AN ABSTRACT OF THE THESIS OF

Christopher A. Letchworth for the degree of Master of Science in Food Science and Technology presented on April, 24 2020.

Title: Reduction of *Salmonella* spp. on In-shell Hazelnuts Using Continuous Steam Blanching and Prevalence of *Salmonella* spp. on In-shell Oregon Hazelnuts.

Abstract approved:

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Robert J. McGorrin

Tree nuts have been implicated in a number of foodborne outbreaks and recalls in recent years linked to enteric pathogens, particularly *Salmonella*. Therefore, prior to distribution and marketing, it is necessary to understand the biological risks associated with the consumption of tree nuts and to find effective methods to inactivate foodborne pathogens. Steam treatment processes have been validated for use on California almonds, but little research has been conducted for in-shell hazelnuts. Hazelnuts grown in Oregon were recalled nearly annually from 2009 to 2017 and were implicated in an outbreak of *E. coli* O157:H7 that sickened 10 people in the Midwest and Canada in 2011 (Miller et al. 2014). In 2017, an outbreak of *Salmonella* Typhirium sickened 5 people and was traced to an 80-acre Oregon farm and nursery that sold between 32,000 to 48,000 pounds of raw in-shell hazelnuts directly to consumers from a road-side stand (Yada et al. 2019). To help characterize the biological risk of in-shell hazelnuts, we conducted a prevalence and amounts survey of *Salmonella* on in-shell hazelnuts grown in Oregon over two harvest years (2013-2014). In a separate study, we developed a steam treatment process that inactivates a 5-log reduction of *Salmonella* spp. on the surface of in-shell hazelnuts with minimal impact on final product quality.

For the *Salmonella* prevalence study, raw, green-dried in-shell hazelnut samples (n = 472) were collected by six of the largest hazelnut handlers in Oregon's Willamette Valley and tested for the presence of *Salmonella* spp.

using a modified method from the Food and Drug Administration's (FDA) Bacteriological Analytical Manual (BAM). In-shell hazelnut samples (375 g) were enriched in 1:10 (w/v) lactose broth followed by selective enrichment in Rappaport-Vassiliadis Broth (RV) and Tetrathionate Broth (TT). Selective enrichments were streaked for isolation onto Hektoen Enteric (HE) and Xylose Lysine Desoxycholate (XLD) Agars. Suspected colonies displaying *Salmonella* spp. morphology were confirmed on CHROMagar *Salmonella* Plus. A most-probable-number (MPN) method (3 x 333, 33.3, 3.3 g) using the same cultural steps as the initial testing was used to determine *Salmonella* population levels on naturally-contaminated in-shell hazelnuts. The prevalence of *Salmonella* spp. by year was 21.7% (55/254) and 46.8% (102/218) for 2013 and 2014, respectively. *Salmonella* population levels ranged from 0.092 to 30.7 MPN/100 g, with an average of 2.6 MPN/100 g. These data will help support risk assessment strategies for the Oregon hazelnut industry.

In our second study, we evaluated the efficacy of steam blanching on the reduction of *Salmonella* spp. on the surface of in-shell hazelnuts as a potential thermal postharvest treatment for hazelnuts. A pilot-scale steam blancher was used to deliver a continuous steam treatment at atmospheric pressure. In-shell hazelnuts were inoculated (~8.5 log CFU/g) with a five-strain *Salmonella* spp. cocktail and exposed to steam (88ºC) for 15 s, 1, 3, 5, and 10 min. Following steam treatment, hazelnut samples were transferred to 0.1% peptone water (24ºC), hand agitated 1 min, serially diluted, plated on Hektoen Enteric agar and incubated at 37ºC for 24 h. D-values (0.82 to 1.53 min) were calculated based on plate counts. *Salmonella* spp. could not be recovered by enrichment after hazelnuts inoculated at 5 log CFU/g were treated with steam at 88ºC for 10 min. These data will be useful when developing validated postharvest steam treatments for the hazelnut industry.

# Reduction of *Salmonella* spp. on In-shell Hazelnuts Using Continuous Steam Blanching and Prevalence of *Salmonella* spp. on In-shell Oregon Hazelnuts

By

Christopher A. Letchworth

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented April 24, 2020
Commencement June 2020

Master of Science thesis of Christopher A. Letchworth, presented on April 24, 2020

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Christopher A. Letchworth, Author

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude and appreciation to my advisor, Dr. Robert McGorrin, for giving me the opportunity to learn and grow as a scientist as well an individual. His continual support of my professional and personal goals, along with his fundamental understanding of food science and extensive knowledge of food industry needs, has helped make me a better scientist and person. It has been an honor and privilege conducting research under his guidance.

I would also like to acknowledge the support of my other thesis committee members: Drs. Lisbeth Goddik, Si Hong Park, and Bill Braunworth. I wish to thank them for generously offering their time and expertise. I appreciate their assistance, valuable advice and guidance.

I would like to also express my sincerest appreciation to the Oregon Hazelnut Marketing Board, including Polly Owen and Michael Klein for providing funding and resources for this research. The generous support provided by the Hazelnut Marketing Board helped make this research possible.

I am also extremely grateful for the continual support I received from family and friends during my research. I would like to thank my parents George and Frances Letchworth for their continued support of my educational and personal goals, as well as my amazing friends in the lab and the FST department for their assistance and encouragement over the last few years.

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## 1. Introduction

Low moisture foods such as tree nuts have traditionally been considered biologically safe due to their low water activity ($a_w \leq 0.70$) (Danyluk et al. 2007). However, outbreaks of enteric pathogens, particularly *Salmonella* spp., have been associated with tree nuts, including in-shell hazelnuts in recent years, prompting the need for new industry risk assessment strategies. The Oregon hazelnut industry is actively searching to better understand the biological risks associated with hazelnuts and improve the food safety of hazelnuts.

Currently, there are several validated postharvest treatments for the pasteurization of almonds including, propylene oxide (PPO) fumigation, steam, oil roasting, dry roasting, and hot water blanching that show promise of application to other tree nut industries. Steam treatment is an effective alternative for in-shell hazelnuts and other minimally processed tree nuts that cannot be roasted, hot water blanched, or have chemical residues left behind from PPO fumigation. Evaluation of the efficacy of steam treatment using a pilot-scale steam blancher will help determine the potential for steam technologies as thermal postharvest treatments.

In addition, a two-year *Salmonella* spp. prevalence and quantities survey of in-shell hazelnut samples collected from Oregon processors will help quantify the risk of *Salmonella* spp. associated with in-shell hazelnuts. The results of the prevalence study will help guide future industry risk assessment strategies.

## 2. Literature Review

### 2.1 Hazelnuts

#### *2.1.1 Hazelnuts*

The hazelnut, commonly referred to as filbert, is the fruit of the hazel (*Corylus*) tree, a genus of deciduous trees belonging to the birch family *Betulaceaeae* that consists of approximately 17 species (Holstein et al. 2018). While one hazelnut species, *Corylus cornuta var. californica*, is native to the Pacific Northwest, the European Hazelnut, *Corylus avellana*, has been cultivated for commercial use and is primarily used for commercial production world-wide, including the Pacific Northwest (Olsen 2013a). Hazelnuts are a good source of protein, unsaturated fats (oleic acid), magnesium and vitamins B and E (Richardson 1997). According to nutritional research, hazelnuts may potentially be beneficial for the heart, help reduce cancer risks, decrease inflammation, and aid in digestive health (Richardson 1997). The health benefits of hazelnuts have been purported for centuries. A Chinese manuscript dated 2838 BC lists hazelnuts as one of the five sacred foods given to man from heaven (Dreher et al. 1996; Olsen 2013a). The Greek philosopher Theophrastus described the benefits of hazelnuts in his writings, and the Greek physician Dioscorides wrote about using hazelnuts to treat common ailments such as colds and even baldness (Dreher et al. 1996). Hazelnuts have been cultivated for over five centuries in China, and evidence of Mesolithic nut processing on Colonsay, an island in the Inner Herbides of Scotland, where hundreds of thousands of charred hazelnut shells were found and carbon dated to over 9000 years ago, suggests this island community was trading processed hazelnuts with other surrounding communities (Mithen et al. 2001). Cultivation of hazelnuts in the Pacific Northwest and Oregon began in more recent times, and is thought to have first begun in 1858 when Sam Strickland, an English sailor, planted the first hazelnut tree outside Scottsburg, Oregon using the European cultivar, *Corylus avellana* (Olsen 2013a).

#### *2.1.2 Geographic Distribution*

Hazelnut trees grow naturally in a variety of conditions and locations. However, hazelnuts thrive at growing in temperate oceanic climates along the 45$^{th}$ parallel, and this latitudinal line intersects both the Willamette

Valley in Oregon and Turkey, the world's largest producer of hazelnuts (Lupo 2019; Olsen 2013a). The temperate oceanic climates of the Willamette Valley and the Black Sea region of northern Turkey are ideal for the commercial production of hazelnuts (Olsen 2013a). It is no coincidence that the top three hazelnut producing countries – Turkey, Italy and the United States – all have moderate oceanic regions in proximity to the 45$^{th}$ parallel that are ideal for the commercial production of hazelnuts. Turkey produces the majority of hazelnuts world-wide, accounting for approximately 70% of global production, with Italy and the United States being responsible for approximately 20% and 4% of global production, respectively (Kilic et al. 2006; Olsen 2013a). Commercial hazelnut production in the United States is unique to the Pacific Northwest. Specifically, Oregon's Willamette Valley is responsible for approximately 99% of the United States annual crop, with Washington producing the remaining 1% (NASS 2019; Olsen 2013a). While the Pacific Northwest is responsible for about 4% of world hazelnut production, Oregon-grown hazelnuts have gained a global reputation for their large size and robust flavor.

*2.1.3 Growth and Production*

Hazelnut trees are monoecious and self-incompatible, meaning they contain both male and female flowers but cannot self-pollinate (Germain 1994). Hazelnuts pollinate in the winter before ripening in the fall. Harvest typically begins in late September or October after nuts have ripened, turned hazel colored, and have fallen to the ground separated from their husks (HMB 2012), and lasts approximately one month. Mechanical sweepers then align the hazelnuts into uniform rows before a harvesting machine picks up the hazelnuts, sorts the nuts from other soil and plant debris, and deposits the nuts in trailers or large totes where they are then transported to processing facilities throughout Oregon (HMB 2012).

Postharvest processing of hazelnuts includes general washing and drying steps to ensure hazelnuts are clean and of high quality before being distributed to consumers. Washing steps vary commercially, with processors rinsing or spraying water or diluted food-safe sanitizers on in-shell hazelnuts to remove excess dirt and debris from the hazelnut shell. After processing, hazelnuts must contain no more than 0.02 percent (w/w) of

foreign material (CFR 2008b). After washing, clean hazelnuts are then dried over several days to reduce the moisture to less than 6 percent (CFR 2008b). Following drying, hazelnuts can be packaged and distributed as in-shell nuts or undergo additional processing steps such as shelling and roasting. While hazelnuts are commonly shelled, roasted and incorporated into confectionaries, the majority of Oregon hazelnuts are sold in-shell and undergo minimal processing.

### *2.1.4 Oregon Hazelnut Industry*

Oregon's Willamette Valley is the core of the United States hazelnut industry, responsible for producing over 99 percent of the US annual hazelnut crop (Mehlenbacher et al. 1997). Between 2016-2018, Oregon produced 42,333 tons of hazelnuts on average from 40,333 bearing acres for a total utilized annual production value of $94.7 million, according to the National Agricultural Statistics Service (NASS 2019). Historically, the majority of Oregon Hazelnuts (77 percent in 2014) are sold in-shell and undergo minimal processing.

### *2.1.5 Oregon Hazelnut Varieties*

Major varieties of Oregon hazelnuts have traditionally included Barcelona, Lewis, and Ennis, with Barcelona historically being the most popular variety (HGO 2019; Olsen 2013b). However, newly developed cultivars cross-bred for their resistance to the fungal disease, *Anisogramma anoamala*, compose the majority of new tree plantings. The popularity of Barcelona trees has been declining since the 1990s, with only about 1 percent of new plantings using this varietal. A newer hazelnut cultivar – Jefferson – has become the most commonly planted varietal in Oregon due to its resistance to eastern filbert blight (EFB) caused by the fungus *Anisogramma anoamala*, with over 50 percent of new plantings utilizing this EFB-resistant varietal (Olsen 2013b). Eastern filbert blight is a fungal disease that has decimated hazelnut crops over years by causing severe branch die-back and loss of susceptible trees. The Oregon State University hazelnut breeding program has been working to develop new hazelnut varieties that in addition to having EFB-resistance, increase annual production yield, and have desirable kernel characteristics (Olsen 2013b). In addition to Jefferson, other new

hazelnut varieties are becoming popular for planting including, McDonald, Wepster, and most recently Polly O., all of which display resistance to EFB (HGO 2019; Mehlenbacher et al. 2019).

**2.2 Outbreaks and Recalls Associated with Tree Nuts**

Historically, tree nuts have been considered microbiologically safe due to their low water activity. However, recent outbreaks of foodborne illness associated with the consumption of tree nuts has led to a reevaluation of the risks associated with them, with increasing importance placed on preventative control processes. In addition, detection of foodborne pathogens in prevalence surveys and in recall-associated tree nuts has led to several class-1 recalls in the United States and Canada in recent years. In 2009, detection of *Salmonella* in a single hazelnut processing facility led to the recall of 114,350 lb of shelled hazelnuts (FDA 2009). Additional recalls of hazelnuts in 2012, 2013, and 2015 were attributed to *Salmonella* detection in hazelnut samples. Outbreaks of salmonellosis in the United States and Canada in 2000-2001 and 2003-2004 were epidemiologically linked to raw almonds (Chan et al. 2002; CDC 2004). An outbreak of *E. coli* O157:H7 associated with the consumption of in-shell hazelnuts sickened 8 people in the Midwest in 2011 and was the first case of *E. coli* O157:H7 being associated with tree nuts (Miller et al. 2012). In 2017, an outbreak of *Salmonella* Typhirium which sickened 5 people was traced to an 80-acre Oregon farm and nursery that sold between 32,000 to 48,000 pounds of raw in-shell hazelnuts directly to consumers from a road-side stand (Yada et al. 2019). Walnut kernels were additionally implicated in an outbreak of *E.coli* O157:H7 in 2011. Due to the increase of illness associated with the consumption of tree nuts, along with increased detection of foodborne pathogens on tree nuts, a reevaluation of the health risks and efficacy of preventative control measures is necessary. A summary of recent outbreaks of foodborne illness associated with tree nuts is shown in Table 1.

Table 1. Outbreaks of foodborne illness associated with the consumption of tree nuts[a]

| Type | Product (source) | Pathogen | Year | Number of Cases | Outbreak location(s) |
|---|---|---|---|---|---|
| Almond | raw whole (California) | *S*. Enteritidis PT 30 | 2000-01 | 168 | Canada, USA |
| | raw whole (California) | *S*. Enteritidis PT 9c | 2004 | 47 | Canada, USA |
| | raw whole (California) | *S*. Enteritidis NST 3+ (aka PT 30) | 2005-6 | 15 | Sweden |
| | raw whole (Australia) | *S*. Typhimurium | 2012 | 27 | Australia |
| Coconut | desiccated (Pap New Guinea) | *S*. Typhi, *S*. Senftenberg and possibly others | 1953 | >50 | Australia |
| | desiccated (Sri Lanka) | *S*. Paratyphi B | 1960-61 | 3 | England |
| | desiccated (not stated) | *S*. Java PT Dundee | 1999 | 18 | United Kingdom |
| | dried, raw | *S*. Newport, *S*. Typhimurium | 2017-2018 | 15 | USA (CA, CO, CT, MA, NJ, NY, OK, PA, WA), Canada |
| Hazelnut | in-shell (Oregon) | *E. coli* O157:H7 | 2010-11 | 10 | Canada, USA (WI, MN, MI) |
| | raw in-shell (Oregon) | *S*. Typhimurium | 2017 | 5 | USA (OR) |
| Pine nut | whole, bulk (Turkey) | *S*. Enteritidis | 2011 | 43 | USA (MD, NY, NJ, PA, VA) |
| Pistachio | roasted (Calironia) | *S*. Senftenberg | 2013 | 8 | USA (CA, KS, MA, MD, PA, WI) |
| | roasted (California) | *S*. Montevideo, *S*. Senftenberg | 2015-16 | 11 | USA (AL, AZ, CT, GA, MI, MN, ND, VA, WA) |
| Walnut | raw shelled pieces (California) | *E. coli* O157:H7 | 2011 | 14 | Canada |

[a] Data obtained from Yada et al. 2019

*2.2.1 Mandatory Pasteurization of Almonds*

In response to the salmonellosis outbreaks linked to almonds, and with pressure from the FDA, the Almond Board of California (ABC) – a grower-enacted federal marketing association – proposed mandatory pasteurization of almonds (7 CFR Part 981). Beginning in September 2007, almonds grown in California and sold in the U.S., Canada, and Mexico must be subjected to a treatment process or processes that achieve a minimum 4-log reduction of *Salmonella*. Almond handlers are required to use treatment processes that have been recognized by a scientific review panel identified by the Almond Board known as the Technical Expert Review Panel (TERP), or reviewed by the FDA and issued a letter of determination when a process has sufficiently demonstrated its effectiveness to achieve a 5-log reduction of *Salmonella* in almonds. While a minimum 4-log reduction of *Salmonella* on almonds is considered the mandatory treatment criterion, a 5-log reduction is required for labeling almonds as "pasteurized". The TERP currently recognizes several validated postharvest treatments for almonds, including propylene oxide (PPO) fumigation, steam, oil roasting, dry roasting, and hot water blanching (ABC 2007d). The steam treatments recognized by the TERP consist of two proprietary processes – a continuous conveyor system and a batch system – that have been reviewed and accepted by TERP. Each process has established critical control points for one or two sets of operating parameters that have been accepted by TERP, and has undergone extensive validation testing using almonds inoculated with *Salmonella* Enteritidis PT 30 (SE PT 30). The almond industry is currently the only nut industry requiring mandatory use of validated postharvest treatments that inactivate *Salmonella*. Research conducted on almonds has helped define sanitation standards for tree nuts, and technologies used for reduction of *Salmonella* populations on almonds show promise of application to other tree nut industries.

*2.2.2 Salmonella Risk Assessment for Tree Nuts*

Tree nut industries, along with the FDA, now recognize the increased risk of nut-associated foodborne illnesses and began taking steps to improve the microbiological safety of tree nuts, with the almond industry serving as a primary model. Prompted by increasing outbreaks, recalls, and detection of *Salmonella* in tree nuts, the U.S. Food and Drug Administration (FDA) began conducting a risk assessment of *Salmonella*

contamination associated with tree nuts in 2013. The purpose of the risk assessment – a planned, multiyear study – is twofold: to quantify the public health risk associated with eating tree nuts potentially contaminated with *Salmonella,* and to evaluate the impact of interventions currently being used or that could be applied in the future to prevent or reduce *Salmonella* contamination levels (FDA 2013). The results of the risk assessment will help inform public policy on nut safety and help guide nut producers on best practices, according to the FDA.

The Oregon Hazelnut Industry, represented by the Hazelnut Marketing Board, has actively been working to better understand the risk of *Salmonella* contamination associated with hazelnuts and to develop validated processes that inactivate or sufficiently reduce *Salmonella* and other pathogens on in-shell hazelnuts. Working with the Hazelnut Marketing Board, the Oregon State University Food Safety Systems laboratory recently concluded a multi-year (2013-2015) prevalence survey of *Salmonella* on in-shell hazelnuts. Data collected from the *Salmonella* prevalence survey on in-shell hazelnuts will be provided to the FDA for their risk assessment of *Salmonella* contamination in tree nuts. In addition to the prevalence study, our lab has been working with the Hazelnut Marketing Board to investigate the efficacy of postharvest treatments to inactivate or sufficiently reduce *Salmonella* on in-shell hazelnuts, including steam treatment, propylene oxide (PPO) fumigation, and peroxyacetic acid (PAA) washing.

*2.2.3 Prevalence of Foodborne Pathogens on Tree Nuts*

Much of the available data for the prevalence of foodborne pathogens on tree nuts is derived from retail surveys using small sample sizes (25g; Davidson et al. 2015; Harris et al. 2019). Prior to 2017, available data for the prevalence of *Salmonella* and *E.coli* on hazelnuts was restricted to several retail surveys form the UK and Australia that used a limited amount of samples and small sample sizes for determination of prevalence levels (Harris et al. 2019). Several larger retail surveys of hazelnuts sold in the United States and Canada have since found the prevalence of *Salmonella* ranging from 0.0% to 0.43% on in-shell and shelled hazelnuts sold at the retail level in the United States and Canada (Table 2). Despite these more recent retail surveys, a

comprehensive survey of the prevalence of *Salmonella* occurring at the hazelnut processing level in Oregon has not been conducted. However, large prevalence surveys have been conducted on California almonds, pistachios and walnuts, with hundreds to thousands of samples (100-500 g) collected at the processor level over multiple harvest years (Table 2). In order to make a direct comparison to data derived from the California tree nut *Salmonella* prevalence surveys, we devised an in-shell hazelnut *Salmonella* prevalence study with a similar design, collecting 472 samples (375 g) from processors over three harvest years (2013-2015).

Available data prior to 2016 from foodborne pathogen surveys of California tree nuts indicate approximately a one percent contamination level of *Salmonella* on almonds, pecans, and pistachios, with the exception of in-shell walnuts, which had a significantly lower incidence of *Salmonella* contamination (0.14%) (Table 2). A survey of almonds grown in California found a prevalence of 0.98% (137/13,972) *Salmonella* on raw almond kernel samples (100g) over eight harvest years (2001-2007 and 2010) (Bansal et al. 2010; Danyluk et al. 2007; Lambertini et al. 2012). The average prevalence of *Salmonella* on raw in-shell almonds grown in California was 1.5% (100 g; 7/455) in 2006 and 2007 (Bansal et al. 2010). The incidence of *Salmonella* on in-shell walnuts was found to be 0.14% (100 to 375 g; 4/3,838) over three California harvest years (2011-2014) (Davidson et al. 2015). The average prevalence of *Salmonella* contamination on pistachios grown in California was 0.81% (100g; 32/968) between 2011-2013 (Harris et al. 2016). A recent survey of in-shell pecans collected over four harvest years (2010-2014) from seven pecan shelling facilities located across five U.S. states, found 44 of 4,641 (0.95%) samples (100 g) positive for *Salmonella* (Brar et al. 2015). *Salmonella* population levels in naturally-contaminated tree nuts are relatively low, ranging from 0.000095 to 39 MPN/100 g upon retesting (Table 2). The detection of *Salmonella* in California tree nut surveys, along with increased foodborne outbreaks being linked to tree nuts, underscores the need for effective postharvest treatments that are validated to sufficiently reduce (4 to 5 log) *Salmonella* in tree nuts.

Table 2. Prevalence and levels of *Salmonella* on naturally-contaminated tree nuts in North America[a]

| Type of nut | Where Collected | Sample size (g) | No. of samples tested (n) | No. positive for *Salmonella* | Percent positive | Concentration (Avg MPN/100 g) | References |
|---|---|---|---|---|---|---|---|
| Almond, raw kernel | Processor receiving, California | 100 | 14,949 | 146 | 0.98 | 96 samples: 0.0044 to 0.15 for 2002-06; 4 samples: 0.00080, 0.00080, 0.00095, 0.0034 for 2010 | Bansal et al. 2010; Danyluk et al. 2007; Lambertini et al. 2012 |
| Almond, raw in-shell | Processor receiving, California | 100 | 455 | 7 | 1.5 | 18 samples: 1.4 to 18.3 | Bansal et al. 2010 |
| Almond, in-shell | Retail, Canada | 25 | 86 | 0 | 0 | 0 | CFIA, 2017 |
| Almond, shelled | Retail, Canada | 25 | 319 | 0 | 0 | 0 | CFIA, 2017 |
| Hazelnut, in-shell | Retail, US | 375 | 80 | 0 | 0 | 0 | Zhang et al. 2017 |
| Hazelnut, raw shelled | Retail, US | 375 | 577 | 2 | 0.35 | NA[b] | Zhang et al. 2017 |
| Hazelnut, in-shell | Retail, Canada | 25 | 696 | 3 | 0.43 | NA | CFIA, 2017 |
| Hazelnut, shelled | Retail, Canada | 25 | 870 | 0 | 0 | 0 | CFIA, 2017 |
| Macadamia, raw shelled | Retail, US | 375 | 355 | 15 | 4.2 | NA | Zhang et al. 2017 |
| Pecan, raw in-shell | Processor receiving, 5 U.S. states | 100 | 4,641 | 44 | 0.95 | 44 samples: 0.47 to 39; mean of 2.4 | Brar et al. 2015 |
| Pecan, in-shell | Retail, Canada | 25 | 40 | 0 | 0 | 0 | CFIA, 2017 |
| Pecan, shelled | Retail, Canada | 25 | 86 | 0 | 0 | 0 | CFIA, 2017 |
| Pistachio, raw in-shell | Processor receiving, California | 100 | 3,968 | 32 | 0.81 | 11 samples (sinkers): 0.0046 21 samples (floaters): 0.012 to 0.43 | Harris et al. 2016 |
| Pistachio, in-shell | Retail, Canada | 25 | 481 | 0 | 0 | 0 | CFIA, 2017 |
| Pistachio, shelled | Retail, Canada | 25 | 22 | 0 | 0 | 0 | CFIA, 2017 |
| Walnut, raw in-shell | Processor, California | 100 | 935 | 0 | 0 | 0 | Davidson et al. 2015 |
| Walnut, raw in-shell | Processor, California | 375 | 2,903 | 4 | 0.14 | 3 samples: 0.0032, 0.0038, 0.0042 | Davidson et al. 2015 |
| Walnut, in-shell | Retail, Canada | 25 | 792 | 2 | 0.25 | NA | CFIA, 2017 |
| Walnut, shelled | Retail, Canada | 25 | 874 | 0 | 0 | 0 | CFIA, 2017 |

[a]Data obtained from Harris et al. 2019

[b]Data not determined

A risk assessment model designed by Danyluk et al. (2006) was used to characterize the risk associated with consumption of raw almonds. The model was based on Monte Carlo simulations and took into account many of the factors after almonds reached the processors, such as handler and consumer storage times, and pre-process, post-process, retail, and consumer reduction levels on *Salmonella* during storage (Pan et al. 2012).

The model was able to demonstrate that lack of a pasteurization step led to a greater than 78% probability of more than one case of salmonellosis occurring per year; however, introduction of a pasteurization step achieving a minimum 4-log reduction reduced the probability of illness to 0.01% according to the model. Based on this model, the Almond Board's TERP concluded that a 4-log reduction was a suitable standard for almond pasteurization, and recommended that a mandatory treatment program be implemented (Pan et al. 2012). A similar risk assessment model developed by Lambertini et al. (2012) ran a simulation with updated variables such as total amount ingested by consumer, concentration of *Salmonella* in almonds, assumed storage times, and temperature distributions throughout all processing steps. The model showed that, under the current rule mandating a 4-log minimum reduction of *Salmonella* on almonds, the estimated risk of salmonellosis was 0.72 cases annually. The model further showed that if a 3-log reduction was mandated rather than a 4-log reduction, the risk of salmonellosis would be 7.2 cases annually (Pan et al. 2012).

## 2.3 Validated Postharvest Treatments

Several chemical and thermal treatment technologies have been investigated on almonds for their efficacy at inactivating *Salmonella* and other pathogens (i.e. *E. coli* O157:H7). Chemical treatments have widely been used on food commodities for their efficacy at controlling or inactivating microbial pathogens while preserving the original quality of the product. As an alternative to chemical treatments, which may leave residues on the final product, thermal treatments have long been used on food commodities. However, thermal treatments exceeding 60°C may negatively affect final product quality if not well controlled (Pan et al. 2012).

### *Chemical Treatments*

#### *2.3.1 Propylene Oxide*

Propylene oxide (PPO), a registered fumigant in the US for reduction of bacteria, yeasts, and mold on raw nut meats, is among the technologies approved by the US Food and Drug Administration (FDA) for the pasteurization of raw almond kernels (ABC 2008). Research projects funded by the Almond Board of California (ABC) and carried out by Dr. Linda Harris of the University of California, Davis (UCD), and ABC

staff in collaboration with Blue Diamond Growers, Inc. (Sacramento, CA) and Industrial Sterilization (Sparks, NV) demonstrated that PPO fumigation was effective at achieving a 5-log reduction of *Salmonella* Enteritidis PT30 (SE PT30) on inoculated raw almond kernels (Danyluk et al. 2005). The FDA issued a Letter of Determination confirming the validity of PPO as a pasteurization treatment for raw almond kernels in September 2004 after reviewing the research findings from the ABC-sponsored studies on the efficacy of PPO in reducing *Salmonella* in almonds. Almonds which are fumigated in accordance with the PPO treatment parameters described by Danyluk et al. (2005) can be labeled as "pasteurized" (ABC 2008). Similar PPO treatment parameters for in-shell almonds have been conditionally accepted by TERP that achieve a minimum 4-log reduction of *Salmonella.* The PPO treatment conditions for hazelnuts must be the same as those approved for the use in the pasteurization of almond kernels, except that in-shell hazelnuts must be held for a minimum of 5 days at 15-18ºC for post-treatment for ventilation, whereas almond kernels have the option of be held for 2 days at 38-43ºC for post-treatment ventilation.

The US Environmental Protection Agency (EPA) requires that the exposure time to PPO does not exceed 4 h and that the residue on the product is less than 300 ppm (Danyluk et al. 2005). Consequently, the ABC published a standard operating procedure (SOP) for treatment of almonds kernels and in-shell almonds using PPO, establishing parameters that are effective at inactivating *Salmonella* on almonds while following the EPA requirements of no more than 4 h PPO exposure time and a final product residue less than 300 ppm. A brief summary of the SOP is as follows: Almonds are pre-warmed to (30ºC) before being loaded into a sealed and pre-heated chamber (47-51ºC). The pressure of the chamber is lowered to approximately 9.9 kPa before PPO is injected into the chamber at a minimum concentration of 0.5 kg/m$^3$. Following injection of PPO, an inert gas (i.e. nitrogen) is pumped into the chamber to maintain a pressure of 84.3 kPa during the 4-h treatment. A series of aeration cycles (4 to 14 cycles) follow the 4 h process, where a cycle is the decrease of the chamber pressure to 9.9 kPa followed by an increase to atmospheric pressure with an inert gas or air. Following the aeration cycle, almonds are transferred to a post-ventilation treatment room for a minimum of 2 days at 38-43ºC (almond kernels) or for 5 days above 15ºC (almond kernels and in-shell almonds) to

achieve a PPO residue of 300 ppm or less on the final product (ABC 2008). Treatment with PPO provides biologically safe final products while maintaining the integrity and sensory parameters of almonds (Danyluk et al. 2005). However, public acceptance – particularly in export markets – and the high cost of operation make PPO treatment impractical for some nut processors. Foreign markets such as the European Union have strict guidelines on the importation of food commodities treated with PPO and other chemicals, and the Federal Register estimates the cost of a PPO chamber is between $500,000 and $1,250,000, with alternative off-site contract processing costing between $0.04 and $0.05 per pound (CFR 2008a).

*Thermal Treatments*

*2.3.2 Oil Roasting*

Roasting causes almonds to have a more crunchy texture and alters the flavor profile of nut products (Du et al. 2010). Oil roasting is used by the almond industry to obtain crunchy and roasted flavors in almond products (ABC 2007d). Du et al. (2010) demonstrated that immersion of almonds in hot oil (127ºC) achieved a 5-log reduction of SE PT30 in 1.5 min. The authors partly attributed the rapid and large reduction of *Salmonella* to washing-off of loosely attached, less protected, and more heat-sensitive pathogen cells (Du et al. 2010). In addition, the efficacy of oil roasting may also result from the high temperature of the oil (127ºC) and the high rate of heat transfer from the oil to the kernel.

Oil roasting parameters (oil temperature and time) are dictated by the desired degree of roast, throughput rate, initial temperature and initial moisture levels of the almonds, volume of the heated oil, etc. At oil temperatures of 138ºC to 177ºC (280ºF to 350ºF) roasting times of 3 to 15 minutes are typically needed to achieve crunchy and crispy oil-roasted almond products (ABC 2007d). While oil roasting is able to meet pasteurization requirements (5-log reduction of SE PT30), it is only applicable to roasted almonds and not to raw almonds.

*2.3.3 Dry Roasting*

In general, oil roasting is a much faster process than dry roasting using hot air (129° to 154°C). Several studies initiated by the ABC found that certain existing dry-roasting parameters used by the almond industry did not deliver a minimum 4-log reduction of SE PT30 on almonds. Yang et al. (2010) demonstrated that dry roasting almond kernels to a medium level at 130°C was insufficient to achieve a 4-log reduction on SE PT30. The heat resistance of SE PT30 during dry roasting (hot air) is well documented, with SE PT30 having a D-value of 25 min at 121.1°C and a Z-value of 26.1°C (ABC 2007c). While dry roasting typically takes much longer than oil roasting, it has been recognized by TERP as a validated technology to achieve a minimum 4-log reduction of *Salmonella* on almonds. Common temperatures for dry roasting range from 129 to 154°C for 10 to 45 minutes. Dry roasting may not achieve the required 4-log reduction of *Salmonella* on almonds without significantly impacting the sensory properties of almonds, and is therefore not applicable as a treatment for raw almonds or other minimally processed tree nuts such as in-shell hazelnuts.

*2.3.4 Steam Treatment*

Steam at 100°C has a higher heat capacity than the same amount of water at that temperature. One of the advantages of steam pasteurization is the large transfer of heat when steam condenses on the surface of foods, which rapidly raises the surface temperature (James et al. 2000). Another attractive feature of condensed steam team is its ability to penetrate small cavities and condense on cold surfaces that water is unable to reach. Water vapor molecules are much smaller in diameter (mean free path of steam molecule at 140°C is 0.4 μm) than *Salmonella* cells (approximately 0.7 μm), making steam capable of reaching bacteria that occur in cavities (Morgan et al. 1996). Unlike steam, the surface tension of water makes it unable to penetrate pores of this size. Consequently, water cannot reach all the contaminated surfaces that are large enough to contain bacterial cells of this size (Morgan et al. 1996).

Several studies have investigated steam pasteurization of *Salmonella* Enteritidis inoculated on the surface of almond kernels with mixed results. Using the same almond variety (Nonpareil) and same SE strains (*S.*

Enteritidis 43353, ME-13, ME-14), Chang et al. (2010) were able to demonstrate a 5-log reduction of SE after exposure to pressurized steam (143 kPa) at 25 s using a batch style, custom made, almond pasteurizer. In contrast, Lee et al. (2006) were unable to achieve a 4-log reduction of SE even after 35-s exposure to atmospheric steam using conventional steaming methods. The efficacy of the pressurized steam treatment observed by Chang et al. (2010) was partly attributed to the rapid increase of temperature within the pressurized treatment chamber in contrast to the heat dissipation that occurs in open air using conventional steaming, with the author concluding that the efficacy of steam at inactivating SE is dependent on the condition of steam applied (Chang et al. 2010). Both authors noted negative impacts on final product quality, with increasing moisture content and loss in visual quality of almond kernels exposed to steam for prolonged periods (35 s). A separate study conducted by Bari et al. (2010), demonstrated that a combination of superheated steam (115ºC) for 70 s followed by infrared heating for 70 s was able to achieve a 5-log reduction of *Salmonella* on almond kernels without significantly affecting final product quality.

*2.3.4.1 Proprietary Steam Technologies*

There are currently two proprietary steam technologies that have been reviewed and accepted by TERP. Both proprietary processes include specific sets of parameters for the treatment of almond kernels and have undergone extensive validation testing using SE PT30 and established critical control points for one or two sets of operating parameter that have been accepted by TERP (ABC 2007b).

The two accepted proprietary processes are: the FMC JSP-1 pasteurization system installed at Going Nuts (Madera, CA) and the $H_2O$ Express pasteurization system Chamber 1, installed at Stewart & Jasper Company (Newman, CA) (ABC 2007b). The FMC JSP-1 pasteurization unit is an inline, continuous conveyor system that treats almonds prior to packaging. Two sets of processing parameters have been accepted by TERP for the FMC process: one to achieve a 5-log reduction of *Salmonella* and one to achieve a 4-log reduction. The FDA, after reviewing the 5-log reduction validation results, issued a Letter of Determination to acknowledge

that the FMC JSP-1 pasteurization unit achieves a minimum 5-log reduction of SE PT30 on natural almonds when operated at defined parameters including belt speed and loading capacity (ABC 2007b).

The $H_2O$ Express pasteurization system is a batch type system that treats almonds in their final packaging. For this system, TERP accepted operating parameters for a 4-log reduction of *Salmonella* on almonds for three chambers in Newman, CA. The current acceptance only applies to almonds packed in 50-lb cartons for Chamber 1 and 2,200-lb-tri-wall fiber totes for chambers 1,2, and 3 (ABC 2007b).

Steam treatment is an effective alternative to chemical treatments such as PPO fumigation which may leave chemical residues.

*2.3.5 Hot Water Blanching*

Blanching with hot water or steam-injected water is a thermal process used by almond handlers to remove the pellicle (skin) from almond kernels. Typical hot water blanching processes include scalding and drying steps where almonds are exposed to heat. Scalding is the step of interest for validation and involves soaking almond kernels in hot water or steam-injected water (ABC 2007a). Harris et al. (2012) studied the efficacy of heated water on the reduction of *Salmonella* on almonds in a hot water bath. In their study, *Salmonella* could not be recovered by enrichment after almonds inoculated with SE PT30 and *Salmonella* Senfentburg 775W at 5-log CFU/g were heated at 88ºC for 2 min (Harris et al. 2012). Based on information from this study, the TERP determined that a minimum process of 2.0 min or more of exposure to hot water at 88ºC (199ºF) or above will provide a 5-log or greater reduction of *Salmonella* on almonds (ABC 2007a). Subsequently, the FDA reviewed the information and issued a Letter of Determination acknowledging the process was suitable for pasteurization. Almond products processed utilizing those conditions may be labeled as pasteurized (ABC 2007a). While approved for pasteurization of almond kernels, hot water blanching may not be a suitable treatment technology for in-shell hazelnuts and other minimally processed tree nuts due to its effect on quality and sensory characteristics of the final product – particularly moisture content. In a preliminary

trial conducted at the OSU pilot plant, hot water blanching was determined to have negative impacts on the sensory and quality characteristics of in-shell hazelnuts, with a large increase in moisture content compared to steam blanching.

**2.4 Potential Sources of Microbial Contamination During Tree Nut Production and Processing**

Microbial contamination of tree nuts can occur during several stages of nut production and processing. *Salmonella* contamination on almonds has mainly been traced to the orchard and huller/sheller facilities. Contamination in the orchard during harvest was considered the most likely source of *Salmonella* for outbreak-associated almonds and *Salmonella* has been shown to survive and persist in almond huller/sheller processing facilities (Davidson et al. 2015).

Similarly to almonds, there are several points during the harvest and post-harvest handling of hazelnuts when *Salmonella* could easily be introduced. At harvest, hazelnuts fall to the ground before they are mechanically swept up. Any pathogens introduced to orchard soils could potentially contaminate hazelnuts while they are exposed to the orchard floor. Studies on almond orchard soils show that *Salmonella* may persist long-term and even multiply in contaminated soils (Danyluk et al. 2008). Uesugi et al. (2007) observed that a *Salmonella* strain associated with a foodborne outbreak in 2001 was able to persist in an almond orchard for over 5 years. Microbial contamination of orchard soils is a likely source of contamination for hazelnuts and other tree nuts that fall to the orchard floor before harvest.

Following harvest, hazelnuts are washed to remove any dirt and plant debris and then dried. Ineffective washing processes may lead to contamination or cross contamination of hazelnuts because *Salmonella* is able to easily cross-contaminate products in a liquid medium. Effective washing procedures are critical for the biological safety of in-shell hazelnuts and other minimally processed tree nuts.

**2.5 Pathogens Associated with Tree Nuts**

*2.5.1 Salmonella* spp.

*Salmonella* is a genus of gram negative, non-spore forming, rod-shaped (bacillus) bacteria of the Enterobacteriaceae family. *Salmonella* is divided into two species – *Salmonella enterica* and *Salmonella bongori* – with *S. enterica* further divided into six subspecies and over 2,500 serovars. *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium are the most common serotypes isolated from salmonellosis patients in the United States (CDC 2011). Nontyphoidal salmonellosis refers to illnesses caused by all serotypes of *Salmonella* except Typhi, Paratyphi A, Paratyphi B, and Paratyphi C. Nontyphoidal salmonellae are a leading cause of bacterial diarrhea worldwide, causing an estimated 94 million cases of gastroenteritis and 115,000 deaths globally each year (CDC 2011).

Investigations of *Salmonella* Enteritidis outbreaks frequently implicate contaminated poultry and egg products as the source of infection (Patrick et al. 2004). While eggs and poultry products continue to be major vehicles for the transmission of salmonellae, raw fruits and vegetables have been increasingly implicated in outbreaks due to modern agricultural practices, such as irrigation with polluted water or fertilization with manure, sewage sludge, and animal excrement (Beuchat et al. 2013). Outbreaks of *Salmonella* Enteritidis were linked to raw almonds in 2000 to 2001 and 2003 to 2004.

*2.5.2 E. coli* O157:H7

*Escherichia coli* is a gram-negative, facultatively anaerobic, rod-shaped (bacillus) bacterium of the genus *Escherichia* and family Enterobacteriaceae. *E. coli* are a diverse group of bacteria that naturally inhabit the gastrointestinal tracts of people and animals. Similarly to *Salmonella*, over 200 serotypes of *E.coli* have been classified serologically. While most *E. coli* are harmless and an important component of a healthy human intestinal tract, pathogenic types that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animal or persons. Pathogenic *E. coli* strains are categorized into six pathotypes based upon virulence and host clinical symptom and include: (i) enteropathogenic *E. coli* (EPEC),

which causes diarrhea in children and animals; (ii) enterohemorrhagic *E. coli* (EHEC), which is responsible for hemorrhagic colitis and hemolytic-uremic syndrome; (iii) enterotoxigenic *E. coli* (ETEC), which causes traveler's diarrhea and porcine and bovine diarrhea; (iv) enteroaggregative *E. coli* (EAEC), which causes persistent diarrhea in humans, and diffusely adherent *E. coli* (DAEC), a subclass of EAEC which causes diarrhea in children; (vi) uropathogenic *E. coli* (UPEC), which causes urinary tract infections in humans and animals; and (vii) neonatal meningitis *E. coli* (NMEC), which is responsible for meningitis and sepsis (Palaniappan et al. 2006).

EHEC, also referred to as Shiga-toxin producing *E. coli* (STEC), are the most common cause of *E. coli* foodborne illnesses, and are estimated to cause more than 265,000 illnesses each year in the United States, with more than 3,600 hospitalizations and 30 deaths (CDC 2014). Most outbreaks of EHEC infection in the United States have been caused by EHEC O157:H7 (E. coli O157:H7), which is responsible for more than 75 percent of EHEC infections (Jay et al. 2005).

**2.6 Persistence and Survival of Pathogens in Tree Nuts and Other Low-Moisture Foods**

Foodborne pathogens are unable to multiply on low moisture foods (water activity <0.70), but are capable of persisting for long periods on dry surfaces. Studies have shown that *Salmonella* and *E. coli* O157:H7 are able to survive long-term on tree nuts and other low moisture foods, and that pathogenicity may be associated with survival advantages such at desiccation or thermal resistance (Hiramatsu et al. 2005). In addition, low water activity has been shown to increase the resistance of *Salmonella* to thermal (Izurieta et al. 2012) and chemical treatments (Kieboom et al. 2006). Any treatment process designed for mitigation of pathogens such as *Salmonella* on tree nuts will need to address these issues.

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# 3. PREVALENCE AND LEVELS OF *SALMONELLA* SPP. ON IN-SHELL OREGON HAZELNUTS OVER THE 2013 AND 2014 HARVESTS

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*Research Note*

**3.1 Abstract**

A multi-year *Salmonella* spp. prevalence study was conducted to support risk assessment strategies for Oregon's hazelnut industry. During the 2013 and 2014 harvest seasons, raw, green-dried in-shell hazelnut samples (n = 472) were collected by six of the largest industry handlers and tested for the presence of *Salmonella* spp. using a modified method from the Food and Drug Administration's (FDA) Bacteriological Analytical Manual (BAM). Samples of in-shell hazelnuts (375 g) were enriched in 1:10 lactose broth followed by selective enrichment in Rappaport-Vassiliadis Broth (RV) and Tetrathionate Broth (TT). Selective enrichments were isolated onto Hektoen Enteric (HE) and Xylose Lysine Desoxycholate (XLD) Agars. Colonies displaying typical morphology for *Salmonella* spp. were confirmed on CHROMagar *Salmonella* Plus. When a sample was positive for *Salmonella*, the pathogen level was determined by a most-probable-number (MPN) method (3 x 333, 33.3, 3.3 g) following the same cultural steps as the initial testing. *Salmonella* spp. prevalence on in-shell hazelnuts by year was 21.7% (55/254) and 46.8% (102/218) for 2013 and 2014, respectively. Contamination levels averaged 2.6 MPN/100 g with a range of 0.092 to 30.7 MPN/100 g. *Salmonella* prevalence on in-shell hazelnuts is drastically higher compared to prevalence studies for other tree nuts. Further investigation is needed to understand the contributing factors leading to these high rates of contamination as well as mitigation factors to improve the food safety of hazelnuts.

**3.2 Introduction**

Outbreaks of foodborne illness associated with the consumption of tree nuts have increasingly been documented in recent years, including in-shell hazelnuts. Raw almonds were implicated in outbreaks of salmonellosis in the United States and Canada in 2000-2001 (Isaacs et al. 2005) and 2003-2004 (CDC 2004), prompting the California almond industry to voluntarily adopt mandatory pasteurization of raw almonds beginning in 2007. In 2011, outbreaks of *E. coli* O157:H7 were epidemiologically linked to in-shell hazelnuts and walnut kernels (CDC 2011; Davidson et al., 2015). A 2017 outbreak of *Salmonella* Typhirium sickened 5 people and was traced to an 80-acre Oregon farm and nursery that sold between 32,000 to 48,000 pounds of raw in-shell hazelnuts directly to consumers from a road-side stand (Yada et al. 2019). In 2013, prompted by outbreaks and recalls of *Salmonella* contamination in tree nuts, the U.S. Food and Drug Administration (FDA) began conducting a multiyear, planned risk assessment of *Salmonella* contamination associated with tree nuts. The risk assessment is intended to quantify the public health risk associated with eating tree nuts potentially contaminated with *Salmonella* and to evaluate the impact of interventions to prevent *Salmonella* contamination or reduce its contamination levels (FDA 2013). Foodborne pathogen surveys from tree nuts that quantify the prevalence and amounts of pathogens, such as *Salmonella,* provide valuable data when making quantitative risk assessments.

Oregon's Willamette Valley produces approximately 99 percent of the United States annual hazelnut crop. According to the National Agricultural Statistics Service (NASS), between 2016-2018, Oregon produced 42,333 tons of hazelnuts on average from 40,333 bearing acres for a total utilized production value of $94.7million (NASS 2019). Historically, the majority of Oregon hazelnuts (77 percent of total yield in 2014) have been sold in-shell and undergone minimal processing (NASS 2015). However, recent trade tariffs with China have disrupted the supply chain of in-shell hazelnuts that are typically exported to China, and more hazelnuts are being sold shelled. For example, between 2016-2018, almost half (47 percent) of the total yield of Oregon Hazelnuts were sold shelled, compared to only 23 percent in 2014 (NASS 2019).

*Salmonella* is unable to multiply on the surface of tree nuts due to the low water activity (generally less than 0.70), but is able to persist on tree nuts for prolonged periods and has been shown to survive in production and processing environments. There are several points during the harvest and post-harvest handling of hazelnuts when *Salmonella* could feasibly be introduced. Before harvest, hazelnuts ripen and fall to the orchard floor before they are mechanically swept up. Hazelnuts may be exposed to pathogens in soil, water, and manure on the orchard floor. After harvest, hazelnuts are washed to move any dirt and debris and then dried. Inadequate washing procedures may lead to contamination or cross-contamination as *Salmonella* is able to easily contaminate products in a liquid medium. Cross-contamination of hazelnuts may occur during processing, handling and storage (GMA 2016). *Salmonella* populations were found to be stable on inoculated almonds after over a year in cold storage (4°C) (Kimber et al. 2012; Uesugi et al. 2006). In addition, *Salmonella* strains associated with foodborne outbreaks have been shown to survive in contaminated almond orchard soils for over 5 years (Uesugi et al. 2007). Persistence of *Salmonella* has led to recalls of tree nuts, including hazelnuts grown in Oregon. Between 2009 to 2015, there were 4 class-1 recalls of hazelnuts in the United States and Canada due to *Salmonella* contamination (Yada et al. 2019).

Large-scale prevalence surveys of California almonds, walnuts, pecans, and pistachios have been conducted over multiple harvest years with hundreds to thousands of samples (100-500 g) collected at the processor level (Harris et al. 2019). Available data for the prevalence of *Salmonella* on hazelnuts is limited to several retail surveys in the UK and Australia that used a limited amount of samples and small sample size (25g) (Davidson et al. 2015; Harris et al. 2019). This study was undertaken to characterize the likelihood of *Salmonella* contamination among in-shell hazelnuts produced in Oregon over several years. The primary study objective was to determine the prevalence and levels of *Salmonella* in minimally processed in-shell hazelnuts collected from processors throughout Oregon's Willamette Valley.

## 3.3 Materials and Methods

### *3.3.1 Hazelnut Sample Collection*

In-shell hazelnuts from the 2013 and 2014 harvests were collected from six large hazelnut handlers located in the Willamette Valley in Oregon. Hazelnut samples were collected three times a week during active season (middle September – late October) following the first drying stage (green dry). Personnel from the Hazelnut Marketing Board collected samples from the handlers and delivered samples to Oregon State University each afternoon to ensure the identity of the processors and origin of samples remained anonymous to lab personnel. In 2013, no information was collected about any of the samples. In 2014, random numerical codes were used to unmask sample results to handlers after all nuts were out of commerce.

Each handler was asked to provide samples from four separate lots of hazelnuts per collection day. Each sample consisted of four subsamples (>100g/subsample) collected from various locations throughout the lot. Subsamples were collected in sterile Whirl-Pak bags (Nasco, Salida, CA) and placed inside a larger zipper-style plastic bag to maintain sample organization. Sample analysis began upon receipt by the laboratory.

### 3.3.2 *Salmonella* spp. Analysis

#### *3.3.2.1 Presence/Absence*

Enrichment and isolation of *Salmonella* spp. was performed using a modified version of the Food and Drug Administration's Bacteriological Analytical Manual (FDA-BAM) method (Andrews et al. 2020). A total of 375 g of each hazelnut sample (~93.75 g/subsample) were aseptically transferred to a sterile 5.4 L Whirl-Pak bag (Nasco). Lactose broth (3.375L; Neogen, Lansing, MI) was added to each sample bag and vigorously shaken for 20 seconds and incubated at 37°C for 24 ± 2 h. Following pre-enrichment, 0.1 ml and 1.0 ml of samples were transferred to 10 ml of Rappaport-Vassiliadis (RV) broth and Tetrathionate (TT) broth, respectively, and incubated at 37°C for 24 ± 2 h. Enrichments were streaked for isolation onto Hektoen Enteric agar (HE; Neogen) and Xylose Lysine desoxycholate agar (XLD; Neogen) and incubated at 37°C for 24-48 h. Presumptive *Salmonella* colonies (4 per selective enrichment) were transferred to CHROMagar

*Salmonella* Plus (DRG International Inc., Springfield, NJ) and incubated at 37°C for 24 ± 2 h. Colonies displaying pink/mauve coloration were considered to be confirmed as *Salmonella* spp. and were transferred to Tryptic Soy Broth (TSB; Neogen) and incubated 24 hrs. Following incubation, TSB cultures were mixed in a 1:1 ratio of 80% (v/v) glycerol and stored at -80ºC for future analyses. Remaining hazelnut samples were stored at ambient temperature (2013) or at 4ºC (2014) pending testing results.

*3.3.2.2 Most Probable Number (MPN)*

Hazelnut samples determined to be positive for *Salmonella* spp. were further evaluated for enumeration. To determine the level of *Salmonella* contamination, a three-tube MPN analysis was performed. Hazelnuts were divided into nine samples (3 x 3 g; 3 x 33 g; 3 x 333 g) into sterile Whirlpak bags and combined with a 1:10 ratio (w/v) of Lactose broth and incubated at 37°C for 24 ± 2 h. Each MPN bag was analyzed to detect the presence of *Salmonella* as described above.

Concentrations of *Salmonella* were calculated using the Thomas approximation (Blodget et al. 2006; Swanson et al. 2001) of MPN per gram (Bansal et al. 2010) for the three-tube *Salmonella* MPN analysis:

$$\text{MPN/g} = P/\sqrt{NT}$$

where $P$ is the number of positive tubes, $N$ is total grams of sample in all negative tubes, and $T$ is total grams of samples in all tubes. The 95% confidence interval was also estimated using the equation:

$$\log(\text{MPN/g}) \pm (1.96)(0.55)\sqrt{\frac{(\log a)}{n}}$$

where $a$ is the dilution ratio and $n$ is the number of tubes per dilution. The limit of detection (0.090 MPN/100 g) was determined by calculating the value for an MPN of 0/3, 0/3, and 1/3 for the 333 g, 33 g, and 3 g samples, respectively.

**3.4 Results and Discussion**

*3.4.1 Prevalence of Salmonella spp. in Hazelnuts*

A total of 472 in-shell hazelnut samples were analyzed over the 2013 and 2014 harvest, and 157 (33.3%) samples were positive for *Salmonella* upon initial screening (Table 1). The highest annual prevalence occurred in 2014, where 102 (46.8%) of 218 samples were positive for *Salmonella*. In contrast, *Salmonella* prevalence in 2013 was less than half that of 2014, with 55 (21.7%) of 254 samples positive for *Salmonella* upon initial screening.

The average prevalence of *Salmonella* on in-shell hazelnuts of 33.3% (375g; 157 of 472) was determined over the 2013 and 2014 harvests. The prevalence is significantly higher than previously reported for other tree nuts in similarly conducted prevalence surveys (hundreds to thousands of 100 to 375 g samples collected from processors over multiple years). Average *Salmonella* prevalence levels for California tree nuts are comparable, with approximately a 1% prevalence of *Salmonella*, with the exception of in-shell walnuts, which is significantly lower. The average prevalence of *Salmonella* in raw almond kernels collected over eight California harvests (2001 to 2007 and 2010) was 0.98% (100g; 137 of 13,972) (Bansal et al.2010; Danyluk et al. 2007; Lambertini et al. 2012). The prevalence of *Salmonella* for in-shell almonds was determined to be 1.3% (100g; 6 of 455) in a 2006 and 2007 survey (Bansal et al. 2010). A three-year survey of in-shell walnuts found the incidence of *Salmonella* was 0.14% (100 to 375g; 4 of 3,838) between 2011 to 2014 (Davidson et al. 2015). A survey of in-shell pecans collected over four harvest seasons (2010 to 2014) from seven pecan shelling facilities located across five U.S. states, found the average prevalence of *Salmonella* to be 0.95% (100 g; 44 of 4,641). A survey of California pistachios determined the average prevalence of *Salmonella* to be 0.81% (100 g; 32 of 3,968) between 2010 to 2012.

Available data for the prevalence of *Salmonella* on hazelnuts is limited to several retail surveys from Australia and the UK which used limited amounts of samples and small sample sizes (25 g). Two surveys of edible hazelnut kernels (25g; 0 of 233) for sale in England in 2008 and 2010 did not identify any *Salmonella* in retail

samples (Little et al. 2009; Little et al. 2010). A survey of hazelnut kernels (25 g; 0 of 48) collected from Australian processors at the point of receipt, prior to any processing, also did not find any *Salmonella* contaminated product (Eglezos et al. 2008; Eglezeos et al. 2010). A survey of RTE packages collected from an Australian hazelnut processor (25 g; 0 of 51) in 2010 failed to find any *Salmonella* contamination (Eglezos 2010). In another study, hazelnut kernel samples (25 g; 0 of 34) were collected from retailers, processors, and growers and did not document any *Salmonella* contamination on hazelnut kernels (NSW Food Authority, 2012).

### 3.4.2 Concentration of *Salmonella* spp. in Hazelnuts

For MPN analysis, *Salmonella* was recovered in 116 of the 157 samples initially testing positive for *Salmonella* over the 2013 and 2014 harvests. The detection limit of the MPN method was 0.092 MPN/100 g (Table 2). *Salmonella* was recovered from 43.4% and 90.2% of samples originally testing positive in 2013 and 2014, respectively. Average concentration levels of *Salmonella* were 0.879 MPN/100 g in 2013 and 3.103 MPN/100 g in 2014. Hazelnut samples collected in 2013 were stored under ambient conditions for 3 to 5 months before MPN testing was performed. In contrast, 2014 hazelnut samples were stored at 4ºC for approximately one month before MPN analysis was conducted.

Discrepancies in storage time and temperature of *Salmonella*-positive hazelnut samples prior to MPN analysis between 2013 and 2014 likely contributed to differences in *Salmonella* concentration (MPN/100g) and recovery during MPN analysis. In 2013, hazelnuts were stored under ambient conditions for 3 to 5 months prior to MPN testing; in contrast, in 2014 hazelnut samples were stored at 4ºC for approximately one month prior to MPN analysis. Studies have shown that *Salmonella* population levels are stable on nuts stored at 4°C for months with no reduction in population levels, while *Salmonella* population levels decline over time on nuts stored at ambient temperature (Kimber et al. 2012). Samples collected in 2014 were held in cold storage at 4ºC, stabilizing and preventing *Salmonella* populations from declining, likely contributing to higher recovery and concentration rates for 2014 MPN samples versus 2013 MPN samples.

When *Salmonella* is detected in tree nuts, its levels are often near or less than 1 cell per gram, even in outbreak associated product (Danyluk et al. 2007, Lambertini et al. 2012). Lambertini et al (2012) estimated that the levels of *Salmonella* could have been 120 MPN/g during the 2001 almond outbreak. Of the survey hazelnut samples initially determined to be positive for *Salmonella*, 66.92% remained positive in a subsequent MPN analysis. The levels of *Salmonella* estimated for in-shell hazelnuts averaged 0.879 MPN/100g and 3.103 MPN/100g for the 2013 and 2014 harvests, respectively. The levels of *Salmonella* found in hazelnuts are similar to those determined for *Salmonella* in walnuts (0.32 to 0.42 MPN/100g) (Davidson et al.2015) and raw almond kernels (0.79 to 16.0 MPN/100g) (Bansal et al. 2010, Danyluk et al. 2007, Lambertini et al. 2012).

Hazelnuts can be exposed to *Salmonella* at a number of points in the orchard and during harvesting and post-harvest handling. After ripening, hazelnuts fall to the orchard floor where they are mechanically swept into long, narrow piles between rows of trees in the orchard (Waterbury 2016). Harvesting machines pick up the hazelnuts, separate them from other plant debris, and deposit the nuts in tote boxes or trailers (Waterbury 2016). Nuts are transported to processing facilities throughout the region. General processing steps involve washing and drying, with washing methods varying by process company. Washing involves spraying or rinsing the nuts with water or a diluted food-safe sanitizer to remove excess dirt and debris from the hazelnut shells. Washed hazelnuts are immediately dried to reduce the moisture to less than 6 percent (CFR 2008). Hazelnuts can be dried in warm, dry locations over several weeks or over several days in food dryers. Once dried, hazelnuts can be distributed as in-shell nuts or be shelled, roasted and incorporated into confectionaries.

Hazelnut samples used in this survey were collected from processors after washing and during the initial drying stage of processing. In similar large-scale surveys of tree nuts, samples were collected at receipt, prior to any processing (Davidson et al. 2015). Thus, contaminants in those surveys could only have been introduced before any processing steps, namely in the orchard or during harvest or immediate postharvest handling (Davidson et al. 2015). While the orchard remains a likely environmental source for the

introduction of *Salmonella* to in-shell hazelnuts, the process of washing hazelnuts provides several feasible opportunities for the introduction of *Salmonella* to the shell surface as well. Cross-contamination of *Salmonella* during the initial washing of in-shell hazelnuts may have attributed to the significantly higher levels of *Salmonella* prevalence of in-shell hazelnuts compared to other tree nuts.

The comingling of in-shell hazelnuts during washing provides an opportunity for cross-contamination of *Salmonella* between in-shell hazelnuts. If hazelnut lots are not carefully kept separated during washing, a potentially contaminated lot from one orchard could cross-contaminate other lots. In addition, if the water used for washing hazelnuts is continually reused or recycled, it could potentially become infected with *Salmonella* and cross contaminate product. Also, inadequate washing methods that fail to spray or rinse the complete surface of shell surfaces could contribute to *Salmonella* contamination during processing. Lack of lot separation, contaminated washing water, and inadequate washing techniques are some of the potential ways *Salmonella* can contaminate in-shell hazelnuts during the initial washing process.

While hazelnuts can be roasted or blanched, the majority of hazelnuts (76%) are sold in-shell and undergo minimal processing (NASS 2015). There is considerable interest in processes that are able to reduce *Salmonella* in hazelnuts without affecting the quality or sensory characteristics of the nut.

Table 1. Frequency and quantity of *Salmonella* spp. detected on in-shell hazelnut samples from 2013 and 2014 harvests.

| Harvest Year | Samples Analyzed | Samples Positive for *Salmonella* spp. | Prevalence | Number of Samples Containing *Salmonella* spp. in MPN Range | | | | | | | Average MPN/100 g[e] | Frequency of MPN Recovery |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | <0.092 MPN/100 g[c] | 0.092 MPN/100 g | 0.092-1.0 MPN/100 g | 1.0-10.0 MPN/100 g | 10.0-30.0 MPN/100 g | >30.0 MPN/100 g | ND[d] | | |
| 2013[a] | 254 | 55 | 21.7% | 29 | 0 | 17 | 6 | 0 | 0 | 3 | 0.879 | 44.2% |
| 2014[b] | 218 | 102 | 46.8% | 10 | 2 | 46 | 37 | 6 | 1 | 0 | 3.103 | 90.2% |
| **Total** | **472** | **157** | **33.3%** | **39** | **2** | **63** | **43** | **6** | **1** | **3** | **2.643** | **74.7%** |

[a] 2013: Hazelnut samples stored under ambient temperature conditions between initial *Salmonella* analysis and MPN enumeration.

[b] 2014: Hazelnut samples stored at 4°C between initial *Salmonella* analysis and MPN enumeration.

[c] Detection limit for MPN analysis was 0.092 MPN/100 g.

[d] Not determined: MPN analysis not completed due to excessive mold growth during storage.

[e] Average MPN/100 g calculated using only samples with at least one positive sample in MPN analysis (≥0.092 MPN/100 g).

**Table 2.** Recovery frequency of *Salmonella* during MPN analysis

| Harvest year | No. of positive samples | No. of Samples in *Salmonella* MPN range (MPN/100g) | | | | Frequency of recovery% | Avg (MPN/100g) |
|---|---|---|---|---|---|---|---|
| | | <0.092 | 0.092[c] | >0.092 | ND[d] | | |
| 2013 | 55[a] | 29 | | 24 | 2 | 43.64 | 0.879 |
| 2014 | 102[b] | 10 | 2 | 90 | | 90.20 | 3.103 |
| Total | 157 | 39 | 2 | 114 | 2 | 66.92 | 2.643 |

[a] Samples stored under ambient conditions prior to MPN analysis

[b] Samples stored at 4 ˚C prior to MPN analysis

[c] Detection limit (MPN/100g)

[d] Not determined - mold contamination

### 3.5 Acknowledgments

Funding for this research was provided by the Hazelnut Marketing Board. The authors would like to thank Alex Emch, Eva Pearson, Daniel Wright, Joey Minarsich, Selena Callahan, Sam Mertz, and Whitney Nielson for assistance with media preparation and sample analysis. The authors would also like to thank Dr. Linda Harris of University of California, Davis and Dr. Michelle Danyluk at the University of Florida for assistance with sample size calculations and methodological considerations.

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# 4. REDUCTION OF *SALMONELLA* SPP. ON IN-SHELL HAZELNUTS USING CONTINUOUS STEAM BLANCHING AND THE IMPACT ON PRODUCT QUALITY AND SENSORY CHARACTERISTICS

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*Prepared for submission to:*
*Food Research International*

**4.1 Abstract**

This study was conducted to evaluate the efficacy of steam blanching on the reduction of *Salmonella* spp. on the surface of in-shell hazelnuts for Oregon's hazelnut industry. A pilot-scale steam blancher was used to deliver a continuous steam treatment at atmospheric pressure. In-shell Barcelona hazelnuts (size large; 3 g/nut) (50 g /sample) were inoculated (8 to 9 log CFU/g) with *Salmonella* spp. and exposed to steam (88ºC) for 15 s, 1, 3, 5, and 10 min. Following treatment, hazelnuts were transferred to 0.1% peptone water (24ºC), hand agitated 1 min, serially diluted, and plated on Hektoen Enteric agar and incubated at 37ºC for 24 h. D-values of 0.82 to 1.53 min were calculated for exposure of *Salmonella* spp. to steam treatment at 88ºC for different sample positions in the steam blancher. *Salmonella* spp. could not be recovered by enrichment after hazelnuts inoculated at 5 log CFU/g were treated with steam at 88ºC for 10 min. These data will be useful to validated hazelnut industry blanching processes.

**4.2 Introduction**

Tree nuts have been identified as a potential source for enteric pathogens, particularly *Salmonella* spp. Globally, there have been 15 reported outbreaks of salmonellosis and 2 outbreaks of *E. coli* O157:H7 associated with the consumption of tree nuts since 1953 (Harris et al. 2019). Outbreaks of salmonellosis were first linked to tree nuts when outbreak-associated *Salmonella* strains were isolated from coconuts in three separate outbreaks involving coconuts in 1953, 1960, and 1999 that sickened over 75 people. Since 2000, there has been an increase in reported outbreaks of foodborne illness associated with tree nuts, with 12 reported outbreaks of salmonellosis responsible for 383 cases of illness associated with tree nuts including, almonds, cashew, pine nuts, and pistachios. In 2011, in-shell hazelnuts and raw shelled walnuts were implicated in outbreaks of *E. coli* O157:H7 that sickened 24 people in Canada and the Midwest United States (Harris et al. 2019). In addition, since 2001 there have been 101 recalls of tree nuts involving foodborne pathogen contamination or possible contamination (Yada et al. 2019). The majority of product recalls has been for *Salmonella* contamination or possible contamination and has involved numerous types of tree nuts including hazelnuts, almonds, pecans, walnuts, pine nuts, macadamia nuts, and cashews. Detection of

*Salmonella* in hazelnut samples has led to several class I recalls in the United States and Canada since 2009. In 2009, detection of *Salmonella* in shelled hazelnuts was traced to a single hazelnut processing facility and resulted in the recall of 114,350 lb of product (Beuchat et al. 2013; Yada et al. 2019). Detection of *Salmonella* in hazelnuts led to additional recalls in 2012, 2013, 2015, and 2017 (Beuchat el al. 2013; Yada et al. 2019). While *Salmonella* is responsible for the majority of recalls associated with biological contamination in tree nuts, *E. coli* O157:H7 was responsible for recalls of hazelnuts and walnuts in 2001, and walnuts were also recalled twice in 2014 for *Listeria monocytogenes* contamination (Yada et al. 2019). Increasing reports of foodborne illness and outbreaks associated with the consumption of tree nuts underscore the need for effective postharvest treatments that inactivate foodborne pathogens and a reevaluation of the efficacy of technologies currently used for tree nut sanitation.

In response to outbreaks and recalls, the Almond Board of California approved a voluntary action plan making pasteurization mandatory for almonds grown in California beginning in September 2007, with a final rule published in 2009 (7 CFR Part 981). Under the rule, almonds grown in California and sold in North America must be processed using a validated method that achieves a minimum 4-log reduction of *Salmonella*. However, a 5-log reduction of *Salmonella* is the minimum process required for labeling almonds as pasteurized (ABC 2007a). Several validated postharvest treatments for almonds are currently recognized by the Almond Board of California, including propylene oxide (PPO) fumigation, steam, oil roasting, dry roasting, and hot water blanching (ABC 2007b). Research conducted on almonds show promise for application to other nut industries that may incorporate process elements into their own processing procedures. However, postharvest treatments should be designed specifically for each type of nut product based on safety needs and final product quality.

The Oregon Hazelnut industry produces 99 percent of the United States annual hazelnut crop. According to the National Agricultural Statistics Service (NASS), in 2018, Oregon produced 51,000 tons of hazelnuts from 44,000 bearing acres for a total utilized production value of $91.8 million (NASS 2019). Historically, the

majority of Oregon hazelnuts (63 percent of total average yield in 2016-2017) (NASS 2019) are sold in-shell and undergo minimal processing. However, hazelnuts sold in-shell only accounted for 38 percent of total utilized production in 2018 (NASS 2019). Due to the increasing detection of S*almonella* in hazelnuts and other tree nuts, there is a need for postharvest treatments that not only effectively reduce levels of *Salmonella* with minimal impact on final product quality, but are also cost-effective and flexible enough to incorporate into existing processing lines.

Steam pasteurization is effective for reducing naturally occurring and pathogenic bacteria in foods (Nutsch et al. 1998). The efficacy of steam pasteurization is largely due to the large amount of heat transferred to foods when steam condenses, which rapidly increases the surface temperature (James et al. 2000). Steam has a higher heat capacity than the same amount of water at a given temperature (James et al. 1997) and can effectively penetrate small areas such as cracks and crevices on nuts that may protect microorganisms (Morgan et al. 1996). There is a limited understanding of the efficacy of using steam as a postharvest *Salmonella* treatment for in-shell hazelnuts and the effects of such treatments on final product quality.

The purpose of this study was to identify a steam treatment that would effectively pasteurize in-shell hazelnuts (5-log reduction of *Salmonella* spp.) and would have minimal impact on nut quality. To achieve these goals, a variety of steam treatments (time/temperature combinations) were evaluated for their ability to reduce *Salmonella* spp. on in-shell hazelnuts and used to predict treatments that would achieve pasteurization. Predicted time/temperature combinations that would achieve pasteurization were verified. Additional hazelnuts were treated with pasteurizing steam treatments and sensory tests were conducted to evaluate changes in consumer perception of the nuts.

## 4.3 Materials and Methods

### *4.3.1 Hazelnuts*

Raw in-shell hazelnuts (washed, dried, graded, and packaged) were provided by the Oregon Hazelnut Marketing Board (Aurora, OR). Barcelona hazelnuts (size large; 3 g/nut) in bulk 22.7 kg bags were stored at ambient temperature for up to 6 months prior to use.

### *4.3.2 Preparation of Inoculum*

Five strains of *Salmonella enterica* previously associated with tree nuts or peanuts were used in this study (Table 1). Stock cultures were stored at -80$^0$C in Tryptic Soy Broth (TSB; Neogen, Lansing, MI) supplemented with 40% glycerol. Stock cultures were resuscitated by transferring to individual tubes containing TSB and incubated at 37ºC for 24 h. The resulting culture (1 ml) was spread onto several large format (150 x 15 mm) petri dishes containing Tryptic Soy Agar (TSA, Neogen) and incubated at 37ºC for 24-26 h to produce a lawn. Bacterial lawns were harvested by adding 8.0 ml of 0.1% peptone water (Neogen) and loosening with a disposable cell spreader. Cell suspensions for each strain were transferred into 50 ml sterile conical tubes and thoroughly vortexed (1 min). Equivalent volumes of each harvest were combined to create a five strain cocktail. For spot inoculation procedures, Tween 80 was added to the cocktail to achieve a final concentration of 0.5% to reduce surface tension. Cell densities were determined by standard serial dilution and plating on Hektoen Enteric agar (Neogen) plates and enumerated following incubation at 37ºC for 24 h. When necessary, the inoculum cocktail was diluted with 0.1% peptone water to achieve lower targeted inoculation levels. The cocktail was held at 4ºC for up to 2 weeks prior to use as hazelnut inoculum.

Table 1. *Salmonella enterica* strains included in the inoculation cocktail for this study.

| Serotype | Isolate Identifier | Description | Source |
|---|---|---|---|
| *Salmonella* Enteritidis PT30 | ATCC BAA-1045 | Almond isolate | American Type Culture Collection (ATCC) |
| *Salmonella* Enteritidis PT9c | RM4635 | Clinical isolate from almond outbreak | Rob Mandrell USDA-ARS |
| *Salmonella* Montevideo | GRC1 | Pistachio isolate | Food and Drug Administration |
| *Salmonella* Oranienburg | MDD317 | Pecan isolate | Michelle Danyluk University of Florida |
| *Salmonella* Tennessee | MDD319 | Clinical isolate from peanut butter outbreak | Larry Beuchat University of Georgia |

*4.3.3 Immersion Inoculation of Hazelnuts*

In-shell hazelnuts were inoculated by immersion following previously published procedures for almond kernels with minor modifications (Danyluk et al. 2005). Briefly, hazelnuts (400-2400 g) were transferred to a 1.5 L sterile sample bag (WhirlPak, Nasco, Salida, CA). The previously described inoculum was added to the hazelnuts at a ratio of 1:6 (v/w) (25 ml inoculum per 400 g hazelnuts). The bag was closed and mixed by hand shaking and inversion for 1 min to distribute inoculum evenly on the surface of the hazelnuts. Inoculated hazelnuts were transferred to perforated stainless steel baskets (30 x 30 cm) in the biological safety cabinet and air dried 18-24 hrs. Dried, inoculated hazelnuts were aseptically transferred into sterile sample bags and held at 4ºC for up to 1 month prior to steam treatment.

*4.3.4 Spot Inoculation of Hazelnuts*

Individual in-shell hazelnuts (n = 200) were arranged onto sterile baking sheets (33 cm x 45 cm) and inoculated by dispensing 12.5 µl of the *Salmonella* cocktail onto the basal scar or middle shell surface of the hazelnut. Inoculated hazelnuts were air dried for 16-18 hrs., transferred to sterile sample bags, and stored at 4ºC for up to 2 weeks prior to steam treatment.

*4.3.5 Steam Blancher*

A pilot-scale steam blancher was designed and built by GEM Equipment of Oregon, Inc. (Mt. Angel, OR) for this study (Figure 1). The blancher utilized all T304 stainless steel construction with overall blancher treatment chamber dimensions being approximately 180 x 33 x 27.5 cm. The blancher was designed to mimic continuous inline feed conveyor systems designed to treat hazelnuts prior to packaging. An inverter (Sinamics V20 ; Siemens AG, Germany) was used to power and control the conveyor belt of the steam blancher. The feed conveyor consisted of a continuous stainless steel belt used to transport stainless steel hazelnut catches (30 x 30 x 15 cm tall with flat wire bottom; GEM Equipment) through a treatment vessel containing upper and lower manual valve steam pipes above and below the continuous belt. Two control timers (GT3A; IDEC, Sunnyvale, CA), a feed timer and dwell timer, were used to operate the feed conveyor of the steam blancher. A separate automated steam control valve – regulated by a thermocouple that measured the temperature of steam condensate inside the steam blancher – controlled the inlet of steam into the blancher, helping to regulate the vessel's temperature. Pressurized air connected to the automated steam inlet valve and regulated at 20 psi was used to open the automated steam control valve. Temperature in the steam blancher was regulated by a proportional-integral-derivative (PID) controller with a digital user interface (EZ-ZONE PM Express; Watlow, Winona, MN), which is a control-loop feedback mechanism commonly used in industrial control systems.

The manual valves on the upper and lower steam bars were used to influence the output and direction of steam. A feed timer was used to load hazelnut catches into the blancher. When the conveyor was turned on, hazelnut catches would move into the blancher based on the duration of this timer. We found a feed time of 10 s at maximum belt speed was optimum for quickly centering three baskets side-by-side in the blancher. A second dwell timer determined the length of time that baskets remained in the blancher before the conveyor restarted. A start/stop switch controlled the entire process. Turning on the selector switch engaged the conveyor forward for the length of time set by the feed timer. The conveyor would then stop for the length

of time determined by the dwell timer (0-15 minutes), before restarting and moving baskets forward and down an exit chute (outlet).

When the automated steam inlet valve opened, atmospheric steam (100°C) entered the treatment vessel and would begin to condense, raising the temperature of the treatment vessel. In turn, when the automated steam inlet valve closed, the temperature of the treatment vessel would drop. By monitoring the temperature and controlling the input of steam, the steam blancher was designed to calibrate the internal temperature of the treatment vessel, helping achieve targeted treatment temperatures.

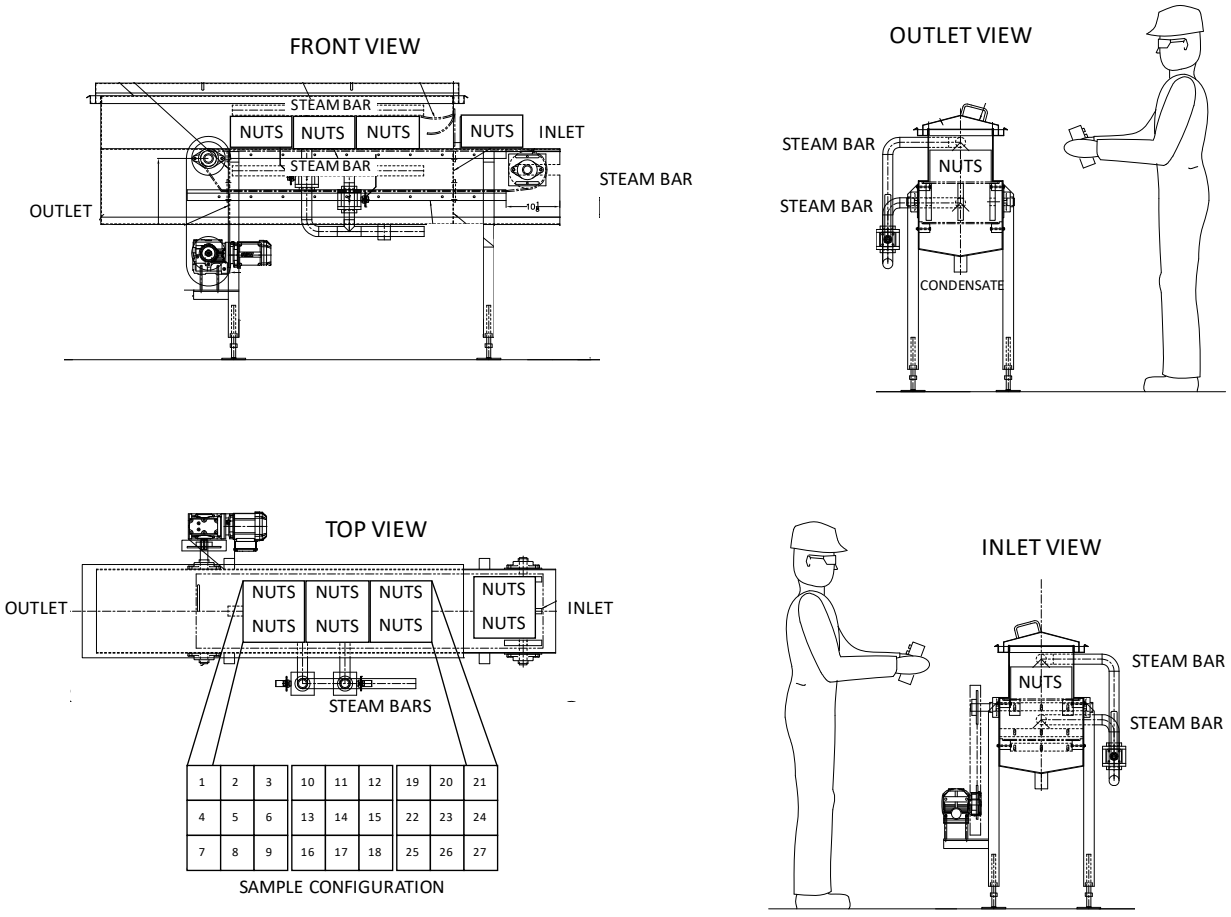



Figure 1. Pilot-scale steam blancher and sample configuration for in-shell hazelnut treatments. (Graphics courtesy of GEM Equipment of Oregon, Inc.)

*4.3.6 Sample Preparation and Arrangement*

Inoculated hazelnuts were removed from refrigerated storage at least 2 hours prior to steam treatment. Immersion-inoculated hazelnuts (50 g) were aseptically transferred to nylon mesh produce bags (Royal, Santa Fe Springs, CA). Spot-inoculated hazelnuts (12 g) were combined with uninoculated hazelnuts (38 g) in produce bags. Samples were placed into stainless steel boxes (30 cm x 30 cm x 15 cm) with flat wire bottoms divided into 9 compartments (9.85 cm x 9.85 cm x 15 cm) by four interlocking stainless steel plates (GEM Equipment). This configuration provided 27 unique sample positions in the chamber for a single steam treatment (Figure 2). For initial steam treatments, inoculated samples were assigned to six compartments (positions 4, 9, 11, 18, 19, 24) with the remaining positions being filled with ~200 g of bulk uninoculated hazelnuts. Later experiments had inoculated samples in all positions with a subset of samples being placed between two layers of bulk, uninoculated hazelnuts (~200 g each) to assess the impact of bed-depth on *Salmonella* cell survival. A temperature data logger equipped with a 559 mm stainless steel flexible probe (OM-CP-HITEMP140-PT; Omega Engineering, Stanford, CT) was placed in the center compartment of the middle basket (position 14) to record the temperature profile of the treatments. On a subset of treatments, additional temperature data loggers equipped with 50 mm stainless steel rigid probes (OM-CP-HITEMP140) or with a 131 mm flexible probe (OM-CP-HITEMP140-5.25) were placed in various positions to measure the temperature variability throughout the chamber.

Figure 2. Sample layout blancher: Location of catch compartments used for treatment validation

Orientation of hazelnut catches and compartments in the blancher

Back

Hazelnut catches (A-C)

| | A | | | B | | | C | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1[b] | 2 | 3 | 10 | **11** | 12 | **19** | 20 | 21 |
| Exit | **4**[a] | 5 | 6 | 12 | 14[c] | 15 | 22 | 23 | **24** |
| | 7 | 8 | **9** | 16 | 17 | **18** | 25 | 26 | 27 |

Entrance

Front

[a] Hazelnut catch compartments used for sample placement are shown in bold

[b] The other 21 compartments are blocked with approx. 200 g/each of uninoculated hazelnuts

[c] Data logger (OM-CP-HITEMP140-PT; Omega Engineering, Stanford, CT)

*4.3.7 Steam Treatments*

The steam blancher was set to a targeted calibrated temperature (79°C or 88°C) and allowed to self-calibrate for at least 20 min prior to treating samples. Once calibrated, the first steam basket (positions 1-9) was placed at the inlet of the steam vessel and the conveyor belt was initiated. The second and third baskets were placed in quick succession on the conveyor to minimize air space between the baskets. Hazelnuts were steam treated for 15 s to 15 min prior to exiting the steam blancher on the outlet end. Each time-temperature steam treatment combination was performed in triplicate.

*4.3.8 Microbiological Analysis*

Baskets were immediately recovered from the outlet of the steam blancher. The entire contents of the hazelnut samples (50 g) were aseptically transferred to sterile Whirl-Pak bags (250 ml; Nasco) and mixed with 50 ml of 0.1% peptone water. Samples were forcibly shaken by hand for 1 min. Serial dilutions were

prepared using 0.1% peptone water and plated in duplicate onto Hektoen Enteric agar (HE; Neogen) using a spiral plater (Autoplate 4000; Advanced Instruments, Norwood MA). Plates were enumerated following incubation at 37°C for 24-48 hrs. Spot-inoculated samples were recovered similarly to immersion-inoculated cells with the following exception. For sample treatments >3 min, the detection limit was improved by dividing 1 ml samples in two, then spread plating using two HE plates (0.5 ml/ HE plate) and incubated as described earlier. Samples were also enriched in lactose broth (200 ml) with incubation at 37°C for 24 h prior to streaking onto HE plates to qualitatively determine the presence or absence of surviving *Salmonella* cells in the entire sample. Colonies displaying typical *Salmonella* spp. morphology on HE (black, no acid production) were considered confirmed. Inoculated, untreated hazelnuts (n = 3) were serially diluted and plated as described above to determine initial populations (time = 0).

*4.3.9 Sensory Analysis*

Additional raw, in-shell hazelnuts were provided by the Hazelnut Marketing Board for sensory evaluation to evaluate the ability of the consumer to detect differences and evaluate attributes between untreated and steam-treated product. To protect consumers from potential cross-contamination from the laboratory, uninoculated, in-shell hazelnuts were processed using a secondary pilot-scale steam blancher (GEM Engineering) in the Oregon State University Food Science Pilot Plant. Hazelnuts were processed at 88°C for 8 and 15 minutes. A data logger (OM-CP-HITEMP) with a 175 mm flexible probe was used to verify temperature and time exposure to steam. Processed hazelnuts were dried at room temperature for 72 h then placed in unsealed cardboard boxes and transported to the Oregon State University Food Innovation Center (Portland, OR).

The sensory tests were conducted by a consumer panel (n = 58) recruited from a database that indicated them as tree nut consumers. A triangle test was conducted using two sets of samples: (i) untreated compared to steam treatment (8 min) and (ii) untreated compared to steam treatment (15 min). In a second session,

panelists were asked to complete an attributes acceptability test consisting of a 20 question ballot to rank attributes such as flavor, aroma, and color by level of acceptability.

*4.3.10 Statistical Analysis*

Triplicate data was used to plot survivor curves. Data were analyzed using GraphPad Prism software (GraphPad Software Inc.; LaJolla, CA). Linear regression analyses of survivor curves with 95% confidence intervals were used to predict D values and times required to achieve 5-log reductions of *Salmonella* on in-shell hazelnuts.

**4.4 Results**

Preliminary data was gathered using only the bottom steam bar (valve fully open). Used alone, steam from the bottom bar was inefficient at penetrating beds of hazelnuts up to 15 cm tall and results were variable making it hard to consistently achieve a 5 log reduction of *Salmonella.* Using both the bottom and top steam bars (valves fully opened) allowed steam to more effectively penetrate hazelnut bed layers from two sides and the variability in results, while not eliminated, was reduced helping to better predict and achieve treatment parameters (time and temperature) that achieve a minimum 5 log reduction of *Salmonella.* All subsequent studies were used using both steam bars (valves fully opened) and any treatments mentioned in this study should be assumed to use both steam bars unless otherwise specified.

*4.4.1 Reduction of Salmonella spp. after Steam Treatment*

Hazelnuts were initially inoculated with a *Salmonella* cocktail (~ 8.5 log CFU/g) following the inoculation methods of Danyluk et al. with minor modifications, as previously described, and subjected to a combination of treatment times and temperatures. At treatment temperatures of 79°C and 88°C, *Salmonella* population levels decreased overtime on in-shell hazelnuts; however, considerable variability was observed between steam treatment runs using the same operating parameters (time and temperature) (Figure 3 and Table 2). *Salmonella* population levels varied by as many as 4 log CFU/g between sample locations after treatments

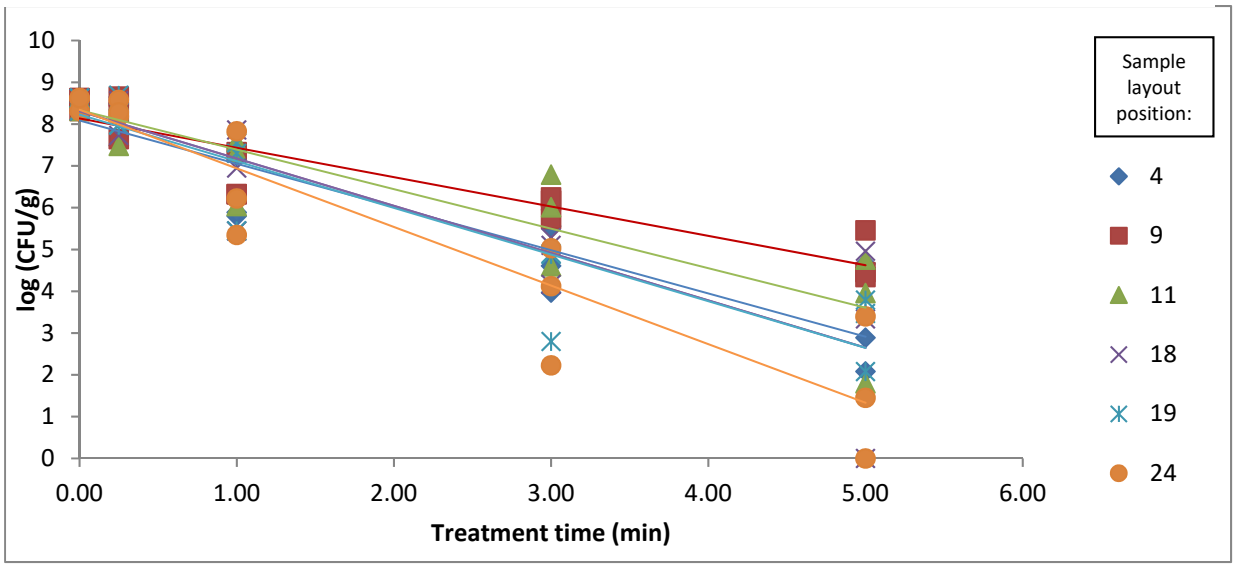
(Figure 3). At 88ºC, the predicted treatment times required to achieve a 5 log reduction of *Salmonella* were 7.67 min and 6.23 min for inoculated samples in locations 9 and 11, respectively, and 4.11 min for location 24 (Table 2). At 79ºC, predicted times to achieve a 5 log kill were longer, with estimates ranging from 8.99 min and 14.90 min for locations 9 and 11, respectively, to 6.39 min for test location 24 (Table 2). Due to treatments at 88ºC achieving a 5- log reduction of *Salmonella* in a shorter amount of time, and with no perceived negligible impact on final product quality (Tables 5 and 6), all further experiments were conducted at 88ºC.

Table 2. Estimated times required to achieve 1 and 5 log reductions of Salmonella with steam treatment at 88°C and 79°C

| Temperature (°C) | Estimate | Time required for 5 log reduction (min) | | | | | | D-value: time required for 1 log reduction (min) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample location | | | | | | Sample location | | | | | |
| | | 4 | 9 | 11 | 18 | 19 | 24 | 4 | 9 | 11 | 18 | 19 | 24 |
| 88 | Best fit | 4.34 | 6.15 | 4.98 | 4.18 | 4.16 | 3.43 | 0.87 | 1.23 | 1.00 | 0.84 | 0.83 | 0.69 |
| | 95% CI[a] | 5.26 | 7.67 | 6.23 | 5.50 | 4.99 | 4.11 | 1.05 | 1.53 | 1.25 | 1.10 | 1.00 | 0.82 |
| 79 | Best fit | 7.07 | 7.38 | 8.51 | 6.47 | 7.81 | 5.65 | 1.41 | 1.48 | 1.70 | 1.29 | 1.56 | 1.13 |
| | 95% CI[a] | 8.58 | 8.99 | 14.90 | 7.98 | 8.62 | 6.39 | 1.72 | 1.80 | 2.98 | 1.60 | 1.72 | 1.28 |

[a] Upper limit of confidence interval

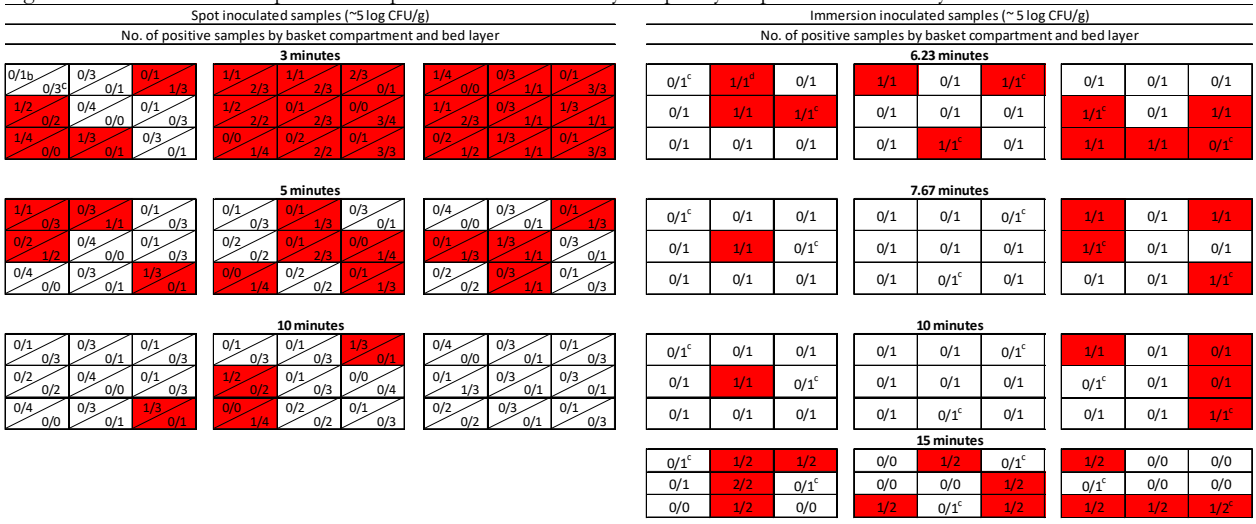
Figure 3. Survival of *Salmonella* spp. on immersion inoculated hazelnuts after exposure to steam at 88°C




*4.4.2 Temperature Profile of Steam Blancher*

Temperature was not uniform throughout the steam blancher during steam treatments. Mapping the temperature profiles of the six hazelnut catch compartments used to predict thermal death times with data loggers did not help to establish a map of potential hot and cold locations within the blancher (data not shown). Rather, temperatures in the blancher appeared to be dynamic and variable, varying by treatment run, and making it challenging to create a temperature profile of different sample positions within the blancher. Mapping the recovery frequency of *Salmonella* after enrichment of the 27 hazelnut catcher locations used for process verification also failed to distinguish a reliable temperature profile pattern or hot/cold spots for the steam blancher (Figure 4).

Figure 4. Ratio of *Salmonella* positive samples after enrichment[a] by sample layout position and bed layer after 88°C steam treatment




[a] Lactose enrichment (37°C 24 h)
[b] Single layer samples
[c] Center samples - 15 cm layer
[d] Red boxes indicate *Salmonella* positive samples

### 4.4.3 Endpoint Determination – 5 Log Verification

#### *4.4.3.1 Immersion Inoculated Hazelnuts*

Hazelnuts were inoculated by immersion into inoculum, as previously described, at 5 log CFU/g to verify results of plate counts and predicted times required to achieve 5-log reductions. Immersion inoculated hazelnuts were unable to consistently inactivate all *Salmonella* cells within a sample and did not adhere to the predicted reduction times derived using plate counts (Tables 2 and 3). At 88ºC, a conservative time of 7.67 minutes (location 9) was required to achieve a 5-log reduction of *Salmonella* (Table 2). However, *Salmonella* was recovered from 5/27 (18.5%) samples treated for 7.67 minutes and 13/54 (24.1%) samples treated for 15 minutes at 88ºC (Table 3). We postulated that the variability in treatments and discordance with the predicted times may have been caused by *Salmonella* cells penetrating split sutures (cracks) in the hazelnut shells during inoculation, which in turn may have harbored and protected *Salmonella* cells from steam contact during steam treatment. To test this, we began spot-inoculating hazelnuts.

Table 3. Qualitative reduction of immersion-inoculated 5 log CFU/g of *Salmonella* spp. on in-shell hazelnuts treated in the steam blancher.

| Sample | Number of positive samples after steam treatment (88°C set point) | | | |
|---|---|---|---|---|
| | 6.2 min | 7.7 min | 10 min | 15 min |
| Single Layer | 6/21 (28.5%) | 3/21 (14.3%) | 2/21 (9.5%) | 12/42 (28.6%) |
| Center Layer[b] | 3/6 (50%) | 2/6 (33.3%) | 1/6 (16.7%) | 1/12 (8.3%) |
| **Combined** | **9/27 (33.3%)** | **5/27 (18.5%)** | **3/27 (11.1%)** | **13/54 (24.1%)** |

[b] Center layer samples were placed in the middle of two layers of uninoculated in-shell hazelnuts. Total depth of the product was 15 cm.

*4.4.3.2 Spot Inoculated Hazelnuts*

Spot inoculated hazelnuts (~ 5 log CFU/g) were able to consistently achieve a 5-log inactivation of *Salmonella* at 88°C for 10 minutes (Table 4). Spot inoculated hazelnuts showed a more consistent reduction of *Salmonella* cells over time compared to immersion inoculated hazelnuts (Tables 3 and 4). At treatment durations of 3 and 5 minutes, hazelnut bed layer had a significant impact on the reduction levels of *Salmonella* after steam treatment, with center layer samples (15 cm layer) exhibiting a higher recovery frequency of *Salmonella* compared to single layer samples (Table 4). At 3, 5, and 10 min treatment durations, the frequency of *Salmonella* recovery for single layer samples after lactose enrichment were 12/54 (22.2%), 3/54 (5.56%), and 3/54 (5.56%), respectively (Table 4). In contrast, *Salmonella* was recovered from center layer samples at a frequency of 31/54 (57.4%), 12/54 (22.2%), and 3/54 (5.6%) for treatment times of 3, 5, and 10 min, respectively (Table 4). At ten minutes, the hazelnut bed layer had no significant impact on the recovery of *Salmonella* after steam treatment (Table 4).

Table 4. Qualitative reduction of spot-inoculated 5 log CFU/g of *Salmonella* spp. on in-shell hazelnuts treated in the steam blancher.

| Sample Placement | Number of hazelnut samples positive for *Salmonella* spp. after steam treatment (88°C set point) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 min | | | 5 min | | | 10 min[a] | | |
| | Basal Scar | Shell | Combined | Basal Scar | Shell | Combined | Basal Scar | Shell | Combined |
| Single Layer | 8/28 (28.6%) | 4/26 (15.4%) | 12/54 (22.2%) | 1/28 (3.6%) | 2/26 (7.7%) | 3/54 (5.6%) | 1/28 (3.6%) | 2/26 (7.7%) | 3/54 (5.6%) |
| Center Layer[b] | 19/26 (73.1%) | 12/28 (42.9%) | 31/54 (57.4%) | 8/26 (30.8%) | 4/28 (14.3%) | 12/54 (22.2%) | 2/26 (7.7%) | 0/26 (0%) | 2/54 (3.7%) |
| Combined | 27/54 (50%) | 16/54 (29.6%) | 43/108 (39.8%) | 9/54 (16.7%) | 6/54 (11.1%) | 15/108 (13.9%) | 3/54 (5.5%) | 2/54 (3.7%) | 5/108 (4.6%) |

[a] All positives for the 10-minute treatment were from the same treatment replicate.

[b] Center layer samples were placed in the middle of two layers of uninoculated in-shell hazelnuts.

The inoculation surface (basal scar or shell) had a significant impact on the recovery frequency of *Salmonella* after steam treatment for 3 min at 88ºC. *Salmonella* was recovered from 27/54 (50.0%) of the basal scar inoculated samples and 16/54 (29.6%) of the shell inoculated samples treated at 88ºC for 3 min (Table 4). At longer treatments of 5 and 10 min at 88ºC, the inoculation surface did not have a significant impact on *Salmonella* recovery (Table 4). Table 4 illustrates how the combination of inoculation surface and hazelnut bed layer significantly impacted the recovery frequency of *Salmonella* for 3 min treatments at 88ºC. At treatment times of 3 min, center layer samples (15 cm layer) inoculated on the basal scar of in-shell hazelnuts had a *Salmonella* recovery rate of 19/26 (73.1%); compared to a 4/26 (15.4%) recovery rate for single layer samples that were spot inoculated on the shell surface (Table 4).

*4.4.5 Sensory Analysis*

Volunteer participants in a sensory test conducted by the Oregon Food Innovation Center (Portland, OR) were unable to distinguish steam processed hazelnuts at 88ºC for 8 and 15 minutes from unprocessed hazelnuts. In a difference test (triangle), 45 out of 58 consumers (78%) could not tell a significant difference (p-value 0.975) between hazelnuts treated at 88ºC for 8 min and control hazelnuts (Table 5). However, only 60% of consumers could not tell a difference (p-value 0.188) between 15 min treated samples and controls (Table 5). The attribute acceptability levels were similar for all treatments except for aroma and color based on an attributes acceptability test consisting of a 20 question ballot (Table 6). Consumers favored the aroma of steam treated samples for their "roasted" aroma and steam treated samples appeared somewhat darker in color (Table 6).

Table 5. Ability of consumers to distinguish steam treated hazelnuts from untreated control samples

| Difference (Triangle) Test | | |
|---|---|---|
| | Control vs. 8 minutes | Control vs. 15 minutes |
| No. of judgments: | 58 | 58 |
| Incorrect: | 45 | 35 |
| Correct: | 13 | 23 |
| Confidence: | 0.025 | 0.812 |
| **Significance[a] (p-value):** | **0.975** | **0.188** |

Table 6. Sensory properties of hazelnuts treated by steam blanching at various holding times. Values presented are the mean score (n = 100). Values within a row with different superscript capital letters are significantly different ($p < 0.05$).

| Parameters | Steam treatment time (88°C set point) | | |
|---|---|---|---|
| | Untreated | 8 min | 15 min |
| Appearance[a] | 3.68 ± 1.07 | 3.69 ± 1.13 | 3.43 ± 1.21 |
| Color[b] | 3.06 ± 0.47$^{A}$ | 3.12 ± 0.56$^{AB}$ | 3.28 ± 0.53$^{B}$ |
| Crunchiness[e] | 2.64 ± 0.72 | 2.84 ± 0.73 | 2.72 ± 0.71 |
| Flavor Strength[c] | 2.51 ± 0.76 | 2.52 ± 0.72 | 2.59 ± 0.77 |
| Sweetness[d] | 2.47 ± 0.69 | 2.49 ± 0.70 | 2.41 ± 0.73 |
| Texture[f] | 2.81 ± 0.65 | 2.85 ± 0.56 | 2.93 ± 0.71 |
| Liking[g]: | | | |
| Aroma | 5.39 ± 0.84$^{AB}$ | 5.33 ± 0.82$^{B}$ | 5.47 ± 0.87$^{A}$ |
| Flavor | 6.81 ± 1.57 | 6.73 ± 1.66 | 6.77 ± 1.46 |
| Texture | 6.20 ± 1.88 | 6.43 ± 1.72 | 6.16 ± 1.85 |
| Overall | 6.75 ± 1.67 | 6.75 ±1.67 | 6.60 ± 1.61 |
| Purchase Intent[h] | 3.49 ± 1.09 | 3.49 ± 1.12 | 3.39 ± 1.25 |

[a] Appearance was evaluated on a scale from 1 (very unappealing) to 5 (very appealing).
[b] Color was evaluated on a scale from 1 (much too light) to 5 (much too dark).
[c] Flavor strength was evaluated on a scale from 1 (much too weak) to 5 (much to strong).
[d] Sweetness was evaluated on a scale from 1 (not at all sweet enough) to 5 (much too sweet).
[e] Crunchiness was evaluated on a scale from 1 (not at all crunchy enough) to 5 (much too crunchy).
[f] Texture was evaluated on a scale from 1 (much too soft) to 5 (much too hard).
[g] Liking was evaluated on a scale from 1 (dislike extremely) to 9 (like extremely).
[h] Purchase intent was evaluated on a scale from 1 (would definitely not buy) to 5 (would definitely buy).

## 4.5 Discussion

### *4.5.1 Steam Treatment Efficacy – This Study Compared to Previous Studies*

The primary objective of thermal pasteurization of foodstuffs is to inactivate pathogenic and spoilage microorganisms to produce a shelf-stable product in which the original properties of the raw material are retained as much as possible (Da Silva et al. 2009). In this study, we show that steam treatment at 88ºC for 10 minutes achieves a 5-log reduction of *Salmonella* on in-shell hazelnuts with negligible and minimal impact on final product quality.

Several similar studies have evaluated the efficacy of steam pasteurization at inactivating *Salmonella* Enteritidis on the surface of inoculated raw almond kernels with conflicting results. Using the same variety of almonds (Nonpareil) and same SE strains (*S*. Enteritidis 43353, ME-13, ME-14), Chang et al. (2010) observed a 5-log reduction of *Salmonella* after 25-s exposure to steam at 143 kPa, whereas Lee et al. (2006) was unable to achieve a 4-log reduction even after 35-s exposure to atmospheric steam. Chang et al. (2010) attributed the disparity in results to differences in the condition of steam applied, noting that the effectiveness of steam treatment on *S*. Enteritidis is dependent on the type of steam technology used for treatment, with pressurized and atmospheric steam treatments yielding different results (Chang et al. 2010). Chang et al. (2010) used a custom built steam pasteurizer with a pressurized steam treatment chamber, whereas Lee et al. (2006) used conventional steaming by placing inoculated almonds directly above boiling water. The enhanced reduction of *S*. Enteritidis observed by Chang et al. (2010) may be because of rapid increases in temperature within the enclosed chamber compared to heat dissipation in open air with conventional steaming. Both authors noted that prolonged exposure to steam (35 s) had negative impacts on the quality of almond kernels, resulting in increased moisture content and loss in visual quality. In a separate study, a combination of superheated steam (115ºC) for 70 s followed by infrared heating for 70 s completely eliminated *Salmonella* Enteritidis without resuscitation in enrichment (Bari el al. 2010) and with minimal impact on product quality. Due to the dissipation of heat in an open system, steam blanching at atmospheric pressure typically requires longer exposure times to steam to sufficiently inactivate *Salmonella* on tree nuts than pressurized or

superheated steam processes which require an enclosed treatment chamber. However, inline continuous atmospheric steam blanching systems often are able to more efficiently treat tree nuts and have a greater product throughput rate than batch style pasteurization systems that use enclosed chambers to administer pressurized and superheated steam. In addition, prolonged atmospheric steam blanching of in-shell hazelnuts does not appear to affect the final product quality.

There is a limited understanding of the heat resistance of *Salmonella* spp. on hazelnut shells. *Salmonella* Oranienburg and *S*. Enteritidis PT30 were extremely resistant on dry (4% w/w moisture), crushed (1mm) hazelnut and cocoa shells, with $D_{100^{o}C}$ values ca. 2.5 min in crushed cocoa bean shells and 7-11 min in crushed hazelnut shells, respectively. Addition of moisture to ca. 7% w/w markedly reduced D-values ($D_{80^{o}C}$ of 2-4.5min) for both strains in the two matrices (Izurieta et al. 2012). Heating in hazelnut shells resulted in significantly higher ($p<0.05$) D-values than in cocoa bean shells, possibly due to the matrix effect and possibly cocoa shell components, such as polyphenols and flavonoids. However, it is worth noting that crushed cocoa bean shells had significantly higher ($p<0.05$) z-values than in crushed hazelnut shells (Izurieta et al. 2012). The heat resistance of *S*. Oranienburg and *S*. Enteritidis PT30 under dry conditions (4% w/w) gives rise to concerns about the efficacy of mild or dry roasting conditions that may be insufficient to eliminate *Salmonella* in nut processing (Izurieta et al. 2012). Inactivation of *Salmonella* using steam helps decrease the heat resistance of *Salmonella* on low moisture foods by increasing the moisture content. During preliminary work, we found the moisture content of in-shell hazelnuts to be ca. 10% w/w after exposure to steam (88ºC) for 1 min (data not shown).

*4.5.2 Steam Treatment Efficacy – Variability at Pilot Scale*

At shorter treatment times (1 to 3 min) the efficacy of the steam blancher at reducing *Salmonella* levels was inconsistent, varying by steam run. This inconsistency is partly explained by temperature variations within the steam blancher during steam treatment runs. In addition to temperature fluctuations, there is likely unequal dispersal of steam condensate in the steam blancher. Jeong et al. (2009) found moisture status at the surface

of almonds rather than the humidity of the bulk air was the primary factor controlling the rate of *Salmonella* in a moist-air convection heating process. Fluctuations of steam flow within the pilot-scale steam blancher may have led to unequal dispersal of moisture among inoculated samples which would likely influence the inactivation rates of *Salmonella* spp. While longer treatment times are able to reduce the inconsistences in *Salmonella* inactivation, commercial level hazelnut steam blanchers should be better optimized to have more consistent temperatures and even steam flow.

*4.5.3 Predicted Model versus Verification Results*

In this study, we were able to demonstrate that steam treatment at 88ºC for10 minutes achieved a 5- log reduction of *Salmonella* spp. on in-shell hazelnuts. However, the 10 min required to achieve a 5-log reduction is longer than the predicted 7.67 minutes (Table 2), and considerably longer than seen in other studies using steam to inactivate *Salmonella* on almonds (Chang et al. 2010; Lee et al. 2006; Bari et al. 2010). A number of factors can affect the inactivation and recovery of *Salmonella*. The effectiveness of steam treatment is dependent on the condition of steam applied (Chang et al. 2010), and factors such as inoculation method, drying time, growth medium and treatment affect *Salmonella* recovery (Lang et al. 2004). Factors that affect *Salmonella* inactivation and recovery should be carefully considered when designing thermal validation studies and comparing validation processes.

*4.5.4 Physical Characteristics of the Nut and Immersion Inoculation versus Spot Inoculation*

Inoculation method, drying time and treatment affect *Salmonella* recovery (Lang et al. 2004). Two methods of inoculating in-shell hazelnuts were used in this study – immersion and spot inoculation. Initially, we used an immersion inoculation procedure designed for almond kernels to derive D-values, but later found that liquid inoculum likely penetrated small cracks in the hazelnut shells, potentially protecting *Salmonella* cells from exposure to steam and making results unpredictable and inconsistent. Spot inoculation was used for enriched samples to verify inactivation of *Salmonella* after immersion inoculated samples. Studies suggest that immersion inoculation provides the most rigorous test of chemical sanitizer efficacy. *Salmonella* inoculated on

egg shells was more resistant to chemicals when eggs were inoculated by immersion vs. spot inoculation or fecal smear (Musgrove et al. 2010). Higher levels of *E.* coli were recovered on lettuce leaves using immersion inoculation compared to spot inoculation (Singh et al. 2010). Lang et al. (2004) recommend spot inoculation with a drying time of 24 h at 22ºC for inoculation of tomatoes. They found significantly larger populations of *Salmonella* were recovered from the surface of raw tomatoes subjected to water and chlorine washes that were inoculated by immersion compared to spot and spray inoculated treatments. The observed differences were attributed to the larger number of cells adhering to the surface of tomatoes during immersion inoculation (Lang et al. 2004). Similar to these studies, we recovered significantly higher levels of *Salmonella* after exposure to steam from immersion inoculated hazelnuts compared to spot inoculated hazelnuts (data not shown).

*4.5.5 Sublethally-Injured Foodborne Pathogens*

Sublethally-injured foodborne pathogens pose a serious food safety risk. Selective media such as HE contain agents that can inhibit heat-injured *Salmonella* from growing, whereas tryptic soy agar (TSA) does not. To facilitate recovery of heat-injured *Salmonella* cells while also providing selectivity of isolation of *Salmonella* form other bacteria, several methods use a combination of TSA and selective media. A traditional overlay method (OV) involves pouring selective agar on top of resuscitated cells on TSA agar 3-4 h after incubation. A thin agar layer (TAL) procedure consists of pouring nonselective media (TSA) onto a solidified selective (XLD) medium in a petri dish. In both methods, heat-injured Salmonella cells are resuscitated with TSA and a selective medium such as XLD or HE is used for the isolation of *Salmonella.*

In the current study, the selective medium HE was used for plate counts. Thus, heat-injured *Salmonella* cells may have survived steam treatment and not been recovered on HE media plates. The inability to recover heat injured *Salmonella* using HE may have led to artificially low plate counts and an underestimation of D-values and time required to achieve a 5-log reduction of *Salmonella.* In addition, any *Salmonella* surviving below the limit of detection, would also not be seen on HE, but would be resuscitated after enrichment in

lactose, helping further underestimate the predicted time required to achieve a 5- log reduction. Studies have compared the use of selective and nonselective media on the recovery of *Salmonella*. Lee et al. (2006) found no significant difference in recovery of *Salmonella* on OV XLD and XLD. Counts on BSA were consistently, but not significantly lower than on TSA (Harris et al. 2012). In contrast, Bari et al. (2011) found significant differences in plate counts on TSAR vs BSAR, suggesting superheated steam (115ºC) may thermally injure *Salmonella*. In the current study, sublethally injured *Salmonella* cells may have survived at shorter treatment times and potentially skewed D-values. However, after a 10 minute steam treatment no survivors were found in enrichment medium, suggesting *Salmonella* spp. cells were completely inactivated after 10 minutes.

**4.6 Conclusion**

Steam is an effective and practical alternative for inactivating *Salmonella* spp. on in-shell hazelnuts. A 5-log reduction of *Salmonella* spp. can be achieved after 10 min at 88ºC in a steam blancher without deteriorating final product quality.

**4.7 Acknowledgements**

Funding for this study was provided by the Oregon Hazelnut Marketing Board. The pilot-scale steam blancher was designed and built by GEM Equipment of Oregon, Inc. The authors would like to thank Dr. Linda Harris at University of California, Davis for providing *Salmonella* strains used in this study. The authors would like to additionally thank Alex Emch, Daniel Wright, and Joey Minarsich for media and sample preparation.

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**5. Overall Conclusion**

The aim of this research was to help the Oregon hazelnut industry characterize the hazards of *Salmonella* contamination associated with in-shell hazelnuts and to evaluate the efficacy of steam blanching as a postharvest thermal inactivation step for *Salmonella* spp. on the surface of in-shell hazelnuts. Hardly any prior studies have been conducted on hazelnuts to assess their biological safety and postharvest treatments used to inactivate *Salmonella* and other foodborne pathogens. Therefore, this research consisted of a two-year *Salmonella* prevalence survey, quantified contamination on in-shell Oregon hazelnuts, and determined a steam blanching treatment process that would reduce contamination while maintaining quality of in-shell hazelnuts.

The average prevalence of *Salmonella* on in-shell hazelnuts (33.3%) over the 2013 and 2014 harvests is dramatically higher than seen on other tree nuts (~1.0%). It is rather dramatic and disconcerting from an industry standpoint to see such high prevalence levels of *Salmonella* on in-shell hazelnuts compared to other tree nuts, and raises concerns about harvest and postharvest handling practices that may contribute to *Salmonella* contamination. There are a number of points during the harvest and postharvest handling of hazelnuts when *Salmonella* could feasibly be introduced, making it difficult to identify and mitigate sources of contamination. However, it is our opinion that ineffective postharvest washing procedures likely contributed to cross contamination of in-shell hazelnuts resulting in the high prevalence levels of *Salmonella* observed. Effective washing procedures should be used to ensure hazelnuts are clean and of high quality prior to market distribution, but washing should not be used as a standalone postharvest treatment for the inactivation of *Salmonella*. In addition to washing, validated postharvest treatments such as steam treatment should be used to ensure 5-log reductions of *Salmonella* on in-shell hazelnuts.

Steam treatment is a practical technology for the inactivation of *Salmonella* on raw, in-shell hazelnuts. We were able to demonstrate that a continuous steam treatment at 88°C for 10 min achieved a 5-log reduction of *Salmonella* spp. on in-shell hazelnuts with minimal impact on final product quality. For many low-moisture foods, including almond kernels, prolonged exposure to steam may have detrimental effects on product

quality, limiting the application of steam as a cost-effective postharvest thermal process. However, prolonged exposure to steam had a negligible impact on the product quality of in-shell hazelnuts, with consumers actually favoring the "roasted" flavor and aroma of in-shell hazelnuts treated with steam at 88°C for up to 15 min in a consumer sensory test conducted by the Food Innovation Center (Portland, OR). The fact that in-shell hazelnuts can be exposed to steam for prolonged periods without degradation of product quality indicates that treatment parameters that achieve a 5-log reduction of *Salmonella* without affecting product quality should be achievable at an industry level. In addition, inline continuous steam blanchers can be incorporated into existing hazelnut processing lines. Steam treatment technology shows promise of application to Oregon hazelnut processors in the form of a flexible technology for the inactivation of *Salmonella* spp. and other foodborne pathogens on the surfaces of in-shell hazelnuts.