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Effect of hot water conditioning on microbial safety and quality of in-shell pecans

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EFFECT OF HOT WATER CONDITIONING ON MICROBIAL SAFETY AND
QUALITY OF IN-SHELL PECANS

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ABSTRACT

Hot water conditioning of in-shell pecans is one common practice followed by industries to facilitate shelling. It also acts as a preventive control for potential microbiological contamination that might occur during pre- and post-harvest processes. However, heat treatment may have an effect on the eating quality of nuts. The main objectives of the study were to develop a post-harvest hot water treatment intervention as a kill step to destroy foodborne pathogens on in-shell pecans and evaluate the effect of treatments on physicochemical properties, consumer acceptance and purchase intent of dehulled and roasted pecans. The time (1-5 min) and temperature (70, 80, and 90 ℃) treatments to achieve a 5-log reduction of *Salmonella enterica*, *E. coli* O157:H7 and *Listeria monocytogenes* as well as non-pathogenic *Enterococcus faecium* were studied. The D-value of organisms showed that *Salmonella* and *Listeria* were the most and least resistant pathogens, respectively, and *Enterococcus faecium* was found to be the suitable surrogate for *Salmonella enterica*. As calculated from the D-value, hot water treatment for 8.6, 6.6 and 4.6 min at 70, 80 and 90°C, respectively, gave 5 log CFU/g reduction of the most heat resistant pathogen. In-shell nuts were subjected to these treatments, shelled and roasted at 160°C for 10 mins. The effect of hot water treatment on the physicochemical properties (% moisture content, water activity, color, and texture) of the roasted pecans was determined. Sensory evaluation studies using a 9-point hedonic scale were performed by serving the samples of roasted pecans to consumers (N=112). Hot water treatment alone had no significant effect on the physicochemical properties of shelled pecans. However, roasting the treated pecans decreased the moisture content ($P<0.05$), water activity ($P<0.05$) and hardness values ($P>0.05$). Hot water treated pecans became darker on roasting which was liked by consumers; pecan hot water treated at 90°C was the darkest with

lowest L* value ($P<0.05$). Pecans hot water treated at 70°C for 8.6 min followed by roasting were most liked by the consumers (liking >6.3 for all attributes). Thus, hot water conditioning of pecans is an effective method as it improves its microbial safety, quality and sensory liking.

1. INTRODUCTION

Pecans are one of the tree nuts that are native to North America with a history dating back to 5000 year (Beuchat & Pegg, 2013). It is scientifically known as *Carya illinoinensis* (Wangenh.) K. Koch and is a member of hickory family (Juglandaceae) (Maness, 2016). Pecan production increased from 184 million in 1980 to an estimated 255 million pounds in 2015 (USDA, 2016). And, on an average, production of pecans contributes approximately $12 million to Louisiana's economy each year (LSU AgCenter, 2017).

Contamination on in-shell pecans and nutmeats can occur in pre-harvest, during harvesting and throughout post-harvest handling and processing(Beuchat & Pegg, 2013). Pecan harvesting includes shaking the tree or naturally allowing the pecans to drop on the ground which rests there for several days until collected(Beuchat & Mann, 2010b; Brar, Strawn, & Danyluk, 2016). Pecans absorb moisture from soil that can be potentially contaminated with food-borne pathogens from wild and domestic animal feces, inadequately composted manure, irrigation or run-off water from land grazed by livestocks (Beuchat & Mann, 2010b). Cattle grazing in the orchards is still practiced in some parts of the U.S.(Beuchat & Pegg, 2013) and cattle manure has been found to be the main source of foodborne pathogens such as *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* (Alam & Zurek, 2006; Pell, 1997). To date there have not been any outbreaks associated with pecans but it has been associated with frequent recalls for potential *Salmonella* contamination (Beuchat & Mann, 2010b; Brar et al., 2016).

A conditioning step is one of the essential pecan processing steps as it is carried out to reduce kernel breakage and improve shelling efficiency. Hot water soaking (3-5 mins at 85°C, holding 12-24 hr before cracking) is one of several ways it can be carried out (Santerre, 1994c). In addition, hot water conditioning can also aid in the decontamination of pecans (Beuchat &

Mann, 2011a). Treating the in-shell pecans with hot water at 82 or 93°C for 2 mins was unable to eliminate the *Salmonella* Senftenberg 775W inoculated at 5.8 log CFU/g, it was only reduced by 3.5 log CFU/g (Beuchat & Heaton, 1975). Recent studies on the effect of conditioning on inactivation of surface-inoculated *Salmonella* on pecans showed that the treatment at 90 or 95°C for 80 s was able to achieve a reduction of ≥6.42 log CFU/g. However, treatment at 95°C for 5 mins could reduce the immersion-inoculated load by only ≥4.82 log CFU/g but prolonging the treatment to ≥10 mins at 75 to 95°C gave reduction >5.12 log CFU/g (Beuchat & Mann, 2011a). This showed that inoculation methods affected the level of reductions achieved. There are studies carried out to evaluate the efficacy of hot water treatment on inactivation of *Salmonella* on various nuts like almonds and pecans. However, its efficacy against other potential pathogens as *E. coli* O157:H7 and *Listeria monocytogenes* are yet to be studied.

Even though hot water treatment is advantageous in numerous ways, application of heat on nuts can cause degradation of its quality by bringing change in its physicochemical and sensory properties. Thus, developing new technologies and processing parameters that improve microbial safety of nuts without affecting the quality of raw products is essential. A study on hot water treatment (85°C for 40s) of almonds demonstrated that even though the moisture content increased after hot water treatment, there was no significant difference in the color. However, non-treated almonds were firmer than hot water treated but the overall quality for both treated and non-treated was within acceptable limits(Bari et al., 2009). There are limited studies determining the physicochemical changes pertaining to hot water treatment of pecans and its consumer acceptance.

Thus, the objectives of the research were: (i) to determine optimum hot water treatment conditions to achieve a 5 log reduction of several foodborne pathogens/surrogates, (ii) to determine the rate of thermal lethality of tested organisms, (iii) to determine the effect of heat treatment of

pecans on physico-chemical properties, and iv) to evaluate consumer acceptability and purchase

intent of the treated pecans.

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2. LITERATURE REVIEW

2.1   General Introduction

2.1.1   Pecans

Pecans [*Carya illinoinensis* (Wangenh.) K. Koch], a member of hickory family (Juglandaceae) (Maness, 2016) are native to North America with a history dating back 5000 years (Beuchat & Pegg, 2013). Pecan production increased from 184 million in 1980 to an estimated 255 million pounds in 2015 (USDA, 2016) . According to the annual 2016 Agricultural survey by USDA, the major states producing pecans in 2015 were Georgia (89 million pounds), New Mexico (72 million pounds), Texas (28 million pounds), Arizona (22.5 million pounds), Oklahoma (20 million pounds) and Louisiana (11 million pounds)(USDA, 2016b). And, on an average, production of pecans contributes to approximately \$12 million to Louisiana's economy each year (LSU AgCenter, 2017).

Major pecan cultivars that are commercially produced are 'Western', 'Desirable', 'Stuart', 'Wichita' and 'Pawnee'. Pecan trees are mechanically shaken and the nuts are harvested from the orchard floor during late fall and early winter. On maturation of seed, the shuck that surrounds the edible seed opens where pecan halves are covered by the shell. The color of the pecan kernels range from yellow-golden to light-brown. Nuts become brittle if the moisture content is <2% thereby causing breakage of kernel during handling and storage. This induces cracking of testa which results in oxygen permeation and thus supports rancidity in nuts. So, it should be dried to a moisture of <4% so as to avoid mold development and check development of rancidity. Pecan nuts are considered to be matured when the accumulation of oil is complete and shucks split.(Maness, 2016).

In general, a mature pecan kernel composes of 70% oil (ranges from 55-75%), 10% protein (varies from 8-18%), 10% easily available carbohydrate, 2.5% water and 7.5% fiber and ash(Santerre, 1994b). The oil content of pecan kernels range from 55-75% and as the oil content increases there is decrease in shelf-life and vice-versa and the amount of oil varies as per the variety, location and the year grown (Wells & McMean, 1978). Kernel oil is composed mainly of 16 and 18 carbon chain fatty acids with 0-3 double bonds(Santerre, 1994b). High amount of mono unsaturated fatty acids (MUFAs) and low amount of saturated fatty acids in kernel oils are linked with reduction in risk of heart diseases (Beuchat & Pegg, 2013; Rajaram, Burke, Connell, Myint, & Sabate´, 2001). Pecans also have a high antioxidant capacity against free radicals due to the presence of phenolic compounds, condensed tannins and hydrolysable tannins (FloresCordova et al., 2017). Studies have shown the potential of phenolics to lower the frequency of several chronic diseases like cancer, Alzheimer's disease, Parkinson's disease and other degenerative diseases (Mertens-Talcott & Percival, 2005; Tam et al., 2006).

2.1.2   Food safety risks: potential on-farm sources of contamination

Pecans are highly valued and nutritious nuts, however, contamination on in-shell pecans and nutmeats can occur in pre-harvest, during harvesting and throughout post-harvest handling and processing (Beuchat & Pegg, 2013). Pecan harvesting includes shaking the tree or naturally allowing the pecans to drop on the ground which rests there for several days until collected (Beuchat & Mann, 2010b; Brar et al., 2016). Pecans can absorb moisture from soil that can be potentially contaminated with food-borne pathogens from wild and domestic animal feces, inadequately composted manure, irrigation or run-off water from land grazed by livestock (Beuchat & Mann, 2010b).

Even though there is raised awareness of potential risk of food-borne pathogen to the nuts from grazing cattle in the orchards, the practice is still prevalent in some regions of U.S. (Beuchat & Pegg, 2013). It is one of the most common forms of ground cover management in native pecan groves. The advantages of cattle grazing are it provides a second source of income from the same parcel of land (i.e. pecans and beef as meat source) and a significant reduction in orchard mowing costs. However, cattle manure is the main source of foodborne pathogens such as *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* (Alam & Zurek, 2006; Pell, 1997). In an almond associated outbreak in 2000-2001, the orchard floor was found to be one of the potential sources for *Salmonella* contamination (Brar et al., 2016). It is recommended that the pecan orchards be kept free from grazing domesticated animals, clean and free of debris as much as possible (Santerre, 1994a). A study done by Marcus and Amling (1973) found that higher levels of *E. coli* on pecans samples from a cattle-grazed farm than non-grazed farm. Reportedly, 23% of pecans from grazed orchards and 4% from non-grazed orchard were positive for *E. coli.*

During rainfall, shucks along with pecans get wet and thus can be one of the potential sources for microbial growth. Studies have shown that *Salmonella* survived on the surface of high-moisture shucks owing to higher amount of sugar, protein, oil and suitable pH (6.08) in it favoring microbial growth. When the nuts mature, four shucks surrounding the in-shell pecans dry and eventually fall on the ground. *Salmonella* has been found to survive on dry soil for up to 18 weeks posing a potential risk of contamination. During heavy rainfall, shuck components can leach out and get mixed in the soil. Studies have shown that when almond hull extract was added on orchard soil it promoted the growth of *Salmonella.* But, pecan shuck extracts on soil showed an opposite effect on the pathogen which could be because of low concentrations of water-soluble nutrients, an acidic pH, higher concentration of poly-phenolic compounds and antimicrobials in the extract.

Likewise, studies have also shown the growth of *Salmonella* on high moisture nutmeats, in-shell pecans and shells(Beuchat & Mann, 2010b). Thus, pecans should be minimally exposed to water in pre-harvest and post-harvest environments and should be dried rapidly to appropriate moisture content to prevent bacterial, mold growth and rancidity.

2.1.3   Outbreaks in nuts

Low water activity foods are an unsuitable growth medium for bacteria, however, they have been frequently linked with recalls and outbreaks(Zhang et al., 2017). Recalls of low water activity foods by U.S. Food and Drug Administration from 2004-2011 showed that 43.6% of the total recalls was nut, seeds or nut products. Various nuts that were recalled included almonds, hazelnuts, peanuts, pecans, pine nuts, pistachios and walnuts (Beuchat, Mann, & Alali, 2013). *Salmonella* was detected in pecans, peanuts, almonds (Center for Disease Control and Prevention, 2004; Isaacs et al., 2005), pistachios, walnuts and pine nuts(Center for Disease Control and Prevention, 2011b), *Escherichia coli* O157:H7 in peanuts, pecans, hazel nuts (Center for Disease Control and Prevention, 2011a), cashew nuts and walnuts, and *Listeria monocytogenes* in peanuts, pecans and mixed nuts(Beuchat et al., 2013; Brar, Proano, Friedrich, Harris, & Danyluk, 2015; Zhang et al., 2017). Nut outbreaks are most commonly associated with pathogens like *Salmonella, E. coli* O157:H7 and *Listeria monocytogenes* (Zhang et al., 2017). Among all, *Salmonella* has been found to be the major recurring organism for outbreaks and recalls and many of the outbreaks have lasted for months (Brar et al., 2015).

Currently there have been no outbreaks of food borne illness associated with pecans however, pecans have been recalled for potential *Salmonella* contamination(Beuchat & Mann, 2010b; Brar et al., 2016). There were 25 recalls issued in 2015 in United States because of

Salmonella contamination where pecans was one among walnuts, macadamia nuts, pine nuts, almonds and hazel nuts(Zhang et al., 2017).

Various outbreaks associated with nuts and nut products and the organisms associated for the disease are: raw shelled and in-shell walnuts (*E. coli* O157:H7), peanut butter (*Salmonella* Bredeney, *Salmonella* Typhimurium,*Clostridium botulinum, Salmonella* Tennessee), almonds (*Salmonella* spp.), raw, roasted pistachios (*Salmonella* Montevideo). There have been various recalls for hazelnuts and mixed nut products (*Salmonella* spp., *E. coli* O157:H7), shelled walnuts (*Salmonella* spp.)(Farakos & Frank, 2014).

2.1.4   Prevalence/persistence of microbial pathogens

2.1.4.1 Prevalence of pathogens in nuts

From a survey conducted over 4 harvest years (2011-2014) on prevalence of *Salmonella* on North American in-shell pecans, 44 samples among 4641 samples were positive for *Salmonella* on initial screening from which 31 serotypes were isolated. Most of the serotypes isolated from pecans were found to be resistant to the antibiotics streptomycin and tetracycline, thus raising public health concerns. Thus, the presence of *Salmonella* cells on high fat content and low water activity foods possess likely chances of outbreaks (Brar et al., 2016). Likewise, peanuts from various growing regions (southwest, southeast, Virginia-Carolina) had 2.33% (22 out of 944) of its samples positive for *Salmonella* as collected for 3 harvest years (2008 to 2010)(Calhoun, Post, Warren, Thompson, & Bontempo, 2013). As for a study on almonds in California for 5 years (2001-2005) 35 serotypes were identified from 81 *Salmonella*-positive samples and 23 serotypes from 53 *Salmonella*-positive samples(Danyluk et al., 2007). These results show there is a high probability of occurrence of bacterial pathogens in nuts, which necessitates efficient processing steps for its inactivation.

2.1.4.2 Shelf-life

The shelf life of pecans is affected by the initial moisture content in the nut as well as humidity and temperature of storage. As it is high in fat content it can undergo oxidation if not stored well and thus degradation of quality might occur. Growers or pecan handlers usually store bulk raw products at controlled conditions in order to maintain the quality(Brar et al., 2015). On storage at -10°C, 0°C, 10°C and 20°C in-shell pecans stay for 24, 18, 9 and 4 months respectively. Whereas, pecan kernels when stored at -10°C, 0°C and 20°C can stay until 18, 10 and 3 months respectively. In-shell nuts have 25-50% longer shelf-life than kernels depending on the type of packaging material used against moisture and oxygen or on the nut type. Also, intact nutmeats stay twice as long as nutmeat pieces and, roasted nuts have a shelf-life of about 1/4th of raw nutmeats(Cantwell, 2014). The common storage temperature and storage times for pecans (in-shell and nutmeats) are -18°C (up to 6-8 years), 1-5°C (approx. 1 year) or ambient (up to 6 months)(Picha & Pyzner, 2017).

2.1.4.3 Survival of pathogens in nuts

Generally, *Salmonella* survives better than *E. coli* O157:H7 and *Listeria monocytogenes* on nuts and without significant decline at lower temperatures of storage (i.e. refrigeration or freezer conditions) however, there is significant but slow reduction when stored at ambient temperatures (21-25°C) (Harris, Uesugi, Abd, & McCarthy, 2012; Kimber, Kaur, Wang, Danyluk, & Harris, 2012).

Pathogens (*Salmonella, E. coli* O157:H7 and *Listeria* monocytogenes) inoculated on pecans stored under refrigerated and frozen conditions were stable, however, *Listeria monocytogenes* showed a slight reduction over a 365 day time period (0.03 log CFU/g/30 days at 4±2°C)(Brar et al., 2015). A study on the survival of *Salmonella* on the surface of dry in-shell

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pecans stored at -20, -18, -7, 4, 21 and 37°C showed it could persist for up to 78 weeks, however, bacterial populations were stable at lower temperatures and it declined significantly when at ambient temperature (21 and 37°C) (Beuchat & Heaton, 1975; Beuchat & Mann, 2010a). Studies have also shown that *Salmonella* can survive on surface of high-moisture (a_w 0.96-0.99) pecan nutmeats, in-shell pecans, shucks and shells, however, it gets inactivated in the middle septum and shuck extract owing to presence and leaching out of antimicrobial compounds(Beuchat & Mann, 2010b).

Similar studies on various nuts observed that, *Salmonella* on peanuts showed stability at -24 and 4°C however, *E. coli* O157:H7 and *Listeria monocytogenes* declined at a rate of 0.03 to 0.12 log CFU/g/30 days. Slower decline rates of pathogens were observed on pecans than on peanuts, likewise pathogens were more persistent on pistachios than on almonds (Brar et al., 2015) suggesting that the decline rate of pathogens is dependent on nuts' physical structure or their shell structure. A survival study of *Salmonella, E. coli* O157:H7 and *Listeria monocytogenes* on other nuts showed these organisms survived for 365 to 550 days at -19,4,24 and 35°C on almonds (Kimber et al., 2012), for at least 365 days at -19,4 and 24°C on pistachios (Kimber et al., 2012) and for at least 365 days at -20,4 and 23°C on walnut kernels (Blessington, Mitcham, & Harris, 2012). Thus, pecans, if contaminated during harvesting, can possess various pathogens which survive during the storage conditions and persists over a year or more. Thus, effective post-harvesting processing treatments are necessary.

2.1.5   Pecan processing (Post-harvest treatments)

A general flow diagram of pecan processing is shown in Fig 2.1. The primary objective of a grower is to remove pecans as soon as possible from the orchard floor, to reduce moisture, and prevent molds and rancid flavor development. Pecans dried on trees or mechanically harvested

pecans can have a moisture content as high as 8-30%, thus it is generally advised to dry the pecans right after harvest to less than 4.5% (w.b.) so as to maintain its quality.

2.1.5.1 Storage of in-shell pecans

After drying, in-shell pecans are stored at a temperature controlled facility in order to prevent mold growth, insect damage, discoloration, rancid flavor development and absorption of volatiles. Discoloration can occur when nuts are exposed to UV lights, migration of color from shells to kernels or oxidation of iron containing pigments in testa. Likewise, a high lipid content in pecans helps in absorption of lipophilic compounds thus causing absorption of volatiles. Flavor of pecans are adversely affected when rancidity initiates in the nuts by oxidative reactions. Thus, in-shell pecans can be stored for 6 months (at 22°C), 9 months (8°C), 18 months (0-3°C), 30 months (-6 to -4°C) and 6-10 years (-18°C) without significant loss in quality. Care should be taken while removing pecans from the extreme cold storage temperatures as it is prone to mechanical damage because of its brittleness. After storage at cold temperature, pecans should be placed sequentially at rooms with proper air flow, humidity and temperatures of 5-16°C for several days to prevent condensation (Santerre, 1994c).

2.1.5.2 Conditioning

The conditioning step is one of the essential steps in the process as it is carried out to reduce kernel breakage and improve shelling efficiency. Before shelling, pecans nutmeats are moistened by water or steam which is absorbed through the vascular system at the base and apex and eventually enters through the middle partition to the kernel. The kernel moisture increases from 4 to 8% which makes it more flexible and reduces kernel breakage while cracking the nut(Santerre, 1994c). Different techniques of conditioning includes: hot water soaking (3-5 mins at 85°C, holding 12-24 hr before cracking), steam processing (atmospheric steam for 3 min, holding for 20

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mins before cracking), cold water soaking, immersing or spraying chlorinated water (1000ppm for 1-2 hr, holding for 12-24 hr before cracking) or moisture equilibration in a humidity controlled storage room(Beuchat & Pegg, 2013; Forbus & Senter, 1976; Santerre, 1994c). Apart from facilitating the cracking process, conditioning is also one of the decontamination steps for pecans.

2.1.5.3 Shelling

Commercially, shellers or nut cracking equipment are used where pecans are fed through a hopper to rotating feed that orients the position of pecans correctly to receive an impulse force striking at each end of pecans. The so broken or cracked nuts are passed into sheller (a rotating drum with cylindrical rings to assist separation of shells and nuts). After shelling, pecans should be dried to a moisture content of 3-4% for a good quality for consumers. Excessive drying causes the lipids to come to the surface and become less stable to oxidative reactions thus causing shortened shelf-life(Santerre, 1994c).

2.1.5.4 Storage of pecan nutmeats

Temperature control of storage rooms are one of the best ways to extend shelf-life of pecans however, other techniques are also necessary. Pecan nutmeats are less stable than pecans in shell thus shelled pecans can only be stored for 3-4 months (22°C), 6 months (8°C), 12 months (0-3°C), 18-24 months (-6 to -4°C) and 6-10 years (-18°C). Apart from temperature, moisture levels should also be controlled for prevention of mold and bacterial growth and rancidity. Additionally, the migration of tannin is also likely to occur due to moisture migration from the shell lining to the kernel resulting in darkening of nutmeats. Pecans should also be stored away from sunlight as UV light initiates lipid oxidation and affects the color quality. As for the packaging materials, it is suggested that packaging materials with oxygen transmission rates above 0.08 cc O_2 100 cm^{-1} 24 hr^{-1} is suitable to use for pecan storage(Santerre, 1994c).

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                    Harvesting (Hand/mechanically)
                                 ↓
                               Clean
                                 ↓
                            Wash, Dry
                                 ↓
        Dehull  ←──────────────  Storage
          │                       ↓
          │                Conditioning (Wetting)
          ↓                       ↓
    Polish/ Bleach            Crack/Shell
          │                       ↓
          │                    Separate
          │                       ↓
          │        Roast  ←──────  Dry
          │          ↘             ↓
          └────────→ Storage and Marketing
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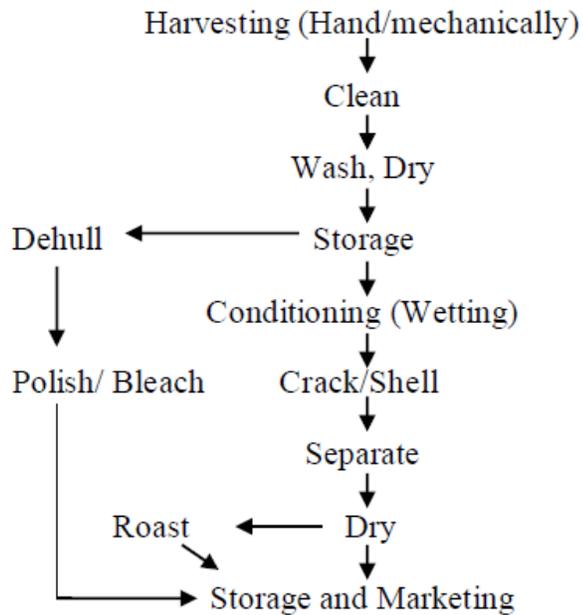
Figure 2.1. General steps in harvesting and processing of pecans

2.2   Post-harvest intervention methods

2.2.1   Hot water treatment

As described earlier, hot water treatment is one of the ways of conditioning pecans for ease of kernel separation but it also facilitates in the inactivation of pathogens. Various studies have tested the efficacy of hot water treatment to decontaminate nuts. Reduction of *Salmonella* due to conditioning treatments are found to be dependent on inoculation techniques (less effective on immersion-inoculation than surface inoculation) and if it has been exposed to stress conditions like drying between inoculation to treatment phase. Inoculation techniques simulate contamination of nuts as a result of its contact with any source with pathogens such as immersion in water containing *Salmonella* in pre or post-harvest conditions or during storage or cleaning (Beuchat & Mann, 2011a). Treating the in-shell pecans with hot water at 82 or 93°C for 2 mins was unable to eliminate the *Salmonella* Senftenberg 775W inoculated at 5.8 log CFU/g, it was only reduced by 3.5 log CFU/g (Beuchat & Heaton, 1975). Recent studies on the effect of conditioning on inactivation of

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surface-inoculated *Salmonella* on pecans showed the treatment at 90 or 95°C for 80 s was able to achieve a reduction of ≥6.42 log CFU/g. However, treatment at 95°C for 5 mins could reduce the immersion-inoculated load by only ≥4.82 log CFU/g but prolonging the treatment to ≥10 mins at 75 to 95°C gave reduction >5.12 log CFU/g. Likewise, hot water treatment of pecans and the sequential treatment of pecans with chlorinated water, soak water followed by hot water showed similar reductions of the inoculated *Salmonella* cocktail. Stressed *Salmonella* (exposed to dessicated conditions) were found to show greater resistance to heat treatment i.e. conditioning treatments (Beuchat & Mann, 2011a).

When almonds were treated with hot water at 85 and 88°C for 40 and 20s respectively it was successful in eliminating *Salmonella* by 5.73 log CFU/g with no significant effect on its quality (color and firmness)(Bari et al., 2009). However, at 70, 80 and 88°C showed only a reduction of 1.1, 2.9 and 4.7 log CFU/g respectively within 30s (Harris et al., 2012). Thus, hot water treatment of nuts has been found to be an effective decontamination step against *Salmonella* but studies on efficacy of hot water treatment for inactivation of various other pathogens is also important.

2.2.2   Cold water dip

Dipping pecans in the water for 20 mins did not show significant reduction of *Salmonella*; reduction increased as exposure of pecan nutmeats in the water (21°C) was prolonged achieving a maximum reduction of 1.03 log CFU/g. The reduction is believed to be due to removal of *Salmonella* from nutmeat surface rather than water being lethal to cells on surface(Beuchat et al., 2013).

2.2.3   Chlorine and other sanitizer solutions

Chlorine treatment is another common way to reduce surface contamination of pathogens on the produce. In-shell pecans when treated with 1000µg/ml chlorine for 20 mins reduced

Salmonella population by 2.8 log CFU/g (Beuchat, Mann, & Alali, 2012). Pecans immersion-inoculated with *Salmonella* and dipped in chlorinated water (400µg/ml) could only reduce the pathogen by 1.6 log CFU/g. Infiltrated *Salmonella* into the undamaged in-shell pecans survives for longer time once it reaches the kernel. Nuts when soaked in chlorine water will also infiltrate the water into the undamaged in-shell pecans but lethality of chlorine gets reduced because of change in its form (Beuchat & Mann, 2011a). Also, reduction is increased when pecans are pre-cleaned before sanitizer treatment. When nuts are harvested it contains various materials high in organic content attached to the nut surface like soil, dust. Thus, free chlorine in cleaning water decreases sharply on interaction with these organic compounds on repeated use, necessitating frequent stocking up of cleaning water(Beuchat & Pegg, 2013). This makes usage of chlorine water a less effective decontamination method for nuts since it hasn't been found to give reductions as higher as 4-5 log CFU/g. However, if it is paired with hot water treatment, dipping in chlorine solution 400 µg/ml for 1 min, soaking in water for 2 h at 21°C followed by hot water treatment at 85°C for 10min, it could reduce *Salmonella* by >5.1 log CFU/g(Beuchat & Mann, 2011a).

Other sanitizers like levulinic acid or 2% lactic acid reduces *Salmonella* by 3.3 and 2.1 log CFU/g on in-shell pecans; while the addition of other sanitizers like 0.05% sodium dodecyl sulfate (SDS) gave greater reductions (3.7 and 3.4 log CFU/g respectively)(Beuchat et al., 2012). Similar potential for reduction were observed when SDS was paired with lactic acid or levulinic acid for the treatment of nutmeats. Regardless of sanitizer concentration and treatment time only 1.1 log CFU/g reduction of *Salmonella* was observed in immersion-inoculated pecan pieces/halves whereas it ranged from 0.7 to 3.6 log CFU/g for surface-inoculated ones (Beuchat et al., 2013). Thus, dipping heavily contaminated pecans in chlorine solution cannot be an effective process for inactivation of pathogens unless paired with other sanitizer or processes.

2.2.4   Steam treatment

Apart from improving the whole kernel extraction, steam conditioning has also been shown to preserve the sensory quality of pecans during storage. Dielectric heating is an alternative heating method where pecans are placed in between parallel electrodes attached to dielectric heater (43 MHz) for 1-3 minutes(Santerre, 1994c). Dielectric heating and steam treating pecans were found to retain a pleasing flavor even better than the raw pecans. Steam treatments darkened the kernel color however, dielectric heating (at about 90-156°C) did not show any effect on color change. Moreover, they were also found to reduce rancidity problems as confirmed by PV value on storage (21°C, 65% RH)(Nelson, Senter, & Forbus, 1985; Senter, Forbus, Nelson, & Horvat, 1984). Steam treatment was effective in reducing *Salmonella* Enteritidis on almonds by 4-5.7 log depending on the variety of almonds used when exposed for 65 s(S. Lee et al., 2006) whereas, it took only 25 s to achieve a 5-log reduction of *Salmonella* PT 30 on almonds without any loss of visual quality(Chang, Han, Reyes-De-Corcuera, Powers, & Kang, 2010). Although literature is available on quality changes due to steam treatment there haven't been many studies on inactivation of pathogens by steam treatment on pecans.

2.2.5   Irradiation

Irradiation of food is one of the effective methods in reducing the post-harvest food losses and ensuring food safety. It is found to be effective on most of the food pathogens like *Salmonella*(Prakash, Lim, Duong, Caporaso, & Foley, 2010). High amounts of unsaturated fatty acids in nuts make it susceptible to lipid oxidation and irradiation can be one of the inducers. Higher irradiation doses caused high peroxide values (PV), increase in rancidity and decreased sensory quality. Treating the almonds with ionizing radiation showed that it required a dose of 5 KGy for 4 log CFU/g reduction of *Salmonella* while a dose of 2.98-5.25 KGy caused unacceptable

changes in the sensory quality of the nut(Prakash et al., 2010). This shows that even though irradiation is an effective method for decontamination, it severely affects the sensory quality giving a negative impression on consumers' taste.

2.3 Effects on quality

Until now it is seen that conditioning of nuts is a crucial step in inactivating pathogens. However, application of heat on nuts can cause degradation of its quality by bringing change in its color, texture, moisture content, water activity and sensory properties. Thus, developing new technologies and processing parameters that improve microbial safety of nuts without affecting the quality of raw products is essential.

A study done on hot water treatment (85°C for 40s) of almonds showed that even though the moisture content increased on hot water treatment, there was no significant difference in the color. However, non-treated almonds were firmer than hot water treated but the overall quality for both treated and non-treated was within acceptable limits(Bari et al., 2009). Thus it is necessary to evaluate physico-chemical characteristics and sensory quality of pecans when treated with hot water.

2.3.1 Color

Heat treatments enhance the color, flavor, texture and appearance of the product while severe ones can have negative effect too. Roasting, a form of heat treatment, changes color, flavor and texture by inducing non-enzymatic browning like Maillard reaction, caramelization, chemical oxidation of phenols and others (Kalkan, Gariepy, & Raghavan, 2016). The L* value measures lightness (0=black and 100=white); a positive a* value represents redness and a negative a* represents greenness; a positive b* value represents yellow and a negative b* value represents blue. The hue angle ($\tan^{-1}$ (b*/a*) represents an actual color, and chroma $(a*^2+b*^2)^{1/2}$ evaluates

purity or intensity of the color which are calculated based on L^*, a^*, and b* values (Moncada-Reyes, 2013). Roasting of hazel nuts at increasing temperatures gave a darker color due to decreased L* values in the range of 36.46 – 47.09 (Kalkan et al., 2016). However, on hot water treating almonds it resulted in almost similar L* (49.9, 50), a* (15.3, 15.2) and b* (31, 29.7) values of control and treated samples respectively (Bari et al., 2009).

2.3.2 Moisture and water activity

Moisture content in pecan nutmeats plays an important role in mold development, rancidity and bacterial contamination. A good quality pecan kernel of 4.3-4.5% moisture will have water activity in the range of 0.65-0.70 (Santerre, 1994a). Following hot water treatment of almonds, moisture content was found to significantly ($P<0.05$) increase from 5% (w.b.) to 6.4% (w.b.)(Bari et al., 2009).

Water activity (a_w) is defined as $Aw = p/p_o$ where p is vapor pressure of water in the substance, and p_o is vapor pressure of pure water at the same temperature (Rockland & Beuchat, 1987).

2.3.3 Texture

Texture properties of nuts like hardness, compression energy, chewiness, cohesiveness, resilience, springiness and fracturability can be measured with the help of texture analyzers (Anzaldúa-Morales, Brusewitz, & Anderson, 1999). Usually the cutting force, which is related to the hardness of the nuts, is an empirical indicator of the force needed to cut a particular food (Hojjati, Noguera-Artiaga, Wojdyło, & Carbonell-Barrachina, 2015). It was found that on hot water treatment of almonds, firmness of non-treated nuts was significantly ($p<0.05$) higher than the hot water treated almonds (Bari et al., 2009). This indicates that hot water treatment affects the textural quality of nuts.

2.4　　Knowledge gaps

Pecans are highly nutritious nuts with high MUFAs, particularly, oleic acid and low saturated fatty acids which contributes positively to a good heart health and blood lipids. It also consists of antioxidants, vitamins, minerals and bioactives like flavonoids, stilbenes, and phytosterols that have numerous health benefits (Beuchat & Pegg, 2013). Louisiana is one of the top pecan producing states and these nuts contribute greatly to the state's economy. Although it is an important crop, pecans have been recalled by U.S Food and Drug Administration for potential contamination of pathogens (Brar et al., 2016) thus potentially causing economic loss to growers. Still, at some places, pecan farming includes grazing of cattle on the orchard which possess a severe risk of contamination of *Salmonella, E. coli* O157:H7 and *Listeria monocytogenes* through cattle manure (Alam & Zurek, 2006; Pell, 1997; Santerre, 1994a).

Conditioning the pecans is basically done for ease of separation of kernels but it is also one of the decontamination steps which when administered at optimum time and temperature can lead to efficient inactivation of the pathogens of public concern. Currently, studies on inactivation of *Salmonella* on pecan nutmeats, nut pieces and in-shell pecans through hot water treatment have been carried out but evaluation of the behavior of all three potential pathogens (*Salmonella, E. coli* O157:H7 and *Listeria monocytogenes*) when subjected to hot water treatment is yet to be done. Thus it is necessary to evaluate the time-temperature of hot water treatment required for inactivation of all three pathogens. Heat treatment is one of the decontamination steps but it also affects the quality of food products thereby hampering the consumers' perception. This necessitates the evaluation of quality and liking of hot water treated pecans and its' purchase intent for development of successful processing parameters.

2.5   References

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3. EFFICACY OF HOT WATER TREATMENT ON IN-SHELL PECANS TO DESTROY *E. coli* O157:H7, *Salmonella spp., Listeria monocytogenes,* AND *Enterococcus faecium*

3.1 Introduction

In general, low-moisture foods such as tree-nuts with water activity lower than 0.7 are presumed to be associated with lower risk of pathogen contamination (Blessington, Theofel, & Harris, 2013; Harris, 2012). However, in the past few years tree nuts such as pecans, almonds, walnuts, pine nuts, pistachios, mixed nuts as well as peanuts have been frequently associated with various recalls and outbreaks due to recurrent contamination with foodborne pathogens such as *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Zhang et al., 2017). Moreover, relatively high amount of fats in the nuts may protect the organisms from the highly acidic condition of stomach thereby helping live organisms pass to the intestine and cause illness even at low numbers (Harris, 2012). Thus, nuts when contaminated with microbial pathogens pose a higher risk of foodborne illness.

Pecans are one among the several most favored tree nuts consumed worldwide in different forms. However, pecans are susceptible to pre and post-harvest microbial contamination (Beuchat & Pegg, 2013). Usually pecan harvesting involves shaking the trees to let the mature nuts fall on the orchard ground. This action may pose potential risk of contamination of pecans from the soil.

In addition, cattle grazing in the pecan orchards is still prevalent in some parts of the United States which is one of the potential sources of pathogen contamination (Beuchat & Pegg, 2013; Maness, 2016; Worley, 1994). In an almond associated outbreak in 2000-2001, the orchard floor was found to be one of the potential sources for *Salmonella* contamination (Brar et al., 2016). Marcus and Amling (1973) reported higher levels of *E. coli* on pecans samples from a cattle-grazed farm than non-grazed farm. Field studies conducted by our group detected the presence of

E.coli O157:H7 and *Listeria* spp. on in-shell pecans collected from the pecan orchards that were also used for cattle grazing (Unpublished).

Post-harvest processing of in-shell pecans is one way to mitigate the risk associated with pre-harvest contamination. Conditioning of pecans aid in kernel separation, minimize kernel breakage and increase the shelling efficiency (Santerre, 1994) as well as aid in decontamination of pecans (Beuchat & Pegg, 2013). Common methods used for conditioning are either soaking in hot water at 85°C for 3-5 minutes or immersing in 1000 ppm of chlorinated water for 1-2 hours followed by 12-24 hours holding before shelling; or steam processing in an atmospheric steam in retort for 3-4 minutes. (Forbus & Senter, 1976).

Several studies by Beuchat et al (2011, 2012, and 2013) concluded that individual treatment of in-shell pecans to inactivate *Salmonella* with hot water at 90 or 95ºC for 80 sec (>5 log CFU/g), chlorinated water at 1000µg/ml (2.8 log CFU/g), lactic and levulinic acids at 2% concentration with or without sodium dodecyl sulfate (2.1-3.7 log CFU/g) resulted in different degrees of reduction. While sequential treatment with chlorinated water and hot water or hot water alone did not show significant difference (Beuchat and Mann 2011). Similarly, almonds treated with hot water at 88°C for 1.6 and 2 min showed a 4 and 5 log reduction of *Salmonella* serovars, respectively. (Harris et al., 2012). These studies demonstrated that hot water treatment of nuts such as pecans and almonds are effective in reducing the levels of *Salmonella*. However, determination of optimum time-temperature treatment conditions to achieve a minimum of 5-log reduction of several potential pathogenic bacteria on in-shell pecans is critical for the post-harvest process development and validation as an efficient kill-step. Hence the main objectives of this study are to: (i) determine hot water treatment conditions to achieve a 5 log reduction of several foodborne pathogens/surrogates, and (ii) determine the rate of thermal lethality of tested organisms.

3.2   Materials and methods

3.2.1   Selection of pecans

Raw in-shell pecans (*Carya illinoinensis)* harvested from several Louisiana orchards during the October/November season of 2015-2016 were stored at 4°C until they were used in experiments.

3.2.2   Selection of bacteria

Several different out-break strains of *Salmonella, E. coli* O157:H7, *Listeria monocytogenes* as well as non-pathogenic strains of *Enterococcus* spp. were used in this study (Table 3.1). These pathogenic strains were provided by Dr. Michelle D. Danyluk at University of Florida and were similar to those used by Brar et. al. (2015) for their study on peanuts and pecan kernels. *Enterococcus faecium* ATCC 8459, a non-pathogenic organism, was used as a surrogate organism for *Salmonella enterica.* Mutant strain of *Enterococcus faecium* resistant to nalidixic acid was developed according to the protocol used by Parnell et al (2005). Frozen culture of *Enterococcus faecium* were sub-cultured twice in tryptic soy broth (TSB) followed by incubation at 37°C for 24 h. Then, 100 µl of the overnight culture was spread onto plate count agar (PCA) containing 0-50 µg/ml nalidixic acid and incubated at 37°C for 24 h. The isolated colonies seen on the plate with the highest concentration of the antibiotic was selected and cultured overnight in TSB. The process was repeated until colonies resistant to 50µg/ml nalidixic acid were obtained. The isolates were stored in TSBN supplemented with 20% glycerol at -20°C.

Table 3.1. Bacterial strains used in the study

Pathogen	Strains	Source
Salmonella enterica	Anatum, strain 1715	isolated from an almond survey
	Enteritidis PT 30, strain ATCC BAA-1045	isolated from raw almonds associated with an outbreak
	Enteritidis PT 9c, strain RM4635	a clinical isolate from an almond-associated outbreak
	Tennessee, strain K4643	a clinical isolate from a peanut butter- associated outbreak
E. coli O157:H7	Odwalla strain 223	Clinical isolate from apple juice associated outbreak
	CDC 658	Clinical isolate from cantaloupe associated outbreak
	H1730	Clinical isolate from lettuce associated outbreak
	F4546	Clinical isolate alfalfa associated outbreak
	EC4042	Clinical isolate from spinach associated outbreak
Listeria monocytogenes	101M (serotype 4b)	isolated from beef from a beef-associated outbreak
	Scott A (serotype 4b)	clinical isolate from a milk-associated outbreak
	V7 (serotype 1/2a)	isolated from milk associated with an outbreak
	LCDC 81-861 (serotype 4b)	Isolated from raw cabbage associated with an outbreak
Enterococcus faecium	ATCC 8459	ATCC ; mutant strain resistant to nalidixic acid was developed

3.2.3 Inoculum preparation

Frozen cultures of *Salmonella enterica, E. coli* O157:H7, *Listeria monocytogenes* and *Enterococcus faecium* that were nalidixic acid resistant were subcultured twice in TSB or TSBY (TSB with 0.6% yeast extract for *Listeria monocytogenes)* supplemented with nalidixic acid (TSBN) at 50µg/ml with incubation at 37°C for 24 h. Then, 1 ml of the each overnight bacterial culture was plated on tryptic soy agar supplemented with 50µg/ml nalidixic acid (TSAN) and incubated at 37°C for 24 h. The inoculum was grown on agar plates to develop increased stress resistance of the bacteria as suggested by Useugi et. al. (2006). Each strain of the pathogens *Salmonella enterica* and *E. coli* O157:H7 were grown in two TSAN plates, *Listeria monocytogenes* in three TSAN plates while *Enterococcus faecium* was plated in eight TSAN plates. The resultant lawn of bacteria on TSAN was loosened with the help of a sterile glass rod using 7 ml of 0.1% sterile peptone water. For each strain, a total of 5 ml was collected from each plate thereby making the final volume of each organism's inocula as 100 ml using 0.1% sterile peptone water. The bacterial cocktail was collected and mixed in a 400ml stomacher® bag (Control Numero 5, Seward, UK)

3.2.4 Inoculation of pecans

Whole, undamaged in-shell pecans were selected and kept overnight inside the bio-safety cabinet to dry and bring it to room temperature prior to running the experiment. Approximately 28 pecans weighing 310±10 g were added into the stomacher bag containing 100 ml of test bacterial suspension. The bag was shaken to facilitate wetting of all the pecans in the bacterial suspension and hand massaged for a minute ensuring complete coating of pecans with the inoculum. The pecans in the bag were allowed to stay immersed in the inoculum for 1 hour with frequent mixing and hand massaging. This process was carried out for each pathogen cocktail separately. The

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immersed pecans were aseptically transferred to large petri dishes (150 by 15 mm) with the help of sterile spoon and allowed to dry for 20 minutes inside the bio-safety hood. After the drying time, 2 pecans were placed in each teabag (t-sac tea filter bags, 1601) making a total of 14 bags for 28 pecans and sealed. Organisms were inoculated at initial load of 6.60-7.96 log CFU/g in the in-shell pecans.

3.2.5 Hot water treatment of inoculated in-shell pecans

Pecans were subjected to hot water treatment in a water bath maintained at 70, 80 and $90\pm1°C$ for 1, 2, 3, 4 and 5 minutes. Five sterile wide mouthed glass bottles of 500 ml capacity completely filled with sterile distilled water were placed in a 12 L water bath (VWR, Radnor, PA, U.S.A.). The water bath was set to a temperature of either 70, 80, or $90+1.5$ °C in order to reach desired test temperatures of 70, 80 and 90ºC for water in the bottles. Individual groups of four inoculated pecans were dipped in hot water and treated at 70, 80, and 90°C for 1, 2, 3, 4 and 5 minutes, respectively. The temperature inside the bottles was monitored continuously during the treatment.

3.2.6 Enumeration

To enumerate the organism in the cocktail, appropriate serial dilutions were prepared in 0.1% peptone water and spread plated on selective media- Xylose Lysine Deoxycholate agar containing nalidixic acid at 50µg/ml (XLDN) for *Salmonella enterica*, Cefixime-Tellurite Sorbitol MacConkey Agar containing nalidixic acid at 50µg/ml (CT-SMACN) for *E. coli* O157:H7, Oxford Listeria Agar base containing nalidixic acid at 50µg/ml for *Listeria monocytogenes* and non-selective media TSAN for *Enterococcus faecium* followed by incubation at 37°C for 24-48 h.

To enumerate the bacterial cells on pecans, two tea bags with 4 treated/untreated pecans was taken in a puncture resistant stomacher® bag (Control Numero 5, Seward, UK) and crushed

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by the use of a sterile pestle. This procedure was followed for each pathogen or surrogate cocktail except for *Listeria monocytogenes* where whole in-shell pecans, without crushing, were directly eluted to enumerate attached cells. This is because our preliminary studies indicated potential bioactive compounds in pecan interfering with the viability of *Listeria*. The resultant suspension of treated was immediately placed in an ice bath to lower the temperature. After that the pecan samples in the bag were hand massaged and shaken for 1 minute to dislodge the organisms. Appropriate serial dilutions of the samples were prepared and enumerated survived organisms as described earlier.

3.2.7   Determination of D and Z-values

Log reduction charts for each organism was plotted with its respective log reduction at 3 different treatment temperatures on y-axis against treatment time on x-axis. The decimal reduction time (D-value) is defined as the time required for 90% reduction or 1 log reduction of initial bacterial population at a particular temperature. D-values were calculated at each test temperature for each organism by taking the inverse of the slope of linear regression line from the log reduction graph and expressed in minutes. Similarly, Z-value is defined as temperature change necessary to bring about a 10-fold change in the D-value. A negative inverse slope of the linear regression line obtained from the graph of log D-values over range of treatment temperatures gave z-vales for each test organisms.

3.2.8   Statistical analysis

All experiments were replicated three times and data were analyzed by ANOVA using SAS software (Version 9.1, SAS institute Inc., Cary, NC). The Fisher's least significant difference test was used to determine the significant differences in mean value with significance considered at $P<0.05$.

3.3   Results and discussion

3.3.1   Effect of hot water treatment on *Salmonella*

The effect of hot water treatment of pecans on the reduction of *Salmonella* is shown in Figure 3.1. At 70°C, increasing the treatment time from 1 to 4 min increased the reduction from 1.79±0.52 to 2.92±0.13log CFU/g with no significant difference ($P>0.05$). However a sharp and significant ($P<0.05$) rise in reduction (4.39±0.38 log CFU/g) was seen on further heating from 4 min to 5 min at 70°C. Treatment at 80°C increased reduction from 2.25±0.5 (1 min) to 4.88±1.8 (4 min) log CFU/g and 90°C showed 2.95±0.03 (1 min) to 5.60±0.19 (4 min) log CFU/g reduction. Furthermore heating to 5 min showed reduction of 4.98±1.87 log CFU/g at 80°C and 6.59±0.95 log CFU/g at 90°C. There was no significant ($P>0.05$) difference observed in the reduction between 4 and 5 min at both 80 and 90°C. This indicates that a minimum of 5 min at 80°C and 4 min at 90°C is required to achieve a 5 log reduction of *Salmonella*. These results can be attributed to the sensitivity of *Salmonella* to higher treatment temperatures in the range of 70 to 90°C. A study conducted by Beuchat and Mann (2011) on the inactivation of *Salmonella* on in-shell pecans during various conditioning treatments reported that hot water treatment could reduce *Salmonella* that were surface-inoculated on in-shell pecans by > 5 log CFU/g within 1 min 20 s at 90 or 95°C and within 4 min at 85°C. Conversely, the results from our study indicate that treatment at 90°C required a longer time (4 min) to achieve a minimum of 5 log- reduction level. Likewise, a hot water treatment study on almonds required 3.75 and 1.95 min time to achieve a 5 log reduction of *Salmonella* PT 30 at 80 and 88°C, respectively as predicted from the calculated D-value (Harris et al., 2012), which is lower than the time required for 5 log reduction in our study. This can be due to exposure of pecans in the inoculums for longer time in our study (1 hour) in comparison to the other study (1 minute) that might have resulted in entry of pathogen through shells to the nutmeats.

It is seen from the heat treatment study on pecans and almonds that movement of organisms from the inoculum to the nutmeat through cracks in the shell and poor heat penetration through shells influence the effectiveness of heat treatment (Beuchat & Heaton, 1975; Harris et al., 2012) The other reasons could also be, because of differences in the pecan varieties used, their shell surface characteristics, or the strains of *Salmonella* used in the study.
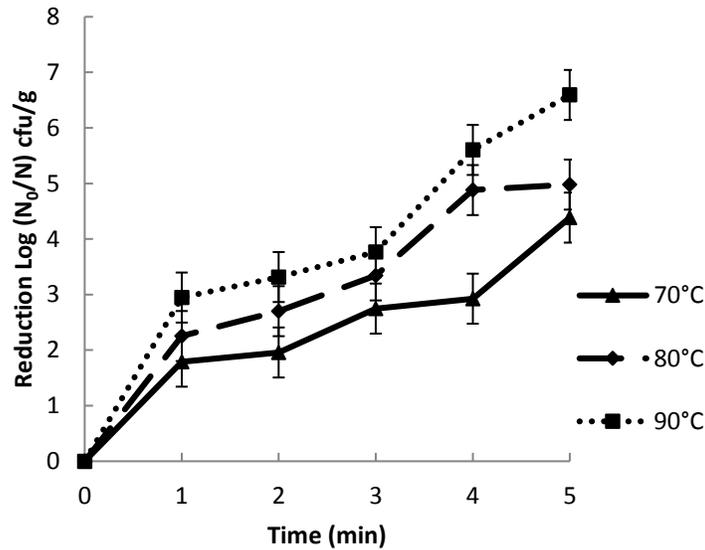
Figure 3.1. Reduction (log CFU/g) of *Salmonella enteritidis* observed in in-shell pecans when treated with hot water at 70, 80 and 90°C for 5 minutes (p<0.05)

Beuchat and Mann (2011) reported that *Salmonella* exposed to stressed conditions (like dessication) were found to be more resistant to conditioning treatment. For example, surface inoculated in-shell pecans that were dried and stored required a minimum of 5 min of hot water treatment at 80 and 90°C to achieve >5 log reduction, longer than the time required for inactivation of non-stressed *Salmonella* in the same study (Beuchat & Mann, 2011a). However, time required for inactivation of non-stressed *Salmonella* in our study was similar to that for stressed *Salmonella* in the previous study. This indicates that both the stressed as well as not stressed *Salmonella* in in-

shell pecans were resistant to the hot water treatment requiring longer treatment times to achieve significant reductions.

3.3.2   Effect of hot water treatment on *E. coli* O157:H7

Inactivation of *E. coli* O157:H7 due to heat treatment at different time and temperature is shown in Figure. 3.2. Hot water treatment of pecans with 7.70±0.07 log CFU/g of *E. coli* O157:H7 was able to achieve a reduction from 0.89 (at 70°C for 1 min) log CFU/g to 7.1 (at 90°C for 5 min) log CFU/g.
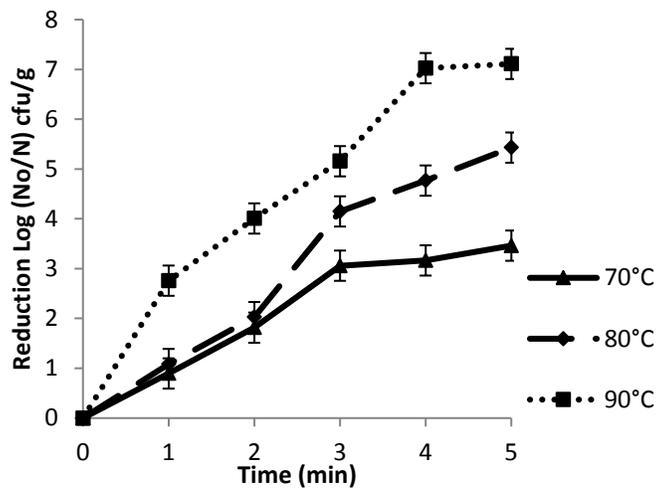
Figure 3.2. Reduction (log CFU/g) of *E. coli* O157:H7 observed in in-shell pecans when treated with hot water at 70, 80 and 90°C for 5 minutes (p<0.05)

Treatment of pecans with hot water at 70°C for 2 min showed a reduction of 1.81±0.45 log CFU/g. Increasing the treatment time to 3 min increased the reduction to 3.05±0.6 log CFU/g. Further increasing the treatment time to 5 min showed no significant difference (*P*>0.05) in the reduction. Similarly, at 80°C *E. coli* were reduced from 1.08±0.11 (1 min) to 4.76±0.20 (4 min) and 5.43±0.38 (5 min) log CFU/g. Increasing the treatment from 4 to 5 minutes at 80°C showed no significant difference (*P*>0.05) in reduction. Further increasing the hot water treatment temperature to 90°C showed a significant increase in the reduction 4±0.07 log CFU/g , 5.16±0.11,

7.02±1.22 log CFU/g at 2, 3, and 4 min respectively. However, there was no significant difference ($P>0.05$) between 4 and 5 minutes of treatment. Our results showed that treatment of pecans at 80°C for 5 min or 90°C for 3 min can achieve a 5 log reduction of *E. coli* O157:H7.

As compared to *Salmonella, E. coli* O157:H7 has not been frequently related with food-borne illness outbreaks associated with low water activity foods (He, Guo, Yang, Tortorello, & Zhang, 2011). However, it has been found that pecans could be a possible carrier of the pathogen from the orchard floor and pose risk of cross-contamination to shelling equipment in processing plant if not inactivated to a desired level. Marcus and Amling (1973) reported there were six times more *E. coli* contaminated pecan samples collected from a cattle grazed farm than from a non-grazed farm necessitating effective conditioning steps during post-harvest treatment. They also suggested that *E. coli* does not contaminate the unbroken shells of pecan but it enters through cracks on the shell, that can occur by water absorption as it sits on the orchard floor or during mechanical harvesting (Marcus & Amling, 1973). Furthermore, it was found that moisture absorption in in-shell pecans takes place through fibro-vascular bundles at its base and suture separations at its apex which act as routes for pathogen entry in the nut (Beuchat & Heaton, 1975). A survival study of pathogens in pecan halves showed that *E. coli* O157:H7 can survive on pecan halves at -24, 4 and 22°C where there was no significant decline in population when the contaminated nuts were stored at first two temperatures however at 22°C there was a reduction by 4.3 log CFU/g over 365 days (Brar et al., 2015). This shows that pecans can be contaminated with pathogenic *E. coli* due to poor pre-harvest practices and can maintain viability for longer storage periods without proper post-harvest intervention. The findings of our study indicate that the pathogenic *E. coli* on in-shell pecans can be destroyed using hot water conditioning treatment prior

to shelling. Hot treatment at 90°C for 3 min was found to be effective in achieving significant reduction. However, the effects of hot water treatment on the quality of nuts need to be determined.

3.3.3 Effect of hot water treatment on *Listeria monocytogenes*

Among all three tested pathogens in the study *Listeria monocytogenes* was found to be most susceptible to heat. Reduction of the organism in response to hot water treatment at different time and temperature is shown in Figure 3.3. Reduction of *Listeria monocytogenes* at three tested temperatures were found to be significantly different ($P<0.05$).
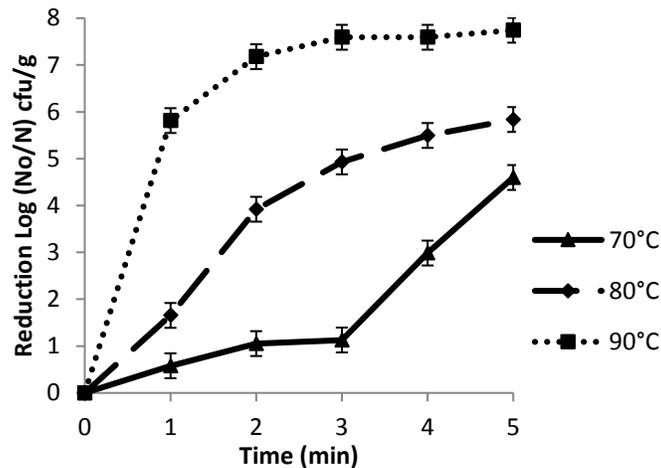
Figure 3.3. Reduction (log CFU/g) of *Listeria monocytogenes* observed in in-shell pecans when treated with water for 5 minutes ($P<0.05$)

The in-shell pecans were inoculated at a level of 7.58±0.26 log CFU/g. Treatment at 70°C showed reduction from 0.58 log CFU/g (1 min) to 4.60 log CFU/g (5 min). Treatments at 70°C until 3 min were not significantly different ($P>0.05$). However, on further treatment, reductions at 4 and 5 min varied significantly (P<0.05). Increasing the temperature to 80°C reduced the pathogen level by 4.93 log CFU/g within 3 min of treatment and ≥5.49 log reduction was achieved within 4 min. However the difference was not significant between 3 and 4 min or 4 and 5 min ($P>0.05$).

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Unlike other organisms, reduction of *Listeria monocytogenes* was greatly reduced (> 5 log CFU/g) within 1 minute at 90°C. Furthermore, there was no significant difference (*P*>0.05) in the reduction (≥ 7.18 log CFU/g) for treatment ≥ 2 min at 90°C. Brar, Proano et al. (2015) reported that inoculated *Listeria monocytogenes* on pecans were stable at -24°C and 4°C; however, at 22°C population fell below the limit of detection by the end of 365 days storage.

In our study, breaking the hot water treated pecans to enumerate *Listeria* attached on the shell and those infiltrated inside nutmeat did not show viability of cells based on the growth on selective and non-selective media. Broken pecans release excess oil in the diluents which when spread plated on to the agar plates makes it slippery and wet. This causes colonies to be smeared. Additionally, inhibitory effect of antimicrobial compounds present in the nut might have had a major effect on the viability of *Listeria*. Pecan shells are believed to contain 5-20 times more polyphenols than kernels that possess antimicrobial effect(Rosa, Alvarez-Parrilla, & Shahidi, 2011). Various studies have shown that pecan shell extracts have been more effective against gram positive than gram negatives. A study by Prado et. al. (2014) found that pecan shell extracts were highly effective against *Listeria monocytogenes* due to the effect of epicatechin gallate extracted from the shell. Similar results were observed when extracts were used against minimally processed lettuce leaves stored at refrigerated conditions where the efficacy was highest on *Listeria* and *Salmonella* but not on *E. coli* (Caxambu et al., 2016). In another study on the effect of pecan shell extracts against *Listeria* spp. when inoculated on poultry skin found that it was able to achieve around 2 log reduction of inoculated cocktail of *Listeria* spp., and >4 log reduction of the native spoilage organism present in the skin (Babu, Crandall, Johnson, O'Bryan, & Ricke, 2014). Thus, when pecans were broken after the hot water treatment phenolic compounds being water soluble in nature must have infused in the diluent from the shell thereby showing inhibitory effect against

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Listeria monocytogenes. However, our revised protocol for the organism i.e. enumeration of *Listeria* without breaking the pecans allowed minimal infusion of bioactive compounds in the diluents giving us the reduction results as only affected by hot water treatment.

3.3.4   Effect of hot water treatment on *Enterococcus faecium*

As shown in Figure 3.4 inactivation of *Enterococcus faecium* significantly ($P<0.05$) increased with increasing the temperature from 70, 80 and 90°C. *Enterococcus* was found to be the most resistant to hot water treatment among all the tested organisms in the study.

The lowest reduction of *E. faecium* was achieved at 70°C which ranged from 0.95±0.4 (1 min) to 2.95±0.10 log CFU/g (5 min) whereas at 80°C it ranged from 1.2±0.56 (1 min) to 4.072±0.21 log CFU/g (5 min). Neither of the temperatures was able to achieve a 5 log CFU/g reduction of the organism.   A resemblance in the reduction pattern was observed between *Enterococcus* and *E. coli* O157:H7 until 2 minutes treatment at 70 and 80°C. Further increasing the temperature to 90° gave a minimum reduction of 2.39±0.48 log CFU/g (1 min) which peaked to 3.96±0.16, 5.29±0.15 and 5.61±0.31 log CFU/g at 3, 4 and 5 min respectively where, reduction at 4 and 5 min didn't differ significantly ($P>0.05$). Our results concluded that a minimum of 4 min at 90°C was required for 5 log inactivation of *Enterococcus faecium* and no other treatment combination used in the study was able to achieve the desired level of reduction. The Almond Board of California requires treatment processes to achieve 4 log reduction of *Salmonella* in almonds and recommends to use *Enterococcus faecium* as a surrogate organism in validation of effectiveness of processing equipment (ABC, 2014). *Enterococcus faecium* NRRL B-2354 (ATCC 8459) has been shown to be just as resistant as *Salmonella* PT 30 (Shah, Asa, Sherwood, & Graber, 2017) and it has been considered safe to be used as a surrogate organism in thermal process validation in the food manufacturing areas (Kopit, Kim, Siezen, Harris, & Marcoa, 2014).

However, it is recommended to validate if the organism can be used as a surrogate for products other than almonds (ABC, 2014) and there have been many studies determining the heat resistance of *Enterococcus faecium* in other foods. A study by Shah et. al. (2017) reported that vacuum steam pasteurization of flaxseed, quinoa and sunflower kernels showed that *Enterococcus faecium* was the most heat resistant among *Enterococcus faecium*, *Salmonella* PT 30 and *E. coli* O157:H7 and it could be used as an effective surrogate for both of the pathogens. Similar results were shown in a study by Bianchini et. al. (2014) on balanced carbohydrate-protein meal where 5 log reduction was achieved at a minimum of 73.7 and 60.6°C for *Enterococcus faecium* and *Salmonella* respectively showing that *Enterococcus faecium* could be used for validation studies for the product during extrusion (Bianchini et al., 2014). Our findings are similar to the previous studies showing that *Enterococcus faecium* was just as resistant as *Salmonella enterica* and more resistant than all pathogens during the heat treatment of in-shell pecans.
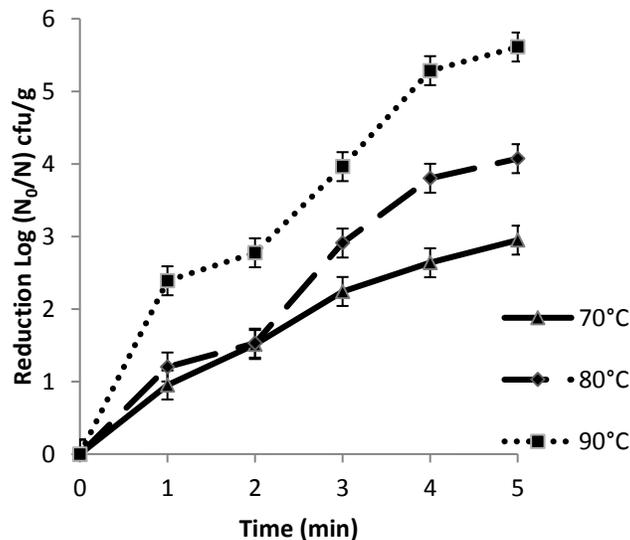
Figure 3.4. Reduction (log CFU/g) of *Enterococcus faecium* observed in in-shell pecans when treated with hot water at 70, 80 and 90°C for 5 minutes (p<0.05)

3.3.5   Heat resistance of organisms

D-value (min) and Z-value (°C) obtained from the log reduction graph for each of the organisms is presented in Table 3.2. As calculated from the D-value table, time required for 5 log reduction is 6.8, 6.25 and 4.25 min for *Salmonella enterica* and 8.6, 5.95 and 4.6 min for *Enterococcus faecium* at 70, 80 and 90°C respectively. Our results indicated that *Enterococcus faecium* was the most heat resistant organism of all at 70 and 90°C whereas at 80°C, *Salmonella* showed higher resistance. However, there was no significant difference (*P*>0.05) in the heat resistance shown by *Enterococcus faecium, Salmonella* spp. and *E. coli* O157:H7 at each temperature. This result suggests that *Enterococcus faecium* is as resistant as or even more resistant than the pathogens of concern in the heat treatment of pecans and can be a potential surrogate organism for hot water treatment validation studies in pecan processing areas. Similar results have been shown by various researchers for the heat resistance of *Enterococcus*. In a study where almonds were heat treated with moist-air, *Enterococccus faecium* had lower reductions and 30% higher D-values in comparison to *Salmonella enteritidis* PT 30(Jeong, Marks, & Ryser, 2011). Likewise, in a hot water treatment study on almonds, *Salmonella* Senftenberg 775 W, *Salmonella* Enteritidis PT 30 and *Enterococcus faecalis* ATCC 29212 required similar time to achieve 4-5 log reduction. D-values (min) reported for *Salmonella enteritidis* PT 30 at 70, 80 and 88°C were 1.2, 0.75 and 0.39 respectively (Harris et al., 2012) which were slightly lower than what we observed in our study. This may be due to the difference in the nuts' physical structures and heat penetration properties through the surface of almonds and pecans.

In our study, *Salmonella* has been found to be the most heat resistant pathogen. In a study on heat inactivation of *Salmonella, E. coli* O157:H7 and *Listeria monocytogenes* in fruit juices, *E. coli* O157:H7 was the most heat resistant among all the pathogens followed by *Listeria* and

Salmonella being the least(Mazzotta, 2001). The higher heat resistivity of *Salmonella* in our study can be attributed to the development of heat resistance by the organism in low water activity food matrices. For example, *Salmonella enterica* achieved lower reduction and was found to be more resistant than *E. coli* O157:H7 in peanut butter(He et al., 2011). In addition, vacuum steam pasteurization at 75°C for 1 s of low moisture foods (flaxseed, quinoa and sunflower kernels) showed that microbial reduction levels were highest for *E. coli* O157:H7 followed by *Salmonella* and *Enterococcus faecium* (Shah et al., 2017).

Although *Enterococcus faecium* showed the highest resistance to heat treatment, *Salmonella* had the maximum z-value of all tested organisms. Higher z-value indicates that at increased temperature organisms behave differently and become more heat resistant at that temperature. It was evident from the D-values (Table 3.2) that at 80°C *Salmonella* required a longer time to get reduced. Among all the organisms, *Listeria monocytogenes* was found to be the least resistant with lowest z-value (4.51°C±0.51) during hot water treatment of in-shell pecans. A metaanalysis study on heat resistance of organisms in liquid growth media calculated z-value (°C) of *Enterococcus faecium* (10.2±3.3), *Salmonella* spp. (5.1 ± 1.6), *Salmonella senftenberg* 775 W (6.2±1.1), *E. coli* (5.4±1.5) and *Listeria monocytogenes* (6.2±1.1) (Sorqvist, 2003) which were similar to the observed values in the study except for *Salmonella* which was higher in our study. This is thought to be due to the organism's resistivity to heat treatment in low water activity food matrix.

Table 3.2. Calculation of D and Z-values from log reduction graphs

Organisms	D-values (min)			Z- value (°C)
	70°C	80°C	90°C	
Enterococcus faecium	1.72±0.16[a]	1.19±0.12[bcd]	0.92±0.02[cde]	7.62±1.36
Salmonella enterica	1.36±0.19[ab]	1.25±0.66[bc]	0.85±0.09[de]	10.18±1.9
E. coli O157:H7	1.38±0.09[ab]	0.87±0.07[cde]	0.73±0.18[ef]	5.80±0.61
Listeria monocytogenes	1.15±0.09[bcde]	0.83±0.02[cdef]	0.41±0.01[f]	4.51±0.51

Table 3.2 represents D-value of organisms at each temperature and z-value of organisms ($P<0.05$). Experiments were run in triplicates. The superscripts represent the significant difference between organisms at each temperature and between different temperatures.

3.4   Conclusion

Five log reduction was achieved for *Salmonella* spp. (5 min at 80°C or 4 min at 90°C), *E. coli* O157:H7 (5 min at 80°C or 3 min at 90°C), *Listeria monocytogenes* (4 min at 80°C or 1 min at 90°C) and *Enterococcus faecium* (4 min at 90°C) upon hot water conditioning of in-shell pecans. Among all the tested organisms, *Enterococcus faecium* was found to be the most heat resistant, and among the tested pathogens, *Salmonella enterica* was the most resistant to heat treatment. However, the highest z-value (°C) was observed for *Salmonella enterica* (10.18±1.9°C) and lowest for *Listeria monocytogenes* (4.51±0.51). As calculated from the D-value table, 5 log reduction of all pathogens can be achieved with hot water treatment for 8.6 min at 70°C, 6.6 min at 80°C and 4.6 min at 90°C. Thus, the hot water treatment was found to be an effective kill-step for different potential pathogens in pecans.

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4. EVALUATION OF PHYSICO-CHEMICAL PROPERTIES AND CONSUMER ACCEPTABILITY OF ROASTED PECANS SUBJECTED TO VARIOUS DEGREES OF HOT WATER PRE-TREATMENT

4.1   Introduction

Pecans are commercially important nut crop that add to agricultural economy of U.S. (Lombardini, Zajicek, Waliczek, & Harris, 2008) and are one of the most favored tree nuts consumed worldwide in different forms. Additionally, they are also nutritionally rich and can be effective against various diseases (Beuchat & Pegg, 2013; Santerre, 1994b). Pecans are found to have a high antioxidant capacity against free radicals due to presence of phenolic compounds, condensed tannins and hydrolysable tannins (FloresCordova et al., 2017). Studies have shown the potential of phenolics to lower the frequency of several chronic diseases like cancer, Alzheimer's disease, Parkinson's disease and other degenerative diseases (Mertens-Talcott & Percival, 2005; Tam et al., 2006). Also, the high amount of monounsaturated fatty acid in pecans plays a crucial role in lowering the LDL cholesterol and minimizing the risk of heart disease (Rajaram et al., 2001).

However, pecans are susceptible to pre and post-harvest microbial contamination (Beuchat & Pegg, 2013) thus making them prone to causing foodborne diseases. Usually pecan harvesting involves shaking the trees to let the mature nuts fall on the orchard ground. This action may pose potential risk of contamination of pecans from the soil. In the past few years tree nuts such as pecans, almonds, walnuts, pine nuts, pistachios, mixed nuts as well as peanuts have been frequently associated with various recalls and outbreaks due to recurrent contamination with foodborne pathogens such as *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Beuchat & Pegg, 2013; Zhang et al., 2017). Post-harvest processing of in-shell pecans is one way to mitigate the risk associated with pre-harvest contamination. Hot water conditioning of pecans aid in kernel

separation, minimize kernel breakage and increase the shelling efficiency (Santerre, 1994) as well as aid in decontamination of pecans (Beuchat & Pegg, 2013). Our previous studies indicate that hot water treatment of in-shell pecans at 70°C for 8.6 min, or at 80°C for 6.6 min, or at 90°C for 4.6 min can successfully achieve a minimum of 5-log reduction of various bacterial pathogens of public concern such as *Salmonella enterica, E. coli* O157:H7, *Listeria monocytogenes*((Kharel, Yemmireddy, Karki, Graham, & Adhikari, 2017, Unpublished).

Nevertheless, heat treatment can also affect the quality of nuts apart from effectively eliminating pathogens. Blanching and roasting can bring significant changes in color, flavor and texture of nuts where, blanching can lead to softening of nut texture whereas roasting can change flavor and skin color (Harris, 2013). A study done by Forbus and Senter (1976) found that in-shell pecans exposed to conditioning treatments gained darker color with steam exposed pecans being the darkest of all. However, it did not hamper the quality except that it was found to gain a slightly cooked flavor. Fried potatoes that were prior subjected to blanching showed more appealing color with increased L* value and improved texture (Agblor & Scanlon, 2000). It is critical to understand quality and consumer acceptability of roasted pecans subjected to hot water pretreatment for the practical implementation. Hence, the main objectives of this study are: i) to determine the effect of heat treatment of pecans on physico-chemical properties ii) to evaluate consumer acceptability and purchase intent of the treated pecans.

4.2   Materials and methods

4.2.1   Selection of pecans

Raw in-shell 'Sumner' pecans (*Carya illinoinensis)* were obtained from Little Eva Pecan Company LLC, Cloutierville, Louisiana. The pecans were stored for a month at 4°C until they were used for analysis.

4.2.2   Hot water treatment of pecans

A 2 kg of undamaged in-shell pecans were first weighed using a balance (Mettler Toledo PG 5001-S, Columbus, OH) for each treatment. A skillet containing water was heated up to temperatures of either 70, 80, or 90±2℃. The weighed in-shell pecans were placed in stainless steel strainers and dipped in the hot water maintained at respective treatment temperatures for 8.6 (70°C), 6.6 (80°C) and 4.6 (90°C) min, respectively. Temperature of skillet surface, water and the nuts was continuously measured using a data logger (ExTech SDL200, Nashua, NH) with K-type thermocouples attached to it. The time-temperature combinations were calculated from our previous study on hot water conditioning of in-shell pecans so as to inactivate potential pathogens. The treatment conditions obtained from D-value table in the previous study could achieve 5 log reduction of the most heat resistant pathogen i.e. *Salmonella enterica.*

4.2.3   Roasting of pecan

Roasting of pecans was selected as a processing step so as to present samples to consumers. Hot water treated in-shell pecans were allowed to air dry and cool down to room temperature (21°C) on metal trays for 1 hour. After that, the hot water treated and control (raw) pecans were de-shelled using nut crackers and were spread on the oven trays. A mini rotating rack convection oven (Baxter, Orting, WA) was preheated to 160±3°C and the trays containing shelled pecans were roasted for 10 minutes at 160°C. These roasting conditions were selected based on one of the treatment combinations used in the study for hot air roasting of pecans (Beuchat & Mann, 2011b). Roasted pecans were allowed to cool to room temperature and vacuum packed in bags (BOPPT/VMCPP-Biaxially-Oriented Polypropylene-Plastics technology/Cast Polypropylene) using a vacuum sealer. The bags were stored at refrigerated conditions (4°C) until used for further analysis.

4.2.4   Physicochemical analysis

Pecans (25 g) were ground using magic bullet blender (Magic bullet, Los Angeles, CA) for the analysis of moisture and water activity of pecans. Moisture content and water activity of pecan samples were measured based on using a moisture analyzer (Mettler Toledo MJ33, Switzerland) and Novasina Labtouch water activity meter (Neutec Group Inc, NY, USA) respectively. For color measurement, 3 pecan halves were placed on the top port of the spectrophotometer (CM-5 Konica Minolta, Inc., NJ, USA) and the L* (0=black and 100=white), a*(+a*= redness, -a*=greenness), b*(+b* =yellow, -b* =blue) were measured for each treatment sample and the untreated control. Readings were taken in triplicates for each sample where samples were rotated at ~90° on the top port after each reading. The chroma $(a*^2+b*^2)^{1/2}$ and hue angles $(\tan^{-1} (b*/a*)$ were calculated based on L^*, a^*, and b* values. The texture of raw, hot water treated and roasted shelled pecans were analyzed using a texture analyzer (TA-XT plus Texture Analyzer, Texture Technologies Corp, NY, USA) with a sharp blade (HDP/BS) following the protocol by Lee and Resurreccion (2006) for roasted peanuts. The blade was lowered with cross head speed of 250 mm/min and 20 mm distance from the platform. The mean value of twenty measurements was reported as hardness (N).

4.2.5   Microbiological analysis

Aerobic plate count and yeast and mold count on the roasted shelled pecans were measured before conducting sensory analysis using 3M^{TM} Petrifilms^{TM} (3M^{TM} Petrifilms^{TM}, St. Paul, MN). A 25 gram of pecan halves was taken in a stomacher® bag (Seward, UK) with 225 ml of 0.1% peptone water and homogenized in a Bagmixer® 400 blender (Interscience Laboratories Inc., MA,, USA). Appropriate serial dilutions were prepared and a 1 ml of sample was placed on the center of bottom film of the petrifilm. After spreading the sample evenly on the petrifilm using a spreader,

the films were incubated at 35±1°C for 48±3 hrs (for Aerobic Plate Count) and 25±1°C for 3-5 days (for Yeast and Mold count). Experiment was performed in duplicates. No growths were observed in any of the plates except for presence of 4-6 colonies at the zero dilution on aerobic plate count for the control.

4.2.6 Consumer liking and purchase intent

The sensory study was approved by the LSU Institutional Review Board with the IRB exempt number of HE 15-9. Consumer test was conducted with 112 panelists (47.3% male and 52.7% female) who were faculty, staff and students at Louisiana State University, Baton Rouge, LA, USA. Sensory booths illuminated with cool, natural, fluorescent lights were used for sensory evaluation and questionnaires were developed through Compusense® five (Compusense Inc., Guelph, Canada) software. Consumers read and electronically signed a consent form (Appendix A) [screening criteria including not allergic to pecans and crackers]. Samples, coded with 3 digit random number, were presented using randomized complete block design where each consumer was presented with 4 pecan samples (roasted raw pecans and roasted pecans that were hot water treated at 70, 80 and 90°C) in 2oz serving size cups in a counterbalanced protocol so as to minimize psychological biasness on the order of sample presentation.

Consumers were instructed to evaluate the acceptability of 5 attributes namely, Appearance /color, aroma, texture (crunchiness), flavor and overall liking using a 9-point hedonic scale (1-dislike extremely, 5=neither like nor dislike, 9=like extremely). After, a purchase intent question was asked using a binomial (yes/no) scales.

Consumers were then informed with a safety disclaimer "The shells of these pecans were treated with hot water making them safer for consumption" for hot water treated samples.

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Consequently, they were again asked to evaluate each sample on their overall liking and purchase intent. Unsalted plain crackers and water were provided to cleanse the palate between samples.

4.2.7   Statistical analysis

The mean difference of physicochemical properties and consumer liking was evaluated using analysis of variance (ANOVA) followed by Tukey's adjustment test for *post hoc* multiple comparisons. Values were considered significantly different at *P<0.05*. McNemar's test was carried out to analyze significant difference in the percentage change in purchase intent before/after (SAS software Version 9.1, SAS institute Inc., Cary, NC).

4.3   Results and discussion

4.3.1   Moisture and water activity

The effect of heat treatment on physicochemical properties of raw, heat treated and roasted pecans are shown in Table 4.1. Statistical analysis of the data indicated that hot water treatments, regardless of temperatures, had no significant (*P>0.05*) effect on the moisture content of pecans when compared to its control (i.e. raw pecans). Roasting significantly decreased the moisture content of the raw and hot water treated pecans from 6.09-6.97% to 2.06-2.94%; while, the water activity significantly dropped from 0.80-0.85 to 0.35-0.44. It could be observed that conditioning in-shell pecans with hot water slightly increased water activity of shelled pecans (*P>0.05*). On subsequent roasting, water activity decreased, however, water activity was comparatively higher for pecans which had undergone hot water treatments (0.44) than raw pecans (0.35) (*P<0.05*). This asserts that conditioned pecans on roasting will have free water available for microbial growth, enzymatic and chemical reactions thus, they are prone to rancidity and bacterial growth.

Table 4.1. Physicochemical properties of raw, hot water treated and roasted pecans

Parameters	Control		Hot water treated Pecans					
			70°C		80°C		90°C	
	Raw	After Roasting	After hot water treatment	After Roasting	After hot water treatment	After Roasting	After hot water treatment	After Roasting
Moisture (%)	6.45 ± 0.65^{a}	2.06 ± 0.24^{b}	6.48 ± 0.22^{a}	2.94 ± 0.34^{b}	6.09 ± 0.40^{a}	2.84 ± 0.09^{b}	6.97 ± 0.83^{a}	2.39 ± 0.1^{b}
a_w	0.81 ± 0.00^{b}	0.35 ± 0.01^{d}	0.82 ± 0.01^{b}	0.44 ± 0.02^{c}	0.83 ± 0.00^{ab}	0.44 ± 0.00^{c}	0.85 ± 0.02^{a}	0.44 ± 0.01^{c}
Texture-Hardness (N)	45.70 ± 13.60^{a}	35.66 ± 7.16^{b}	40.75 ± 9.83^{ab}	40.15 ± 13.05^{ab}	40.86 ± 6.21^{ab}	38.86 ± 5.69^{ab}	43.05 ± 9.42^{ab}	36.14 ± 7.82^{b}
Color								
L*	47.09 ± 0.28^{a}	47.18 ± 0.30^{a}	45.74 ± 0.28^{ab}	44.76 ± 0.07^{b}	45.81 ± 0.30^{ab}	44.69 ± 1.08^{b}	47.05 ± 0.48^{a}	41.87 ± 0.69^{c}
a*	13.06 ± 0.38^{ab}	11.03 ± 0.22^{b}	13.13 ± 0.13^{a}	13.87 ± 0.09^{a}	13.30 ± 0.98^{a}	12.16 ± 1.20^{ab}	13.75 ± 0.32^{a}	13.01 ± 0.33^{ab}
b*	25.83 ± 0.93^{ab}	20.97 ± 0.18^{c}	27.03 ± 0.72^{a}	26.29 ± 0.20^{ab}	27.56 ± 0.66^{a}	23.99 ± 2.53^{abc}	27.43 ± 1.72^{a}	22.61 ± 2.91^{bc}
Chroma	28.95 ± 0.66^{abc}	23.69 ± 0.26^{d}	30.5 ± 0.59^{ab}	29.72 ± 0.22^{abc}	30.60 ± 1.02^{ab}	26.93 ± 2.28^{bcd}	30.69 ± 1.39^{a}	26.12 ± 2.49^{cd}
Hue (°)	63.16 ± 1.51^{a}	62.25 ± 0.26^{a}	64.08 ± 0.82^{a}	62.19 ± 0.03^{a}	64.26 ± 1.11^{a}	63.01 ± 3.42^{a}	63.34 ± 1.97^{a}	59.88 ± 3.31^{a}

Mean Values in the same row by different letters are significantly different ($P<0.05$).

Moisture content of raw pecans observed in our study was higher than the moisture content of raw pecans, 3.5-3.76%, found by Resurreccion and Heaton (1987). In a study by Beuchat and Mann (2011) pecan nutmeats at 2.8-4.1% moisture reached to around 1-2% on hot air roasting (120°C for 10 min). This finding was similar to observed value of moisture for roasted pecans in our study.

Moisture content and water activity are important parameters that affect the shelf-life of nuts. A good quality pecan kernel of 4.3-4.5% moisture will have water activity in the range of 0.65-0.70(Santerre, 1994a). Conditioning increases the moisture of pecan nutmeats from 4 to 8% which makes it more flexible and reduces kernel breakage while cracking the nut. However, it is advised to dry the conditioned shelled pecans to 3-4% moisture content so as to reduce mold development, rancidity, microbial growth and maintain quality that is desired by consumers (Santerre, 1994b).

4.3.2   Texture

Texture of fresh, hot water treated and roasted pecans was measured and expressed as hardness (N) (Table 4.1). Hardness is given by peak force that occurs during the compression of any material. In our study, raw shelled pecans required the maximum force (45.70 ± 13.60 N) to get deformed, followed by shelled pecans hot water treated at 90 (43.05 ± 9.42), 80 (40.86 ± 6.21) and 70°C (40.75 ± 9.83) respectively. On roasting, hardness of raw shelled pecans (35.66 ± 7.16) and hot water treated shelled pecans at 70 (40.15 ± 13.05), 80 (38.86 ± 5.69) and 90°C (36.14 ± 7.82) decreased. Roasting significantly ($P<0.05$) decreased the hardness value in raw pecans but, there was no significant difference ($P>0.05$) in the hardness values among hot water treated and roasted samples. Also, hot water treatment or roasting showed no significant effect on the texture of shelled pecans when compared to its respective control (i.e. raw pecans and roasted raw pecans). Similar

results were reported by Moghaddam et. al. (2016) on roasted pistachio kernels whose hardness ranged from 37.59-82.76 N and as the temperature for roasting increased hardness values decreased owing to decrease in moisture content and strength of the kernels. Thus, our study showed that heat treatment did not have a pronounced effect on the hardness of pecans; however, roasted shelled pecans required less force to get deformed than raw or hot water treated pecans owing to its brittle nature due to removal of moisture.

4.3.3   Color

The effect of heat treatment on color values of pecans is presented in Table 4.1. When raw pecans were roasted it slightly increased ($P>0.05$) the L* value from 47.09±0.28 to 47.18±0.30. As the pecans were hot water treated, L* values decreased to 45.74-47.05 but were not significantly different than that of raw pecans. This indicated that there was no color change on hot water treatment. However, when the pecans were roasted, the L* values of pecans hot water treated at 70, 80 and 90°C significantly decreased to 44.76±0.07, 44.69±1.08 and 41.87±0.69, respectively. This indicated that hot water treated pecans became darker on roasting. Among all the samples, roasted raw pecans was the lightest while roasted pecan that was hot water treated at 90° was the darkest.

The hue angles of pecans ranged from 59.88-64.26° where, on the color wheel, 0° means +a* (red) and 90° means +b* (yellow). Hue angles increased as pecans were treated by hot water whereas it decreased on roasting however, the values weren't significantly different ($P>0.05$). This value indicates that color of the pecan kernels were towards the yellowish shade. Also, chroma values ranged from 23.69-30.69; with an increase in temperature of hot water treatment the chroma values (saturation) of the pecan nutmeat were found to increase but decreased on roasting. Chroma value starts at the 0 in the center of the color wheel and is a distance from the lightness axis.

Observed chroma value in the study indicates that the pecans had darker yellow shade. Color of the food is linked with its' quality attributes like freshness, sensory, nutritional and defects (visual and non-visual). Unwanted changes in color can lead to decreased consumer's acceptance and its' worth in the market thus is one of the important appearance attributes (Xiao et al., 2017). A sensory study on traditionally harvested pecans found the color values of the nut to be 31.58-35.67 (L*), 10.06-10.77 (a*), 13.61-15.92 (b*) and a hue angle of 51.63-52.72°(Resurreccion & Heaton, 1987). The values were similar but slightly lower than values observed in our study which can be attributed to varietal difference. Thus, color of the shelled pecan (dark yellow) was maintained even after hot water treatment and roasting process. However, hot water treatment made the kernels look darker on roasting as seen from their lower L* values as compared to roasted raw pecan.

4.3.4 Consumer liking

The liking scores for different sensory attributes and purchase intent of roasted pecans is presented in Table 4.2. Consumers didn't find any significant (*P*>0.05) difference among the roasted samples (raw and hot water treated) for all tested sensory attributes. Except for the color and aroma, the mean liking scores for the presented roasted pecan samples had no significant difference with control samples. The mean liking scores for the color and aroma of the control (roasted pecan without hot water treatment) were 5.2 (*P*<0.05) and 5.79 (*P*<0.05) respectively, lowest among all the samples indicating that consumers neither liked nor disliked the sample (Table 4.2). This lower liking for color can be related to the samples' L* value which was significantly (*P*<0.05) different and highest than all other roasted pecan samples indicating lighter color (Table 4.1). This indicated that consumers liked darker colored pecans. Statistically, consumers did not find any significant difference among the colors of the treated samples. However, the mean liking scores have slightly increased with increase in the hot water treatment

temperature which can also be attributed to its decreasing L* value. This indicates that increasing the hot water treatment temperature potentially increased the liking of its color among consumers as the kernel color became darker. Likewise, there was an increase in the liking for aroma in the same pattern. As for texture, mean liking scores for the roasted pecan samples ranged from 6.49-6.64 which were not significantly ($P>0.05$) different to each other. Likewise, the hardness values of roasted pecans had no significant difference ($P>0.05$) when measured by the texture analyzer (Table 4.1). Likewise, consumers did not find any significant ($P>0.05$) effect of hot water treatment on the flavor attribute of the pecans with mean scores ranging from 6.17-6.42.

Although there was no significant ($P>0.05$) difference among the overall liking scores of pecans before the disclaimer and also among the overall liking of the samples after the disclaimer was shown, mean overall liking scores were higher for hot water treated pecans. Also, it was seen that the disclaimer about treating pecans with hot water for its safety has shown rise in overall liking from 6.42 to 6.53, 6.29 to 6.43 and 6.46 to 6.52 in 70, 80 and 90°C treated pecans respectively while there was drop in the overall liking from 6.31 to 6.21 when consumers knew about the control pecans. Studies have shown that overall liking increased for products after the health benefit statement or safety disclaimer was shown. For example, a consumer liking and purchase intent study on sponge cakes showed that overall liking of the product increased after the health benefit statement and was one of the important attributes that influenced purchase intent (Poonnakasem, Pujols, Chaiwanichsiri, Laohasongkram, & Prinyawiwatkul, 2016). Likewise, another study on pomegranate juice and green tea blends found that disclaimer about health benefits had positive impact on overall liking of the product(Higa, Koppel, & Chambers IV, 2017). Consumers liked the color and aroma of roasted pecans that were hot water treated at 90°C. However, in general, consumers liked pecans treated at 70°C for its texture, flavor and overall

liking. This indicates that conditioning in-shell pecans with hot water enhanced the color quality, maintained the texture and subsequently roasted pecans were liked by consumers over raw pecans. Lower temperature for longer time (i.e. 70°C for 8.6 min) was best rated by consumers for its texture, flavor and overall liking.

4.3.5   Purchase intent

A drop in purchase intent was observed after the display of disclaimer even though overall liking of hot water treated pecans had increased. The highest purchase intent was observed for the roasted pecans that were hot water treated at 90°C which can be likely caused by its higher appearance/color, aroma and overall liking (Table 4.2). Still, there was a significant drop in purchase intent from 39.29 to 33.04% after the disclaimer was shown. Consumers intended to purchase the control pecans, with rise in purchase intent from 37.5% to 43.75%, despite the lower overall liking scores after the disclaimer. This showed that disclaimer about hot water treating the pecans for its safety had a negative impact on its purchase intent despite consumers' liking the samples. A study on impact of claims on consumer perception about prebiotic enriched breads found that even though there was no change in overall liking of the product when the claim was presented, there was decrease in the purchase intent by one of the clusters of people who were not receptive towards the claims. Consumers found them hard to understand and were skeptical on the truth of the claims (Coleman, Miah, Morris, & Morris, 2014).

Table 4.2. Consumer acceptability scores$^\beta$ and purchase intent before and after of heat treated pecans

Treatment	Appearance/ Color	Aroma	Texture	Flavor	OLb	OLa	PIb$^\mu$ (%)	PIa$^\mu$ (%)
Control	5.2±1.73[b]	5.79±1.77[b]	6.63±1.52[a]	6.29±1.8[a]	6.31±1.75[a]	6.21±1.8[a]	37.50	43.75
70°C	6.46±1.45[a]	6.32±1.47[a]	6.64±1.57[a]	6.42±1.7[a]	6.42±1.58[a]	6.53±1.5[a]	33.04	30.36
80°C	6.70±1.56[a]	6.37±1.51[a]	6.49±1.61[a]	6.17±1.8[a]	6.29±1.71[a]	6.43±1.7[a]	35.71	35.71
90°C	6.79±1.39[a]	6.42±1.66[a]	6.58±1.69[a]	6.21±1.7[a]	6.46±1.62[a]	6.52±1.6[a]	39.29	33.04

β Mean and standard deviation from 112 consumer responses based on 9-point hedonic scale. Mean values in the same column by different letters are significantly different ($P<0.05$).

μStatistically significant p-values in bold print ($P<0.05$) based on McNemar Exact Probability

A safety disclaimer "The shells of these pecans were treated with hot water making them safer for consumption" was displayed

OLb and Ola refers to Overall liking before and after safety disclaimer was displayed

PIb and PIa refers to Purchase intent before and after safety disclaimer was displayed

4.4 Conclusion

As the results of this study, apart from inactivating potential pathogens of public health concern, hot water pre-treatment of pecans enhances or maintains the quality of the pecan nutmeats. Significantly higher water activity values were observed for roasted hot water treated pecans as compared to roasted raw pecans. Heat treatment did not affect the textural quality of shelled pecans; however, hardness decreased for roasted samples owing to its brittle nature due to loss of moisture. As the temperature of hot water treatment was increased the color of kernels darkened. When roasted, L* values was lowest (41.87±0.6) for 90°C treated pecans giving it darker in appearance whereas, L* highest for raw pecans indicated a lighter colored kernel. This was seen from the consumer's mean score liking for color (5.2±1.73) of roasted raw pecans which indicated that consumers neither liked nor disliked the color. Although the likings were not significantly different, with higher values of flavor, texture and overall liking after disclaimer was shown, roasted pecans that were prior treated at 70°C was found to be the most liked product of all. A disclaimer concerning about the safety of pecans associated with hot water treatment displayed to the consumers had positive impact on overall liking which increased its mean scores however, there was drop in purchase intent of the hot water treated products. As for roasted raw pecans purchase intent increased from 37.5 to 43.75%. Thus, conditioning the in-shell pecans with hot water was found to enhance its color on roasting, maintain the texture, enhances the overall quality and was preferred over the roasted raw pecans by consumers.

4.5 References

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5. CONCLUSIONS

Hot water treatment of in-shell pecans at 70, 80 and 90° for 1 through 5 min could achieve different levels of reduction on pathogens. A five log reduction was achieved when pecans were treated for 5 min at 80°C or 4 min at 90°C for *Salmonella,* 5 min at 80°C or 3 min at 90°C for *E. coli* O157:H7*,* 4 min at 80°C or within 1 min at 90°C for *Listeria monocytogenes.* Likewise, the non-pathogenic organism in the study, *Enterococcus faecium* required 4 min when treated at 90°C to achieve desired level of reduction. The D-values (min) at 70, 80 and 90°C were 1.36, 1.25 and 0.85 for *Salmonella,* 1.38, 0.87 and 0.73 for *E. coli* O157:H7, 1.15, 0.83, 0.41 for *Listeria monocytogenes* and 1.72, 1.19 and 0.92 for *Enterococcus faecium* respectively. Our results indicated that *Enterococcus faecium* was the most heat resistant organism of all at 70 and 90°C whereas at 80°C, *Salmonella* showed higher resistance. However, there was no significant difference ($P>0.05$) in the heat resistance shown by *Enterococcus faecium, Salmonella* spp. and *E. coli* O157:H7 at each temperature. *Enterococcus faecium* possessed similar heat resistance as that of *Salmonella* and thus could be considered safe to be used as a surrogate organism in thermal process validation in the pecan manufacturing areas. The z-values (°C) of *Enterococcus faecium, Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes* were found to be 7.62 ± 1.36, 10.18 ± 1.9, 5.80 ± 0.61 and 4.51 ± 0.51 respectively. As calculated from the D-value table, time required for 5 log reduction of most heat resistant organisms were 6.8, 6.25 and 4.25 min for *Salmonella enterica* and 8.6, 5.95 and 4.6 min for *Enterococcus faecium* at 70, 80 and 90°C respectively. Thus, the hot water treatment was found to be an effective kill-step for different potential pathogens in pecans.

As calculated earlier, hot water treatment of in-shell pecans at 70, 80 and 90°C for 8.6, 6.6 and 4.6 min respectively was found to achieve 5 log reductions of all pathogens in the study. Thus,

in-shell pecans that were exposed to the treatments were monitored for any change in quality and consumer liking. Hot water treatments had no significant ($P>0.05$) effect on the moisture content and water activity of pecans when compared to its controls (i.e. raw pecans). Roasting significantly decreased the moisture content of the raw and hot water treated pecans from 6.09-6.97% to 2.06-2.94%; while, the water activity significantly dropped from 0.80-0.85 to 0.35-0.44. Heat treatment didn not have a pronounced effect on the hardness of pecans; however, roasted shelled pecans required less force to get deformed which can be due to its brittle nature due to removal of moisture. When in-shell pecans were hot water treated, no effects were observed on the kernel color. However, when the pecans were roasted, the L* values of pecans hot water treated at 70, 80 and 90°C significantly decreased to 44.76±0.07, 44.69±1.08 and 41.87±0.69 respectively. This indicated that hot water treated pecans became darker on roasting. Among all the samples, roasted raw pecans was the lightest while roasted pecan that was hot water treated at 90° was the darkest. The hue angles of pecans ranged from 59.88-64.26° and chroma values ranged from 23.69-30.69. This indicated that pecan kernels had a dark yellow shade on the color wheel.

The mean liking scores for the color and aroma of the control (roasted raw pecan) were 5.2 ($P<0.05$) and 5.79 ($P<0.05$) respectively, lowest among all the samples indicating that consumers neither liked nor disliked the sample. Increasing the hot water treatment temperature increased the liking of its color among consumers as the kernel color became darker. Likewise, there was an increase in the liking for aroma in the same pattern. Also, it was seen that the disclaimer about treating pecans with hot water for its safety has shown a rise in overall liking from 6.42 to 6.53, 6.29 to 6.43 and 6.46 to 6.52 in 70, 80 and 90°C treated pecans respectively while there was drop in the overall liking from 6.31 to 6.21 when consumers knew about the control pecans. Also, a drop in the purchase intent of the hot water treated products was seen after display of disclaimer.

As for roasted raw pecans purchase intent increased from 37.5 to 43.75%. Lower temperature for longer time (i.e. 70°C for 8.6 min) was rated best by consumers for its texture, flavor and overall liking. Thus, the hot water treatment not only effectively inactivated potential pathogens in pecans but it also enhanced or maintained the quality and sensory liking.

APPENDIX: IRB APPROVAL FORM

LSU AgCenter Research & Extension

LSU AgCenter Institutional Review Board (IRB)
Dr. Michael J. Keenan, Chair
School of Human Ecology
209 Knapp Hall
225-578-1708
mkeenan@agctr.lsu.edu

Application for Exemption from Institutional Oversight

All research projects using living humans as subjects, or samples or data obtained from humans must be approved or exempted in advance by the LSU AgCenter IRB. This form helps the principal investigator determine if a project may be exempted, and is used to request an exemption.

- Applicant, please fill out the application in its entirety and include the completed application as well as parts A-E, listed below, when submitting to the LSU AgCenter IRB. Once the application is completed, please submit the original and one copy to the chair, Dr. Michael J. Keenan, in 209 Knapp Hall.

- A Complete Application Includes All of the Following:
  - **(A)** The original and a copy of this completed form and a copy of parts B through E.
  - **(B)** A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1 & 2)
  - **(C)** Copies of all instruments and all recruitment material to be used.
    - If this proposal is part of a grant proposal, include a copy of the proposal.
  - **(D)** The consent form you will use in the study (see part 3 for more information)
  - **(E)** Beginning January 1, 2009: Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing and handling data, unless already on file with the LSU AgCenter IRB.
    Training link: (http://grants.nih.gov/grants/policy/hs/training.htm)

1) Principal Investigator: _Witoon Prinyawiwatkul___ **Rank:** _Professor___ **Student?** _No___
School of Nutrition and Food Sciences   **Ph:** _8-5188_
E-mail: wprinyawiwatkul@agcenter.lsu.edu and **wprinya@lsu.edu**

2) Co-Investigator(s): please include department, rank, phone and e-mail for each   NONE
- If student as principal or co-investigator(s), please identify and name supervising professor in this space

3) Project Title: Consumer Acceptance and Perception of New and Healthier Food Products
4) Grant Proposal?(yes or no) _NO___   If Yes, Proposal Number and funding Agency_____
  Also, if Yes, either: **this application completely matches the scope of work in the grant Y/N__**
  OR
  more IRB applications will be filed later Y/N___
5) Subject pool (e.g. Nutrition Students)_ LSU Faculty, Staff, Students and off-campus consumers
- Circle any "**vulnerable populations**" to be used: (children<18, the mentally impaired, pregnant women, the aged, other). Projects with incarcerated persons cannot be exempted. **NONE**
**6) PI signature_____ **Date _3-12-2015___ (no per signatures)
**I certify that my responses are accurate and complete. If the project scope or design is later changed I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU AgCenter institutions in which the study is conducted. I also understand that it is my responsibility to maintain copies of all consent forms at the LSU AgCenter for three years after completion of the study. If I leave the LSU AgCenter before that time the consent forms should be preserved in the Departmental Office.

Committee Action: Exempted___ Not Exempted __✓__ IRB# _HE15-9_
Reviewer _Michael Keenan_ Signature _Michael Keenan_ Date _3-16-2015_

VITA

Karuna Kharel was born and raised in Kathmandu, Nepal. She received her Bachelor's degree in Food Science and Technology from Tribhuvan University, Nepal in 2014. She came to the US for her higher education and is currently a Master's student in Food Science and Technology at Louisiana State University. She is a graduate research assistant in the School of Nutrition and Food Sciences (SNFS). She plans to graduate in the December of 2017. Upon completion of her master's degree she will begin work on her doctorate at LSU.