CONDITIONS FOR OPTIMAL SHELLING, MICROBIAL REDUCTION, AND KERNEL QUALITY IN PECANS

by

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(Under the Direction of William Kerr)

ABSTRACT

Tree nuts such as pecans can be contaminated with pathogens including *Salmonella enterica* subspecies *enterica* during harvest, processing, and post-processing. Thus, a microbial reduction step such as heat treatment is recommended for pecan processors to produce a safe product. However, the quality of the final product may be affected by a thermal treatment. This research seeks to achieve a 5 log CFU/g reduction in a surrogate organism for *Salmonella enterica*: *Enterococcus faecium* on in-shell pecans using hot water or saturated steam. This research also studies the effects of these heat treatments on quality parameters of pecans including cracking efficiency, color, and moisture content. A 5 log CFU/g reduction in *E. faecium* was achieved using a hot water treatment at 90 °C for at least 3 min or saturated steam treatment at 100 °C for at least 4 min. No significant changes in moisture percentage or pecan testa color were found.

INDEX WORDS: Processing treatment, pecan color, *Enterococcus faecium*, *Salmonella enterica*, pecan quality, saturated steam, hot water treatment

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CHAPTER 1

INTRODUCTION

The United States produces approximately 300 million tons of pecans annually which accounts for approximately 80% of the world's pecans. Georgia is one of the top pecan-producing states in America, in 2019 growing 69 million pounds of pecans (in-shell basis). The demand for pecans worldwide is growing, especially in Asia. To keep up with demand, Georgia needs to enable the operation of more pecan sheller and grower facilities. New shellers need to know the best and most economical ways to produce a safe and high-quality product for consumers both domestically and abroad. The United States Department of Agriculture (USDA) (2019) found that pecan production in the U.S. increased in 2019 by 14% and demand is expected to continue to grow. The market is variable, but with current health food trends in mind, it is important for Georgia to stay ahead of the curve and provide information regarding production and processing of high-quality pecans. A critical factor in this endeavor is food safety, since there have been outbreaks of foodborne illness linked to tree nuts (FDA Archive, 2017; Harris et al., 2019) and an excess of caution is recommended. However, in the pursuit of food safety, the impacts of processing activities on the quality and appearance of the product and its ultimate consumer acceptability must still be considered. This research study attempts to link optimizing food safety in Georgia pecans while also preserving the best quality possible considering typical pecan quality indicators.

CHAPTER 2

REVIEW OF LITERATURE

Pecans and Pecan Processing

Pecan Health Benefits

The health benefits of pecan consumption are becoming more widely recognized in the health food market. Pecans contain more than 19 vitamins and minerals and are composed of approximately 70% lipids (Sabate, 2003). Most of this fat is monounsaturated (66%) with some polyunsaturated fat (26%) and very little saturated fat (8%) (Sabate, 2003). Studies have also shown that consuming pecans this frequently can help to decrease cancer risk by up to 11% (Hudthagosol et al., 2011; McKay et al., 2018). McKay et al. (2018) determined that consumption of pecans as 15% of total calories consumed could be linked to a concurrent and clinically significant effect in overweight and obese adults on markers of cardiometabolic risk such as serum insulin, insulin resistance, and beta cell function. With the pecan diet, insulin decreased by 1.31 μIU/mL, glucose decreased by 97 mg/dL, and total cholesterol decreased by 0.98 mg/dL (McKay et al., 2018). A study by Morgan and Clayshulte (2000) determined that in a group of individuals consuming ~68g of pecans per day for 8 weeks resulted in a lowering of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in adults with normal lipid levels compared to the control group who avoided nuts and nut products for 8 weeks. Pecans contain a sterol called β-sitosterol which can inhibit uptake of cholesterol in the body and can also contribute to lower levels of LDL-C (NMSU, 2018).

Pecans are also an excellent source of antioxidants because they are rich in phytochemicals including β-carotene, vitamin E, lutein, and ellagic acid (NMSU, 2018). The antioxidant power of pecans contributes to a reduction of cancer risk by binding free radicals. In addition, one ounce (28.35g) of pecans contains about 10% of the average person's recommended daily value of fiber and contributes high-quality protein and low amounts of carbohydrates (NMSU, 2018).

A study by Robbins et al. (2015) determined the predominant phenolic compounds in pecans to be gallic acid, ellagic acid, their derivatives, and proanthocyanins. A study by Hudthagosol et al. (2011) found that bioactive constituents of pecans such as γ-tocopherol and flavan-3-ol monomers (compounds with known antioxidant properties) are absorbable and can contribute to postprandial antioxidant defenses in plasma in healthy adults. γ-tocopherol is a form of vitamin E that is found in pecans and other seeds and nuts (NMSU, 2018). These phenolic compounds contribute to the overall antioxidant behavior of pecans and consuming pecans may help to decrease risk of cancer, heart disease, and stroke (NMSU, 2018).

A study by Rajaram et al. (2001) found that no increase in body fat occurred from consuming a pecan-enriched diet (20% pecans) compared to a nut-free control diet, despite the high fat content of pecans. They found that the subjects on the pecan-rich tended to be hungrier and required more caloric intake to maintain their body weight (Rajaram et al., 2001). Another study found that a 3 kg weight reduction occurred in the nut-fed group over a 6-month period (O'Byrne et al., 1997). These studies suggest that the isocaloric replacement of pecans for other foods does not lead to an increase in body weight (Sabate, 2003).

Pecan Harvest and Storage

Pecans are harvested from trees using mechanical shakers to deposit the pecans onto the ground. After a time, the pecans are swept into windrows and picked up and transported in trailers

to their next destination (Santerre, 1994). The pecans are transported to holding locations where their moisture level is reduced due to low relative humidity of the storage areas. Moisture level, water activity, and relative humidity (RH) of the storage environment are critical factors in the storage of pecans. All three of these factors are correlated and if in-shell pecans are stored improperly, conditions may become suitable for possible mold or bacterial growth and other quality deterioration such as lipid oxidation. Water activity levels of 0.65 to 0.7 a_w equate to approximately 4.3-4.5% moisture (Santerre, 1994). The recommended moisture level for storing pecans in-shell is approximately 3.5-4.5%. An RH of 55-65% is ideal for storage, as any higher can contribute to mold or bacterial growth (Prabhakar et al., 2020). Pecans should be stored at refrigerated temperatures to further prevent quality deterioration including lipid oxidation and testa darkening (Beuchat & Heaton, 1975; Prabhakar et al., 2020). Freezing in-shell nuts can lead to nutmeat shattering, so storage temperatures of 1-10 °C are recommended (Beuchat & Pegg, 2013). Prevention of light exposure during storage also contributes to preventing lipid oxidation.

Cultivars, Conditioning, and Treatment

There are over 500 varieties of pecan cultivars, but most in the commercial pecan industry are from 5 major cultivars: ‘Cape Fear’, ‘Desirable’, ‘Moreland’, ‘Stuart’, and ‘Natives’ (Grauke & Thompson, accessed 2021). These cultivars vary in size, shape, and structure and also have large variability within their cultivars based on the conditions before harvest and care and upkeep of the pecan trees and their health. Pecan nut length to height ratio determines its general shape, and the apex, cross-section, and base shape also vary by cultivar (Wells & Conner, 2015). It is important to note the high variability in pecan structure when evaluating the effectiveness and efficiency of processing, treating, cracking, and shelling pecans in the industry.

Following storage, pecans are conditioned to prepare them for cracking and shelling and to reduce possible microbial load. In the pecan industry, in-shell pecans may first be submerged in water ~23 °C to wash debris from their surfaces. This is customarily followed by a first drying step at 60 °C followed by a microbial reduction treatment such as saturated steam, cold chlorinated water soaking, or hot water soaking treatment. Another drying step should be done after these treatment processes to drain excess water. These treatments serve as a microbial reduction step, but also serve to increase the total moisture level of the pecan to about 8% after the drying step (Santerre, 1994). The increase in moisture is necessary because if the pecan is too dry, the nutmeat can shatter during cracking and shelling which is undesirable.

Cracking and Shelling

Pecans are cracked and shelled using machines made of steel. The cracker machine orients the nuts horizontally and then strikes the nut from both ends with approximately 200-600 pounds of force (Santerre, 1994). This cracks the outer shell while usually leaving the inner nutmeats intact. Most pecan crackers can be adjusted to approximately the size of the variety or cultivar that is being cracked. After cracking, the nuts are transferred to a sheller machine which contains steel plates through which the pecans tumble and are gently separated from the shell pieces. Following this step, the nutmeats and shell fragments go through a blower which blows the lighter shell fragments away from the heavier nutmeats (Santerre, 1994).

Parameters of Quality Pecans

Cracking Efficiency

In the American pecan market, whole (intact) halves of pecans have the most value and the highest retail price. Whole halves have a longer shelf life than smaller pecan pieces due to less exposed surface area, and they are less susceptible to insect infestation and other quality-

deteriorating problems because there is less accessibility for oxygen and light which can lead to quality deterioration (Forbus & Senter, 1976; Brooks et al., 1965). Also, whole halves can be further broken down into smaller pieces as the consumer prefers. As mentioned above, the conditioning treatments also serve to increase yield of whole halves by increasing the moisture content to ~8%. The nutmeat moisture level increase helps to make the nutmeat more pliable which prevents breaking into smaller, less desirable pieces. Presently, yield of whole halves in processing plants ranges from 50-80% of total yield. Conditioning and heat treatment temperature and duration also play a role in cracking efficiency.

A study by Forbus and Senter (1976) established that saturated steam treatment yielded 12-17% more whole halves compared to chlorinated water soaking and hot water treatment. They were able to obtain 19% more whole halves than the untreated control pecans (Forbus & Senter, 1976). There is no present research on hot water treatment at levels sufficient for microbial reduction on cracking efficiency of in-shell pecans. Beuchat and Heaton (1975) were able to show that when in-shell pecans are submerged in hot water, the internal nutmeat temperature increases slowly. They concluded that this is due to the poor heat conductivity of the pecan nut interior packing material and the high lipid content of the nutmeat (Beuchat & Heaton, 1975). Lipids have slower heat transfer coefficients compared to water and higher moisture foods, and the low water activity of the pecan nutmeat means the nutmeat heats more slowly. This finding could suggest that minimal effect of hot water treatment on kernel quality or acceptability parameters such as color, texture, or flavor can be expected.

A study by Kharel et al. (2019) found that hot water treatment of pecans at temperatures 70, 80, and 90 °C for 4.6, 6.6, and 8.6 min did not significantly affect the moisture content of the raw pecan nutmeats. The pecan nutmeats were subsequently roasted at 160 °C for 10 min and

moisture content was decreased from 6.09-6.97% to 2.06-2.94%. However, the Kharel et al. (2019) study and a study by Beuchat and Mann (2010) both found that water activity is affected by the temperature of the hot water treatment. As the temperature of treatment increased, the resulting water activity increased. They also found that if the starting pecans were at a lower temperature than the water in which they are being immersed, the water activity increased less (Beuchat & Mann, 2010). A study by Senter et al. (1984) evaluated the effects of saturated steam on pecan kernel storage stability and found that after treatment at normal steam temperature (100 °C), moisture content of the pecans was not significantly affected until the steam temperature increased to 136 °C and 156 °C. They also found that steam treatment can help decrease lipid oxidation when pecans are treated before storage (Senter et al., 1984).

Color

Pecan nutmeat testa color is considered to be one of the most important factors to estimate quality of the pecan, according to pecan shellers. The testa is the outer layer or "skin" of the pecan nutmeat and is usually golden brown or amber colored. Pecan processors consider darkening of the testa to be undesirable, because it can indicate other quality problems or improper storage conditions. The USDA has a four-color standard system for pecan testa color with four classes: golden, light brown, medium brown, and dark brown (Thompson et al., 1996). However, in the pecan industry, they do not consider this classification system to be useful since they consider high quality pecans to be even lighter in color than 'golden'. In the industry, dark color (i.e., USDA dark brown or dark amber) can indicate age and rancidity and is classified thus when greater than 25% of the nutmeat surface is 'dark brown' (Thompson et al., 1996). Pecan testa color darkening is affected by other factors such as cultivar, time of harvest, moisture content, and storage time

and conditions including whether they are stored in-shell or shelled (Thompson et al., 1996). It is also known that heat treatment can affect nutmeat darkening.

There are many different color systems that can be used to evaluate and compare pecan testa color, but it is challenging because pecans do not have smooth surfaces and the color of one whole half may vary over its surface. Colorimeters can be used to obtain L*a*b color values. Lightness (L*) is the most accurate of these measures as a* (green-red) and b* (blue-yellow) influence each other and indirectly reflect hue and chroma (Thompson et al., 1996). Color can also be interpreted by comparing pecans to standards using color chips such as the Munsell color system of value, chroma, and hue using soil color chips, though this method is also open to interpretation (Thompson et al., 1996). Because of the subjectivity of color, it has been recommended to use a Konica-Minolta chromameter or similar device to gauge colors numerically using the L*a*b* color space for horticultural food samples (ASHS, 2016). These values can be converted to chroma and hue angle (°) which are more easily visualized using a two- or three-dimensional color space. The study by Kharel et al. (2019) analyzed color of pecan nutmeats after hot water treatment and roasting using a Konica-Minolta chromameter which analyzed the L*a*b* values. They found that the L* values showed no significant difference based on the hot water treatment. After roasting, compared to the unroasted hot water-treated nuts, they found that the L* significantly decreased, concluding that hot water treatment followed by roasting results in testa darkening (Kharel et al., 2019). The darkening was attributed to non-enzymatic Maillard browning and caramelization (Kharel et al., 2019). The further darkening of the testa of the hot water treated and roasted nuts compared to the roasted control is due to the higher water activity of the nutmeats after hot water treatment (Kharel et al., 2019). The water activity increase results in higher mobility of reactants which increases the reaction rate of the Maillard browning reaction (Kharel et al., 2019). Sensory

analysis was also performed in the Kharel et al. study (2019), and they found that consumers preferred pecans (based on aroma and color) that had been treated with hot water and then roasted over pecans that were only roasted, due to the darker color and more roasted aroma of the pecans treated with hot water first (Kharel et al., 2019). The increase in this aroma and color change resulted from the water activity increase allowing the roasting step to cause more Maillard browning and resulting flavor compounds to emerge. They did not find any significant difference between flavor and texture preference due to the hot water treatment alone. However, they found that overall, when the consumers knew that hot water treated pecans were safer, their acceptability increased (Kharel et al., 2019).

A study by Ban et al. (2018) evaluated the effect of superheated steam on pecan testa color. Superheated steam is steam under high pressure, increasing its temperature, in this case up to 120-180 °C. Ban et al. (2018) observed little change in color using a Konica-Minolta chromameter with L*a*b* for evaluating color of pecan testa. They also observed a small change in moisture resulting from the treatment at all the temperatures but found that a smaller moisture change was shown after treatments at 180 °C compared to those at lower temperatures. They hypothesized that this was due to the increased temperature of the pecans creating a higher heat energy and the moisture escaping more quickly after the treatment as a result (Ban et al., 2018).

Kays (1980) studied the influence of iron content in pecan testa color. They determined that an iron-containing fraction of the testa was causing the color changes when pecan nutmeats were exposed to an oxidizing environment or ammonia vapor (Kays, 1980). The total iron contents of four pecan cultivars ranged from 78.3 to 99.3 ppm (Kays, 1980). They determined that the iron in pecans is largely concentrated in the testa which is why the testa is so color-reactive (Kays, 1980). There is little to no iron content in the light-colored interiors, which usually do not show

signs of darkening. Ammonia damage can cause reversible or irreversible darkening of the testa due to the iron reacting with the ammonia and making brown $Fe(OH)_3$. This reaction can be reversed using sulfur dioxide or phosphoric acid, but only for pecans that were treated with ammonia vapor for less than 10 min (Kays, 1980). Extensive ammonia damage leads to only partly reversible darkening with these treatments (Kays, 1980). These findings may explain why pecan processors tend to consider darkened kernels to be damaged or of low-quality.

Microbiology of Tree Nuts

Contamination

Contamination of pecans with *Salmonella enterica* subspecies *enterica* or other enteric pathogens can occur at various points in the growing, harvesting and production process. Potential sources include the soil when the pecans are shaken onto the ground, wildlife and grazing animals in or around the pecan trees, or contaminated irrigation water or runoff (USDA, 2013). Cross-contamination can also occur between treated and untreated pecans, and facilities may accidentally contaminate their product by lack of sufficient cleaning and sanitation or environmental contamination (USDA, 2013). Beuchat and Mann (2010) studied the rate of infiltration and survival of *Salmonella* on in-shell pecans. They found that water infiltration into in-shell pecans is dependent on the extent of damage to the pecan shell and that the rate of infiltration is higher at higher temperatures (Beuchat & Mann, 2010). In-shell pecans immersed in 8.66 log CFU/mL *Salmonella* inoculum containing a mixture of 5 serotypes for 1 h enumerated levels of 6.94-6.99 log CFU/g of *Salmonella* (Beuchat & Mann, 2010). They also inoculated in-shell pecans at lower levels of 2.82 log CFU/mL *Salmonella* cocktail inoculum for 1 h resulting in 1.53 log CFU/g on the in-shell nuts (Beuchat & Mann, 2010). They determined that if *Salmonella* bacteria reached the kernel of the pecan, for both high and low *Salmonella* populations, the *Salmonella* cells

remained viable after drying and storage at 4 ℃ or -20 ℃ for 78 weeks (Beuchat & Mann, 2010). Their results emphasize the importance of developing process treatments that reduce populations of *Salmonella* at low or high levels regardless of storage time or temperature before delivering the product to the market.

Outbreaks

While there have not been any outbreaks of foodborne illness in humans directly linked to pecans, other tree nut-associated outbreaks indicate that caution must be practiced when bringing tree nuts to consumers. Pecans have been included in recalls in an abundance of caution due to a positive sample for *Salmonella* on pecan pieces produced by the American Pecan Co, though no consumers experienced illness (FDA Archive, 2017). Several outbreaks of Salmonellosis have been linked to almonds in several countries including the United States. Raw almonds are the most often associated with outbreaks due to lack of a roasting treatment, but there have also been outbreaks in almond butter and sprouted spreads (Harris et al., 2019). The definition of "raw" almonds means unroasted, since it is against the law to produce almonds without a pasteurization step, following the expansion of USDA rule 7 CFR Part 981 in 2016. Other tree nut products produced with coconut, cashew, hazelnut, and walnut have also been recalled due to presumptive presence of pathogens (Harris et al., 2019) With these outbreaks in mind, it is important to consider the risk of Salmonellosis from all types of tree nuts and develop risk-based controls to reduce risk of illness.

Tree Nuts Regulations

The National Pecan Shellers Association has compiled guidance for pecan producers based on standards set in related industries. These protocols are highly recommended but are not legally binding Hazard Analysis Critical Control Point (HAACP) regulations. However, pecan processors

as well as other tree nut producers are regulated by FSMA "Preventive Controls" or "Produce Safety" rules (FDA, 2020). Depending on their activities, they fall under either of these FSMA rules. A "Primary Production Farm" falls under the "Produce Safety" rule and would apply to pecan growers. A pecan shelling operation which grows a majority of its pecans would be considered a "Secondary Activities Farm" and this would also fall under the "Produce Safety" rule. A pecan shelling operation which does not produce or own the majority of nuts that it is shelling would be considered a processor that falls under the "Preventive Controls" rule. The "Produce Safety" rule dictates science-based standards for safe production and harvest of fresh produce. The standards include regulations regarding worker health and hygiene, agricultural water, soil standards, domesticated and wild animals, and equipment sanitation (FDA, 2020). The "Preventive Controls" rule requires Current Good Manufacturing Practices (CGMPs) including employee certifications, food safety training, employee health and hygiene, allergen cross-contact standards, by-products, and an approved food safety plan (FDA, 2020). The food safety plan should include hazard analysis, preventive controls, supply chain programs, a recall plan, and oversight and management of these (FDA, 2020).

The California Department of Health and US Food and Drug Administration (FDA) dictate that a minimum 4 log CFU/g *Salmonella* reduction must be achieved on all almonds grown in California (7 CFR Part 981). This regulation was motivated by the 2001 and 2004 *Salmonella* outbreaks in raw almonds. In addition, the FDA recommends a 5 log CFU/g reduction in pathogens for pistachios and peanuts (FDA, 2009). While there are no legally binding regulations concerning log-reduction in pathogens for pecans, nor any FDA regulations or recommendations specifically concerning pecans, the National Pecan Shellers Association set forth the following guidance to pecan producers: "a 4 to 5 log reduction in pathogens by use of a pasteurization step is

recommended at this time for all pecan products" (2009). According to their protocol, this reduction may be achieved using hot water treatment for 2 min at 190 °F (~87 °C), oil roasting for 2 min at 260 °F (~126 °C), or a treatment of propylene oxide gas at 0.5 ounces per square foot. However, they proceed to mention that these processes should be verified for use on pecans and further recommend verifying that the reduction is constantly being achieved. Annual process audits are also recommended. Process validation must be conducted by "an authority who has the educational requirements and experience to evaluate the effectiveness of a process to reduce the level of *Salmonella*" (p. 2) (NPSA, 2009).

Salmonella on Pecans

According to a study by Beuchat et al. (2011), the use of cold chlorinated water (400 µg /mL chlorine) alone decreased *Salmonella enterica* on in-shell pecans by no more than 1.6 log CFU/g. Soaking in cold chlorinated water (200 µg/mL) followed by soaking in fresh water for 2 h at 21 °C then treating for 10 min in 85-95 °C water reduced *Salmonella enterica* by >5.12 log CFU/g (Beuchat & Mann, 2011). They determined that a combination of chlorinated water soaking, water soaking, and hot water treatment proved most effective at eliminating *Salmonella enterica* (Beuchat & Mann, 2011). In pecan processing facilities, the nuts are not typically dried completely between treatments, but they found that a drying step after inoculation could hinder some growth or survival of *Salmonella* cells between 0.42 log and 1.23 log CFU/g (Beuchat & Mann, 2011). Surface inoculated pecans experienced higher log reductions after treatment than immersion inoculated pecans, due to pecan shells allowing bacteria into the interior of the shell through cracks, damage, or small pores (Beuchat & Mann, 2011).

A study by Farakos et al. (2017) found through situational modeling that hot conditioning, such as hot water or steam, had a significant impact on the likelihood of foodborne illness, in

"typical" situations of contamination (i.e. contamination that is unlikely to be easily avoided). In "atypical" cases, such as cattle grazing below the pecan trees, recontamination of the nuts after treatment, or delay in drying post-conditioning had much higher risk of illness (Farakos et al., 2017). They concluded that delay of drying post-processing had the greatest impact on risk for both typical and atypical situations due to the higher moisture level and water activity creating an environment suitable for any surviving *Salmonella* cells to grow (Farakos et al., 2017) A study by Beuchat and Heaton (1975) examined high amounts (>5 log CFU/g) of *Salmonella* on in-shell pecans. They concluded that there was a lack of uniformity of the contamination on/in the nuts as well as a lack of uniformity for heat penetration into the interior of the pecan. They examined heat penetration within the interior of the shell and concluded that there is only a 2 °C difference between the temperature of the outer shell and packing material and the nutmeat itself (Beuchat & Heaton, 1975). The pecan packing material is porous and thus conducts heat poorly, and if *Salmonella* cells were able to reach the lipid-rich nutmeat, they may remain viable even after up to 2 min treatment in hot water at 82-93 °C (Beuchat & Heaton, 1975). In-shell pecans can absorb liquid through fibrovascular bundles on the ends of the pecan and through suture separations at their apex (i.e., where the pecan was attached to the tree). In addition, the internal packing material of the in-shell pecan has some bactericidal properties, probably due to levels of tannins and polyphenolic compounds (Beuchat & Heaton, 1975).

A study by Beuchat and Mann (2011) evaluated *Salmonella* survival using hot air roasting, hot oil roasting, and dry air roasting on shelled pecan halves. These are recommended treatment types by the National Pecan Shellers Association, as mentioned above. Beuchat and Mann (2011) used high moisture (10.5-11.2%) and low moisture (2.8-4.1%) nutmeats and compared the treatments' microbial reduction of immersion-inoculated nutmeats (~7 log CFU/g). They found

that hot air roasting at 120 °C, regardless of moisture content, did not eliminate *Salmonella*, and they found that hot air treatment produced undesirable sensory qualities (Beuchat & Mann 2011). However, dry roasting of the pecan halves resulted in a >7 log CFU/g reduction of *Salmonella* at 160 °C for 15 min and a 5 log CFU/g reduction was accomplished when dry roasting at 140 °C for 20 min, 150 °C for 15 min, or 170 °C for 10 min (Beuchat & Mann 2011). Hot oil roasting in peanut oil produced a 5 log CFU/g reduction after 1.5 min at 127 °C or 1 min at 132 °C (Beuchat & Mann 2011).

Propylene oxide gas (POG) treatment is also a treatment suggested by the National Pecan Shellers Association. However, no studies have been published regarding how *Salmonella* reacts to propylene oxide treatment on pecans. A study by Blanchard and Hanlin (1973) evaluated how the general microflora of pecans reacts to propylene oxide gas treatment, but while they achieved some reduction in bacteria and fungi, neither could be eliminated completely. Saunders et al. (2018) studied propylene oxide inactivation of *Salmonella* using cashews and macadamia nuts. They found that *Salmonella* was reduced by 7.3 log CFU/g on macadamias and 5.4 log CFU/g on cashews after a treatment of POG in a commercial facility (Saunders et al., 2018). While these findings are promising, it is unknown how pecan halves quality and consumer acceptability would be altered by exposure to POG, and how effective it would be against high levels of *Salmonella* and other bacterial contamination. Ban et al. (2018) evaluated the effects of superheated steam (high pressure steam) on inactivation of *Salmonella enterica*. They concluded that superheated steam is effective at reducing populations of *Salmonella typhimurium* and *Salmonella enteritidis* on pecan halves (Ban et al., 2018). They found that the higher the temperature of the treatment, the inactivation efficiency increased (Ban et al., 2018). Their treatment of pecan halves at 180 °C for 13 s caused 6 to 7 log CFU/g reduction of *Salmonella* populations (Ban et al., 2018). They also

evaluated quality effects and determined that no quality deterioration of moisture content, color, or texture occurred as a result of the superheated steam treatment (Ban et al., 2018).

E. faecium as a Surrogate Organism

Applications

The Almond Board of California allows for use of a surrogate organism for process validation of log reduction of *Salmonella* for almonds. This surrogate organism, *Enterococcus faecium*, has also been validated for use in peanut and pecan thermal processing by various studies. Kopit et al. (2014) verified that *E. faecium* is genetically non-virulent and that it has high resistance to acidic conditions (~pH 2.4) and high temperatures (>60 °C). *E. faecium* was also shown to be able to grow in up to 8% ethanol (Kopit et al., 2014). They found that the thermal tolerance of *E. faecium* on almonds is comparable to that of *Salmonella enterica* (Kopit et al., 2014). Brar and Danyluk (2019) verified that, on in-shell pecans, *E. faecium* reductions for hot water treatments were not significantly different than reductions of three different *Salmonella* serotypes. The z-values they obtained with hot oil roasting of pecans were 44.8 °C for *Salmonella* and 45.2 °C for *E. faecium*. Liu et al. (2018) found that both *Salmonella* and *E. faecium* had log-linear increased d-values when processed at a fixed temperature and a_w was decreased. This finding shows that *E. faecium* and *Salmonella* react similarly to changes in a_w on low-moisture foods. These studies verify that *E. faecium* is an appropriate surrogate organism for *Salmonella* in low-moisture foods. The use of a surrogate nonpathogenic organism like *E. faecium* is beneficial if one is utilizing a food processing facility or equipment onto which a pathogen should not be introduced.

Heat Tolerance and Antibiotic Resistance

A study by Pangaea and Chadwick (1996) studied 27 *E. faecium* isolates in water and sought to determine heat resistance of wild-type (no adapted resistance) *E. faecium* and strains that

were resistant to Vancomycin, an antibiotic. They found that *E. faecium* cultures exposed at 71 and 80 °C in a water bath resulted in >6 log CFU/g reduction for all isolates (Pangaea & Chadwick, 1996). Twenty-four percent of the isolates survived at 65 °C for 10 min. They also compared the *E. faecium* isolates to two *E. faecalis* reference isolates and found that the *E. faecium* isolates were more resistant to heat than the *E. faecalis* isolates (Pangaea & Chadwick, 1996). They observed no difference between heat resistance of the antibiotic-resistant strains and the wild-type strains of *E. faecium* (Pangaea & Chadwick, 1996). *E. faecium*, like other enterococci, are naturally resistant to certain low levels of antibiotics due to resistance genes on their chromosomes (Rathnayake et al., 2012). *E. faecium* also readily develops resistance to antibiotics due to their ability to acquire resistance determinants on plasmids or transposons (Rathnayake et al., 2012).

As mentioned above, *E. faecium* is more resistant than *Salmonella* in terms of heat tolerance. Liu et al. (2018), evaluated *E. faecium*'s general tolerance and utility in a low-moisture food environment. They compared two substrates to mimic food matrices and found that the $\log D_{80°C}$ overlapped for both *S. enteritidis* and *E. faecium*. They concluded that this means that the d-values of these bacteria are more dependent on the water activity than the surrounding matrix (Liu et al., 2018). Kharel et al. (2018) calculated the z-value at 75.86 °C for *E. faecium* on in-shell pecans in hot water treatment. They also found the d-value at 70 °C to be 1.72 min and at 90 °C to be 0.92 min. Lee et al. (2006) found d-values for *Salmonella enterica* on almonds treated with steam (~100 °C) to be 12.22 min for one variety of almond and 16.13 min for the other. D- and z-values have not been determined for saturated steam treatment (100 °C) of in-shell pecans.

CHAPTER 3

EVALUATION OF MICROBIAL REDUCTION OF A SURROGATE ORGANISM ON IN-SHELL PECANS AFTER HEAT TREATMENT

Introduction

This portion of this research study focused on evaluating microbial reduction of *Enterococcus faecium*, a surrogate organism for *Salmonella enterica* subspecies *enterica*. There have been outbreaks of *Salmonella* on tree nuts such as almonds and as a result, pecan processors are attempting to add a reliable microbial reduction step to their processing methods before bringing the product to consumers in order to prevent possible foodborne illness. Based on previous studies mentioned above by Beuchat et al. (2011), Beuchat and Heaton (1975), and Brar and Danyluk (2019), we hypothesize that at least a 5 log CFU/g reduction in *E. faecium* can be accomplished using a thermal treatment on in-shell pecans such as hot water soaking in a steam kettle or other heating vessel at 80 or 90 °C or saturated steam treatment in a steam cabinet at approximately 100 °C. It is important to learn at which temperature, duration, and processing method is most effective at reducing *E. faecium* by at least 5 log CFU/g and relay this to pecan growers and shellers. The processors can then determine which method is most economical for their situation and their product and conduct process verification to ensure that it is consistent and reliable. With this information, processors can attempt to modify and conduct their processing methods effectively and produce safe pecans for consumers.

Materials and Methods

Inoculum Preparation

Microbiological methods were adapted from Kharel et al. (2018). The treatment groups were 70 °C, 80 °C, 90 °C, and 100 °C (steam) for 2-7 min. Each treatment was processed 6 times and results were averaged (n=6). *E. faecium* culture was revived from glycerol stocks for three subsequent transfers in 10 mL Tryptic Soy Broth (BD Bacto: Sparks, MD) for 24 h at 37 °C. These were gradually brought to be resistant to the antibiotics Rifampicin (Research Products International: Mount Prospect, IL) and Nalidixic Acid (Alfa Aesar: Haverhill, MA) at 50 ppm by increasing antibiotic concentrations by 5-10 ppm each subsequent transfer. Glycerol stocks of the antibiotic resistant culture were stored at -80 °C. Prior to use, culture was transferred into 10 mL TSB with 50 ppm Rifampicin (Rif) and Nalidixic Acid (Nal) for 24 h at 37 °C. A 10 µL loopful was transferred into fresh TSB with 50 ppm Rif+Nal and incubated two subsequent times. A 10 µL loopful was transferred into 1000 mL TSB with 50 ppm Rif+Nal and incubated at 37 °C for ~24 h. After 24 h, culture was then transferred to two sterile centrifuge bottles (ThermoScientific: Waltham, MA) and centrifuged (ThermoScientific: Waltham, MA) at 3400 rpm for 10 min. The supernatant was poured off and about 100 mL of 0.1% peptone (BD Bacto: Sparks, MD) was added to each centrifuge bottle and the culture was resuspended by hand shaking. Centrifugation was repeated for 10 min at 3400 rpm (356.05 radians/s), supernatant removed, and the pellets were resuspended in 50 mL of 0.1% peptone each, by shaking. The two 50 mL aliquots were combined into one final inoculum sterile bottle. This final inoculum was approximately $6x10^9$ CFU/mL (9 log CFU/mL) and was used to inoculate pecan samples.

Sample Preparation

Three-hundred pounds of in-shell pecans were obtained from a farm in Tifton, GA. The cultivars of pecans used for this experiment are Oconee and Desirable. Oconee and Desirable pecans have similar sizes, structures, and harvest dates (Wells & Conner, 2015) and were used interchangeably. These pecans were stored in mesh bags in a refrigerated setting[1] (2 °C, 55% RH) for approximately one year. For each treatment ~310 grams of pecans (~30 nuts) were weighed and added to a 4.25 L sterile sample bag (Whirlpak: Madison, WI). The 100 mL of *E. faecium* inoculum was poured over the pecans and hand massaged for 1 min. The inoculated pecans were incubated at room temperature in the inoculum in a biosafety hood for 1 h, with additional hand mixing occurring every 10-20 min.

Sample Processing and Treatment

After 1 h, the pecans were drained and spread in a single layer on a sterile foil pan in the biosafety cabinet for 20 min leaving space between each nut as a drying step. After 20 min, two nuts (~20 g) were aseptically added to a sterile 1.63 L filtered sample bag (Whirlpak: Madison, WI) with filter and sealed loosely at the top to approximately 10 bags per treatment cycle. The bags were submerged in the water bath (ThermoFisher Precision: Waltham, MA) at the specified temperature in 500 mL Naglene™ (ThermoScientific: Waltham, MA) beakers filled with water and held in place with round weights. The temperature within one bag was measured with a Type K thermocouple (Bestdo: Dobel, DE) to determine when the contents of the bag came to the desired temperature, at which point timing was started. After the specified time, the bags were removed

[1] The pecans used in the microbiology portion of this research developed mold due to extended storage over the COVID-19 lockdown. However, the presence of this mold should not have impacted the results of this study due to the inclusion of antibiotics Rifampicin and Nalidixic Acid at 50 ppm to eliminate background flora. The negative controls had no growth.

from the water bath and dried off. No drying step was done following hot water treatments since the pecans were only submerged in the hot water within a filter bag.

For steam treatments, pecans were weighed and then placed on a sterile double-layer aluminum foil pan. This pan, with the in-shell pecans, was placed in the pre-heated steam cabinet (Pyramid: Stilwell, KS) and processed for the specified time, not including come up time (~100 °C). The steam cabinet takes approximately 15 s to come to temperature and time was started after coming to temperature. No drying step was performed for steam treatments. For a visual representation of the steam cabinet design, see Figure 1.1.

Thermal curves were created by monitoring the surface temperature of a pecan in the different conditions used for this experiment. These are shown in Figures 3.1-3.3 and serve to illustrate the change in temperature over time before and after the treatment temperature was reached. Figure 3.2 shows how heat would have transferred if a filter bag was used inside the steam cabinet. It was decided to allow the pecans to be exposed to the steam directly and placed on a foil pan instead of within a filter bag because the internal temperature of the filter bag within the steam cabinet did not reach temperature (99-100 °C) even after 10 min. The thermal curve for steam treatment of a pecan on a foil pan is shown in Figure 3.1. The thermal curve for a pecan inside a filter bag within a hot water bath at each treatment temperature is shown in Figure 3.3.

Microbial Enumeration

For all samples, the pecans were crushed by hand inside the bag using a hammer. To each bag, 100 mL of peptone was added, and the bags were shaken for 20 s. The resulting bacterial suspension was then diluted into 9 mL peptone tubes and 3 dilutions per sample were plated with spiral plater (EddyJet 2W: Barcelona, ES) onto TSA+RN (TSA agar with 50 ppm Rif+Nal) (BD Bacto: Sparks, MD). The plates were incubated at 37 °C for 24-48 h. Plates were counted using

the spiral plate count method (SPLC) for bacterial enumeration counting grid (SPLC method of APHA and AOAC).

Two negative controls (pecans with no inoculum added and no treatment applied) and two positive controls (pecans with inoculum added and no treatment applied) were also conducted and plated for each sample group. Statistical analysis of this data was completed using one-way ANOVA with JMP statistical analysis software (Tukey's HSD, $p<0.05$), and the limit of detection for this experiment was calculated to be 1.35 log CFU/g.

Results

To evaluate the effectiveness of each treatment against the surrogate organism, *Enterococcus faecium*, log reductions of the organism were calculated and are shown in Figure 2.1. The average (n=3) log CFU/g of *E. faecium* recovered from the positive controls of this experiment ranged between 6.33-6.91 log CFU/g and are expressed as the time zero for each treatment in Figure 2.1. The 70 °C treatment achieved less than a 2 log CFU/g reduction in *E. faecium* after 6 min. At 80 °C, *E. faecium* was reduced by less than 3 logs. At 90 °C, there was a 5 log CFU/g reduction after only 2 min. The saturated steam treatment at 100 °C achieved a 5 log CFU/g reduction after 3 min. The limit of detection for this experiment was 1.35 log CFU/g Decimal reduction values were calculated for some temperatures: $D_{70}=4.8 \pm 0.4$ min, $D_{80}=3.0 \pm 0.5$ min, and $D_{100}=0.66 \pm 2.4$ min. The d-value for 90 °C was not able to be calculated due to the extreme dip in the data at the first timepoint. The z-values were not able to be calculated due to limited data.

Discussion

As illustrated in Figure 2.1, the 70 °C treatment achieved less than a 2 log reduction in *E. faecium* after 6 min. At 80 °C, *E. faecium* is reduced by less than 3 log CFU/g after 6 min. These two treatment temperatures are not viable options to achieve a 5 log reduction within a fast-paced production facility. At 90 °C, a 2 min treatment resulted in a 5 log reduction. This finding is consistent with work by Beuchat and Heaton (1975) that found that after a 2 min treatment in hot water at 82-93 °C, some *Salmonella* cells survived, resulting in a >4 log reduction. Therefore, it is recommended based on this research to exceed the minimum amount of treatment to achieve at least 5 log reduction and treat in-shell pecans for at least 3 min at 90 °C. A drying step was not performed after the hot water or steam treatments before microbial enumeration, but it would be recommended to accomplish a drying step in a production facility before pecans would be stored, cracked, or shelled. Farakos et al. (2017) emphasized that the drying step post-processing was a critical step to prevent growth of surviving bacteria. As Liu et al. (2018) found, water activity of the food matrix has a great influence on microbial reduction, survival, and growth. Therefore, it is recommended to thoroughly dry in-shell pecans after the conditioning step. Treatment in hot water increases the water activity, which decreases the decimal reduction time for the bacteria, but the water activity should be reduced again to prevent any surviving bacteria to grown during storage. The ideal water activity for in-shell pecans after the drying step should be approximately 0.5 a_w for storage (Santerre, 1994).

The saturated steam treatment at 100 °C achieved a 5 log CFU/g reduction after 3 min (Figure 2.1). This treatment was sufficient to reduce bacteria and pathogens by at least 5 log CFU/g but is recommended based on this research for in-shell pecans to be processed for at least 4 min due to the need to exceed the minimum treatment required to achieve at least a 5 log reduction

every time. When comparing steam to hot water treatments in an industry setting, the 90 °C treatment could be performed in a continuously heating hot water soaking tank which is kept at 90 °C. While the hot water treatment would need to be accomplished in batches in order to monitor the treatment time, the come-up time of the water would be little to none, and the nuts would reach 90 °C almost immediately after being submerged in the water. However, the recommended drying time would be longer (overnight) than the drying time after a saturated steam treatment (1-2 h). A saturated steam treatment can be accomplished in a steam cabinet or a continuous steam blancher in a pecan processing plant. There are no modern studies involving pathogen microbial reduction and saturated steam conditioning of in-shell pecans, but Lee et al. (2006) studied the effects of steam on *S. enterica* on the surfaces of raw shelled almonds. They found that a reduction between 4.0 and 5.8 log CFU/g occurred after 65 s of steam treatment. The structure of a shelled almond is quite different from an in-shell pecan, so it can be hypothesized that while steam would be effective against microbial load, the duration of treatment may need to be somewhat longer to be able to penetrate the pecan shell and packing material.

It is evident that this data in Figure 2.1 is counterintuitive in that steam treatment (100 °C) should achieve a reduction more quickly than hot water treatment at 90 °C. However, the thermal curves created in the conditions used for this experiment (Figure 3.1-3.3) show that the increase in temperature for steam treatment with a foil pan (i.e., the conditions used for this experiment) occurs much faster (Figure 3.1) than the heat penetrating the filter bag in the hot water treatment experiment. Figure 3.3 shows the change in temperature over time for the hot water treatment occurred more slowly and gradually. Based on the data in Figure 2.1, during treatment at 70 and 80 °C, some microbial reduction is able to occur at those temperatures. Therefore, it can be

concluded that some reduction in *E. faecium* probably was able to occur during the 90 °C treatment before time even started (i.e., before the temperature within the filter bag reached 89-90 °C).

At 70 °C, microbial reduction at all timepoints were not significantly different. The 80 °C treatment values were not significantly different from the 70 °C treatment except for the 5 min timepoint at 80 °C. For 90 °C, all timepoints were not significantly different from each other and to the 1, 2, 3, and 4 min timepoints of 100 °C. The 0.5 min timepoint at 100 °C was not different from to the 70 and 80 °C treatments (Figure 2.1). The d-values for this experiment were D_{70}=4.8 ± 0.4 min, D_{80}=3.0 ± 0.5 min, and D_{100}=0.66 ± 2.4 min. The d-value for 90 °C was not able to be calculated due to the extreme dip in the data after the zero timepoint. The z-values were not able to be calculated due to limited data. The d-values listed above are close to the d-values for hot water treatment of in-shell pecans from Kharel et al. (2018).

The variation in the d-values and notable differences in the data from this experiment versus the data from Kharel et al. and other studies can possibly be explained by the high variety of different cultivars of pecans and their structure. As mentioned by Wells and Conner (2015), the varieties used for this experiment, ‘Oconee’ and ‘Desirable’, are very similar in their structure. Kharel et al. (2018) utilized pecans of the ‘Sumner’ variety. According to Grauke and Thompson, the ‘Sumner’ variety has tight dorsal grooves and a greater weight but lower kernel percentage than ‘Oconee’ and ‘Desirable’. The ‘Sumner’ variety also has a higher alternate bearing index which means that every other year the quality of the resulting pecan varies. Their harvest date is also somewhat later in the season. The variety of pecan could affect its uptake of organism and its protection and reaction to heat treatment.

Kharel et al. (2018) processed their pecans in hot water on a hot plate or stovetop as opposed to this experiment which utilized a steam kettle. It is possible that the variation in heating

method, conductive versus convective, may influence the heating of the pecans. The steam kettle is heated by steam which heats convectively, while a hot plate or stovetop heats the water conductively. They dried their hot water treated pecans for 1 h (Kharel et al., 2018) while in this experiment the hot water treated pecans were only dried for 20 min and the steam treated pecans did not undergo an extensive drying step.

Beuchat and Mann (2010) hypothesized that the degree of damage to a pecan can cause high variability in its ability to absorb bacteria into its interior and cause it to be protected from a treatment method. There was possibly some difference in the degree of damage sustained by the pecans used for this experiment and those used by other studies. Because the nutmeat of the pecan is so rich in lipids, the water activity within the pecan is somewhat lower than the pecan shell which readily absorbs moisture. Therefore, if a bacterium is able to reach the lipid-rich interior, it may be more resistant to heat treatment. The pecans used in this research were inspected for damage, but it is still possible there was some imperceptible damages pecans used. In addition, Beuchat and Mann (2010) found that there was a significant difference between the water activity change in pecans that were at similar temperature to the water into which they were immersed, as opposed to if they were cooler than the treatment water. The greater the difference between the temperatures, the less increase in water activity would occur from treatment. This could explain the variability between temperatures and the quick microbial reduction that was observed.

Finally, Kharel et al. (2018) stored their pecans in mesh bags at 4 °C for approximately a month. The pecans used for this experiment were also stored in mesh bags in a refrigerated environment, but Kharel et al. (2018) did not specify the relative humidity (RH) of the chamber where they stored their pecans. As mentioned above, the pecans used for this experiment were stored in a walk-in refrigerator at 2 °C with an RH of 55%. It can be assumed that if pecans are

stored in an open-air container, they can absorb or release moisture and their water activity may fluctuate which can influence bacterial survival or growth.

As a surrogate organism, *E. faecium* has been shown to be somewhat more resistant to heat treatment and other stressors compared to *Salmonella enterica* (Pangaea & Chadwick, 1996; Rathnayake et al., 2012). While Pangaea and Chadwick (1996) found that there was no difference between wild-type *E. faecium* and antibiotic-resistant *E. faecium* in terms of heat resistance, the Rifampicin- and Nalidixic Acid-resistant *E. faecium* used in this experiment may have developed more stress resistance than the wild-type used by Kharel et al. (2018).

Figures

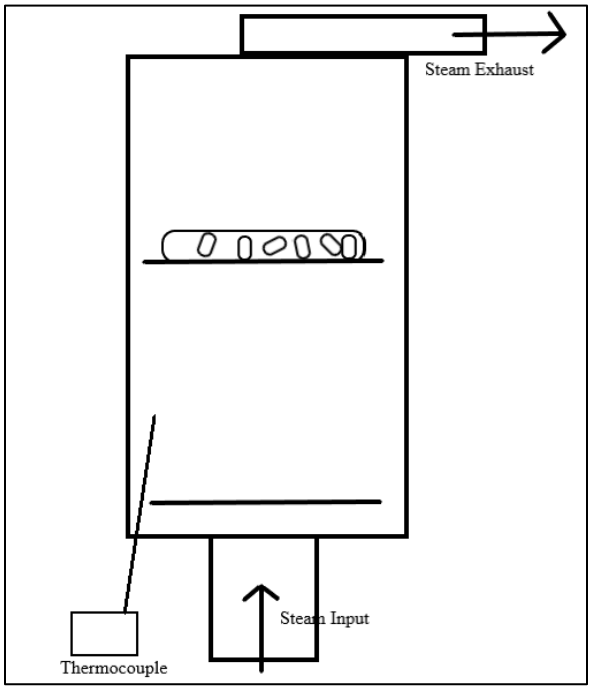

Figure 1.1: Steam cabinet diagram. This diagram illustrates the structure of the steam cabinet used in this experiment and the locations in which the pecans and thermocouple were placed.

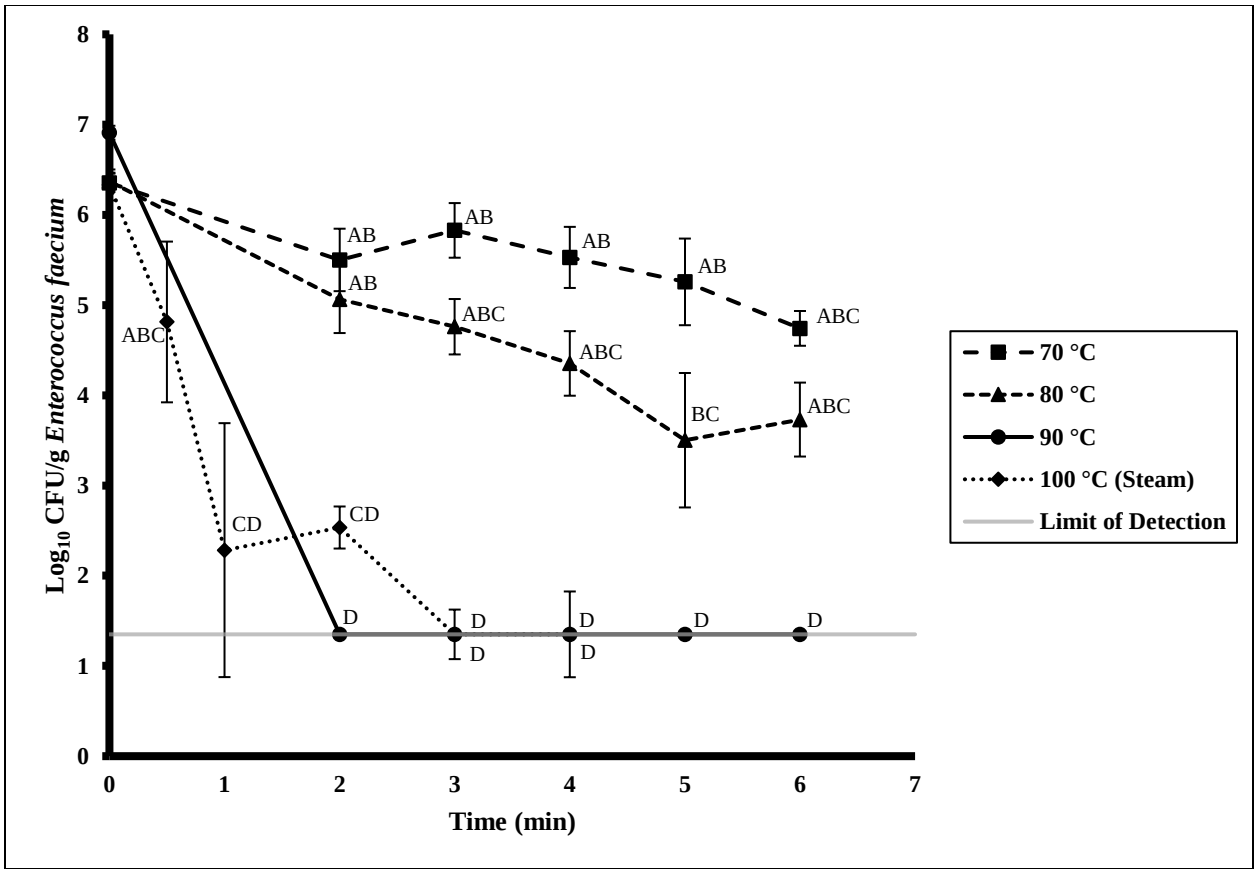

Figure 2.1: Log_{10} CFU/g Reduction of *E. faecium* after thermal treatment. (n=6). This figure shows the log_{10} reduction of the organism *Enterococcus faecium* for each temperature treatment (70, 80, 90, and 100 °C) over time 0.5 min-6 min on in-shell pecans. The limit of detection for this procedure is 1.35 CFU/g. Error bars were calculated by determining the standard error which is the standard deviation divided by the square root of the sample size.

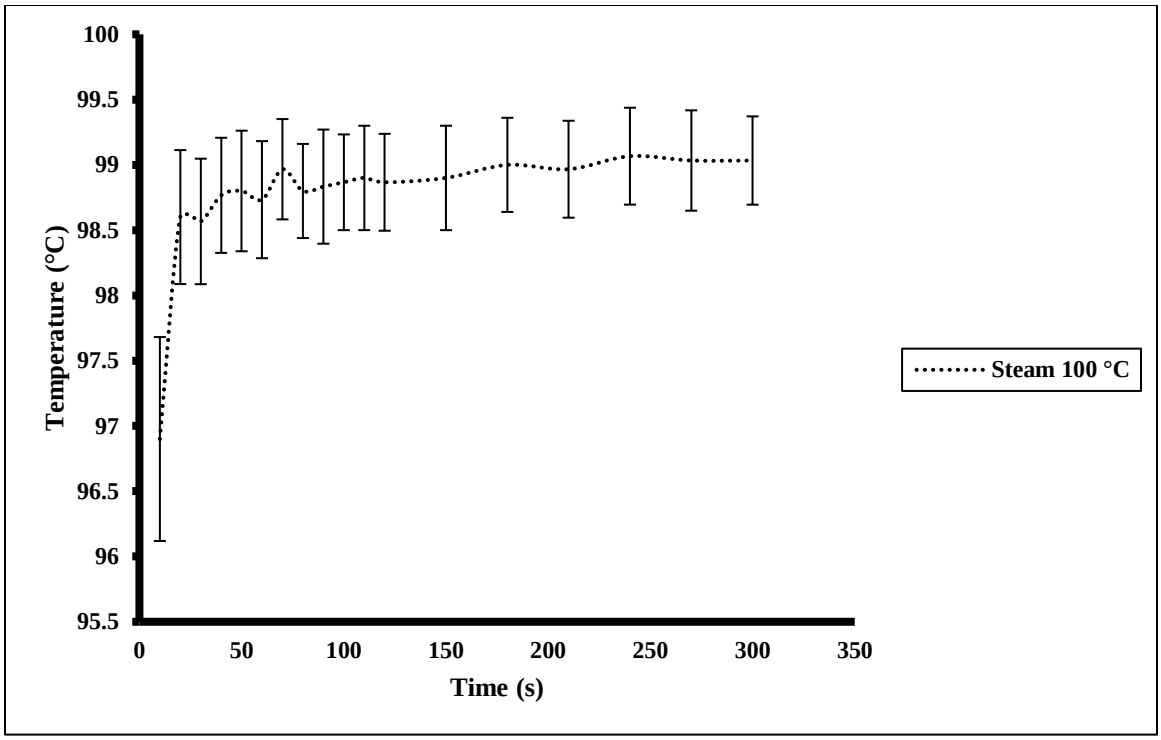

Figure 3.1: Thermal curve for steam cabinet with foil pan. This figure shows the increase in temperature over time of the surface of an in-shell pecan on a foil pan inside the steam cabinet at 100 °C. (n=3)

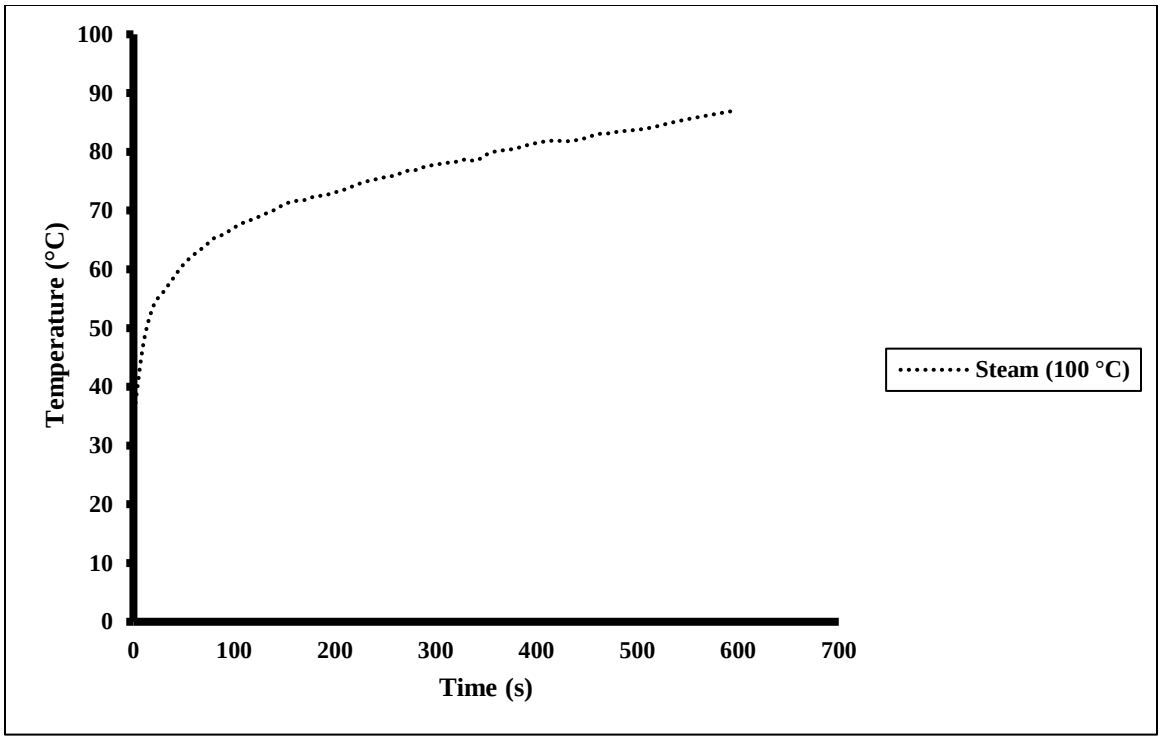

Figure 3.2: Thermal curve for steam cabinet with filter bag. This figure shows the increase in temperature over time of the surface of an in-shell pecan inside a filter bag inside the steam cabinet at 100 °C. No error bars are included due to only processing one sample. (n=1)

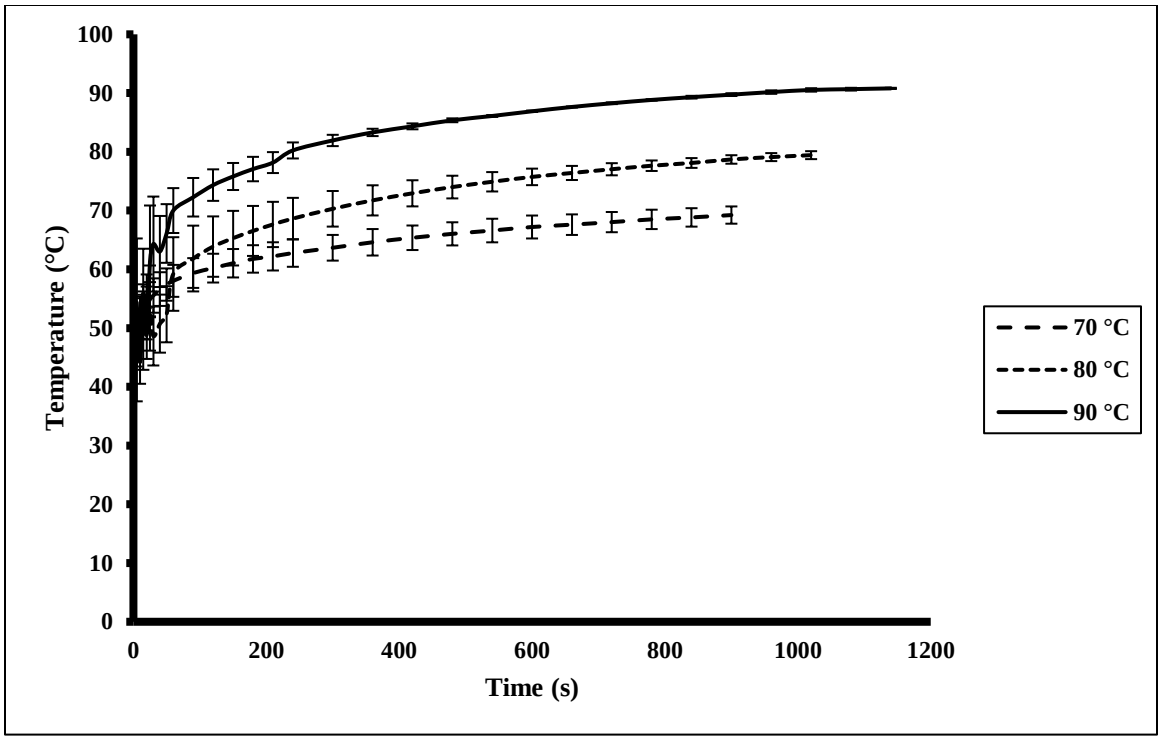

Figure 3.3: Thermal curve for hot water bath with filter bag. This figure shows the increase in temperature over time of the surface of an in-shell pecan inside a filter bag in the hot water treatment experiment for temperatures 70 °C, 80 °C, and 90 °C. (n=3)

CHAPTER 4

PROCESSING OF IN-SHELL PECANS AND EVALUATION OF PECAN NUTMEAT QUALITY

Introduction

Pecan processors are interested to know how conditioning and thermal treatments meant to increase cracking efficiency affect the quality of their final product. While safety is a priority, processors are conducting business and thus are in the position to value shelf life, quality, and consumer acceptance of their products. To pecan processors, the main indicators of quality pecans are testa color, which impacts consumer acceptance; moisture, a critical factor in storage and shelf life of pecans both in-shell and shelled; and cracking efficiency, because whole halves are the most valuable commodity and can be broken down further into whatever size pecan pieces that the consumer is seeking. Because of these important factors, this experiment evaluates how thermal treatments of hot water soaking or saturated steam treatment affect these three quality indicators. We hypothesize that there will be a resulting darkening of the pecan testa from thermal treatment, that moisture will increase due to hot water treatment, and that cracking efficiency will increase due to saturated steam treatment based on studies by Forbus and Senter (1975), Kharel et al. (2019) Beuchat and Heaton (1976), and Senter et al. (1984). As a result of this research, pecan processors can evaluate and weigh their processing options to produce safe, high-quality pecans for consumers.

Materials and Methods

Sample Preparation

Two-hundred pounds of in-shell pecans were obtained from a farm in Tifton, GA. The cultivars of pecans used for this experiment were ‘Oconee’ and ‘Desirable’. These pecans were stored in a humidity-controlled environmental chamber (13 °C, 71% RH) with constantly circulating UV-sterilized air filtration (Bissell: Walker, MI) for about 1 month. For processing treatments, 5-10 pounds of pecans were used for each treatment and 1 sample was processed per treatment. Hot water treatments were performed in a steam kettle (Groen: Conyers, GA) filled with water which was heated by a controlled mixture of steam and water, with the temperature monitored by thermocouple (Bestdo: Dobel, DE). Steam treatments were performed in a steam cabinet (Pyramid: Stilwell, KS) with the temperature controlled by the steam pressure and monitored by thermocouple (Bestdo: Dobel, DE). The pecans were directly added to the steam kettle and processed for the desired time. The water in the steam kettle was reused for multiple sample treatments. For steam treatment, the in-shell pecans were placed on a perforated tray in the steam cabinet in a single layer. The steam cabinet was pre-heated, and the pecans were processed for the specified time, not including the come up to temperature (~100 °C). The steam cabinet took approximately 15 s to come up to temperature at which point the timing was started. After each treatment, the pecans were removed and spread on trays with paper towels to dry overnight (~18 h).

Moisture Analysis

After drying, moisture analysis was performed using AOAC method 925.40, in which in-shell nuts are weighed, crushed, and dried in a vacuum oven (VWR Scientific Products: Radnor, PA) overnight to constant weight. The vacuum oven was set to 100 °C and ≤ 100 mm Hg (≤ 3.9

in Hg). Statistical analysis of this data was completed using one-way ANOVA with JMP statistical analysis software (Tukey's HSD, $p<0.05$).

Color Analysis

Pecans were cracked at North GA Pecan Company (Athens, GA) after drying overnight. A pecan cracker (The Champion Pecan Machine Co.: San Antonio, TX) was used for cracking followed by a sheller/blower combo (unbranded) to separate the nuts and remove large shell fragments. Color values were obtained for each treatment using a ColorMuse (Variable: Chattanooga, TN) mini colorimeter. Ten intact whole halves from each treatment were randomly selected and the colorimeter was applied at the smoothest area of the half. Measurements were obtained using the L* a* b* color scale. These color values were converted to chroma, hue angle (°), ΔE, ΔL, ΔH, and ΔC for interpretation and comparison. Statistical analysis of this data was completed using one-way ANOVA with JMP statistical analysis software (Tukey's HSD, $p<0.05$).

Cracking Efficiency

Cracking efficiency was evaluated for each treatment by sorting the resulting shelled pecan pieces by size. Approximately 50% of the weight of the in-shell pecans is made up of the larger shell pieces which were removed from the shelled pecans. One pound (~475 g) of pecans were randomly selected from each sample and whole halves were separated by hand and weighed. In addition, waste such as bad nuts, smaller shell pieces, and other debris were removed by hand and weighed. Next, the pecan pieces were separated by size using varying sized plastic sieves (Raytek: Santa Cruz, CA): 1 inch, ½ inch, and ¼ inch. The pecans were broken down into these categories and their mass recorded: whole halves, > ½ inch, ½ - ¼ inch, < ¼ inch, and waste. Statistical analysis of this data was not able to be completed due to only obtaining one sample per treatment.

Results

Moisture

Table 1 shows the results of moisture percentages for each heat treatment and negative control. For hot water treatment at 60 °C, the moisture levels were 10.5% for a 3 min treatment, 11.67% for a 4 min treatment, 9.92% for 5 min, 10.48% for 6 min, and 9.53 for 7 min. The 70 °C treatment had 11.5% at 2 min, 10.76% for 3 min, 10.17% at 4 min, 10.22% at 5 min, and 11.24% at 6 min. At 80 °C, the moisture percentage was 12.19% for a 2 min treatment, 11.38% for a 3 min treatment, 11.35% for 4 min, 10.98% for 5 min, and 11.43% for 6 min of treatment. The moisture percentage values for 90 °C are 11.33% at 2 min, 10.63% at 3 min, 12.83% at 4 min, 13.14% for 5 min, and 12.07 for 6 min in hot water at 90 °C.

The steam treatment was performed at 100 °C and the moisture percentage for a treatment at 0.5 min was 9.95%. A 1 min treatment had a moisture percentage of 9.13%, and 2 min at 9.35%. The negative control experienced no treatment, and the moisture was 8.81%. The maximum moisture was 13.14% for the treatment at 90 °C for 5 min. The minimum moisture overall was the negative control (8.81%), but excluding the control, the minimum moisture was the 1 min treatment at 100 °C at 9.13%. One water activity (a_w) reading was taken of a negative control of crushed, in-shell pecans. The water activity before treatment of the pecans used was 0.740 a_w.

Color

The color measurements are displayed in Table 2. The initial measurements of L*, a*, and b* were recorded and also converted into hue angle (°) and chroma. These values were also compared to the negative control and values of ΔL, ΔC, ΔH, and ΔE were calculated. The range of values for hue angle (°) were 43.5-69.6. The range of values for chroma were 29.1-33.7. The

negative control had a value of 68.7 for hue angle (°) and 31.0 for chroma. The comparative values of ΔL, ΔC, ΔH, and ΔE ranged from -2.8-3.1, -1.9-2.7, 0.2-8.2, and 0.5-85.4, respectively.

Cracking Efficiency

Figures 4.1-4.6 show the cracking efficiency for each treatment temperature and the negative control. To determine cracking efficiency, one 1-pound (453.6 g) sample of cracked and shelled pecan nutmeats was broken down by size into categories of whole pecan halves, pieces > ½ inch, pieces ½ - ¼ inch, pieces < ¼ inch, and waste. The more whole halves are obtained equates to a better cracking efficiency of the treatment. At 60 °C, treatments of 3-7 min were completed. The percentages of whole halves for the 60 °C treatment were 44.4% at 3 min, 50% at 4 min, 46.0% at 5 min, 47.1% at 6 min, and 50.7% at 7 min. For the 70 °C treatment, the timepoints ranged from 2-6 min. The percentages of whole halves were 35.2% for 2 min, 42.2% at 3 min, 42.1% at 4 min, 52.8% at 5 min, and 51.5% at 6 min. At 80 °C, the percentages were 35.6% at 2 min, 33.6% at 3 min, 40.6% at 4 min, 46.8% at 5 min, and 51.3% at 6 min. For 90 °C, the percentages were 39.6% at 2 min, 29.5% at 3 min, 41.7% at 4 min, 35.6% at 5 min, and 51.4% at 6 min.

For the steam treatment at 100 °C, timepoints of 0.5, 1, and 2 min were evaluated. The percentages of whole halves for these treatments were 31.8% for 0.5 min, 21.7% at 2 min, and 23.5% at 2 min. The negative control experienced no treatment and 33.6% of whole halves was obtained. Statistical analysis of the cracking efficiency experiment was not able to be completed due to a single sample for each treatment resulting from limited pecan supply.

Discussion

Moisture

Table 1 shows the moisture content averages for each thermal treatment and the negative control. Statistical analysis shows that the negative control is significantly different from the 90 °C treatments at 4 and 5 min. Additionally, the 100 °C treatment at 1 min is significantly different from the 90 °C 5 min treatment. All other treatment values are not significantly different. The highest moisture recorded was 90 °C for 5 min and the lowest recorded was the negative control. The saturated steam (100 °C) treatments moisture contents were all lower than the hot water treatments which indicates that the in-shell pecans did not absorb as much moisture during the steam treatments or that the drying step was too long for this type of treatment. As mentioned, this drying step is critical for preventing bacterial growth of surviving cells (Farakos et al., 2017; Beuchat et al., 2011), but as steam treatment does not introduce as much moisture as hot water treatment, the drying step can be shorter at about 1 h. Since the drying step for the steam treatments for this experiment was the same length as the drying step for the hot water treatments (~18 h), the pecan moisture was lower. However, since the negative control is not significantly different from most of the other treatment values, including the hot water treatments, it cannot be concluded that a notable change in moisture occurs as a result of any of these treatments.

Color

Table 2 shows the color values (averages of 10-20 samples) corresponding to each temperature and time treatment. No significant differences in any of the color values were detected due to process treatment. A more evident color change may have been obtained if the pecans had been roasted after treatment and shelling as shown in the study by Kharel et al. (2019) and pecan processing facilities may roast pecan pieces for some of their products before packaging. Kharel

et al. (2019) hypothesized that the color change occurred after roasting due to the increase in water activity of the nutmeats after treatment, leading to an increase in reactant mobility and an increase in the reaction rate of non-enzymatic Maillard browning and caramelization reactions. In addition, they found that the roasting step provided a more acceptable aroma and color to consumers when compared to the unroasted, hot water treated nuts (Kharel et al., 2019). Therefore, it may be beneficial to add a roasting step in optimization of a pecan processing method, though there would be a notable color change.

Cracking Efficiency

No statistical analysis was able to be achieved for cracking efficiency evaluation due to only using a single sample for each treatment (due to limited pecan supply). Because of this, the following data discussion cannot be confirmed by statistical analysis and the following discussion is mostly conjecture and thus not reliable. As shown in Figure 4, treating the pecans in water at moderate temperature that is 70 or 80 °C resulted in obtaining slightly more whole halves than measured when treating at 90 °C. This difference could be due to an increase in moisture content and water activity occurring at the hottest water temperature which may excessively soften the nutmeats due to an increase in water activity and prevent the whole pecan halves from separating from the shells effectively. As Beuchat and Mann (2010) showed, the water activity is affected more by a higher temperature hot water treatment. In addition, a higher water activity can enable bacteria to survive within the pecan (Farakos et al., 2017), which is not ideal for producing a safe product. At 60 °C, recovery of whole halves was close to the negative control and recovery of whole halves stays consistent across all timepoints. Figures 4.1-4.6 also show that with an increase in whole halves, typically the >½ inch (12.7 mm) portion decreases indicating that if nutmeats are not recovered as whole halves, they are mostly recovered as larger pecan pieces instead of smaller

pieces and meal. The levels of waste and pecan pieces of ½ (12.7 mm)-¼ inch (6.35 mm) and <¼ inch (6.35 mm) stay relatively consistent for each treatment. As mentioned, in pecan processing facilities, their total yield using conventional methods is approximately 50-80% whole halves (Forbus & Senter, 1976), so these values are consistent with industry standards.

Contrary to previous findings by Forbus and Senter (1976), the saturated steam treatment (100 °C) seems to have a detrimental effect on obtaining whole halves. They found that saturated steam treatment for a short time may lead to more whole halves recovery (Forbus & Senter, 1976). This disparity could be explained due to differences in drying treatments after processing. For example, the Forbus and Senter (1976) experiment performed a drying step after steam treatment for only 20 min after treatment whereas in this experiment, the nuts were dried overnight for approximately 18 h. If the pecans had been cracked and shelled after a shorter drying period, the moisture content may have been greater and within a range that allowed for more whole halves to be obtained. Also, Forbus and Senter (1976) utilized pecan varieties of 'Stuart' and 'Schley'. The pecan cultivar 'Schley' has a higher percentage of kernel and a thinner shell, while 'Stuart' has a similar makeup to 'Oconee' and 'Desirable'. Forbus and Senter (1976) noted that there was no significant difference between the two varieties in terms of shelling efficiency, but it is likely that the difference in variety makes a difference in a nutmeat's ability to be released from the shell. In addition, Forbus and Senter (1976) did not adjust their pecan cracker based on the two varieties of pecans used, which could contribute to applying too much or not enough force on the ends of the pecan during cracking.

An increase in treatment duration for this experiment resulted in more whole halves recovered for the 70, 80, and 90 °C treatments. Duration did not seem to influence whole halves recovery for the 60 °C treatments. For steam treated pecans, somewhat more whole halves were

obtained for the shortest duration (0.5 min), but hot water treatment appears to lead to more whole halves after a 24 h drying period than any of steam treatments in this experiment. As mentioned above, drying is a critical step in the processing procedure, and optimization of the drying time for the type of treatment should be simple to achieve.

When comparing the treatment figures (Figure 4.1-4.5) to the negative control (Figure 4.6), it is evident that processing at 60-80 °C helps to obtain more whole halves compared to no hot water treatment prior to cracking. A longer processing time at these temperatures generates more whole halves due to increased moisture versus the negative control (see Table 1). As discussed, the ideal moisture level for cracking and shelling in-shell pecans is approximately 8%, but when comparing the moisture (Table 1) to the cracking efficiency (Figure 4), moisture levels of 9 or 10% may in fact serve to generate more whole halves than at 8%, but moisture levels of 12-13% are detrimental to obtaining whole halves.

Conclusions

It is recommended to exceed the minimum conditioning treatment duration in order to ensure the full >5 log CFU/g reduction in microorganisms. Because of this, the recommended hot water treatment to eliminate >5 log CFU/g of *E. faecium*, a surrogate organism for *Salmonella enterica* on in-shell pecans is at least 3 min at 90 °C. For a saturated steam treatment, it is recommended to process in-shell pecans for at least 4 min at 100 °C. It is also advisable to include a sufficient drying time for the in-shell pecans before cracking and shelling. For steam treatment, a drying time of about 1 h is sufficient, but for a hot water treatment, a longer drying time is needed. The necessity of a satisfactory drying step is due to the ability of microorganisms such as harmful enteric pathogens to continue to grow after thermal treatment if there is not total elimination of

bacteria as a result of treatment. If a pathogen is able to reach the interior of the pecan close to the lipid-rich nutmeat, it may be able to multiply if there is available moisture, so a drying step is critical.

However, when considering the effects on pecan nutmeat quality and cracking efficiency, a 90 °C treatment may not be ideal. For the 90 °C treatments in this experiment, there was sufficient moisture uptake of the pecan nutmeat to bring the moisture level up to 12-13%, which resulted in a poorer recovery of whole halves due to excessive softening of the nutmeat. In addition, it has been found that the higher the temperature of a hot water treatment, the more the water activity of the nutmeat increases, which can also lead to a suitable environment for bacterial growth. This result could possibly be avoided by extending the drying time or by choosing a lower temperature of conditioning treatment and choosing a different microbial reduction step such as roasting, hot oil roasting, or propylene oxide gas (POG) treatment, but these should be confirmed by further research. Since testa color has an impact on perceived quality of the nutmeat by processors, it is important to note that color change of the testa does not result from hot water or steam treatment on its own. Testa darkening can result from a following roasting step or from improper storage of in-shell or shelled pecans, so one must consider the consumer preference of such a color change if utilizing a different microbial reduction method.

Further Research

Further research should be done to evaluate storage conditions for optimal quality of shelled and in-shell pecans including color change, peroxide value, and sensory testing. Moisture is also a key factor in cracking efficiency, but it is possible to increase or reduce moisture using conditioning and drying steps. Sensory testing of pecans following the processing steps of this

experiment would be beneficial to conduct. An experiment involving adding a roasting step in hot oil or dry air following the hot water or steam treatment and shelling and conducting consumer testing on its effects would be a beneficial future study. An interesting experiment would also be conducting a POG gas treatment followed by or preceding conditioning with or without further roasting and evaluating the effects on microbial reduction and consumer preference. Optimizing pecan processing methods to ensure a routinely safe, stable, and acceptable product to consumers is possible, and this information will be of great interest to pecan growers and shellers, both new and established.

Figures

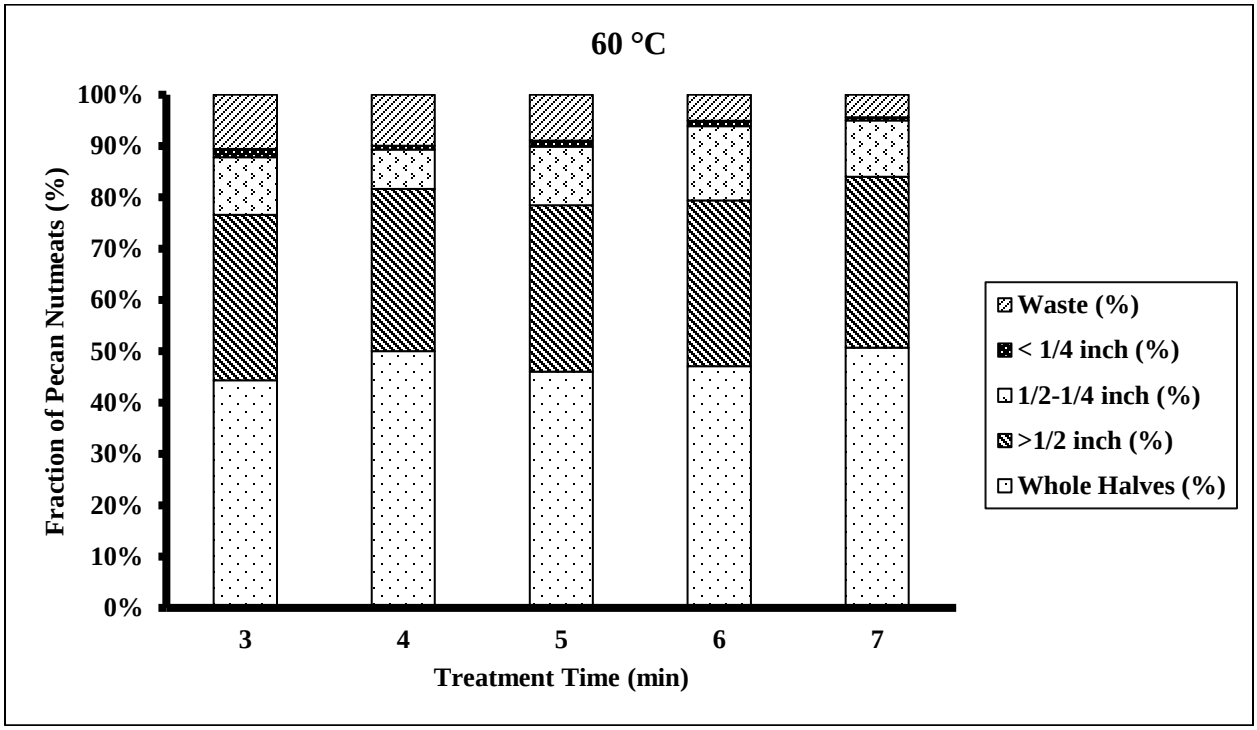

Figure 4.1: Cracking Efficiency at 60 °C

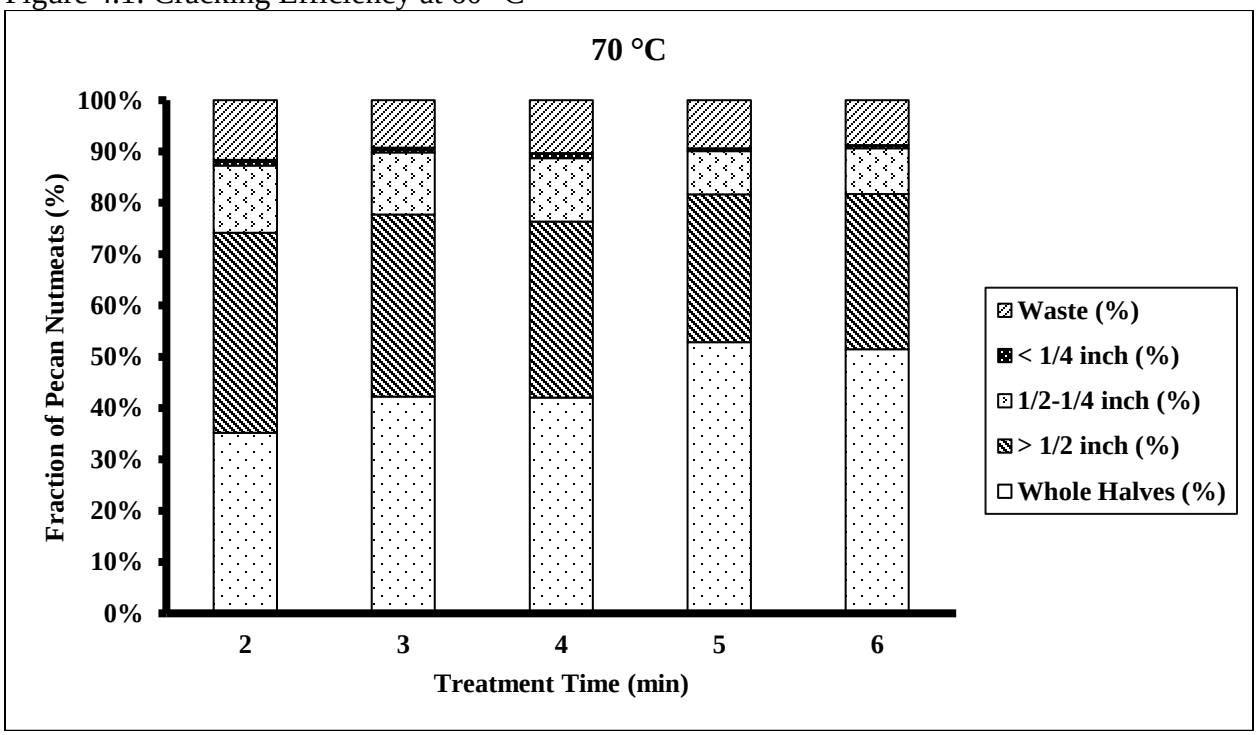

Figure 4.2: Cracking Efficiency at 70 °C

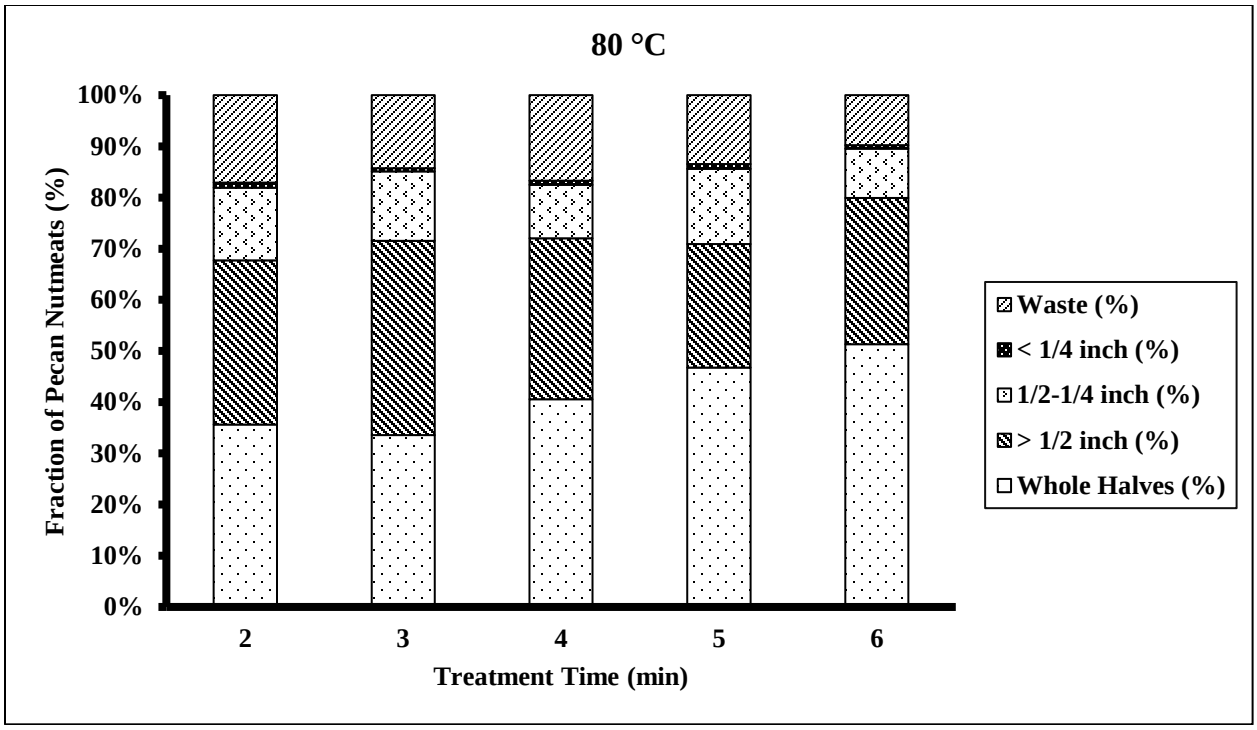

Figure 4.3: Cracking Efficiency at 80 °C

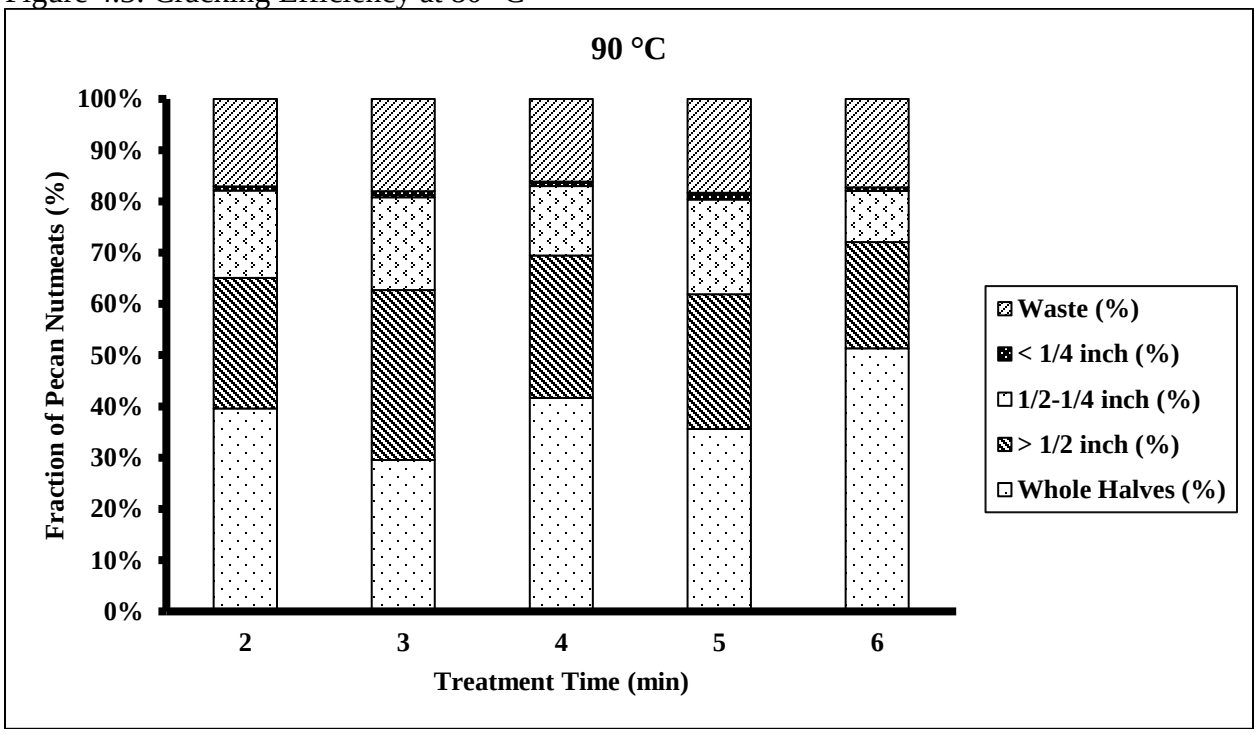

Figure 4.4: Cracking Efficiency at 90 °C

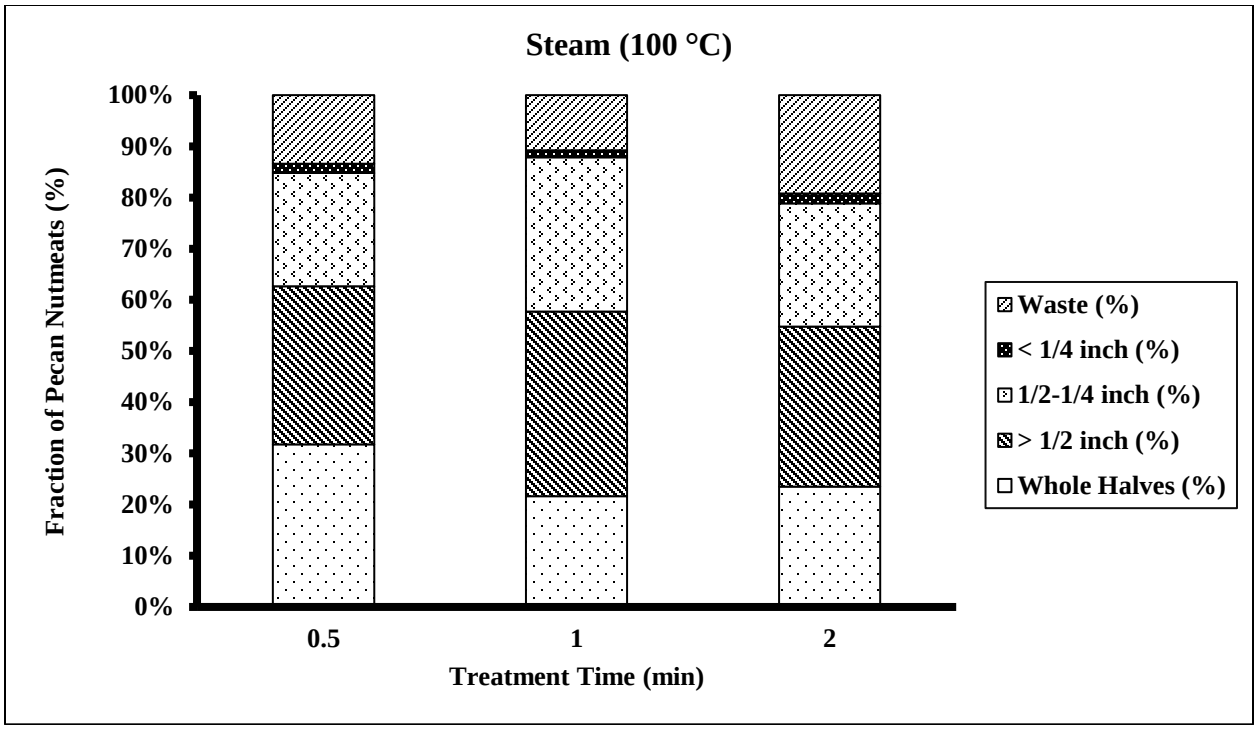

Figure 4.5: Cracking Efficiency at 100 °C (Steam)

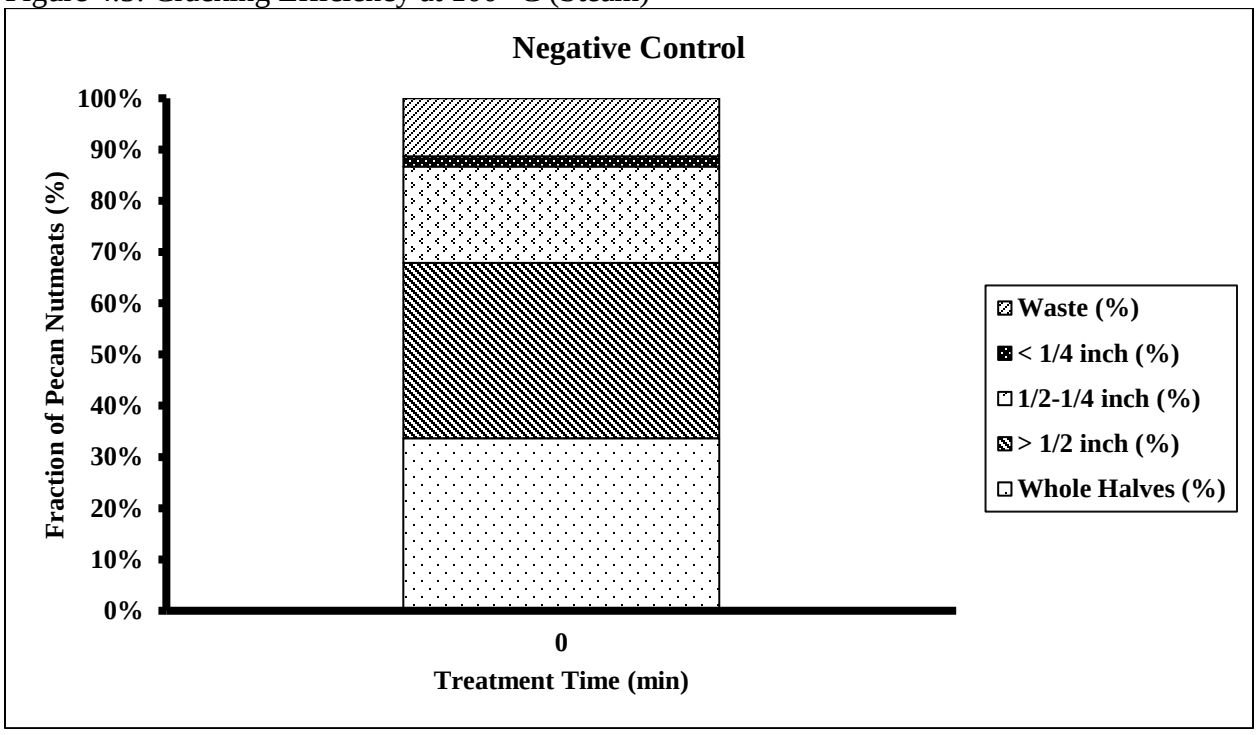

Figure 4.6: Cracking Efficiency Without Treatment - Negative Control

Figure 4: Cracking Efficiency of Pecans after Thermal Treatment. 60 °C, 70 °C, 80 °C, 90 °C, 100 °C (steam), and negative control (no treatment). (n=1) 1-pound samples of shelled pecans were broken down by size and weighed.

Tables

Table 1.1: Moisture content for in-shell pecans after thermal treatment. (n=3) The negative control experienced no treatment.

Temperature (°C)	Time	Average Moisture (%)	SD	Statistics
60	3	10.5	1.6	ABC
	4	11.7	0.0	ABC
	5	9.9	0.9	ABC
	6	10.5	1.1	ABC
	7	9.5	1.1	ABC
70	2	11.5	1.2	ABC
	3	10.8	0.8	ABC
	4	10.2	0.9	ABC
	5	10.2	1.2	ABC
	6	11.2	0.8	ABC
80	2	12.2	0.4	ABC
	3	11.4	0.6	ABC
	4	11.3	2.5	ABC
	5	11.0	2.0	ABC
	6	11.4	0.8	ABC
90	2	11.3	1.2	ABC
	3	10.6	0.6	ABC
	4	12.8	0.6	AB
	5	13.1	3.1	A
	6	12.1	0.4	ABC
100	0.5	10.0	0.5	ABC
	1	9.1	0.1	BC
	2	9.3	0.3	ABC
Negative Control		8.8	0.4	C

Table 2.1: Pecan Testa Color. This table shows the color values from each treatment. (n=10) ΔL, ΔH, ΔC, and ΔE values were calculated using L*, a*, b*, hue (°), and chroma and compared to the negative control means (at bottom). No statistically significant differences were found between any color values or the negative control.

Temp (°C)	Time (min)	mean L*	SD (L*)	mean a*	SD (a*)	mean b*	SD (b*)	Chroma	Hue (°)	ΔL	ΔC	ΔH	ΔE
60	**7**	51.2	4.5	10.6	5.2	28.5	3.5	30.5	69.5	-0.3	-0.5	0.9	0.9
	6	51.7	5.2	17.6	4.6	27.0	3.9	32.3	56.9	0.1	1.3	6.8	42.5
	5	51.4	4.1	13.9	7.3	27.8	4.8	31.2	63.2	-0.1	0.1	2.8	7.9
	4	52.4	4.1	16.0	4.6	27.9	3.6	32.2	60.2	0.9	1.2	4.9	23.8
	3	52.3	3.4	14.2	6.0	28.8	2.7	32.3	63.8	0.8	1.3	3.2	9.6
70	**6**	53.4	4.4	16.5	5.5	28.2	3.2	32.7	59.7	1.9	1.7	5.5	31.2
	5	52.0	4.8	17.3	3.6	26.9	3.5	32.0	57.3	0.4	1.0	6.4	38.8
	4	52.5	3.8	13.0	7.2	28.4	4.3	31.4	65.2	0.9	0.4	1.8	4.2
	3	52.3	3.9	14.5	5.0	29.1	4.3	32.5	63.6	0.8	1.5	3.5	11.0
	2	51.9	2.9	15.2	5.6	26.6	3.0	30.6	60.3	0.4	-0.4	4.6	17.8
80	**6**	54.6	5.4	15.2	5.7	28.9	5.1	32.7	59.7	3.1	1.7	4.3	25.2
	5	51.7	5.4	13.9	4.3	29.4	4.1	32.5	64.5	0.2	1.5	3.1	7.5
	4	53.2	4.1	17.5	5.4	28.8	3.7	33.7	58.8	1.6	2.7	6.8	41.8
	3	50.4	5.5	14.6	7.0	26.1	4.7	29.9	60.7	-1.1	-1.1	4.5	15.3
	2	52.0	3.9	17.0	3.6	28.2	3.9	32.9	58.9	0.5	1.9	6.1	33.9
90	**6**	52.3	3.4	14.1	6.6	29.3	2.7	32.5	64.2	0.8	1.5	3.2	9.1
	5	51.0	3.7	18.9	5.0	26.4	4.5	32.5	54.4	-0.6	1.5	8.1	60.9
	4	52.1	3.9	17.3	4.6	24.7	4.5	30.2	55.1	0.6	-0.8	7.3	40.5
	3	52.2	5.8	11.1	4.2	29.0	5.8	31.0	69.0	0.6	0.0	0.2	0.5
	2	50.3	3.7	15.5	2.4	24.7	4.4	29.1	57.9	-1.3	-1.9	6.2	23.4
Steam (100)	**0.5**	48.7	2.7	16.2	6.2	27.6	2.7	32.5	60.1	-2.8	1.5	7.4	76.2
	1	51.3	4.7	10.2	7.2	28.8	5.0	31.5	52.1	-0.2	0.5	7.9	71.2
	2	49.9	4.3	15.2	7.3	28.0	3.9	32.7	43.5	-1.7	1.7	8.2	85.4
Negative Control		51.5	2.3	11.3	3.8	28.9	2.6	31.0	68.7				

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