Gelting and others 2011). Research also suggests that once *E. coli* O157:H7 contaminate soil the cells may remain viable for long periods of time. Islam and others (2004) found that *E. coli* O157:H7 was able to persist in soil for at least 5 months when applied with contaminated compost or irrigation water.

Salmonella is a common environmental bacterium, but is primarily found in the gastrointestinal tract of birds, reptiles, and mammals. Ubiquitous *Salmonella* species have been recovered from the environment (Thomason and others 1977), but many pathogenic strains are also widespread and frequent due to the number of host types infected by S*almonella* (Hilbert and others 2012). Rodents and insects are common carriers and vectors of *Salmonella* and can promote widespread environmental contamination (Hilbert and others 2012; Wales and others 2010). Studies on almond orchard soils show that *Salmonella* can persist in the environment long-term, enabling continuous contamination of any agricultural foods grown in the soil. Uesugi and others (2007) found that a *Salmonella* strain that caused a foodborne illness outbreak in 2001 was able to persist in an almond orchard for over 5 years.

Fecal contamination of soil is assumed to be the main source of pathogen contamination of raw agricultural commodities. However, postharvest factors such as unsanitary facility conditions and poor handing practices can also result in pathogen contamination (Beuchat 2002; Suslow and others 2003). These sources of contamination can be prevented with good sanitation practices. Oregon hazelnut farmers and processors are required to adhere to good agricultural practices and good manufacturing practices in order to prevent

contamination of postharvest nuts and cross contamination between clean, processed nuts and newly harvested nuts (HMB 2012a; HMB 2010). Detailed Good Manufacturing Practices (GAPs) and Good Agricultural Practices (GMPs) used by the hazelnut industry can be found on OregonHazelnuts.org.

2.3.2 Persistence and Survival

Due to the relationship between water and food spoilage, drying has been one of the most popular methods of food preservation in history. Consequently, the microbiological safety of low moisture foods (water activity <0.70) has been a low concern until recent years. Unfortunately, dried agricultural commodities such as tree nuts, peanuts, and spices have been the source of a number of recent foodborne illness outbreaks, especially salmonellosis (Zweifel and Stephan 2012; Blessington and others 2012; Palumbo and others 2009). *Salmonella* is the predominant cause of most illnesses associated with low moisture foods, but *E. coli* O157:H7 outbreaks have also been reported over the last few years (Palumbo and others 2009; Scott and others 2009). Unique survival characteristics and low infectious doses result in *Salmonella* and *E. coli* O157:H7 posing the greatest health risks in low moisture foods (Izurieta and Komitopoulou 2012; Scott and others 2009; Jay and others 2005). Studies show that *Salmonella* and *E. coli* O157:H7 have the ability to survive long-term on dry surfaces (Kieboom and others 2006; Gruzdev and others 2012; Blessington and others 2012), and that pathogenicity may be associated with survival advantages (Hiramatsu and others 2005). In addition, low water activity has even been shown to increase the resistance of *Salmonella* to thermal (Izurieta and Komitopoulou 2012) and chemical treatments (Kieboom and others 2006).

2.4 MICROBIAL REDUCTION METHODS FOR NUTS

2.4.1 General Sanitation Overview

Nut industries are aware of the risk factors associated with low-moisture foods (i.e. nuts) and have adopted a variety of methods for controlling microorganisms. Employment of management systems such as Hazard Analysis & Critical Control Points (HACCP) and

Pathogen Environmental Monitoring Programs (PEMP), as well as adherence to Good Manufacturing Practices, increases the safety of nut products. The Industry Handbook for Safe Processing of Nuts, a valuable resource provided by the Grocery Manufacturers Association (GMA 2010), outlines safety requirements for nut industries. The handbook also provides examples of programs and procedures that can help ensure product safety. Key elements of preventing foodborne illness outbreaks are elimination of pathogens and prevention of post-processing recontamination. Adherence to good handling practices can significantly reduce the risk of post-processing contamination, but most nut industries are still in need of validated processing procedures that can eliminate pathogens introduced via pre-harvest contamination. Appropriate log unit reductions have been recommended for several nut industries, and various sanitation methods have been investigated (or implemented) for achieving these reductions on tree nuts and peanuts.

2.4.2 Microbial Reduction Requirements and Recommendations

Adequate reduction of *Salmonella* can be determined by nut industries or by the FDA using scientific investigations of the microbiota of nuts as well as thorough risk assessments. The almond industry is the only nut industry with a required U.S. standard for reduction of *Salmonella*. The Federal Register and the Code of Federal Regulations require that all almonds go through treatment processes that utilize "technologies that have been determined to achieve in total a minimum reduction of 4 log units of *Salmonella* bacteria in almonds" (FR 2009; CFR 2008a). The FDA also recommends that the peanut (FDA 2009a) and pistachio (FDA 2009b) industries implement processes that achieve reductions of 5 log units of *Salmonella*, but further research is needed to determine a final rule.

2.4.3 Sanitation Methods for Pathogen Reduction

2.4.3.1 Almonds

Validated processes for use on almonds include oil roasting, blanching, steam pasteurization, and propylene oxide pasteurization (ABC 2007d; FR 2009). All almond

processors must have their treatment procedures and equipment validated, and guidelines for implementation and validation are provided by the Almond Board of California's Technical Expert Review Panel (ABC TERP). Based on scientific studies, the ABC TERP review panel determined that oil roasting almonds for 2 min in ≥260°F oil will provide 5 log reductions of *Salmonella* on whole almond kernels (ABC 2007c). The efficacy of oil roasting almonds was also confirmed by Du and others (2010) when exposure of *Salmonella* inoculated almonds to 127°C (260°F) oil for 1.5 min resulted in ≥5 log reduction. *Salmonella* has shown more resistance to dry roasting; the current estimations for designing treatment procedures for achieving 4 log reductions on almonds are: 100 min at 250°F, 50 min at 265°F, 23 min at 280°F, 12 min at 295°F, and 9 min at 300°F (ABC 2007b). Harris and others (2012) found that exposure of *Salmonella* inoculated almond kernels to 88°C (190.4°F) water for 2 min resulted in ≥5 log reductions of *Salmonella*. This research supports the ABC TERP blanching recommendations of exposing almond kernels to hot water at 180°F for 3.09 min or 190°F for 2 min to achieve 5 log reductions of *Salmonella* (ABC 2007a). Chang and others (2010) found that ≥25 sec of steam pasteurization of almonds resulted in ≥ 5 log reductions of *Salmonella*. Variations of steam and hot air are approved for almond treatments by the ABC TERP (ABC 2007d). Propylene oxide (PPO) fumigation has been validated for use on raw almond kernels and in-shell almonds (ABC 2008b; ABC 2008a), and approved in the CFR for use as a fumigant on cocoa, gums, processed spices, and tree nuts (not peanuts) under certain conditions (CFR 2000). Treatment of almonds with PPO involves injecting vaporized PPO into a chamber at 140-160°F to achieve concentrations of 0.05 oz PPO/ft^3. Fumigation takes 4 h, but post-treatment ventilation takes 5 d at 59-65°F for in-shell nuts, and 2 or 5 d at 100-110°F or 59°F, respectively, for kernels (ABC 2008a; ABC 2008b). The CFR requires that final PPO residues on tree nuts be less than 300 ppm at the end of the ventilation period (CFR 2000). Treatment with PPO maintains the integrity and sensory parameters of almonds and provides biologically safe final products (Danyluk and others 2005; ABC 2007e). However, public acceptance and high cost make this treatment method unappealing to some nut

processors. The Federal Register estimates that the cost of a PPO chamber is between 500,000 and 1,250,000 dollars, and that alternative off-site contract processing costs between 0.04 and 0.05 dollars per pound (CFR 2008a).

Some research has been conducted using acidic sprays for almond sanitation. Pao and others (2006) inoculated in-shell almonds with *Salmonella enterica* and found that spraying with acidic solutions (10 % acetic acid, 10% citric acid, ≤400 ppm acidified sodium chlorite, 500 ppm peroxyacetic acid, and a 500 ppm acid mix) reduced the total aerobic plate count by 0.48 to 1.88 log units. However, results from trials with varying acid concentrations, treatment contact times, and multiple treatment applications resulted in reductions between 3 and 4 log units; and 4 applications of 15 % citric acid resulted in >4 log unit reductions (Pao and others 2006).

2.4.3.2 Other Tree Nuts and Peanuts

No regulations have been published that define log unit reduction requirements for peanuts (CFR 2002), pistachios (CFR 2008c), hazelnuts (CFR 2008b), or walnuts (CFR 2008d). However, the FDA published a non-binding document with recommendations for reducing the risk of *Salmonella* in peanut products called "Guidance for Industry: Measures to Address the Risk for Contamination by *Salmonella* Species in Food Containing a Peanut-Derived Product as an Ingredient" (FDA 2009a). The FDA released a similar document for pistachios but currently it is listed as a "draft guidance" and is intended only for comment purposes at this time (FDA 2009b). The peanut guidance and pistachio draft guidance documents both suggest that processors utilize procedures that achieve 5 log unit reductions of *Salmonella*. The documents provide information regarding increased thermal resistance of *Salmonella* in low moisture foods, as well as links to resources for designing treatment processes. The FDA states that "Control of *Salmonella* In Low-Moisture Foods," a document published by the GMA, may be a good resource for "manufacturers that use a [pistachio/peanut]-derived product as an ingredient" (FDA 2009a; FDA 2009b).

Processing procedures validated to achieve 4 or 5 log unit reductions of *Salmonella* on almonds offer foundations for validating thermal processes for the peanut, pistachio, walnut, and hazelnut industries (GMA 2009). However, little research has been published on the efficacy of postharvest treatments (thermal or non-thermal) on nuts other than almonds. Ma and others (2009) found that 49 min at 90°C (194°F) was needed to reduce *Salmonella* by 5 log units in peanut butter, but the matrix of peanut butter is significantly different than the surface of peanut shells. Akbas and Ozdemir (2006) discovered that 360 min of exposure to 1 ppm ozone reduced *E. coli* and *Bacillus cereus* populations on pistachios by 3.5 and 3 log units, respectively, but additional studies are needed to confirm the efficacy of ozone against *Salmonella*. Gamma irradiation is unlikely to become a popular method for nut sanitation as Al-Bachir (2004) found that irradiation of walnuts resulted in negative sensory characteristics. Prakash and others (2010) also found that levels of ionized irradiation high enough to reduce *Salmonella* by >4 log units on almonds resulted in extremely strong off-flavors. Jeong and others (2012) found that X-ray irradiation levels able to achieve 5 log unit reductions of *Salmonella* on almonds and walnuts caused off flavors in walnuts, but no off-flavors were detected in treated almonds.

2.5 CHEMICAL SANITIZERS

Lack of research pertaining to non-thermal sanitation treatments for nut products prompts the need for further hazelnut sanitation research to be based on literature from other agricultural industries. Fresh produce industries have investigated a number of chemical sanitizers in order to increase the safety and prolong the shelf lives of raw agricultural products. Several chemical sanitizers effective for reducing *E. coli* O157:H7 and *S. enterica* on fruits and vegetables show potential for application on hazelnuts. Hazelnut processing already involves washing procedures to remove excess dirt, making incorporation of chemical sanitizers the most convenient method of increasing food safety.

2.5.1 Sodium Hypochlorite (NaOCl)

Sodium hypochlorite (NaOCl) is a common inorganic chemical used for eliminating and reducing microorganisms in water and food. Sodium hypochlorite ionizes in water and forms a solution of chlorine gas (Cl_2), hypochlorite ions (OCl^-), and hypochlorous acid (HOCl) (Estrela and others 2002; Suslow 1997). Hypochlorous acid is an oxidizing agent and the main compound involved in sodium hypochlorite oxidative reactions that kill bacteria (Suslow 1997). Esterla and others (2002) reported that reactions with hypochlorous acid and hypochlorite ions result in hydrolysis and neutralization of amino acids. In general, reactions with amino acids that interfere with cell metabolism and enzyme functions lead to bacterial cell death (Estrela and others 2002). The reactivity of NaOCl increases as the pH of solutions decrease, due to maximizing the concentration of HOCl in solution (Suslow 1997). The optimum pH for sanitation purposes is 6.5-7.0 because HOCl concentrations are high, but the solution is still stable; sodium hypochlorite solutions are less stable at pH 6.0 and chlorine gas is released (Suslow 1997). The main disadvantage of using NaOCl for food sanitation is its reactivity to organic matter (McDonnell and Russell 1999). NaOCl reacts easily with organic matter (such as soil or plant tissues), which results in rapid deactivation of its efficacy as a sanitizer (Suslow 1997; Estrela and others 2002; Harrison and Hand 1981).

Sodium hypochlorite is Generally Recognized As Safe (GRAS) and approved by the FDA for use as a chemical used for washing fruits and vegetables (CFR 1999). The EPA declares sodium hypochlorite residues exempt from tolerances when used as preharvest or postharvest crop treatments (CFR 2009b). In addition, sodium hypochlorite is approved by the National Organic Program for use in or on processed foods labeled as "organic" (CFR 2012c) as long as residual chlorine in the washing solutions do not exceed the maximum disinfectant level for drinking water (4 ppm) (CFR 2001; ICF 2011).

The efficacy of sodium hypochlorite varies based upon agricultural product and application method. Velázquez and others (2009) found that 200 ppm sodium hypochlorite resulted in a 4.77 log unit reduction of *Yersinia enterocolitica* on tomato surfaces, but Allende and others (2009) found that 200 ppm only resulted in a 1 log unit reduction of *E. coli* O157:H7 on fresh cilantro. Kim and others (2006) found that treating apples, tomatoes, and lettuce inoculated with *Enterobacter sakazakii* with 50 ppm chlorine for 5 min resulted in reductions of 4.15, 3.20, and 4.62 log units, respectively. In addition, no *E. sakazakii* was detected in the wash water after treatment. Parnell and others also found that chlorine (200 ppm) was effective in eliminating *Salmonella* from wash water used on contaminated cantaloupes and honeydew melons. These studies indicate that chlorine may be an effective sanitizer to use in wash water for preventing cross-contamination of agricultural commodities.

2.5.2 Peroxyacetic Acid (PAA)

Peroxyacetic acid (PAA) is a synthetic chemical produced from the reaction of acetic acid and hydrogen peroxide (NOP 2000; McDonnell and Russell 1999). The reaction will produce up to 15 peroxyacetic acid, 35 percent acetic acid, 25 percent hydrogen peroxide, and 25 percent water (NOP 2000). Similar to sodium hypochlorite, PAA is a strong oxidizing agent effective against bacteria, yeast, and molds. PAA inactivates microorganisms by transferring electrons to cell membranes and enzymes (NOP 2000; McDonnell and Russell 1999). PAA remains active in the presence of organic matter, making maintenance of active wash water more convenient than using sodium hypochlorite (McDonnell and Russell 1999).

Peroxyacetic acid is approved as a GRAS substance and can be used for washing or assisting in the peeling of fruit and vegetables (CFR 1999). The FDA requires that no greater than 80 ppm PAA be used on fruits and vegetables, and that washing must be followed by potable water rinsing (CFR 1999). However, the Environmental Protection Agency (EPA) states that PAA is exempt from residue tolerances when no more than 100

ppm PAA is used as an antimicrobial treatment on fruits, vegetables, tree nuts, grains, herbs, and spices. Under the same EPA ruling, up to 500 ppm PAA can be used as a sanitizer on food processing equipment (CFR 2009a). Organic producers can use PAA in food washes or on food contact surfaces when using the sanitizer according to FDA guidelines (CFR 1999; CFR 2012c).

Peroxyacetic acid has been investigated in a number of agricultural products, but as with sodium hypochlorite the efficacy against microorganisms appears to vary by food type. Narcisco (2005) found that exposure of spore-inoculated oranges to 100 ppm PAA reduced the microbial load by 2.1 log units. Pao and others (2006) found that spraying in-shell almonds inoculated with *S. enterica* with 500 ppm PAA only resulted in a 1.27 log reduction of *Salmonella*. Chang and Schneider (2012) found that 60 sec in a spray/roller combination process using 80 ppm peroxyacetic acid reduced *Salmonella* on tomatoes by 5.5 log units. This was significantly higher than the results from Narcisco and others (2005) and Pao and others (2006) and could be due to incorporation of physical agitation during treatment.

2.5.3 Acidified Sodium Chlorite (ASC)

Acidified sodium chlorite (ASC) is a synthetic chemical produced from the reaction of sodium chlorite and acid (CFR 2012a; NOP 2008). Sodium chlorite and acid mix in solution to form chlorous acid, which is the main antimicrobial agent in ASC (NOP 2008). Chlorous acid is a strong oxidizing agent that disrupts cell membranes, enzymes, and proteins to cause cell death (NOP 2008). ASC should be acidified immediately before application because a) chlorous acid is unstable and quickly breaks down into chloride and oxygen and b) concentrations of chlorous acid are highest when the pH of chemical solutions are decreased (NOP 2008). The main advantage of using ASC is its ability to maintain antimicrobial activity in the presence of organic matter. As chlorous acid oxidizes cell constituents (or breaks down from reacting with other organic

substances) excess chlorite and acid react to maintain chemical equilibrium and result in generation of additional chlorous acid (NOP 2008).

The ability of ASC to maintain antimicrobial activity in the presence of organic matter makes ASC popular for many food industries. The FDA provides thorough guidelines for using ASC for processing poultry, red meats, processed meats, seafood, raw agricultural commodities, and processed fruits and vegetables (CFR 2012a). ASC can be used as a spray or dip sanitizer on red meat and processed red meat products at concentrations between 500 and 1200 ppm and pH levels between 2.5 and 2.9. Poultry wash solutions must also be 500-1200 ppm, but the pH can be as low as 2.3-2.9. If ASC is used in a chiller or pre-chiller solution for poultry it must be diluted to 50-150 ppm and the pH must be 2.8-3.2 ppm. The seafood industry utilizes ASC for food sanitation as well as in the ice and water used for transporting, thawing, and storing seafood. ASC must be 40-50 ppm and pH 2.5-2.9 for seafood processing, and must be followed with a potable tap water rinse on products that are intended to be consumed raw. ASC is also used for sanitizing, packing, or holding raw and processed agricultural commodities (e.g. fruits, vegetables, legumes, etc.) (CFR 2012a). Both raw and processed agricultural commodities can be sanitized with ASC solutions that are 500-1200 ppm and pH 2.3-2.9. However, treatment of raw agricultural commodities must be followed with either potable water rinsing, blanching, cooking, or canning; and processed commodities must be rinsed with potable tap water and held for 24 h before consumption (CFR 2012a). With the exception of solutions used in organic processing, all ASC solutions used in the food industry can be acidified with any GRAS acid (CFR 2012a). The National Organic Program authorizes the use of ASC as a secondary direct antimicrobial food treatment and indirect food contact surface sanitizer for organic processing, but the ASC can only be acidified with citric acid (CFR 2012c).

ASC is used in many industries to increase food safety and quality and generally results in higher microbial reductions than sodium hypochlorite and PAA. Yuk and others

(2005) found that 60 and 120 sec exposures to1200 ppm ASC (pH 2.5) resulted in reductions of 3.31 and 3.72 log units (respectively) of *Salmonella* inoculated onto the stem scars of tomatoes. Liao and others (2009) observed comparable reductions of 3.9 log units when 800 ppm ASC was used against *Salmonella* on alfalfa seeds. Allende and others found that 1000 ppm ASC induced >3 log unit reductions of *E. coli* O157:H7 on fresh cilantro. Pao and others (2006) found that ≤400 ppm ASC only reduced *Salmonella* on in-shell almonds by 0.17 log units, but the lower reduction may have been the result of using significantly lower concentrations.

2.6 SUMMARY

Enhanced awareness of the numerous health benefits of nuts has resulted in an increased demand for raw in-shell hazelnuts. Oregon, one of the top global producers of hazelnuts, is proud to produce hazelnuts prized for their large size and mellow nutty flavors. Unfortunately, recent foodborne illness outbreaks associated with tree nuts and peanuts have prompted the need for nut industries to enhance current sanitation procedures. The cultivation of hazelnuts inevitably results in microbial contamination from orchards, making postharvest sanitation a crucial component for ensuring food safety.

Numerous research studies analyze the effects of thermal treatments on almonds, but minimal research has been conducted for other nut industries. Additionally, current thermal treatments used for some nut processing either result in alteration of nut properties (i.e. roasting and blanching) or are extremely expensive and time consuming (i.e. PPO fumigation). Chemical sanitation is a popular method of maintaining quality of many agricultural commodities, but little research has been conducted for chemical sanitation of nuts. Treatment with sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite could further increase the efficacy of hazelnut processing while maintaining the ability to offer raw, organically produced products. Direct application of chemical solutions to freshly harvested hazelnuts will help demonstrate their ability to enhance current washing procedures and offer more insight to the natural microbial load

of postharvest hazelnuts. Continued investigations of testing the chemicals on hazelnuts inoculated with *Salmonella* will illustrate the efficacy of sanitizers to eliminate pathogens on the surfaces of hazelnut shells. Results from the examination of chemical sanitization of hazelnuts may also provide groundwork for other nut industries wanting low-cost alternatives to thermal processing procedures.

3. EFFECTS OF WATER, SODIUM HYPOCHLORITE, PEROXYACETIC ACID, AND ACIDIFIED SODIUM CHLORITE ON MICROBIAL POPULATIONS OF POSTHARVEST IN-SHELL HAZELNUTS

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3.1 ABSTRACT

Recent foodborne disease outbreaks involving tree nuts and peanuts generate a need for improving agricultural sanitation procedures. Chemical spray treatments have shown promise for reducing pathogenic organisms on fresh produce, but little research has been conducted for in-shell hazelnuts. Investigation of the effectiveness of three food-safe chemicals as sanitizers for use on postharvest in-shell hazelnuts took place during the fall 2011 harvest. Treatments of water, sodium hypochlorite (NaOCl; 25 ppm and 50 ppm), peroxyacetic acid (PAA; 80 ppm and 120 ppm), and acidified sodium chlorite (ASC; 990 ppm) were applied to freshly harvested hazelnut samples (14 in-shell hazelnuts/50±0.50 g sample) to examine their effects on total aerobic plate count (APC) populations. Separate collection, treatment, and analysis of two experimental groups of hazelnuts allowed for a comparison of the treatments on hazelnuts gathered from different harvest times (Group 1 = early season, dry weather; Group 2 = late season, rainy weather). Treatment groups analyzed against untreated controls included hazelnuts that underwent a tap water rinse (Rinse Only), a tap water rinse followed by a water spray (Water), and a tap water rinse followed by a chemical spray. Post-treatment log population means (log CFU/hazelnut) determined the population reductions of each treatment compared to untreated controls. Group 2 hazelnuts revealed higher initial populations than Group 1, likely due to excess dirt on the shell surfaces from harvest rain and mud. All treatments resulted in significant APC reductions compared to the controls ($P \leq 0.05$). Rinse Only treatments reduced microbial populations by 0.38 log units in both groups, and Water treatments reduced populations by 0.83 and 0.73 log units in Group 1 and Group 2, respectively. None of the chemical treatments in Group 1 resulted in population reductions significantly greater than the Water treatment; however, the highest concentrations of each chemical in Group 2 produced significantly greater reductions than Water ($P \leq 0.05$). ASC treatment produced the greatest reductions in both groups compared to the control (1.22 and 2.08 log units). Physical removal of excess dirt appeared to have the greatest effect on microbial population reductions of in-shell hazelnuts, but adherence to the shell during dry weather may increase chemical resistance of some soil microorganisms.

3.2 INTRODUCTION

The Oregon hazelnut industry, which produces 99 percent of the United States hazelnut crop, saw a 39 percent increase in production from 2010 to 2011 (NASS 2012b). As with other tree nuts, hazelnuts are commonly sold roasted and shelled, or incorporated into consumer products such as ice creams, nut butters, and fruit and nut medleys. However, the majority of Oregon hazelnuts (76 percent of total yield in 2011(NASS 2012b)) are sold in-shell and undergo minimal processing before distribution. Recent foodborne disease outbreaks associated with contaminated peanut (CDC 2009a; Kirk and others 2004; FDA 2012) and tree nut products (CDC 2011b; CDC 2004; CDC 2009b; CFIA 2011a; Kirk and others 2004; Miller and others 2012; Isaacs and others 2005) have prompted the need for improved sanitization procedures. In 2009, detection of *Salmonella* in a hazelnut processing facility resulted in a recall of shelled hazelnuts (FDA 2009c). The following year, in-shell hazelnuts were associated with a multi-state outbreak of *Escherichia coli* O157:H7 (CDC 2011b; Miller and others 2012).

Thermal processing procedures (e.g. roasting, blanching) are used to create many nut products. Common thermal and novel processing procedures have been validated for use in the almond industry to achieve ≥4 log reductions of *Salmonella* (Danyluk and others 2005; Du and others 2010; Harris and others 2012). However, demand for raw in-shell hazelnuts limits the use of thermal processing procedures in the hazelnut industry. A number of chemical sanitizers have been investigated for use on raw agricultural commodities but no investigation has been published about the efficacy of sanitizers on in-shell nuts. Pao and others (2006) inoculated in-shell almonds with *Salmonella enterica* and found that spraying with acidic solutions (10% acetic acid, 10% citric acid, ≤400 ppm acidified sodium chlorite, 500 ppm peroxyacetic acid, and a 500 ppm acid mix) reduced the total aerobic plate count by 0.48 to 1.88 log units. The surfaces of hazelnuts are smoother than almond shells, and may allow chemicals better access to microorganisms. For example, Parnell and others (2005) found that soaking in water (no

chemical) reduced *Salmonella* on the smooth surfaces of honeydew melons by 2.8 log units, but only reduced populations on rough cantaloupe surfaces by 0.7 log units.

Hazelnuts fall to the ground before harvest where they are exposed to soil and ground flora. The amount of dirt on postharvest hazelnuts can vary depending on the muddiness of the ground, and adhesion of soil allows for attachment of microorganisms from the environment. Orchard soil naturally contains a variety of microorganisms, but contamination with pathogens via animals, insects, irrigation or a number of other factors increases the risk of foodborne disease outbreaks (Beuchat 2002; Gelting and others 2011; Hilbert and others 2012; Wales and others 2010).

This study investigated the efficacy of sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite due to a combination of interest expressed by Oregon hazelnut processors and the results from previous studies on their efficacy on raw agricultural products. Reports on the efficacies of these chemicals vary dramatically depending on the methods of application, target organisms, and food characteristics. Velázquez and others (2009) found that 200 ppm sodium hypochlorite produced a 4.77 log unit reduction of *Yersinia enterocolitica* on tomato surfaces, but Allende and others (2009) found that the same concentration resulted in a 1 log unit reduction of *E. coli* O157:H7 on fresh cilantro. Narcisco (2005) found that exposure of oranges inoculated with a spore cocktail to 100 ppm PAA reduced the microbial load by 2.1 log units, and Pao and others (2006) showed that spraying in-shell almonds (inoculated with *Salmonella enterica*) with 500 ppm PAA resulted in a 1.27 log unit reduction of *Salmonella*. Liao (2009) found that exposing alfalfa seeds to 800 ppm of ASC for 45 min reduced *Salmonella* by 3.9 log units, but Pao and others (2006) reported that ≤400 ppm ASC only reduced *Salmonella* on in-shell almonds by 0.17 log units. Inclusion of water as a treatment also allowed for investigation of the importance of physical removal of microorganisms. Chang and Schneider (2012) found that water resulted in significant reductions of microorganisms (3.0-3.8 log units) when used in a spray/roller processor. To date, no research has been

published that determines the total microbial population of postharvest in-shell hazelnuts, or the effects that these treatments have on reducing the population.

3.3 MATERIALS AND METHODS

3.3.1 Raw Materials

Willamette Hazelnut Growers of Newberg, OR, provided the hazelnuts for this study. The hazelnuts were large (19.4-22.2 mm) but of undetermined variety and weighed 3.4 g to 3.6 g each. Two separate sets of hazelnuts, tested independently as Group 1 and Group 2, were stored in woven plastic bags at room temperature prior to use. Group 1 hazelnuts arrived 6 d before commencement of the Group 1 experiment, and Group 2 hazelnuts arrived 3 d before commencement of the Group 2 experiment. Visual inspection ensured that all hazelnuts included in the sample units were free of cracks, holes, and other abrasions. All hazelnut samples used in the experimental groups consisted of 14 nuts and weighed 50±0.5 g each.

3.3.2 Preparation of Treatments

Preparation of chemical treatments took place ≤30 min prior to treating the hazelnut samples. The chemicals and the deionized water used to dilute the chemicals were stored at room temperature prior to preparation. Potable tap water used for the Rinse Only and the Water treatments adjusted to room temperature for at least 1 h before use.

Sterile deionized water was used to dilute 5.7% available chlorine Baker Analyzed® sodium hypochlorite (VWR International, LLC, San Francisco, CA) to 25 ppm and 50 ppm (NaOCl-25, NaOCl-50), and BioSide™ HS 15% (Enviro Tech Chemical Services, Modesto, CA) to 80 ppm and 120 ppm peroxyacetic acid (PAA-80 and PAA-120). A 30% (w/v) citric acid solution was used to lower the pH of the NaOCl solutions to pH 6.5±0.10, and lower the pH of the acidified sodium chlorite (ASC; Alliance Analytical Laboratories, Inc. Coopersville, MI) treatments to pH 3.1±0.10. The citric acid titration into the ASC solution resulted in treatment concentrations of 990 ppm (ASC-990). The

30% citric acid solution was created using BDH 99.5% anhydrous citric acid (VWR Int., LLC) dissolved in sterile deionized water. The pH of each NaOCl and ASC treatment solutions were monitored during acidification using a pH meter and electrode set (VWR® symphony™, SB70P, VWR Int., LLC). The pH of each PAA solution was determined immediately after dilution of the stock solution using the same pH meter.

3.3.3 Treatment of Hazelnut Samples

All hazelnut samples except the untreated control samples (Control) were transferred to previously sterilized 1 L glass beakers containing 500 mL potable tap water (hazelnuts:water = 1:10 w/w). Agitation of the samples involved hand stirring for 30 sec in a clockwise motion at 60 rpm. The samples were decanted and aseptically transferred to sterile test tube racks to dry. The samples dried for 5 min in a Class II biosafety hood (NuAire, Inc. Plymouth, MN) before further treatment.

After drying, the Rinse Only samples were aseptically transferred to sterile 250 mL glass jars to await microbial analysis. The water treatment samples and the chemical treatment samples were sprayed with corresponding treatment solutions (Water, NaOCl-25, NaOCl-50, PAA-80, PAA-120, or ACS-990) using 250 mL hand held spraying bottles with the nozzles set to "SPRAY" (VWR Int., LLC). The trigger of each spray bottle was compressed just enough to produce a gentle spray, never fully compressed. The spraying technique resulted in each hazelnut receiving ~1.8 mL of treatment over the course of 14 sprays per hazelnut. Gentle hand rotation during spraying ensured complete coverage of the hazelnuts.

Untreated hazelnuts served as a negative control and represented the average microbial population found on hazelnut surfaces just after harvest, as they would generally enter a processing facility. The experiment was independently repeated twice as Group 1 and Group 2, and the individual sample trials within each group were repeated 5 times for each chemical treatment, at least 4 times for the Water treatment, and at least 7 times for

the Control and Rinse Only treatments. Appendix Table I and Appendix Table II list the incidence formats for the two experiment groups.

3.3.4 Population Decrease Over Time

The decrease of total aerobic populations was investigated from Oct 24, 2011 until Dec 19, 2011. Five samples were used to determine the population on Oct 24 (4 d after the nuts were collected), and three samples were used to determine the populations on Nov 04, Nov 16, Dec 09, and Dec 19 (15, 27, 50, and 60 d after harvest, respectively).

3.3.5 Microbial Analysis

Hazelnut samples of known weights and number of hazelnuts were placed into sterile 250 mL glass jars. Butterfield's phosphate-buffered water (PW; 50 mL) (VWR Int., LLC) was added to each sample jar. The samples were shaken 50 times through a 30 cm arc, rested 5 min, shaken 5 times more through a 30 cm arc, and then diluted serially with sterile PW. Sample dilutions were plated in duplicate using the pour-plate method (Maturin and Peeler 2001) with standard plate count agar (PCA; VWR Int., LLC) and incubated at 35°C for 36-48 h.

The aerobic plate count population means for each sample were calculated using exactly two plates from their dilution scheme. The selected plates were generally from the lowest dilution plates containing ~25-250 colonies, unless the lowest countable dilution exceeded 250 and appeared more reliable than higher dilutions (Maturin and Peeler 2001). The plating results reported in colony forming units per gram (CFU/g) were later converted to CFU per hazelnut to determine the total aerobic microbial load of postharvest hazelnuts.

3.3.6 Statistical Analysis

The data were analyzed using the General Linear Model (GLM) in the Statistical Analysis System (SAS) version 9.2 (SAS Institute Cary, NC., USA). Significant

differences between treatments were determined using Tukey's Studentized Range (HSD) Test with the significant level set at $P \leq 0.05$ for all the samples and treatments. The standard errors of the means within each treatment were determined using Microsoft Excel (2010).

3.4 RESULTS AND DISCUSSION

3.4.1 Populations on Hazelnuts after Treatment

The population means for treatments in Group 1 are shown in Figure 3.4-1. Pair wise comparisons show significant differences between the mean of the untreated Control compared to samples rinsed with water (Rinse Only), rinsed with water then sprayed with water (Water), or rinsed with water then sprayed with any of the five chemical treatments ($P \leq 0.05$). There was no significant difference between population means from the Water samples compared to any of the chemical treatment samples. The NaOCl-25 treatment was the only chemical treatment with a mean statistically comparable to the Rinse Only treatment.

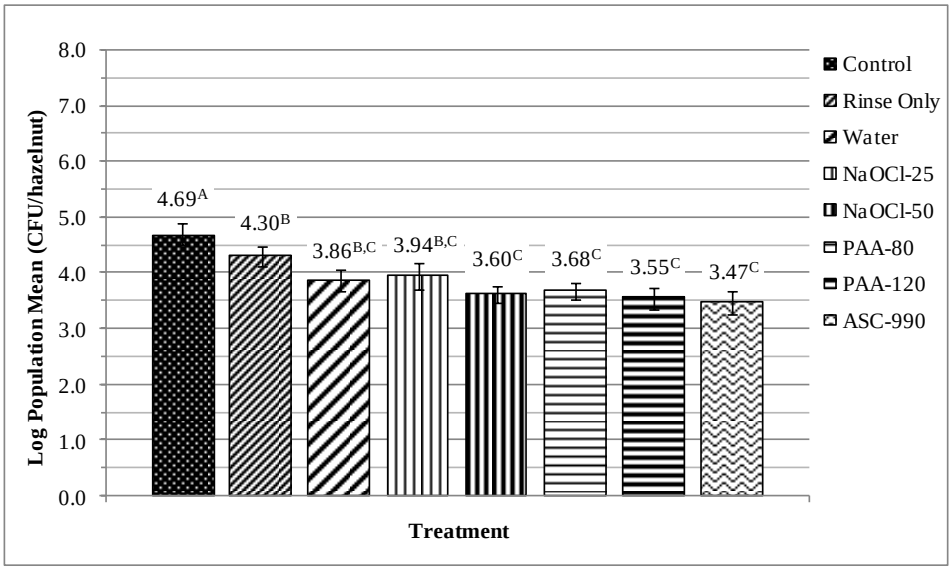

Figure 3.4-1: Total Aerobic Plate Count Means on Hazelnuts (Group 1)
Error bars represent the 95% confidence intervals of the mean of *n* samples.
[A-C]Values with different letters differ significantly ($P \leq 0.05$).
Control *n*=10, Rinse Only *n* =10, Water *n* = 4, NaOCl-25 *n* =5, NaOCl-50 *n* =5, PAA-80 *n* =5, PAA-120 *n* =5, ASC-990 *n* =5.

The population means for treatments in Group 2 are shown in Figure 3.4-2. The population means of NaOCl-25, NaOCl-50, PAA-80, and PAA-120 were all statistically similar to one another, but significantly higher than the ASC-990 population mean ($P \leq 0.05$). The Rinse Only and the Water treatments were statistically similar, as were the Water and the NaOCl-25 treatments. The NaOCl-25 and PAA-80 treatments were not significantly different from the Water treatment. Population means of NaOCl-50, PAA-120, and ASC-990 were all significantly lower than the Water treatment ($P \leq 0.05$).

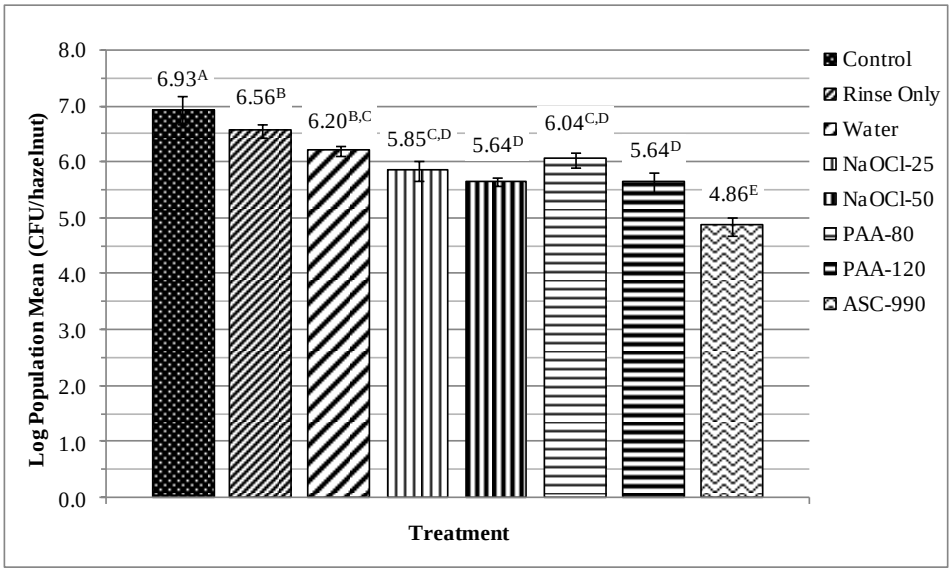

Figure 3.4-2: Total Aerobic Plate Count Means on Hazelnuts (Group 2).
Error bars represent the 95% confidence intervals of the mean of *n* samples
$^{A-E}$Values with different letters differ significantly ($P \leq 0.05$)
Control *n*=8, Rinse Only *n*=7, Water *n*= 7, NaOCl-25 *n*=5, NaOCl-50 *n*=5, PAA-80 *n*=5, PAA-120 *n*=5, ASC-990 *n*=5

Separate analysis of the treatments from the two experimental groups allowed for comparison of the total aerobic populations on hazelnuts collected at different times in the 2011 harvest. Group 1 hazelnuts were gathered early in the fall season while the ground was still relatively dry. Group 2 hazelnuts, which resulted in treatment population means 1.39 to 2.36 log more than Group 1, were collected late in the fall season after rain had increased the moisture of the soil. In addition, the population of individual untreated Control samples ranged from 4.22 to 5.39 log units in Group 1, and from 6.47 to 7.56 log units in Group 2. The increase in aerobic plate count from Group 1 to Group 2 could be due to an increase in excess soil attached to hazelnuts during harvest. Since soil contains an estimated 10^9 CFU/g of bacteria, the variation of the individual sample populations within each group could be the result of inconsistent amount of excess soil attached to the hazelnuts.

3.4.2 Reduction of Total Aerobic Plate Counts on Hazelnuts

Figure 3.4-3 shows the reduction in population means of each of the treatments in Group 1 compared to the Control. The five chemical treatments resulted in population reductions between 0.74 and 1.22 log units, but all of the treatments were statistically comparable to the Water treatment (0.83 log units). The ASC-990, PAA-120, PAA-80 and NaOCl-50 treatments resulted in the greatest reductions, with differences of 1.22, 1.14, 1.01, and 1.08 log units, respectively. The NaOCl-25, Water, and Rinse Only treatments resulted in the lowest population declines of 0.74, 0.83, and 0.38 log unit reductions, respectively. The NaOCl-25 was the only chemical treatment to produce a lower reduction than Water.

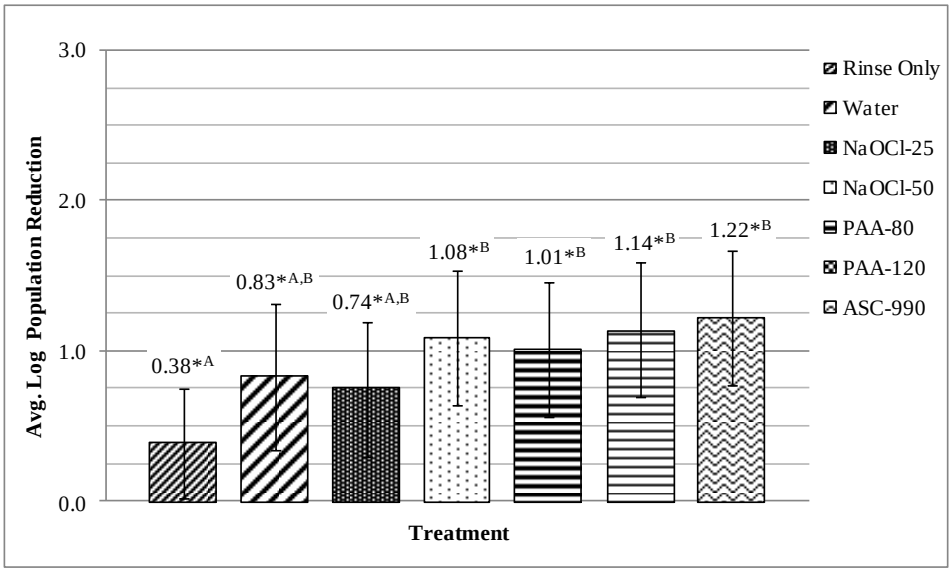

Figure 3.4-3: Reduction in Aerobic Plate Count Compared to Control (Group 1)
Error bars represent simultaneous 95% confidence intervals of the difference between the treatment mean and the Control mean.
*All values are significantly lower than the Control population
A-BValues with different letters differ significantly ($P \leq 0.05$)

Figure 3.4-4 shows the reduction in population means of each of the Group 2 treatments compared to the Control. All of the treatments significantly lowered the microbial

population on the surfaces of the hazelnuts. The NaOCl-50, PAA-120, and ASC-990 treatments also showed significant population reductions compared to the Water treatment ($P \leq 0.05$). The ASC-990 reduced the population by 2.08 log units compared to the Control, which was a significantly greater reduction than the other treatments. The NaOCl-25, NaOCl-50, PAA-80, and PAA-120 resulted in statistically comparable population reductions that ranged from 1.09 to 1.29 log units less than the Control mean. However, the NaOCl-25 and PAA-80 treatments were also statistically similar to the Water treatment, which achieved a 0.73 log reduction.

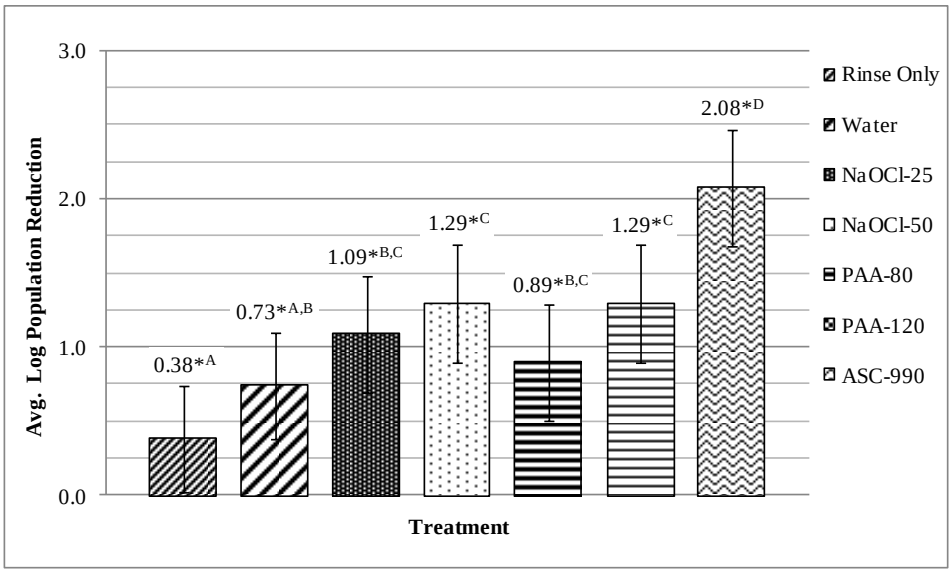

Figure 3.4-4: Reduction in Aerobic Plate Count Compared to Control (Group 2)
Error bars represent simultaneous 95% confidence intervals of the difference between the treatment mean and the Control mean.
*All values are significantly lower than the Control population
$^{A-D}$Values with different letters differ significantly ($P \leq 0.05$)

Group 2 showed greater population means and overall reductions than Group 1. Singh and others (2002) found contrary results demonstrating that lower inoculation levels of *E. coli* O157:H7 on lettuce leaves corresponded to greater log reductions when using aqueous ClO_2, ozonated water, and thyme oil as sanitizers. Differences seen in this study and that of Singh and others (2002) may be accounted for by the amount of bacteria that

were attached to the surface of the hazelnut verses in the dirt attached to the hazelnut. It has been well documented that bacteria dried to surfaces are more resistant to chemical treatments than free-living cells or cells that are not attached tightly to surfaces (Møretrø and others 2012). Musgrove and others (2010) found that *Salmonella* on egg shells was more resistant to chemicals when eggs were inoculated with a dip method than by a fecal smear. The additional excess dirt on hazelnuts in Group 2 may have prevented the bacteria from adhering as tightly to the hazelnut shells, thus making them more susceptible to chemical damage. Alternatively, low water activity during dry weather (Group 1) may have induced chemical resistance of some microorganisms. Keiboom and others (2006) found that low water activity resulted in morphological changes in *Salmonella* that increased its resistance to sodium hypochlorite treatments. Despite the additional susceptibility of Group 2, the total populations after treatment were still higher than Group 1. This shows excess soil on hazelnuts can result in higher microbial populations, and that the lowest microbial populations will be achieved by applying the sanitizers to nuts lacking excess debris.

Rinsing with water produced equivalent logarithmic reductions in both groups, with reductions of 0.38 log units compared to their individual Control treatments. Continuing water treatment with additional water sprays resulted in total reductions of 0.83 and 0.73 log units for Group 1 and Group 2, respectively. Similar reductions of 0.7 log units were seen by Neal and others (2012) when spraying spinach leaves inoculated with *Salmonella* and *E. coli* O157:H7. Narisco (2005) found that agitation of spore-inoculated oranges in water for 1 min also resulted in a 0.7 log unit reduction.

Results from Group 1 showed no significant difference in log population reductions between hazelnuts sprayed with water, and hazelnuts sprayed with any of the five chemical treatments. In Group 2, the only chemicals that resulted in significantly greater reductions than the Water were the NaOCl-50, PAA-120, and ASC-990 treatments. These three treatments showed the greatest log reductions within both experimental

groups, but their exact efficacies for killing microorganisms on hazelnuts probably varies depending on the amount of excess soil present and their ability to attach to nut shell surfaces (Møretrø and others 2012). When accounting for the physical removal of microorganisms (using Water as control), NaOCl-50; PAA-120; and ASC-990 treatments in Group 1 and Group 2 produced logarithmic reductions between 0.26 and 0.56, 0.31 and 0.56, and 0.39 and 1.39 log units, respectively.

3.4.3 Decrease in Total Aerobic Plate Counts Over Time

Figure 3.4-5 shows the log population decrease of natural hazelnut microflora on hazelnuts stored over a two month period. These hazelnuts came from the same harvest bag as the hazelnuts used in Group 2. The population means were recorded based upon days elapsed since the hazelnuts were collected. On Day 4, the logarithmic population mean of five samples was 7.16±0.11. On Day 15, Day 27, Day 50, and Day 60 the population means of three samples were 7.00±0.11, 6.62±0.19, 6.36±0.05, and 6.28±0.04 log units, respectively. After 56 days (Day 4-Day 60) the population mean had decreased by 0.88 log CFU/hazelnut. The population declined by 0.54 log units from Day 4 - Day 27, and by 0.34 log units from Day 27 - Day 60, indicating that the rate of log population decline slowed during the second half of storage.

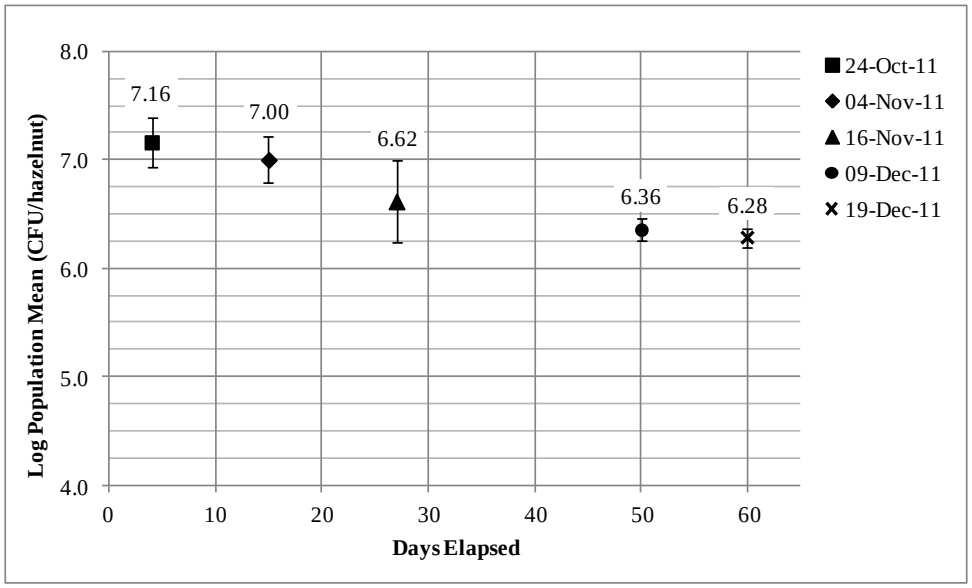

Figure 3.4-5: Aerobic Plate Count Means Over Time.
Error bars represent the 95% confidence intervals of *n* samples.
24-Oct-11 *n*=5, 04-Nov-11 *n*=3, 16-Nov-11 *n*=3, 09-Dec-11 *n*=3, 19-Dec-11 *n*=3

No studies have been conducted on the survival of natural microorganisms on nut surfaces, but Blessington and others (2012) studied the population decreases of pathogenic bacteria inoculated onto the surfaces of walnut kernels and stored at ambient temperatures (23°C). After 49 d of storage, *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* declined by 0.5, 0.3, and 2.2 log CFU/g, respectively (Blessington and others 2012). These decreases were comparable to the 0.80 log CFU/hazelnut decrease in the APC population on hazelnuts from Day 4 to Day 50. However, Blessington and others (2012) based decline values on populations recorded from the end of a 7 d drying period until the end of storage. This was due to dramatic population decreases observed during drying (i.e. reduction of water activity) of inoculated walnut kernels. When *Salmonella* was inoculated onto walnuts, the population decreased by 0.8-1.7 log CFU/g within the first 24 h of drying (23°C), but only decreased by an additional 0.2 log CFU/g between 24 h and 7 d of drying (Blessington and others 2012). Once the water activity stabilized during drying the *Salmonella* population declined slower. Our study did not

monitor the microorganism population until 4 d after harvest, but the results from Blessington and others (2012) support the conclusion that microbial populations will tend to decline more slowly over time when stored at ambient temperatures.

3.5 CONCLUSION

The significant reductions by the Rinse Only treatments compared to the Control, and the similarity of the log reductions from the Water and chemical treatments suggest that the decrease in microbial populations was mostly the result of physical removal of excess soil. Adherence to hazelnut shells or low water activity during dry weather may also have an effect on the chemical resistance of some soil microorganisms. To achieve the lowest final microbial populations, hazelnut processors should attempt to remove as much excess soil from hazelnut surfaces before applying a chemical sanitizer. The most promising sanitizer in this study appeared to be acidified sodium chlorite solution, as it consistently resulted in the greatest population reductions and it is known to be unaffected by the presence of organic matter. A study conducted on hazelnuts lacking excess soil may provide more conclusive results about the lethality of these sanitizers against microorganisms.

3.6 ACKNOWLEDGEMENTS

Thanks to Dr. Cathy Durham of OSU for assisting with the SAS software and statistical analysis and Dr. Mark Daeschel of OSU for assisting with the experimental design.

4. EFFECTS OF WATER, SODIUM HYPOCHLORITE, PEROXYACETIC ACID, AND ACIDIFIED SODIUM CHLORITE ON IN-SHELL HAZELNUTS INOCULATED WITH *SALMONELLA ENTERICA* SEROVAR PANAMA

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4.1 ABSTRACT

Recent foodborne disease outbreaks involving tree nuts and peanuts generate a need to improve food sanitation procedures. Chemical spray treatments have shown promise for reducing pathogenic organisms on fresh produce, but little research has been conducted for in-shell hazelnuts. This study analyzed the effectiveness of three food-safe chemicals as sanitizers for reducing *Salmonella* on in-shell hazelnuts. Treatments of water, sodium hypochlorite (NaOCl; 25 ppm and 50 ppm), peroxyacetic acid (PAA; 80 ppm and 120 ppm), and acidified sodium chlorite (ASC; 450 ppm, 830 ppm, and 1013 ppm) were sprayed onto hazelnut samples (45 hazelnuts/sample) inoculated with *Salmonella enterica* serovar Panama. Hazelnut samples were soaked in pure cultures of *S.* Panama for 24 h, air dried, then sprayed with water and chemical treatments. Inoculation achieved *S.* Panama populations of ~8.04 log CFU/hazelnut. Surviving *S.* panama populations were evaluated using a non-selective medium (tryptic soy agar), followed by a selective overlay (xylose lysine deoxycholate agar) after a 3 h incubation period. Tight adhesion prevented significant population decreases from physical removal and allowed for demonstration of chemical effectiveness against cells in a chemical resistant state (dried on surface). All of the chemical treatments significantly reduced the *S.* Panama population ($P \leq 0.05$). The most effective concentrations of ASC, PAA, and NaOCl treatments resulted in mean population reductions of 2.65, 1.46, and 0.66 log units, respectively. Acidified sodium chlorite showed the greatest potential for use as a postharvest sanitation treatment.

4.2 INTRODUCTION

Recent outbreaks of foodborne illness have been associated with consumption of numerous nut products, including in-shell hazelnuts. In 2009, product recalls ensued from *Salmonella* contamination of hazelnuts (FDA 2009c) and in-shell pistachios (CDC 2009b). In addition, two U.S. outbreaks of salmonellosis were associated with raw almonds in 2000-2001 (Isaacs and others 2005) and 2003-2004 (CDC 2004), and in-shell peanuts were associated with an international outbreak of salmonellosis in 2001 (Kirk and others 2004) and a national (at time of report) outbreak in 2012 (FDA 2012). In 2011, in-shell hazelnuts (CDC 2011b; Miller and others 2012) and raw walnuts (CFIA 2011a; CFIA 2011b) were associated with outbreaks of *Escherichia coli* O157:H7.

In 2011, Oregon's 29,500 acres of hazelnut crops produced 38,500 tons of nuts. The total utilized production value of 2011 was 89.7 million dollars, 76 percent of which was sold in-shell (NASS 2012b). Moreover, the 4-year average utilized production value increased 21 percent from 2004-2007 to 2008-2011. With Oregon producing 99 percent of the U.S hazelnut crop, development of effective sanitation processes for in-shell hazelnuts remains a nationwide concern. Lack of scientific research pertaining to the sanitation of in-shell tree nuts leaves hazelnut processors with little foundation for process modification.

Exposure to microorganisms from soil and plants is inevitable during the cultivation and harvest of hazelnuts. Hazelnuts, similar to almonds and walnuts, fall to the ground from trees before harvest. Contact and with soil allows for attachment of natural yeast, mold, viruses, and bacteria, and can act as a pathway for pathogens. S*almonella* spp. live ubiquitously in the environment (Thomason and others 1977) and investigations of almond orchard soils have shown that some pathogenic strains may persist long-term in contaminated orchards (Uesugi and others 2007; Danyluk and others 2008). *Salmonella* is the leading cause of bacterial foodborne illness in the United States (Scallan and others 2011; CDC 2011a), as well as the leading pathogen for fatal foodborne illness. The ease

of pathogenic contamination, the severity of foodborne illness, and the frequency of recent recalls and outbreaks associated with raw and in-shell nuts prompts the need for improvement of postharvest sanitation procedures.

Various methods have been investigated for sanitizing the surfaces of peanuts and tree nuts, but the only defined U.S. standard for nut sanitation at this time is a 4 log reduction of *Salmonella* for almonds (FR 2009). The FDA recommends implementation of processes resulting in 5 log reductions of *Salmonella* on peanuts (FDA 2009a) and pistachios (FDA 2009b), but no additional requirements have been created. The design of processing methods to eliminate *Salmonella* must be customized for each type of nut based on safety needs and final product quality. For many products, incorporation of a "kill step" that results in a >4 log reduction of *Salmonella* followed by safe handling procedures (GMA 2010) can be sufficient for ensuring safe final products. Research shows that certain roasting procedures for shelled almonds are sufficient for producing at least 4 and 5 log reductions of *Salmonella* (Du and others 2010). Alternative methods validated for almonds include blanching (Harris and others 2012) and pasteurizing (Danyluk and others 2005) procedures. Research conducted on almonds show promise for application to other nut industries that can incorporate thermal treatment methods into their processing procedures. All pistachios and most peanuts receive thermal treatments (Matoian 2010) and the Grocery Manufacturers Association provides an industry handbook (GMA 2010) that describes the necessary procedures for validating a thermal treatment process. However, most in-shell hazelnuts sold directly to consumers do not receive thermal processing, leaving the hazelnut industry in search of efficient non-thermal sanitation methods.

This study investigated the ability of sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite to reduce *Salmonella* on in-shell hazelnuts. Sodium hypochlorite is commonly used in washing solutions on fruits and vegetables due to its low cost and GRAS (generally recognized as safe) status (CFR 2009b). However, the

effects of sodium hypochlorite on reducing pathogenic populations vary in literature, possibly due to deactivation in the presence of organic matter (Harrison and Hand 1981). Velaquez and others (2009) found that 200 ppm sodium hypochlorite produced a 4.77 log unit reduction of *Yersinia enterocolitica* on contaminated tomatoes, but Allende and others (2009) found that the same concentration only produced about a 1 log reduction of *E. coli* O157:H7 on fresh cilantro. Peroxyacetic acid (PAA), a solution made from the reaction of hydrogen peroxide and acetic acid, is approved for use on fruits and vegetables (CFR 2009a) and has shown slower reactivity to organic matter than sodium hypochlorite. Chang and Schneider (2012) found that 60 sec in a spray and roller combination process using 80 ppm peroxyacetic acid, and 25 ppm or 50 ppm sodium hypochlorite reduced *Salmonella* on tomatoes by 5.5, 4.2 and 5.0 log units, respectively. Narciso (2005) found that 100 ppm peroxyacetic acid produced a 2.1 log unit reduction of spores inoculated onto the surfaces of oranges, which was more than the 1.27 log unit reduction seen by Pao and others (2006) when using 500 ppm PAA on in-shell almonds inoculated with *Salmonella*. Acidified sodium chlorite (ASC), a sanitizer prepared by reacting sodium chlorite with a GRAS organic acid, is approved for use on meat, poultry, seafood, and raw agricultural commodities (FDA, 2012). Review of previous research suggests that ASC shows promise for use in the hazelnut industry, as it maintains chemical activity in the presence of organic matter. Yuk and others (2005) found that1200 ppm acidified sodium chlorite resulted in a 3.72 log unit reduction of *Salmonella* inoculated onto the stem scar of tomatoes, and Liao and others (2009) found a comparable reduction of 3.9 log units when 800 ppm was used against *Salmonella* on alfalfa seeds.

4.3 MATERIALS AND METHODS

4.3.1 Raw Material

Willamette Filbert Growers of Newberg, OR, provided the hazelnuts for this study. The hazelnuts were large (19.4-22.2 mm) but of undetermined variety, and were collected,

processed (process undisclosed), and dried during the 2011 hazelnut harvest. The hazelnuts were stored for 8-10 mo. in large woven-plastic bags prior to testing.

4.3.2 Preparation of Hazelnut Samples

Batches of ~800 hazelnuts were sprayed with 70% (v/v) ethanol to reduce the background microbial population of the shells. Triplicate rinses with sterile deionized water (1 L/rinse) removed residual ethanol. The clean hazelnuts dried for 24 h in sterile 13”x9” Pyrex pans in a bio safety hood. Visual inspection before ethanol treatment and after drying ensured that all hazelnuts included in the sample units were free of cracks, holes, and other abrasions. Each hazelnut sample contained 45 hazelnuts.

4.3.3 Bacterial Strain and Morphology

The Food Microbiology laboratory at the Oregon Department of Agriculture (Portland, OR) provided the *S.* Panama culture used in this study. Use of *Salmonella* allowed for selection and differentiation of the inoculum bacteria from other organisms on the hazelnuts. Cultivation of hazelnut sample bacteria with xylose lysine deoxycholate agar (XLD; VWR International, LLC, San Francisco, CA) resulted in selection of enteric bacteria, and differentiation of *Salmonella*. When incubated for 24 h at 35°C on XLD agar, the *S.* Panama grew as black colonies imparting yellow color in the red agar. Colony pigmentation and yellowing of the agar was due to thiosulfate metabolism and xylose fermentation, respectively.

4.3.4 Bacterial Growth

A sample of *S.* Panama was streaked for isolation on tryptic soy agar (TSA; VWR Int., LLC) and incubated for 24 h at 35°C. A single selected colony incubated for 24 h at 35°C in 10 mL tryptic soy broth (TSB; VWR Int., LLC) created a stock culture, which was maintained via 0.10 mL daily transfers to fresh TSB. For each hazelnut batch inoculation, an aliquot of 0.1 mL stock culture was transferred to 10 mL TSB and incubated for 24 h at 35°C. After 24 h, 3 mL aliquots were transferred to two 500 mL shake flasks, each containing 300 mL of sterile TSB. The flasks shook at 100 rpm for 18

h at 37°C. The two flasks were aseptically combined and the optical density (OD_{600}) was confirmed at 0.80±0.01 to maintain cell concentrations at ~9.2 log CFU/ mL. (Plate counts and OD_{600} readings from two preliminary growth procedures were used to estimate the cell concentration of each inoculation preparation sample using individual OD_{600} values.) The optical density of the culture was measured using a spectrophotometer provided by Oregon State University, Department of Food Science and Technology. Cultures were centrifuged in 50 mL centrifuge tubes and the resulting pellets were brought to 2700 mL with phosphate-buffered water (PW; VWR Int., LLC). Final logarithmic population means of *S.* Panama inoculum suspensions were estimated at ~8.35 CFU/ mL. Samples plated on TSA and XLD agar determined final cell counts and inoculum purity.

4.3.5 Inoculation of Hazelnuts with *Salmonella* Panama

Each inoculation batch, which contained ~800 selected hazelnuts (procedure: 4.3.2), was placed into a previously sterilized stainless steel pot. The prepared *S.* Panama suspension (procedure: 4.3.4) was immediately poured over the hazelnuts. The hazelnuts were mixed with a sterile stainless steel spoon at 0 h (immediately after the *S.* Panama was added), 6 h, 21 h, and 27 h (time of removal). The hazelnuts were placed in 4 sterile 13 in x9 in Pyrex® pans lined with sterile paper towels and allowed to dry in a Class II biosafety hood (NuAire, Inc. Plymouth, MN) for 66 h.

After drying, the hazelnuts were placed in a large sterile stainless steel pot and stored in the corner of a Class II biosafety hood until testing. The inoculation process was repeated every week to maintain consistent *S.* Panama populations. Unused hazelnuts were autoclaved and discarded at the end of testing each week.

4.3.6 Preparation of Chemical Treatments

Preparation of chemical treatments took place ≤30 min prior to treating the hazelnut samples. The chemicals and the sterile deionized water used to dilute the chemicals were

stored at room temperature prior to preparation. Deionized water, used for the Water treatment, adjusted to room temperature for at least 1 h before use.

Sterile deionized water was used to dilute 5% available chlorine Baker Analyzed® sodium hypochlorite (VWR International, LLC, San Francisco, CA) to 25 ppm and 50 ppm (NaOCl-25, NaOCl-50), and BioSide™ HS 15% (Enviro Tech Chemical Services, Modesto, CA) to 80 ppm and 120 ppm peroxyacetic acid (PAA-80 and PAA-120). A 30% (w/v) citric acid solution was used to lower the pH of the NaOCl-25 and NaOCl-50 solutions to pH 6.5±0.05, and lower the pH of the acidified sodium chlorite (Alliance Analytical Laboratories, Inc. Coopersville, MI) treatments to pH 2.85±0.05. Citric acid was titrated into the acidified sodium chlorite solutions (ASC) resulted in ASC treatment concentrations of 450 ppm, 830 ppm, and 1013 ppm (ASC-450, ASC-830, and ASC-1013) The 30% (w/v) citric acid solution was created using BDH 99.5% anhydrous citric acid (VWR Int., LLC) dissolved in sterile deionized water. The pH of each NaOCl and ASC treatment solution was monitored during acidification using a pH meter and electrode set (VWR® symphony™, SB70P, VWR Int., LLC). The pH of each PAA solution was determined immediately after dilution of the stock solution using the same pH meter.

4.3.7 Treatment of Inoculated Hazelnuts

The water treatment samples and the chemical treatment samples were sprayed with corresponding treatment solutions (Water, NaOCl-25, NaOCl-50, PAA-80, PAA-120, ACS-450, ACS-830, or ACS-1013) using 250 mL hand held spraying bottles with the nozzles set to “SPRAY” (VWR International, LLC). The trigger of each spray bottle was compressed just enough to produce a gentle spray, never fully compressed. The spraying technique resulted in each hazelnut receiving ~1.8 mL of treatment over the course of 14 sprays per hazelnut. Gentle hand rotation during spraying ensured complete coverage of the hazelnuts.

Untreated hazelnuts served as a negative control and as the baseline of the *S.* Panama population. Experimentation took place on 15 individual days, each day consisting of 1 Control sample, 1 Water treatment sample, and 3 replicate samples of 1 chemical treatment type. Appendix Table III shows the incidence format for the 15 experimental days. Each chemical treatment was repeated 6 times, except NaOCl-50, which was repeated 9 times.

4.3.8 Microbial Analysis

After treatment, each sample unit of 45 hazelnuts was placed into a sterile 500 mL glass bottle containing 135 mL sterile Butterfield's phosphate-buffered water (PW; VWR Int., LLC) and 22 g of 425-600 μm glass beads (Sigma-Aldrich). The samples were shaken vigorously by hand 100 times, soaked for 3 min, shaken 100 more times, then diluted serially with sterile PW.

S. Panama populations were determined using a two-step overlay (OV) resuscitation method similar to that described by Kang and Fung (2000). Sample dilutions were plated in duplicate on sterile non-selective TSA plates, incubated for 3 h at 35.7°C, then overlayed with 14 mL selective xylose lysine deoxycholate agar (XLD). Black colonies were counted after additional 21 h incubation at 35.7°C. The logarithmic plate count population means for each sample were calculated using exactly 2 dilution plates. The selected plates were generally from the lowest dilution plates containing ~25-250 colonies, unless the lowest countable dilution exceeded 250 and appeared more reliable than higher dilutions. The plating results reported in colony forming units per 1/3 of a hazelnut (CFU/(1/3) hazelnut) were later converted to CFU per hazelnut to estimate the total microbial population of each hazelnut.

Sample dilutions were also plated in duplicate on a second set of TSA plates that were incubated for 24 h at 35°C, but received no selective overlay. These data were used to ensure that the selective overlay step was effective in inhibiting background microflora.

4.3.9 Recovery of Injured Cells with Selective Overlay

Prior to experimentation, pure cultures of *S.* Panama suspended in PW were exposed to 2 ppm peroxyacetic acid and plated according to the procedure described in section 4.3.8. The results confirmed the ability of the OV resuscitation method to allow full recovery of injured cells. No in-depth study was performed to determine the percent of injured cells caused by the chemical treatments. The XLD-OV method was similar to the two-Step OV method investigated by Kang and Fung (2000), and the efficacy was confirmed by the results shown in section 4.4.1.

4.3.10 Statistical Analysis

Data obtained were analyzed using the General Linear Model (GLM) in the Statistical Analysis System (SAS) version 9.2 (SAS Institute Cary, NC., USA). Significant differences between treatments were determined using Tukey's Studentized Range (HSD) Test with the significant level set at $P \leq 0.05$ for all the samples and treatments. Normal Q-Q Plots were generated in Revolution R Community version 6.0 (Revolution Analytics, Palo Alto, CA). The 95% confidence intervals for the means of each treatment were determined using Microsoft Excel (2010).

4.4 RESULTS AND DISCUSSION

4.4.1 Recovery of Injured Cells with Selective Overlay

Prior to experimentation, pure cultures of *S.* Panama suspended in phosphate-buffered water were exposed to 2 ppm PAA and plated according to the procedure described in section 3.3.5. Table 4.4-1 shows that chemically treated cells plated directly onto XLD agar resulted in lower and more inconsistent recovery than cells plated onto TSA, with and without selective overlays. Injured cells plated on TSA and plated with the selective overlay procedure (TSA+XLD) showed no significant population differences within each trial, implying incubation on TSA (3 h, 35°C) prior to exposure to the XLD overlay allowed for repair of sub-lethally injured cells, which agreed with the results observed Kang and Fung (2000).

Media	Control (log CFU/mL)	PAA-1 (log CFU/mL)	PAA-2 (log CFU/mL)
TSA	8.47	8.11	8.15
TSA+XLD	8.48	8.11	8.16
XLD	8.46	7.86	7.80

Table 4.4-1: Media vs Log Population Recovery of Chemically Treated *S.* Panama

4.4.2 Inoculation Procedure Results

The growth procedure described in 4.3.4 resulted in *S.* Panama suspensions averaging 8.42±0.02 log CFU/ mL (mean±95% CI) when plated on TSA, and 8.43±0.03 log CFU/ mL when plated with the selective overlay technique. Comparability of the populations confirms that uninjured *S.* Panama cells were uninhibited by the selective overlay technique. Additionally, the small 95% confidence intervals illustrate the consistency of the growth and suspension preparation procedure.

Before inoculation, the population mean of background microorganisms (APC) was 3.20±0.19 log units and lacked evidence of background *Salmonella* species. After inoculation, the hazelnut APC mean was 8.07±0.07 log CFU/hazelnut, and the *S.* Panama population (determined via selective XLD overlays) was 8.03±0.04 log CFU/hazelnut. The normal distribution and statistical comparability of individual Control populations illustrate the consistency of the *S.* Panama populations across the 15 experiment days. Table 4.4-2 shows the comparability and variation of *S.* Panama populations for all samples. The two highest populations were significantly greater than the lowest sample population, but all three samples were statistically similar to the other 12 samples ($P \leq 0.05$). Linearity of the points in a Q-Q plot in Figure 4.4-1 reveals a normal distribution of the Control sample populations.

Tukey Grouping		Mean	N	Day	Batch
	A	8.19	2	July 10	3
	A	8.15	2	July 17	4
B	A	8.12	2	July 09	3
B	A	8.10	2	July 31	6
B	A	8.09	2	July 24	5
B	A	8.04	2	July 25	5
B	A	8.03	2	Aug 14	7
B	A	8.03	2	June 18	2
B	A	7.99	2	June 13	1
B	A	7.98	2	June 19	2
B	A	7.97	2	Aug 13	7
B	A	7.97	2	June 12	1
B	A	7.96	2	Aug 01	6
B	A	7.95	2	June 20	2
B		7.87	2	June 11	1

Table 4.4-2: *S.* Panama Log Populations on Control Hazelnut Samples

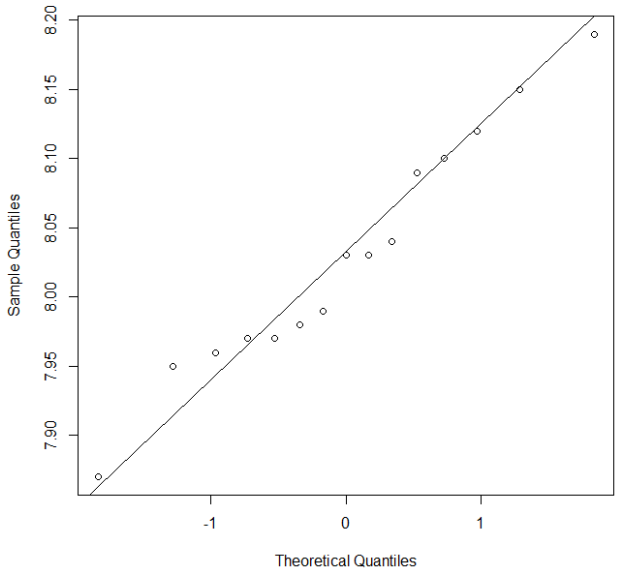

Figure 4.4-1: Normal Distribution Q-Q Plot of Control Sample Populations

The immersion procedure resulted in high and consistent *S.* Panama populations on Control samples with a small 95% confidence interval over 15 treatment days (8.03±0.04 log units). Many similar projects use drop inoculation procedures (Singh and others

2002; Musgrove and others 2010) which allow for known amounts of bacterial cells to be localized on the surfaces of food products, but are not practical for inoculating hundreds of foodstuffs simultaneously. The use of an immersion inoculation procedure and long drying time was ideal for this research project because it enabled inoculation of ~800 hazelnuts simultaneously, resulted in high initial *Salmonella* populations, and may have optimized the chemical resistance of the *Salmonella*. Musgrove and others (2010) found that *Salmonella* on egg shells was more resistant to chemicals when eggs were inoculated with a dip method than by a fecal smear or drop method. Results from Singh and others (2002) showed higher bacterial populations on lettuce leaves inoculated with EC O157:H7 using a dip method verses a drop method with little difference in the standard deviations. Additionally, *Salmonella* dried to surfaces are more resistant to chemical treatments than suspended cells or cells that are not attached tightly to surfaces (Møretrø and others 2012). The main disadvantage of using an immersion inoculation on hazelnuts was the tendency of the hazelnut shells to crack during drying (after inoculation soak). A drop inoculation method may reduce losses, as about 30 percent of hazelnuts were discarded due to cracking when using the immersion procedure.

4.4.3 *Salmonella* Panama Log Populations on Hazelnuts after Treatment

Figure 4.4-2 shows the *S.* Panama population means for each treatment. Pair wise comparisons show significant differences between the mean of the untreated Control compared to all seven chemical treatments ($P \leq 0.05$). There was no significant difference between the Water population mean and the Control mean. NaOCl treatments revealed the highest population means, with NaOCl-50 significantly higher than NaOCl-25. PAA-120 was comparable to PAA-80 and ASC-450, but ASC-450 was significantly lower than PAA-80. ASC-830 and ASC-1013 resulted in the lowest populations, which were 5.73 and 5.38 log CFU/hazelnut, respectively.

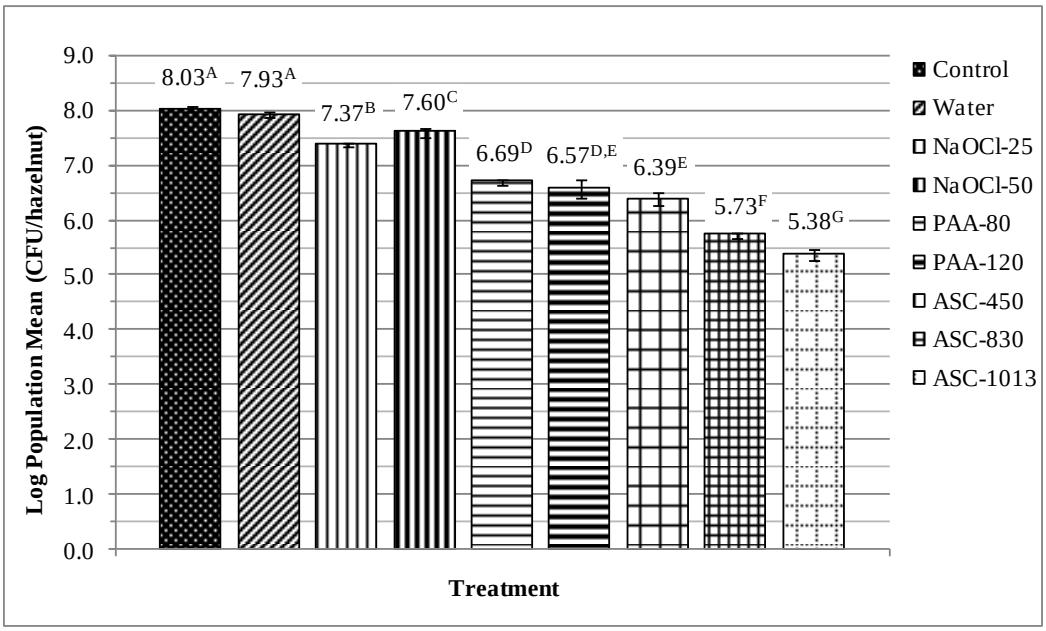

Figure 4.4-2: *Salmonella* Panama Log Population Means on Hazelnuts.
Error bars represent the 95% confidence intervals of the mean of *n* samples
[A-G]Values with different letters differ significantly ($P \leq 0.05$)
Control *n*=15, Water *n*= 15, NaOCl-25 *n*=6, NaOCl-50 *n*=9, PAA-80 *n*=6, PAA-120 *n*=6, ASC-450 *n*=6, ASC-830 *n*=6, ASC-1013 *n*=6

4.4.4 Ability of Treatments to Reduce *S.* Panama Populations on Hazelnuts

The mean from the untreated Control samples was used as the baseline for determining the population reductions produced by the chemical treatments. The Water treatment was originally intended to represent a population baseline that accounted for physical removal of microorganisms caused by treatment application, but tight adherence of *S.* Panama prevented significant reduction by physical removal[2]. The Water population mean was only 0.10± 0.12 log units less than the Control mean (Control mean-Water mean ± simultaneous 95% CI), and the two means were not significantly different ($P \leq 0.05$). Additionally, the Control sample population showed greater consistency and less skew than the Water treatments, making the Control a more reliable baseline. Appendix Table

[2] Glass beads (425-600 μm) shaken with the hazelnuts and buffer during microbial enumeration ensured that the *S.* Panama unattached from shell surfaces prior to serial dilution.

IV and Appendix Figure I show the population variance and distribution of the Water treatment samples. Appendix Figure II shows the log unit reduction of each of the chemical treatments compared to the Water treatment.

Figure 4.4-3 shows the reduction in population means of each of the treatment groups. All chemical treatments significantly lowered the *S.* Panama population compared to the Control and Water samples ($P \leq 0.05$). The ASC treatments showed the greatest population reductions compared to the Control, resulting in reductions of 1.64, 2.30, and 2.65 log units for ASC-450, ASC-830, and ASC-1013, respectively. Each increase in ASC concentration resulted in a significantly greater population reduction, which was similar to the results from Allende and others (2009) that found that 1 min exposure to 250 ppm, 500 ppm, and 1000 ppm ASC caused about 2, 2.5, and 3.5 log unit reductions of *E. coli* on cilantro. However, the final populations in the present study were more comparable to the 2.1 and 2.4 log unit reductions achieved by Liao (2009) when soaking *Salmonella* inoculated alfalfa seeds in 800 ppm ASC for 15 and 30 min, respectively. Liao (2009) found that extending the soak time to 45 min resulted in a 3.9 log unit reduction. Yuk and others (2005) found that floating tomatoes inoculated with *Salmonella* in 1200 ppm ASC for 120 sec resulted in a 3.72 log unit reduction on the stem scar of tomatoes. The increase in reduction seen by Yuk and others (2005) could be the result of using a drop inoculation method and dip sanitizer application. As previously addressed, drop inoculations have resulted in less chemical resistance than dip inoculations (Musgrove and others 2010). In addition, Musgrove and others (2010) found that spraying *Salmonella*-inoculated eggs with chemical solutions resulted in higher resistance than exposure via dipping. These results suggest that dipping hazelnuts in ASC 1200 ppm may increase reduction of *Salmonella*, and that use of dip inoculations likely result in more conservative representations of chemical lethality.

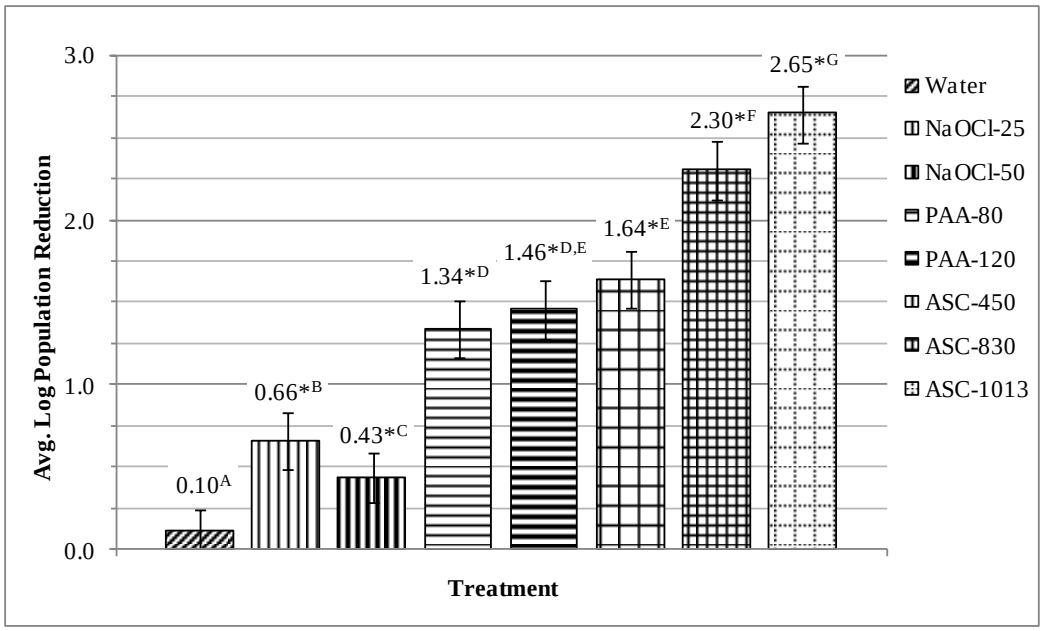

Figure 4.4-3: Treatment Log Reductions of *S.* Panama Compared to Control
Error bars represent simultaneous 95% confidence intervals of the difference between the treatment mean and the Control mean.
*All values are significantly lower than the Control population except Water
$^{A-G}$Values with different letters differ significantly ($P \leq 0.05$)

The PAA-80 ppm and PAA-120 ppm treatments resulted in population reductions of 1.34 and 1.46 log units, respectively, and were statistically similar to each other. These results were comparable to the 1.27 log unit reduction Pao and others (2006) achieved when spraying in-shell almonds inoculated with *Salmonella* with 500 ppm PAA. Neal and others (2012) found that 80 ppm PAA only resulted in 0.8 and 1.1 log unit reduction of *Salmonella* and EC O157:H7 (respectively) on spinach leaves. Yuk and others (2005) found that floating *Salmonella* inoculated tomatoes in 87 ppm PAA for 60 and 120 sec resulted in 2.12 and 2.72 log unit reductions, respectively. Similar to ASC treatments, comparative results suggest that dipping in 100 ppm PAA may result in slightly greater reductions of *Salmonella* than spraying. However, PAA 100 ppm is unlikely to result in reductions as high as ASC when using comparable methods of application.

The NaOCl-25 and NaOCl-50 treatments produced the smallest population reductions resulting in 0.66 and 0.43 log units, respectively. NaOCl was significantly less effective than PAA and ASC ($P \leq 0.05$). Many studies have also found that NaOCl is significantly less effective than other chemical alternatives due to deactivation in of NaOCl the presence of organic matter (Harrison and Hand 1981; Allende and others 2009). Allende (2009) found that dipping cilantro inoculated with *E. coli* into 200 ppm NaOCl for 1 min resulted in about a 1 log unit reduction. Posada-Izquierdo and others (2012)found that submerging lettuce inoculated with *E. coli* O157:H7 in 150 ppm NaOCl for 30 sec resulted in a 1.23 log unit reduction. Due to the environment of hazelnut shells, deactivation of sodium hypochlorite should be expected and alternative solutions would likely provide better sanitization.

Studies that found unusually high reductions using chemical sanitizers generally incorporated significant amounts of physical agitation into treatment processes. Velázquez and others (2009) was able to achieve 2.29 and 4.77 log unit reductions of *Y. enterocolitica* on tomatoes using water and 200 ppm NaOCl, respectively. The procedure involved exposing tomatoes to treatment solutions while agitating them by hand for 1 min, then rinsing the tomatoes with water for 1 min before drying them with paper towels. Chang and Schneider (2012) found that 60 sec in a spray/roller apparatus resulted in 3.8 and 5.5 log unit reductions of *Salmonella* on tomatoes when using water and 100 ppm NaOCl, respectively. These studies show that surface bacteria can be significantly reduced by physical removal. However, the best sanitation practices should involve the most effective chemicals as well as physical removal in order to prevent cross contamination. Parnell (2005) found that scrubbing melons in water resulted in significant reductions of *Salmonella*, but cross contamination of uninoculated samples occurred due to contamination of the rinse water.

4.5 CONCLUSION

Immersion of clean in-shell hazelnuts in high levels solutions of *S.* Panama cells (followed by thorough drying) resulted in tight adhesion of *S.* Panama to shell surfaces. Strong adhesion allowed for clear demonstration of chemical treatment lethality by preventing significant population reductions from physical removal. Acidified sodium chlorite was significantly more effective at killing *S.* Panama than peroxyacetic acid (when both chemicals were used near maximum legal concentrations), and both chemicals were significantly more effective than sodium hypochlorite. Acidified sodium chlorite shows the most potential for use in a postharvest in-shell hazelnut sanitation process. Further studies may reveal the ability of dip sanitation or physical agitation to optimize the microbial reduction capability of ASC.

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5. OVERALL SUMMARY AND FUTURE WORK

Developing effective non-thermal sanitation procedures will help ensure the safety of in-shell hazelnuts and enable processors to continue providing raw products. This study determined the effects of food-safe sanitizer sprays on the microbial populations of postharvest in-shell hazelnuts, as well as populations of *Salmonella* on inoculated in-shell hazelnuts. Results from the first phase showed that soil attachment impacted the microbial population counts, and that physical removal of dirt caused significant population decreases. Determination of specific log unit reduction capabilities of the treatments against natural microbial populations was not possible due to inconsistent amounts of excess dirt between Group 1 (early season, dry weather) and Group 2 (late season, rainy weather). However, the variation in weather, and subsequent excess soil attachment, allowed for examination of chemical performance through different stages of harvest. Greater chemical efficiency was observed on hazelnuts with higher initial populations, which suggests that either dry weather optimized the chemical resistance of some soil microorganisms, or that direct attachment of microorganisms to hazelnut shells (Group 1, little excess soil) makes microorganisms more resistant than microorganism attached via excess soil (Group 2, more excess soil on shells).

Information regarding chemical efficacy in relation to weather and soil attachment is valuable for future nut sanitation projects, but specific chemical efficacy for pathogen elimination could only be determined by controlling for physical removal of microorganisms. The second phase demonstrated the ability of sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite to kill pathogenic bacteria because physical removal was insignificant. The chemicals were applied to hazelnuts lacking excess debris and the inoculated *Salmonella* cells were dried for 66 h to allow for tight adherence to shell surfaces. High concentrations of acidified sodium chlorite (1013 ppm, pH 2.85±0.05) were significantly more effective than the other treatments and resulted in an average reduction of 2.65 log CFU/hazelnut.

Removal of excess soil produced significant reductions of microorganisms, and hazelnut processors should attempt to remove as much soil as possible before applying sanitizers. Acidified sodium chlorite shows potential for significantly increasing the microbial safety of postharvest nuts. An investigation of the microbial reduction resulting from industrial implementation of thorough rinsing and ASC spraying would determine the maximum potential of this sanitizer. Ideal processing procedures lacking a "kill step" should result in >4 log reductions of *Salmonella* between the time of harvest and the end of processing. Although a single spray application of ASC is unlikely to result in a 4 log unit reduction of *Salmonella*, exploratory research (not shown) suggests that the drying step in hazelnut processing may also result in significant microbial reduction. Combined effects of thorough rinsing, ASC spraying, and industrial drying may result in sufficient microbial reductions worthy of whole-process validation as a substitute for a validated kill step.

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APPENDIX

Appendix A: Experiment Incidence Tables for Treatment of Postharvest Hazelnuts

Incidence Table for Group 1								
	Treatment							
Test Day	Control	Rinse Only	Water	PAA-80	PAA-120	NaOCl-25	NaOCl-50	ASC-990
1	5	5	-	-	-	-	-	-
2	1	1	-	5	-	-	-	-
3	1	1	1	-	5	-	-	-
4	1	1	1	-	-	5	-	-
5	1	1	1	-	-	-	5	-
6	1	1	1	-	-	-	-	5
Total	10	10	4	5	5	5	5	5

Appendix Table I: Incidence Table for Group 1 Treatments.
Values represent the number of samples treated per experiment day (14 in-shell hazelnuts/50±0.50 g sample).

Incidence Table for Group 2								
	Treatment							
Test Day	Control	Rinse Only	Water	PAA-80	PAA-120	NaOCl-25	NaOCl-50	ASC-990
1	5	4	-	-	-	-	-	-
2	1	1	5	-	-	-	-	5
3	1	1	1	5	5	-	-	-
4	1	1	1	-	-	5	5	-
Total	8	7	7	5	5	5	5	5

Appendix Table II: Incidence Table for Group 2 Treatments.
Values represent the number of samples treated per experiment day (14 in-shell hazelnuts/50±0.50 g sample)

Appendix B: Experiment Incidence Table for Treatment of Hazelnuts Inoculated with *S.* Panama

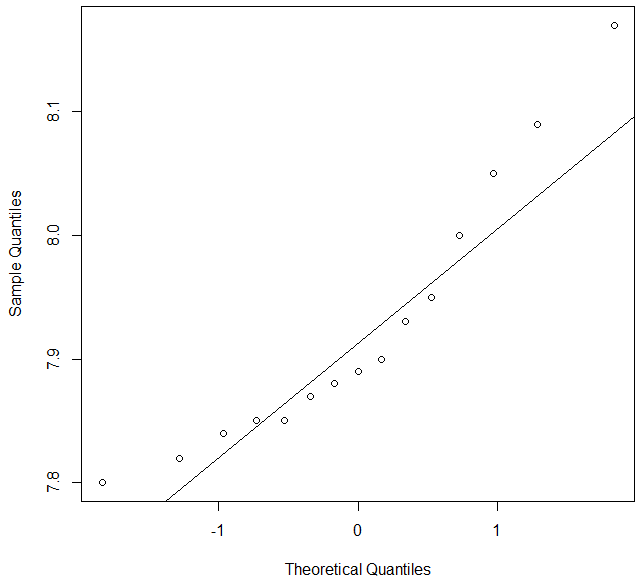
Incidence Table for Treatment of Hazelnuts Inoculated with *Salmonella*									
	Treatment								
Test Day	Control	Water	PAA-80	PAA-120	NaOCl-25	NaOCl-50	ASC-450	ASC-830	ASC-1013
1	1	1	3	-	-	-	-	-	-
2	1	1	3	-	-	-	-	-	-
3	1	1	-	3	-	-	-	-	-
4	1	1	-	3	-	-	-	-	-
5	1	1	-	-	3	-	-	-	-
6	1	1	-	-	3	-	-	-	-
7	1	1	-	-	-	3	-	-	-
8	1	1	-	-	-	3	-	-	-
9	1	1	-	-	-	3	-	-	-
10	1	1	-	-	-	-	3	-	-
11	1	1	-	-	-	-	3	-	-
12	1	1	-	-	-	-	-	3	-
13	1	1	-	-	-	-	-	3	-
14	1	1	-	-	-	-	-	-	3
15	1	1	-	-	-	-	-	-	3
Total	1	1	6	6	6	9	6	6	6

Appendix Table III: Incidence Table for Treatment of Hazelnuts Inoculated with *Salmonella* Panama
Values represent the number of samples treated per experiment day (45 in-shell hazelnuts/sample)

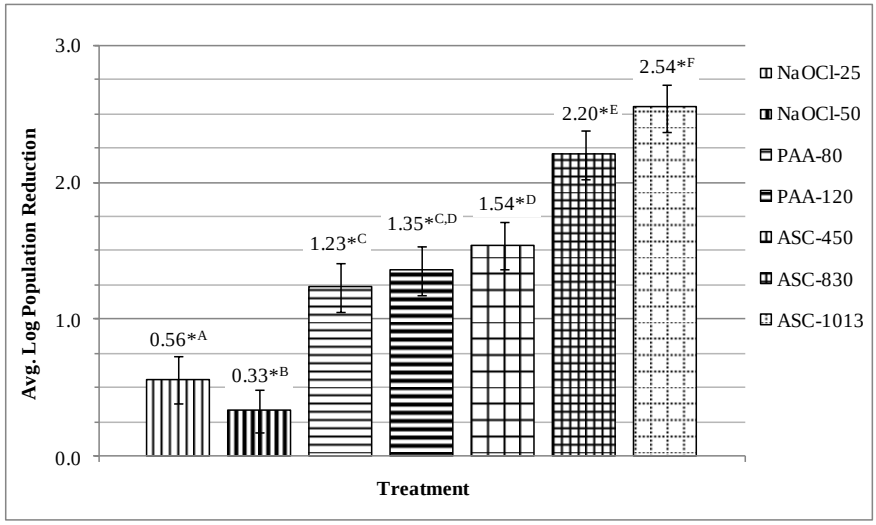
Appendix C: Effect of Water on *S.* Panama Populations on Hazelnuts

Tukey Group				Mean	N	Day	Batch
		A		8.17	2	July 10	3
B		A		8.09	2	July 31	6
B		A	C	8.05	2	June 18	2
B		D	C	8.00	2	July 17	4
E		D	C	7.95	2	June 20	2
E	F	D	C	7.93	2	Aug 01	6
E	F	D		7.90	2	July 09	3
E	F	D		7.89	2	July 25	5
E	F	D		7.88	2	June 19	2
E	F	D		7.87	2	June 12	1
E	F			7.85	2	June 11	1
E	F			7.85	2	July 24	5
E	F			7.84	2	June 13	1
E	F			7.82	2	Aug 14	7
	F			7.80	2	Aug 13	7

Appendix Table IV: *S. panama* Populations on Water Treatment Hazelnut Samples

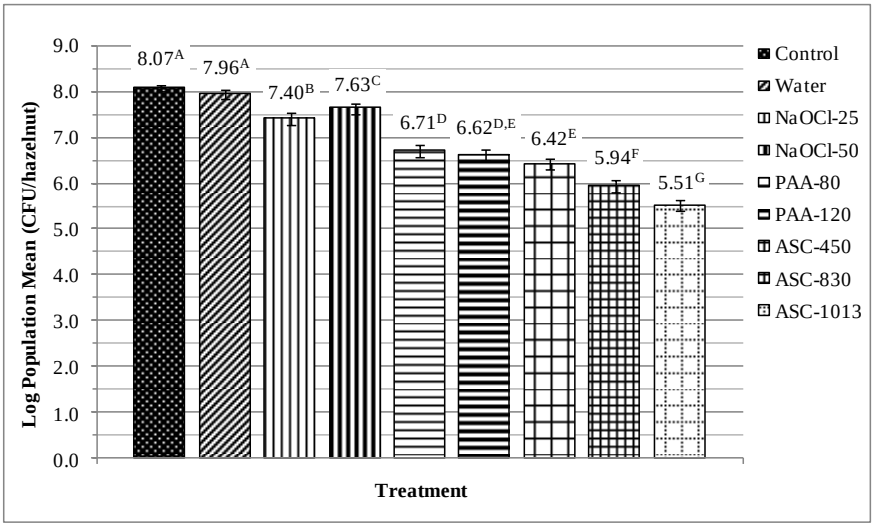


Appendix Figure I: Normal Distribution Q-Q Plot of Water Sample Populations



Appendix Figure II: Reduction in *Salmonella* Panama Compared to Water
Error bars represent simultaneous 95% confidence intervals of the difference between the treatment mean and the Water mean.
*All values are significantly lower than the Water
$^{A\text{-}F}$Values with different letters differ significantly ($P \leq 0.05$)

Appendix D: Aerobic Populations on Hazelnuts Inoculated with *S.* Panama



Appendix Figure III: APC Means on Hazelnuts Inoculated with *Salmonella* Panama
Error bars represent the 95% confidence intervals of the mean of *n* samples
$^{A-G}$Values with different letters differ significantly ($P \leq 0.05$)
Control *n*=15, Water *n*= 15, NaOCl-25 *n*=6, NaOCl-50 *n*=9, PAA-80 *n*=6, PAA-120 *n*=6, ASC-450 *n*=6, ASC-830 *n*=6, ASC-1013 *n*=6