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Efficacy of Selected Powdered Floor Treatments and Turmeric Rhizome Powder Against Salmonella, E. coli, and L. monocytogenes on Polyurethane-Concrete Flooring Material Carriers

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Efficacy of Selected Powdered Floor Treatments and Turmeric Rhizome Powder Against *Salmonella*, *E. coli*, and *L. monocytogenes* on Polyurethane-Concrete Flooring Material Carriers

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Abstract

The flooring in a food processing environment can become contaminated with pathogenic bacteria in many ways including foot and equipment traffic, incoming materials, and drain backups. Turmeric (*Curcuma longa L.*) rhizome powder and seventeen other commercially available powdered floor treatments with a variety of active ingredients including quaternary ammonium compounds (QAC), sodium percarbonate and similar perhydrates (SPC), urea, and borax, were investigated for reducing the levels of pathogens on flooring thereby reducing the risk of cross-contamination from the floor to food contact surfaces. Some of the commercially available floor treatments were Environmental Protection Agency (EPA) registered antimicrobials while others were not but contain chemicals that might provide an antimicrobial effect despite their actual labeled purpose. These substances were evaluated to determine their relative effectiveness against cocktails of *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* dried onto the surfaces of carriers made from polyurethane-concrete commercial flooring material. Aqueous test solutions were prepared from the minimum mass of the treatment required per m^2 from the manufacturer's instructions diluted in 300 mL sterile water. Potential synergy between turmeric and a percarbonate based commercial floor treatment was investigated at half the previous concentration of each. The inoculated carriers were exposed to the treatment solutions or sterile water control for 10 minutes at room temperature. Viable bacteria were enumerated and $\log_{10}$ reductions versus sterile water were calculated for each treatment and inoculum combination. Mean $\log_{10}$ CFU/carrier reductions (with standard deviations) for *Salmonella* ranged between 4.29±0.34 for a sodium percarbonate (SPC) based treatment and 0.004±0.23 for turmeric. Reductions of *E. coli* ranged between 4.81±0.16 for an SPC based treatment and -0.16±0.62 for turmeric. Against *L. monocytogenes*, reductions ranged

from 4.88±0.6 for an SPC based treatment and -0.16±0.15 for turmeric. The results support that among the treatments tested, those containing sodium percarbonate were more effective, turmeric powder alone was less effective, and a percarbonate containing treatment including turmeric powder was not more effective than the commercial treatment alone at half the recommended application rate against the three cocktails of organisms tested.

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Chapter 1: Introduction

1.1 Introduction

The World Health Organization (WHO) estimates that every year nearly 10% of the world's population, approximately 600 million people, become ill from eating contaminated food and 420,000 die (World Health Organization, 2020). Diarrhea causing agents are responsible for 550 million of the 600 million illnesses and 230,000 of the 420,000 deaths (World Health Organization, 2015). The Centers for Disease Control (CDC) estimates that each year in the United States 9.4 million people are sickened, 55,961 people are hospitalized, and 1,351 people die from foodborne illnesses. Of these cases, bacteria were estimated to cause 39% of the illnesses, 64%, of the hospitalizations, and 64%, of the deaths (Scallan et al., 2011). Of the illnesses, non-typhoidal *Salmonella* spp. was estimated to cause the most followed by *Clostridium perfringens*, *Campylobacter* spp., *Staphylococcus aureus, E. coli* (including STEC O157, STEC non-O157, ETEC, and other diarrheagenic *E. coli*), and *Shigella* spp.. Deaths were most likely to be caused by non-typhoidal *Salmonella* spp. with 378 deaths and *L. monocytogenes* with 255 deaths annually (Centers for Disease Control and Prevention, 2018; Scallan et al., 2011). Bearing a disproportionate burden, forty percent of foodborne illnesses and 125,000 deaths are from children under the age of 5 (World Health Organization, 2020).

Approximately 25% of foodborne outbreaks have been linked to recontamination or cross-contamination events according to World Health Organization (WHO) studies in Europe with leading causes of pathogen contaminated prepared foods being unsatisfactory hygiene practices, cross-contamination, processing or storage in unsuitable areas, contaminated equipment, and contamination by personnel (Carrasco et al., 2012; Reij & Den Aantrekker, 2004; Tirado & Schmidt, 2001). Food processing environments are complex with many

potential niches in both equipment and structural elements that can harbor pathogenic organisms and other bacteria that can contribute to human illness, food spoilage, and shortened shelf-life (Carpentier & Cerf, 2011; Hultman et al., 2015; Mettler & Carpentier, 1999; Stellato et al., 2016). Numerous studies have shown that flooring is a high risk area for pathogen contamination in the food processing environment (Barros et al., 2007; Campdepadrós et al., 2012; Carpentier & Cerf, 2011; Dzieciol et al., 2016; Eisel et al., 1997; El-Shenawy, 1998; Lecoq et al., 2017; Muhterem-Uyar et al., 2015). Processing environment flooring can provide an especially difficult challenge in that microorganisms are continually introduced via foot and equipment traffic and on incoming raw materials and ingredients and the flooring must have enough texture to prevent slips and falls which impedes cleanability (Cook, 2011; Mettler & Carpentier, 1999). Food processing environment flooring is also susceptible to damage via heavy equipment traffic, natural settling of the structure which can introduce cracks, chemicals used for processing and cleaning that can erode the flooring material, equipment and process changes which create and leave penetrations into the floor surface, and many other destructive processes (Cook, 2011; Cramer, 2013). Damage to the flooring creates harborages that are difficult to clean and sometimes difficult to detect (Dzieciol et al., 2016; Mettler & Carpentier, 1999; Overney et al., 2017).

Contamination is easily moved within the food processing environment from the floor to other surfaces via a variety of means including spraying the floor with water, personnel touching the floor then touching food contact surfaces or food itself, splashes from standing water, and parts or ingredients or other items being placed on the floor then handled or placed on other surfaces (Berends et al., 1997; Berrang & Frank, 2012; Borch et al., 1996; Botteldoorn et al., 2003; Carrasco et al., 2012; El-Shenawy, 1998; Kang & Frank, 1989, 1990; Muhterem-Uyar et

al., 2015; Reij & Den Aantrekker, 2004). Promptly repairing flooring damage and good manufacturing processes (GMP) such as requiring handwashing after touching floor surfaces and maintaining food contact surface separation can help keep microorganisms that are found on the floor from migrating to food contact surfaces, but require constant vigilance and compliance by plant personnel to enforce and follow as part of a facility's food safety systems and compliance must be part of the food safety culture at the facility in order to be effective (Carpentier & Cerf, 2011; Yiannas, 2009).

To combat microorganisms, several types of environmental sanitizers are commonly used in food processing environments as part of routine sanitation, including quaternary ammonium compounds (QACs), hydrogen peroxide, iodophors, ozone, peroxyacetic acid (PAA), chlorine (sodium hypochlorite), and chlorine dioxide (Carpentier & Cerf, 2011; Cramer, 2013; Lopes, 1986; Minbiole et al., 2016). Along with effective sanitation and good manufacturing practices (GMPs), antimicrobial floor treatments that are applied after sanitation and are maintained on the flooring throughout the production day may be able to reduce the number of pathogens on flooring surfaces.

1.2 Hypothesis

Turmeric rhizome powder and commercially available floor treatments can decrease the levels of *Salmonella*, *E. coli*, and *L. monocytogenes* inoculated onto carriers made from typical food processing environment flooring material.

1.3 Objectives

The experiment was designed to measure the antimicrobial effect of turmeric rhizome powder and seventeen commercially available floor treatment powders against dried inocula of cocktails of *Salmonella*, *E. coli*, and *L. monocytogenes* and is adapted from Environmental

Protection Agency (EPA) methods MLB SOP MB-20 Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilms and MLB SOP MB-05-14 AOAC Use Dilution Method for Testing Disinfectants (EPA, 2016, 2017). The objectives are:

Objective 1: Determine if Hi-Cap neutralizing buffer can effectively neutralize the antimicrobial action of turmeric and other floor treatment chemicals without itself being toxic to *E. coli, Listeria, and Salmonella* to assess the suitability not only for this study, but to support potential fitness as a neutralizing media for these commonly used powdered antimicrobials for environmental sampling of treated flooring.

Objective 2: Determine the antimicrobial effect of quaternary ammonium compound (QAC) based treatment 1, sodium bicarbonate (SBC) and QAC based treatment 2, sodium percarbonate (SPC) and QAC based treatment 3, SPC and QAC based treatment 4, QAC based treatment 5, SPC/QAC treatment 6, SPC based treatment 7, sodium carbonate (SC) and QAC based treatment 8, SPC based treatment 9, urea and QAC based treatment 10, SPC and SC based treatment 11, Urea/QAC based treatment 12, SBC and QAC based treatment 13, Urea based treatment 14, SPC based treatment 15, borax based treatment 16, SPC based treatment 17, and turmeric rhizome powder treatment 18 against 5 constituent cocktails of *L. monocytogenes*, *Salmonella*, and *E. coli* inoculated onto carriers made from Tufcrete (Tufco Flooring/Arkotex inc.), a hybrid polyurethane-concrete material commonly used as a floor topper in food processing environments.

Objective 3: Evaluate potential synergism between turmeric and SPC based treatment 7 against 5 constituent cocktails of *L. monocytogenes, Salmonella*, and *E. coli* inoculated onto carriers made from typical food processing environment flooring.

Chapter 2: Literature Review

2.1 Burden of foodborne illness

Based on data collected between 2000 and 2008 and an estimated US population of 299 million people, a Centers for Disease Control and Prevention (CDC) study in 2011 estimated that each year in the United States 9.4 million people are sickened, 55,961 are hospitalized, and 1,351 die from foodborne illnesses. Of these cases, bacteria are estimated to cause 3.6 million, or 39%, of the illnesses, 35,796, or 64%, of the hospitalizations, and 861, or 64%, of the deaths. Of the illnesses, non-typhoidal *Salmonella* spp. was estimated to cause the most illnesses with 1,027,561 followed by *Clostridium perfringens* with 965,958, *Campylobacter* spp. with 845,024, *Staphylococcus aureus* with 241,148, *E. coli* (including STEC O157, STEC non-O157, ETEC, and other diarrheagenic *E. coli*) with 205,781, and *Shigella* spp. with 131,251. For hospitalizations, non-typhoidal *Salmonella* spp. again caused the most with 19,336 followed by *Campylobacter* spp. with 8,463, *E. coli* (including STEC O157, STEC non-O157, ETEC, and other diarrheagenic *E. coli*) with 2,429, *Shigella* spp. with 1,456, and *L. monocytogenes with* 1,455. Deaths were most likely to be caused by non-typhoidal *Salmonella* spp. with 378 and *L. monocytogenes* with 255 deaths annually (Scallan et al., 2011).

2.2 Sources and routes of contamination in food processing facilities

Approximately 25% of foodborne outbreaks have been linked to recontamination or cross-contamination events according to World Health Organization (WHO) studies in Europe with leading causes of pathogen contaminated prepared foods being unsatisfactory hygiene practices, cross-contamination, processing or storage in unsuitable areas, contaminated equipment, and contamination by personnel (Carrasco et al., 2012; Reij & Den Aantrekker, 2004; Tirado & Schmidt, 2001).

In an environmental study survey of 21 food processing plants, *Listeria* spp. was found in 71% of drains, 43% of standing/condensed water, 39% of floors, 30% of processing equipment, 20% of miscellaneous sites, and 14% of residues. Of these, 18% of the drains, 9% of the process equipment, 6% of the floors, 5% of the residues, 3% of the standing/condensed water, and 2% of the miscellaneous sites tested positive for *L. monocytogenes*. In the environment outside around the plant, 97% of soil and effluent samples tested positive for *Listeria* spp. and 13% contained *L. monocytogenes*. Food handlers were each swabbed three times, one each inside their nasal passages, on hands, and on faces, and 33% were found to carry *Listeria* spp. and 19% carried *L. monocytogenes*. Interestingly, for the *L. monocytogenes* carriers, all the positive samples originated from inside their noses and not from their hands or faces (El-Shenawy, 1998). One of the biggest challenges with *Listeria* is its ubiquity, as illustrated by this study. In another *L. monocytogenes* environmental swab study, based on the sampling results and using the facility plans to map the positives, the authors were able to observe three specific contamination scenarios: between hygienic zones due to lack of effective barriers and traffic patterns, hard to clean niches and harborage "hot spots," and widely distributed contamination caused by hygiene and sanitation failures, Surprisingly, the researchers did not find a correlation between widespread positive environmental non-food contact surface (NFC) swabs and product positives (Muhterem-Uyar et al., 2015).

From data collected during a three-year survey of a meat processing plant in Greece, the researchers found that during a year of higher production, levels of total viable count (TVC), coliforms (TCC) and *E. coli* (EC) increased which they attributed decreased time and attention to sanitation due to increased production demands as a possible cause (Manios et al., 2015). Indeed, according to a study of the impact of environmental factors on *L. monocytogenes*

viability and culturability under processing plant-like conditions, out of several possible factors tested including differing strain tolerance to desiccation, addition of a *Pseudomonas* strain to promote adhesion, tile versus stainless steel surfaces, and differing food soils, the only statistically significant factor in reducing the population was the daily sanitation procedure (Overney et al., 2017).

Recontamination from dirty surfaces to clean surfaces is a significant route of contamination along with contaminated raw materials, poorly designed or hard to clean equipment, employees, mobile equipment, niches, roof leaks, etc., in processing environments (Reij & Den Aantrekker, 2004). Several studies have shown that the cross-contamination from environmental surfaces route in swine slaughter accounts for a large percentage of contaminated carcasses (Berends et al., 1997; Borch et al., 1996; Botteldoorn et al., 2003; Carrasco et al., 2012). In one study to investigate the prevalence of *Salmonella* on pigs, the researchers found when performing environmental swabs that 25% of overshoes before the harvest area, 86% of overshoes in the harvest area, and 40% of the overshoes in the chilling rooms were positive for *Salmonella* (Botteldoorn et al., 2003). Since the overshoe bottoms were positive for *Salmonella* at these rates, the floor was likely contaminated as well. In the same study, cross-contamination was estimated to be the cause of 29% of the *Salmonella* contaminated carcasses (Botteldoorn et al., 2003). Another study found that the *Salmonella* prevalence in drain water samples in two slaughterhouses was 50% and 70% (Swanenburg et al., 2001). Effective GMPs and HACCP plans tend to limit cross-contamination between carcasses and an effective sanitation program limits contamination from the processing environment (Borch et al., 1996). Another study found that while incoming pigs that are already carrying Salmonella are 3 to 4 times more likely to produce *Salmonella* positive carcasses, 30% of positive carcasses are the result of cross-

contamination (Berends et al., 1997). Another study on the routes of *E. coli* contamination in cattle slaughterhouses tested various carcass, tool, and infrastructure including the floor over a three-day period and while several of the samples were *E. coli* O157 positive, none of the floor samples returned positive results (Tutenel et al., 2003).

Additionally, recontamination can occur after a lethality treatment, reintroducing pathogens to product, if adequate cleaning and disinfection procedures are not followed or there is a harborage or uncleanable niche where the pathogens can survive in the post lethality zone (Oliver et al., 2005). Two reviews on the persistence of *L. monocytogenes* in food industry facilities and equipment conclude that there is no "super" *L. monocytogenes* strain that is more resistant to killing by disinfectants, adhere to surfaces more densely, or have any other unique properties that allow them to persist more readily than other *L. monocytogenes* strains. It is therefore likely the simple existence of harborage sites and niches that are unable to be cleaned that is responsible for any persistence in food processing environments (Carpentier & Cerf, 2011; Ferreira et al., 2014).

Flooring and drains are often a significant contamination risk area in food processing plants. In one study of several retail facilities and one processing plant in Brazil, the mean *E. coli* contamination level of floors, platforms, and drains were 1.79, 3.94, and 3.88 $\log_{10}$ CFU/cm^2 respectively (Barros et al., 2007). Mean mesophilic aerobe contamination in this same study was 4.76 $\log_{10}$ CFU/cm^2 for floors, 6.60 $\log_{10}$ CFU/cm^2 for platforms, and 7.26 $\log_{10}$ CFU/cm^2 for drains. Another study performed in one Midwestern United States processing plant found mean aerobic plate counts (APC) for flooring between 3 and 3.6 $\log_{10}$ CFU/cm^2 for processing flooring, with higher counts, 5 $\log_{10}$ CFU/cm^2, in areas where leaking packages were spilling blood onto the floor (Eisel et al., 1997). Both of these studies typically found higher APC levels

on incoming products than on the processing floor, illustrating one of the sources of floor contamination (Barros et al., 2007; Eisel et al., 1997). In the two previously mentioned *Listeria* survey studies, floors and drains were among the sites most often positive for *Listeria* spp. and *L. monocytogenes* (El-Shenawy, 1998; Muhterem-Uyar et al., 2015). Work done on the cleanability of different types of floor surfaces of varying surface profiles found that the main determinant of the ease in removing bacteria from the surface of the material was not the average texture, the average of the highest and lowest points, but the amount of "inwardly-directed" texture, like crevices, cracks, valleys, pores, and holes that trap material (Mettler & Carpentier, 1999). A study of the microbiomes of drains in another plant found that although one out of six drain biofilm samples tested positive for *L. monocytogenes* using the standard ISO cultural-PCR method, the same samples had five out of six positive results from the 16S pyrosequencing results indicating that that biofilm bound *Listeria.* in drains may have been in a viable but not culturable state that traditional methods might not detect (Dzieciol et al., 2016).

Common sources of contamination include biological aerosols, defined by Kang and Frank as aerosolized material that include bacteria, yeasts, molds, spores of bacteria and mold, viruses, and pollen, with sizes ranging generally from 0.5 to 50 μl (1989). These aerosols are produced from spraying water on contaminated surfaces, flooring, and drains, as is common during food production environment sanitation activities, and can linger in the air and spread and settle quite a distance from the source (Berrang & Frank, 2012; Kang & Frank, 1989, 1990). Kang and Frank inoculated floors and drains with *Serratia marcescens* and found that viable aerosolized particles in the air increased 8 to 100 times after rinsing the floor for 90 seconds at a rate of 20.67 gallons per minute and recovered the inoculum from the air above both drains and floor, verifying that the aerosols were coming from the spray sites and illustrating how

contamination (and potentially pathogens if they happen to be present on these surfaces) may be moved from drains and flooring to the air . The researchers additionally found that once these aerosols were airborne, it took 40 minutes or more for the levels of particles in the air to return to normal background levels. In another experiment by Berrang and Frank, the researchers created model drains consisting of PVC pipe, some filled with a liquid *L. innocua* inoculum and others that had an *L. innocua* biofilm grown on the interior and sprayed them with water for 2 seconds to simulate routine sanitation activities, sampling the air periodically and using sedimentation plates positioned at various distances and orientations to simulate equipment surfaces. After just a two second spray, the researchers found that the *L. innocua* persisted in the air for 30 minutes and that while there was more airborne *L. innocua* from the liquid inoculum drains versus the biofilm drains, there was no difference in the sedimentation plates (2012). Berrang and Frank also found from their 2012 experiment that from that two second spray, *Listeria* was detected four meters from the drain horizontally and 2.4 meters vertically on a wall, with the highest amounts of *Listeria* found on the plates closest to the drain. But once beyond 1.2 meters, the counts on the plates no longer decreased indicating a potential widespread plume of aerosolized *Listeria* settling uniformly on the surfaces below with any surface within 4 meters of the drain being equally likely to become contaminated.

Food can become contaminated in the production environment when it comes into contact with contaminated surfaces, as one study determined by looking at how long *E. coli* can survive on different materials and then quantifying how much bacteria is transferred to a product when it comes into contact with a contaminated surface. The researchers found that *E. coli* showed significant decreases on wood and cardboard crates after only an hour of contact and more than a week on both newly manufactured and reused plastic crates. After inoculating these

four types of crates, the researchers found the mean percentage of inoculum transfer of *E. coli* from the crates to the apples was <0.008% for wood and carboard crates, 5.51% for new plastic crates, and 3.98% for the reused plastic crates (Aviat et al., 2020).

For cattle, according to Soon, most *E. coli* contamination caused by cross-contamination in the harvest facility originates from incoming cattle and not from the plant itself, and he recommends rigorous on-farm strategies to reduce the incoming load of *E. coli* including interventions focusing on the animal drinking water, feed, hide, and soil (2011).

2.3 Sanitation of food manufacturing processing equipment and environments

An effective sanitation process is essential in breaking the day-to-day chain of contamination in the processing environment. The sanitation process is typically divided into discrete phases, sometimes referred to as the Seven Steps of Effective Sanitation. In the first step, large pieces of meat and debris are removed from the processing area without the use of water in what is commonly referred to as the dry pickup (Cramer, 2013; Marriott & Gravani, 2006). The second step of the sanitation processes is the hot water rinse, also called the first push, where water hot enough to melt any animal fats present but not so hot that proteins denature and become "baked on" to surfaces is used to rinse from top to bottom any remaining meat from the processing environment to the floor where it can be collected and disposed of properly. Cramer recommends water temperatures between 130°F and 160°F to both melt the fat and keep the proteins from adhering to surfaces (2013).

The third phase is where a chemical detergent is applied to the walls, floor and equipment and the equipment surfaces are scrubbed. The fourth step, called the final rinse, is where the detergent and any remaining solubilized debris is rinsed from the walls first, then the floor, and finally the equipment.

After the detergent is rinsed from the equipment, high pressure water should not be used on the floor as it can aerosolize contaminants and spread contamination from the floor to food contact surfaces via overspray. In one study comparing two different sets of sanitation chemicals, even though both were able achieve zero positive *L. monocytogenes* samples collected from the food contact surfaces, neither was able to completely remove *L. monocytogenes* from non-food contact areas including the floor (Campdepadrós et al., 2012). During this final rinse, sanitation workers should be using flashlights to be able to visualize any remaining debris or residues on the equipment so that if any are found, these areas can be recleaned.

The fifth step in the sanitation process includes removing condensation from overheads and any standing water from equipment, floors, and mezzanines. Condensation removal is typically removed manually as the environmental conditions in the processing environment may not allow for all the water to evaporate in a timely manner, although this can increase the chance of cross-contamination. Lecoq, et al., found that under conditions similar to those during food processing and with the use of a dehumidification system, water in cracks and other areas of the floor that are not perfectly flat will persist for at least several hours continuing to be a potential harborage and contamination risk (Lecoq et al., 2017). The sixth step in a typical sanitation process is a pre-operational inspection where plant personnel use their senses to inspect the equipment, focusing on hard to clean areas, to verify that all the equipment looks, feels, and smells clean and is free from any visible debris. Any equipment found deficient during the pre-operation inspection must be recleaned by scrubbing with soap and rinsing clean with potable water. The seventh step is the application of an EPA registered sanitizer to the walls, floor, and equipment.

There are many different types of sanitation chemicals: cleaners, detergents, disinfectants, and sanitizers and selection of the proper type is primarily based on the soil composition, material used in the construction of the equipment and of the room itself, and organism of concern. One study compared a chlorinated alkaline, low-phosphate detergent paired with a 2600 ppm PAA sanitizer called treatment A with a solvated-alkaline product followed by a 200ppm hypochlorite sanitizer called treatment B against 5 constituent *L. monocytogenes* cocktail biofilms formed on common food processing equipment surface types including 304 and 316L grades of stainless steel, polymers and plastics including Delrin, TURE-2, BUNA-N, silicone, and Polyester 3000, and other environmental surface types including a wall brick material and painted floor resin (Somers, 2004). Somers found that for the environmental surface types, the brick wall material, and the painted floor resin, they were able to get to near the lower limit of detection with combination B, but only inactivate up to 90.88% with combination A. For the equipment surface types, both combinations performed well, but combination B was slightly more effective.

2.4 Methods for verification of cleaning

Pre-operation inspection, step six from the seven steps of effective sanitation mentioned previously, is a subjective organoleptic verification process. The main drawback to organoleptic inspection is that it is limited to what can be sensed by a person. Objective measures include adenosine triphosphate (ATP) and microbiological swabs which can detect if a piece of equipment looks clean when in fact it has either an invisible residue or is contaminated with microorganisms (American Public Health Association, 2001; United States Department of Health, 1967).

From the three-year plant survey in Greece, researchers found that the mean populations of TVC, TCC, and EC were all significantly higher ($P < 0.05$) in samples positive for *L. monocytogenes* and *Salmonella* spp. than those that were negative, potentially indicating that there is a relationship between general hygiene level and the presence of pathogenic bacteria (Manios et al., 2015). The results of another study also found that locations with high aerobic plate counts (APC) were more likely to also test positive for *Enterobacteriaceae* and *L. monocytogenes (*Jessen & Lammert, 2003*).* This provides further support for indicator organism monitoring, such as *Enterobacteriaceae*, along with regular pathogen monitoring to verify that the sanitary operating procedures and any other preventive measures are adequate and being followed (Reij & Den Aantrekker, 2004).

Swabs may also be collected to monitor for food spoilage indicators like blown pack causing *Clostridium gasigenes* and *Clostridium estertheticum* and spoilage causing *Leuconostoc mesenteroides* subspecies. *mesenteroides* and *Leuconostoc citreum* to help locate potential harborages and verify that the sanitation procedures are effective at removing the target organisms (Broda et al., 2009; Hamasaki et al., 2003). For *Clostridium estertheticum*, one study found that even one spore in a vacuum sealed package may cause blown pack spoilage and that as the number of spores increased, the time to spoilage decreased (Clemens et al., 2010). Additionally, microbiome studies may be conducted to describe the complex microbiota of a particular plant in order to better select chemical interventions suited for the particular bacteria, especially for spoilage bacteria (Hultman et al., 2015; Stellato et al., 2016).

2.5 Good manufacturing practices (GMPs) and food safety culture

Good manufacturing practices (GMPs) are best practices that when followed, reduce the chance of food spoilage, foodborne illness, and other types of contamination that include

maintaining the cold chain and avoiding temperature abuse, microbial control measures like pH, water activity, competitive microflora, preservatives, and thermal processing, properly managing and performing a risk assessment on ingredients, product development ensuring that the product is designed properly with food safety and shelf-life in mind, maintaining appropriate hygiene in the processing environment and any storage or cooling locations, proper packaging and storage, and recordkeeping of all of the above (Moberg, 1989).

Frank Yiannas defines Food Safety Culture as, "…how and what the employees in a company or organization think about food safety," and, "…the food safety behaviors that [the employees] routinely practice and demonstrate (Yiannas, 2009)." The Food Safety Culture of an organization can even be measured to identify deficiencies in culture and areas that need improvement in order to maintain high standards of hygiene and food safety (De Boeck et al., 2015). Assessments and audits are another useful tool to uncovering deficiencies and potential routes of contamination that could lead to food safety issues as in one study of the Scottish smoked salmon industry that identified several common food safety problems that when corrected could reduce the level of *L. monocytogenes* contamination in smoked salmon across the industry. Top items identified in this study included the need for better control of processing environment and storage temperatures, eliminating dripping condensation, establishing effective programs for environmental monitoring and finished product testing for *Listeria*, and improving the cleaning and sanitation of food contact surfaces in the entire food processing environment (Rotariu et al., 2014).

Reducing, eliminating, or promptly repairing known *L. monocytogenes* reservoirs in processing environments, like equipment framework, floors, drains, walls (especially areas of damage), ceilings and overhead structures, managing condensate, wet insulation around pipes

and in walls, mobile equipment like forklifts and carts, cleaning tools like sponges, brushes, and floor scrubbers, and maintenance tools, is another example of how implementing GMPs can reduce the chance of cross-contamination (Tompkin et al., 1999).

Carpentier and Cerf make the following recommendations to food manufacturers: 1) Bacterial cells that have recently contaminated a surface are easy to kill with the recommended disinfectant concentration, so the disinfectant concentration should never be lower than the recommended level and the frequency of sanitation should be often enough so that the bacteria does not excessively grow out between cleanings; 2) To limit outgrowth, the processing environment should be kept as dry and cold as possible with no water being used around exposed product; 3) Floors should be cleaned before equipment due to the higher likelihood of the floor being contaminated or containing harborages. If the equipment is cleaned after the floor, any contamination that had splashed from the floor will be washed from the surfaces of the equipment; 4) Equipment should be well hygienically designed and maintained to avoid spaces where *L. monocytogenes* is protected from mechanical action and direct chemical application; 5) Equipment operators should be provided specific information about how to avoid cross-contamination; and 6) To improve hygiene overall, ongoing efforts should be made to improve the hygienic design of the equipment and facility as well as improving the ability to sanitize and disinfect equipment on all surfaces at the correct concentration (2011).

Best practices in plant and equipment design, and verifying and validating cleanability via testing, is also very important to maintaining food safety since proper hygienic design has such a large impact on cleanability and the ability to continue to maintain a high level of hygiene over the life of the equipment and facility (Lelieveld, 1985).

2.6 Antimicrobials used in food processing facilities and active components of common floor treatments

To combat microorganisms in the food processing environment, several environmental sanitizers are commonly used including quaternary ammonium compounds (QACs), hydrogen peroxide, iodophors, ozone, peroxyacetic acid (PAA), chlorine (sodium hypochlorite), and chlorine dioxide (Carpentier & Cerf, 2011; Cramer, 2013; Lopes, 1986; Minbiole et al., 2016).

Quaternary ammonium compounds (QACs) are cationic detergents and generally are composed of a positively charged nitrogen with four bonds to nonpolar alkyl or aryl tails and are generally amphiphilic and water soluble (Gerba, 2015; Jennings et al., 2015). The antimicrobial mechanism of action for QACs is related to their charge and amphipathicity in that the positively charged nitrogen and negatively charged bacterial cell membranes electrostatically interact with the QAC side chains entering the hydrophobic intermembrane space causing membrane disruption, leakage of cytoplasmic material, and cell lysis. Because QACs target the cell membrane, they are active against nearly all bacteria, but work best against Gram-positive bacteria since Gram-positive bacteria have a single phospholipid membrane versus Gram-negative bacteria having two. The second membrane on Gram-negative bacterial cells is thought to be responsible for the decreased antimicrobial activity, typically 8 times less according to the author, of QACs and other antimicrobials that target the cell membrane against Gram-negative bacteria versus Gram-positive bacteria (Jennings et al., 2015).

One study found that *Salmonella* Typhimurium ATCC 7823 was reduced <99.999% when exposed to 100 ppm QAC while *L. monocytogenes* ATCC 13932 was reduced by >99.999% (Lopes, 1986). At 200 ppm QAC, the reductions for both organisms were >99.999%, potentially explained by Gram-negative bacteria being less susceptible to quaternary ammonium

compounds due to their double membrane (Lopes, 1986). Since QACs kill by disrupting cell membranes and Gram-negative bacteria like *Salmonella* and *E. coli* have an additional outer membrane compared to Gram-positive bacteria like *L. monocytogenes*, QACs are generally better at killing Gram-positive bacteria (Minbiole et al., 2016). To reduce the *L. monocytogenes* bacterial population by 5 $\log_{10}$ CFU/mL, QACs tested in another study had a 30 fold difference in the mean minimal effective concentration (MEC) of 66 ml/mL for planktonic *L. monocytogenes* versus 2,023 ml/mL for *L. monocytogenes* in a biofilm (Cruz & Fletcher, 2012).

Other work found that there was a positive correlation between the minimum inhibitory concentration (MIC) of quaternary ammonium compounds (QACs) and biofilm formation among the strains of *L. monocytogenes* tested; they also concluded that the strain most resistant to disinfection had the highest biofilm forming ability (Poimenidou et al., 2016). There are some differences in the levels of QACs required to kill different strains of *L. monocytogenes*, however, and another study found that the MICs for QACs ranged from 10 to 13 ppm benzalkonium chloride (BC) for *L. monocytogenes* that had the efflux pump gene *bcrABC*, 5-12 ppm BC for *L. monocytogenes* that had the efflux pump gene *qacH*, and ~5 ppm BC for *L. monocytogenes* without either, though the MIC for even the *bcrABC* positive strains is far below most manufacturer recommended application levels of QACs which are usually 200 to 1,000 ppm depending on surface type (Moretro et al., 2017).

Another common sanitizer used in food production facilities is hydrogen peroxide (H_2O_2) which is a strong oxidizer that produces hydroxyl free radicals that damage cell membranes, proteins, and DNA (Robbins et al., 2005; Romanova et al., 2002). In one study, a 3% H_2O_2 solution reduced planktonic *L. monocytogenes* by 6.0 $\log_{10}$ CFU/mL and a 3.5% H_2O_2 solution reduced *L. monocytogenes* by 8.7 $\log_{10}$ CFU/mL after 10 minute contact times (Robbins et al.,

2005). An additional experiment conducted with 5 constituent strain cocktails of *Salmonella* Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* inoculated on lettuce leaves and exposed to 2% H_2O_2 at 50°C for 90 seconds found reductions of 4.5, 4.7, and 2.7 $\log_{10}$ CFU per leaf, respectively (Robbins et al., 2005). One study on various sanitizers against *L. monocytogenes* found that the minimum inhibitory concentration (MIC) of H_2O_2 ranged from 9.4 μg/mL to 37.5 μg/mL while the MIC of two QAC products ranged from 0.77 μg/mL to 6.25 μg/mL for 19 different strains of *L. monocytogenes* indicating that *L. monocytogenes* may be more sensitive to QACs than H_2O_2 (Romanova et al., 2002). A dry chemical source of hydrogen peroxide is sodium percarbonate (SPC) a solid peroxygen compound, also called sodium carbonate peroxyhydrate or disodium carbonate hydrogen peroxide, with chemical name sodium carbonate sesquiperhydrate and chemical formula of $2NaCO_3 \times 3H_2O_2$ that is cheap, readily available, has good storage characteristics and safety profile (though should not be mixed with oxidizable substrates), and is commonly found in clothes washing powders (Ando et al., 1986; McKillop & Sanderson, 1995). When exposed to water or moisture, SPC decomposes and releases the hydrogen peroxide (McKillop & Sanderson, 1995).

Urea, chemical formula NH_2CONH_2, is a nitrogenous compound composed of a carbonyl group and two amine groups (PubChem Compound Summary for CID 1176, Urea, 2021). Urea has been long used to denature proteins, but the precise mechanism is unknown but results of one study indicate that it is a combination of urea directly interacting with the proteins and the indirect interaction of urea with the solvent (Bennion & Daggett, 2003). One study looking at the effect of urea added to manure for the reduction of *Salmonella* in compost found that it took 10.2 days for a 2% (w/w) urea and manure mixture to achieve a five-$\log_{10}$ reduction of *Salmonella* (Ottoson et al., 2008). In another compost study, the addition of 3% (w/w) urea

resulted in the reduction of fecal coliforms and *Salmonella* to non-detectable levels in 5 days (Vinnerås, 2007).

Borax, or sodium tetraborate decahydrate (including pentahydrate and other numbers of hydrates) with chemical formula $Na_2B_4O_7 \times XH_2O$, is used in a host of products including the production of borosilicate glass, insecticides, laundry detergents, wood preservatives, and many other products (Murray, 1995). A study on the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of borax found that for Gram-negatives *E. coli* ATCC 3528 and *Pseudomonas aeruginosa* ATCC 27853, both the MIC and MBC was 47.60 mg/mL for each and for Gram-positive *Staphylococcus aureus* ATCC 25923 and Gram-negative *Acinetobacter septicus* DSM 19415, both the MIC and MBC were 23.80 mg/mL (Yilmaz, 2012). Another study in soil found that while boric acid reduced the bacterial biomass of the soil sample, the samples treated with borax in a solution of 0.05 mg/mL showed increased bacterial biomass, both Gram-negative and Gram-positive bacteria relative to the control, consistent with the Yilmaz study which determined the MIC and MBC to be much higher (Vera et al., 2019; Yilmaz, 2012).

Bacteria in biofilms have been shown to enhance survival against antimicrobials in several studies (Aryal & Muriana, 2019; Joseph et al., 2001). A study comparing planktonic versus biofilm bound *Salmonella* found that for biofilms grown on plastic, cement, and stainless steel, it took chlorine (Cl_2) at a concentration of 100 parts per million (ppm) 20, 20, and 15 minutes respectively and iodine (I_2) concentrations of 50 ppm 25 min for cement, 20 minutes for stainless steel, and >25 minutes for plastic to reduce the *Salmonella* biofilm populations to undetectable levels (Joseph et al., 2001). For planktonic *Salmonella* in the same study, the levels were reduced to undetectable levels in 10 min with 10 ppm Cl_2 and 5 minutes with 10 ppm I_2,

indicating that biofilm bound *Salmonella* were much harder to kill than planktonic cells, which agrees with other studies (Joseph et al., 2001). In another study that looked at *Salmonella* biofilms and their resistance to typical cleaning procedures and sanitizers, the researchers found that neither 250 ppm QAC nor 200 ppm Cl_2 with a dwell time of 2 minutes were able to reduce *Salmonella* in biofilm on plastic to undetectable levels alone or in combination with typical cleaning methods that included hot water and alkaline detergents indicating that it is unlikely that typical sanitation procedures will remove *Salmonella* from plastic surfaces in a biofilm (Clayborn et al., 2015).

Another study comparing different sanitizers on biofilms of *L. monocytogenes*, *E. coli*, and *Salmonella* on plastic microplates found that 200 ppm Cl_2 was not able to reduce the recovered bacteria to the lower limit of detection (LOD) while 1000 ppm Cl_2 was able to bring *L. monocytogenes* to the lower LOD in 15 minutes and *E. coli* in 60 minutes but was not able to reduce *Salmonella* to the lower LOD within the 60 minute test (Aryal & Muriana, 2019). In the same study, the researchers found that while 200 ppm QAC was able to reduce the recovered *L. monocytogenes* to the lower LOD in 30 minutes it was not able to decrease *E. coli* and *Salmonella* to the LOD over the 120 minutes test period. 1000 ppm QAC was able to bring *L. monocytogenes* to the lower LOD in 15 minutes, but 1000 ppm QAC was not able to reduce *Salmonella* or *E. coli* to the lower LOD within the 120 minute test period, either (Aryal & Muriana, 2019). Peroxyacetic acid (PAA) at 500 ppm was able to reduce all three organisms in biofilm to the lower LOD, with *L. monocytogenes* and *E. coli* at the lower LOD in 5 minutes and *Salmonella* in 30 minutes (Aryal & Muriana, 2019). Sterilex, a QAC and H_2O_2 based chemical, was able to decrease *L. monocytogenes* to the lower LOD in 2.5 minutes for the 5% concentration and about 1 minute for the 10% concentration, but was not able to completely

reduce *Salmonella* or *E. coli* at either concentration, though *E. coli* was reduces to near the lower LOD (Aryal & Muriana, 2019). Finally, Decon 7, a QAC, H_2O_2, and diacetin blend, was able to decrease *L. monocytogenes* and *E. coli* to the lower LOD in about 2 minutes for the 5% concentration and about 1 minute for the 10% concentration, while the 5% was not able to completely reduce *Salmonella* in 20 minutes at 5% but did reduce *Salmonella* to the lower LOD in about 2 minutes at 10% (Aryal & Muriana, 2019).

2.7 Natural Antimicrobials

Natural antimicrobials, including essential oils, extracts, tinctures, and other plant and animal derived material and products may be preferred over the commonly used antimicrobial chemicals in the previous section because there may be a perception that because they are made from plants or animals, or may have more familiar and easily pronounced names, that they are more environmentally friendly or less toxic to humans and other animals.

In a study exploring the association between attitudes towards chemicals and the preference for natural foods, the researchers found that a person's perceptions of being in contact with chemicals, regardless of dose, as being dangerous combined with negative attitudes about chemicals in general, showed an increased preference for natural foods (Dickson-Spillmann et al., 2011). In other words, if a person had negative feelings towards chemical additives and feels that any exposure to chemicals regardless of dose is dangerous, they are also more likely to not want to ingest chemicals and have a higher preference for natural foods. Another interesting result from the study was that there were gender differences that showed that the males included in the study had less of a sense of danger about chemicals in food in a dose context, lower perceived risk of additives and contaminates in general, so showed less of a preference for natural foods than the female test subjects on average.

In another study, the researchers distilled essential oils from leaves and sprigs of nine different plants, *Eriocephalus africanus, Artemisia absinthium, Santolina chamaecyparissus, Mentha longifolia, Thymbra capitata, Citrus limon, Osbeck, Citrus reticulata Blanco, and Eucalyptus camaldulensis Dehnh,* and performed minimum inhibitory concentration (MIC) testing to see which one had the best performance to perform further testing (Falcó et al., 2019). *Thymbra capitata* essential oil performed the best against both *E. coli* O157:H7 and *S. enterica* with an MIC for both bacteria at 0.05% v/v, the lowest level tested. Carvacrol comprised 91.56% of the identified compounds via GC/GC-MS. Although the results showed better performance on clean surfaces than dirty ones, the 2.5% sanitizing solution was effective against *E. coli* O157:H7 with a >3 log reduction but not *Salmonella* Typhi at 10 minutes, but was effective for both bacteria at 5% all on clean surfaces (Falcó et al., 2019). In another study, the MBC was found to be 1.4 mmol/L for carvacrol and 1.2 mmol/L for thymol for E. coli O157:H7(Burt et al., 2005). Converting the mmol/L to percent gives an MBC for carvacrol of 0.022% and 0.019% for thymol, which is consistent with the Falco MIC for carvacrol that was tested at 0.05% at the lowest level and found to inhibit growth.

One potential drawback for the use of essential oils and other plant derived antimicrobials is that many can impart an odor, flavor, bitterness, or color when directly applied to the product or a food contact surface. According to Burt, this can be partially overcome by appropriately tailoring the incorporation of specific antimicrobial essential oils or components into products or processes that are already similar organoleptically or where it could increase palatability (2004). Burt also asserts that even though essential oils are natural, many plants, especially concentrated components of plants, can be toxic to humans so more toxicology assays should be performed for these products. But as with the results of the Falco study, Burt asserts that there may be

significant difference in antimicrobial activity between different bacteria within the Gram negative and positive groups that should also influence appropriate antimicrobial essential oil selection. Since the most active components of antimicrobial essential oils are likely phenolics that make the cell membrane more permeable, like QACs, Gram-negative bacteria may not be as suspectable as Gram-positives.

Turmeric (*Curcuma longa* in the *Zingiberaceae* family) is widely used as a spice, coloring material, and food preservative in India, China, and Southeast Asia (Amalraj et al., 2017; Gul & Bakht, 2015; Zheng et al., 2020). Curcumin (CUR) is a bioactive component of turmeric and is little soluble in water at acidic or neutral pH and along with demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) make up the curcuminoids (Amalraj et al., 2017). Curcumin has low bioavailability when consumed and a good safety profile with wide therapeutic index, but can interact with some prescription drugs due to inhibition of cytochrome isoenzymes and P-glycoprotein (Bahramsoltani et al., 2017).

Several studies have supported the use of turmeric as an antimicrobial, but some had mixed results or even showed no antimicrobial activity. In one study where turmeric oil extract was prepared by mixing 1 kg of turmeric powder with 5 liters of room temperature water and allowed to steep, 6 µl and 12 µl of turmeric oil placed on a filter paper disk produced a zone of inhibition for *E. coli* of 7 mm and 7 mm and *Salmonella typhi* of 7 mm and 10 mm respectively while having no inhibition for *C. albicans* and *Staph. aureus* for either concentration (Gul & Bakht, 2015). In another study, the zones of inhibition for turmeric extract against *L. monocytogenes* and *E. coli* did not differ from the control at various concentrations from 5 mg/mL to 80 mg/mL (Zhang et al., 2009). Another study determined the MIC of curcumin solubilized in ethanol for methicillin sensitive *S. aureus* at 219 µg/mL, methicillin resistant *S.*

aureus at 217 μg/mL, *Enterococcus faecalis* at 293 μg/mL, *Bacillus subtilis* at 129 μg/mL, *P. aeruginosa* at 175 μg/mL, *E. coli* at 163 μg/mL, and *Klebsiella pneumoniae* at 216 μg/mL (Gunes et al., 2016). For both *Shigella dysenteriae* and *Campylobacter jejuni*, the MIC was found to be 256 μg/mL and MBC were found to be 512 μg/mL (Kareem et al., 2020).

Another study found the MIC for curcumin to be 125 μg/mL for Gram-positive *L. monocytogenes* and *S. aureus* and 250 μg/mL for Gram-negative *Salmonella* Typhimurium and *E. coli* (Altunatmaz et al., 2016). In the same study, when 0.5%, 1%, and 2% (w/w) amounts of curcumin extract were incorporated into veal minced meat medium inoculated with either *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* Typhimurium or *S. aureus*, it was found that after 7 days, the 0.5% treatment reduced *L. monocytogenes* and *S. aureus* by about 2 $\log_{10}$ CFU/g and *E. coli* and *Salmonella* by about 1 $\log_{10}$ CFU/g, the 1% curcumin treatment reduced *L. monocytogenes*, *E. coli*, *Salmonella*, and *S. aureus* by approximately 2 $\log_{10}$ CFU/g, and the 2% treatment reduced *L. monocytogenes* and *S. aureus* but about 3 $\log_{10}$ CFU/g and *Salmonella* and *E. coli* by about 2 $\log_{10}$ CFU/g (Altunatmaz et al., 2016). In another study, chicken breast that was processed into small pieces and inoculated with *E. coli* or *S. aureus* and then treated with 1% (w/w) turmeric powder and the held at 4°C for 48 hours showed no decrease in either *E. coli* or *S. aureus* (Lourenço et al., 2013). A more recent study found the MBC of a crude ethanolic turmeric extract to be 20 mg/mL for Gram-positive *S. aureus* and 80 mg/mL for Gram -negative *P. aeruginosa* and MIC to be 10 mg/mL and 40 mg/mL respectively. Against biofilms, the researchers found that at a concentration of 0.5 to 2 mg/mL this crude ethanolic turmeric extract reduced biofilm formation for *S. aureus* by 49% to 78% and for *P. aeruginosa* 27% to 59% (Suwal et al., 2021).

Natural antimicrobials do not always mean low tech or plant derived, however, they can also incorporate nanotechnology (nanocarriers, nanoemulsions, nanoliposomes, polymeric nanoparticles, nanofibers, and metallic nanoparticles), bacteriophages, be derived from animals like the food lipids C10:0 and C12:0 fatty acids, pleurocidin, lactoferrin, lactoperoxidase, chitosan, and lysozyme, or even other microorganisms such as the bacteriocin nisin that is produced by lactic acid bacteria (LAB), (Cacciatore et al., 2020; Grant & Parveen, 2017; Pisoschi et al., 2018; Quinto et al., 2019).

Chapter 3: Materials and Methods

3.1 Materials

Tufcrete flooring material was obtained from Tufco Flooring/Arkotex, Siloam Springs, AR, digital calipers were Mitutoyo CD-S8" CT, Japan, water cooled diamond blade tile saw was Rigid 7-inch Tile Saw, Emerson Electric Co., Elyria, OH, autoclave sterilization pouches from Cardinal Health, Dublin, Ohio, autoclave Tuttnauer USA, Hauppauge, NY, tryptic soy agar plates and 9 mL Butterfield's dilution blanks were from Edge Biologicals, Memphis, TN, Brain Heart Infusion (BHI) broth from BD Difco, Franklin Lakes, NJ, and centrifuge from ThermoFisher Scientific, Waltham, MA.

Hi-Cap Neutralization Broth (Hi-Cap NB) was World Bio-Products LLC, Bothell, WA, APC Petrifilm was from 3M, Maplewood, MN, commercial antimicrobial floor treatments obtained as samples directly from manufacturers, turmeric powder Swad, Burlington, MA, was obtained from a local grocery store, sample bags from Whirl-Pak, Madison, WI, Synthetic Broth (Wright and Mundy Broth) from HIMEDIA, Mumbai, India, Whatman filter paper #3 from Whatman Plc, GE Healthcare, Buckinghamshire, UK, curcumin determination centrifuge model J2-21, Beckman, Fullerton, CA, curcuminoid reference standard ASB-00003928 (99.7%) from ChromaDex, Irvine, CA, Agilent 1200 series HPLC with DAD detector and Zorbax C18 (4.6 x 250 mm) 5µm column from Agilent, Santa Clara, CA.

The strains in the inoculum cocktails were obtained from the American Type Culture Collection (ATCC) (ATTC, Manassas, VA) or were wild strains: *Salmonella* Typhimurium (wild strain), *Salmonella* Heidelberg (wild strain), *Salmonella* Mbandaka (wild strain), *Salmonella* Kentucky (wild strain), and *Salmonella* Senftenberg (ATCC 43845). The *E. coli* cocktail consisted of *E. coli* P1 (ATCC BAA-1427), *E. coli* P3 (ATCC BAA-1428), *E. coli* P8

(ATCC BAA-1429), E. coli P14 (ATCC BAA-1430), and *E. coli* P68 (ATCC BAA-1431). The *L. monocytogenes* cocktail was composed of *L. monocytogenes* (ATCC 43257), *L. monocytogenes* (ATCC 49594), *L. monocytogenes* (ATCC 19111), *L. monocytogenes* (ATCC 19115), and *L. monocytogenes* 4B #2926 (wild strain).

3.2 Creating the carriers from typical flooring material

Carriers were fashioned from Tufcrete (Tufco Flooring/Arkotex inc., Siloam Springs, AR), a hybrid polyurethane-concrete material commonly used as a floor topper in food processing environments. Tufco provided 42 5-inch by 5-inch tiles of slightly varying thicknesses, so each tile was labeled and measured with digital calipers (Mitutoyo CD-S8" CT, Japan) and was found to range between 9.32 mm and 11.94 mm in height. The yield of 10 mm cubic carriers from each tile was calculated to be 81, so to make at least 1350 carriers, 17 tiles were required. The average standard deviation of each set of 17 tiles among the group of 42 was calculated, and the set with the lowest average standard deviation, 0.169 mm, was selected.

Using a 7-inch water cooled diamond blade tile saw (Rigid 7-inch Tile Saw, Emerson Electric Co., Elyria, OH) and sliding gauge, the distance from the blade to the fixed guard was set at 10 mm. Using a metal square, the guard was verified to be at a 90° angle with the blade. Each tile was cut into 11 strips, (Figure 1), with the two outer strips being discarded so that all carriers would have a newly cut edge to reduce variation between carriers. After the 17 tiles were cut into uniform 10 mm strips, 6 strips at a time were run through the saw, creating 6 carriers with each pass. The first and last set of each carrier produced from each set of strips was also discarded since they had an existing rough edge. The carriers were rinsed well with water and allowed to dry. Each carrier was inspected for cracks and voids and carriers with these defects were rejected. Fifty of the accepted carriers were randomly sampled and the height,

length and width were measured with digital calipers (Mitutoyo CD-S8" CT, Japan). The surface area was calculated for each, and the mean, standard deviation, and other summary statistics of the surface area was calculated, see Table 1. Accepted carriers were washed with laboratory soap in the glassware washing machine, triple rinsed with DI water to remove any residue left from the tile manufacture and carrier cutting process, air dried, and autoclaved at 250°F for 30 minutes (Tuttnauer USA, Hauppauge, NY) in packs of 80 in self sealed sterilization pouches (Cardinal Health, Dublin, Ohio) where they were stored until use.

3.3 Preparation of inoculums

Inoculums were prepared by thawing the frozen culture vials and streaking the contents of each vial for isolation onto a Tryptic Soy Agar (TSA) plate (Edge Biologicals, Memphis, TN). The TSA plates were incubated overnight at 35°C and one isolated colony was picked from each and suspended in 10 mL pre-warmed Brain Heart Infusion (BHI) broth (BD Difco, Franklin Lakes, NJ) in a screw top test tube. This inoculated BHI was incubated at 35°C for 24 hours. After incubation, 0.5 mL of the inoculated BHI was then pipetted into 40 mL pre-warmed BHI broth in a sterile specimen cup and was incubated at 35°C for 24 hours. After the incubation period, the entire contents of the specimen cup was transferred to a sterile centrifuge tube and spun down at 10,000 RPM for 15 minutes at 6°C (ThermoFisher Scientific, Waltham, MA). After pouring off the supernatant, the pellet was resuspended in 40 mL of sterile 0.85% saline solution and was vortexed to resuspend. The vial and contents were centrifuged to pellet and resuspended as above so that the bacteria were washed two times. After resuspension in 40 mL sterile 0.85% saline solution and being transferred to a new sterile specimen cup, 10-fold serial dilutions of the inoculum were prepared with 90 mL portions of chilled 0.85% sterile saline to the 10^{-8} dilution. One hundred µl from each of the 10^{-4} to the 10^{-8} dilutions were plated onto

tryptic soy agar (TSA) in triplicate and incubated overnight to enumerate the culture. After enumeration, the five constituents of each cocktail were mixed to achieve the desired inoculum level. The strains in the Salmonella cocktail were *Salmonella* Typhimurium (wild strain), *Salmonella* Heidelberg (wild strain), *Salmonella* Mbandaka (wild strain), *Salmonella* Kentucky (wild strain), and *Salmonella* Senftenberg (ATCC 43845). The *E. coli* cocktail consisted of *E. coli* P1 (ATCC BAA-1427), *E. coli* P3 (ATCC BAA-1428), *E. coli* P8 (ATCC BAA-1429), E. coli P14 (ATCC BAA-1430), and *E. coli* P68 (ATCC BAA-1431). The *L. monocytogenes* cocktail was composed of *L. monocytogenes* (ATCC 43257), *L. monocytogenes* (ATCC 49594), *L. monocytogenes* (ATCC 19111), *L. monocytogenes* (ATCC 19115), and *L. monocytogenes* 4B #2926 (wild strain).

3.4 Preparation of the test solutions

The test solutions were prepared by mixing turmeric and the floor treatments with sterile deionized water, with a full list of treatments in Table 1. Application rates found on the floor treatment labels were converted to grams per square meter to standardize the mass per area application rates. If a range of use was given on the product label, the lowest concentration of the range was used so that the antimicrobial activity would be measured at the lowest concentration recommended by the manufacturer. If a usage range was not provided, including for the turmeric, the product was uniformly spread across 5 pre-weighed 5-inch by 5-inch square tiles. After the product was uniformly applied to the surface of the tiles, the tiles were weighed again to determine the mass of product per area required for a uniform application and converted to grams per square meter.

The determined application rates for the treatments that did not include specific application instructions were SC and QAC based treatment 8, 61.59 g/m^2; urea and QAC based

treatment 10, 129.37 g/m^2; SPC and SC based treatment 11, 92.38 g/m^2; urea and QAC based treatment 12, 84.73 g/m^2; SBC and QAC based treatment 13, 87.21 g/m^2, urea based treatment 14, 148.80 g/m^2; SPC based treatment 15, 108.71 g/m^2; borax based treatment 16, 136.81 g/m^2; and turmeric rhizome powder treatment 18, 75.23 g/m^2 (Figure 2).

The concentration of the test solutions was determined by the number of grams required per square meter of flooring diluted in 300 mL sterile deionized water (DI), the maximum acceptable amount of water present per square meter of flooring from the EPA label of SPC/QAC based treatment 6. Based on the application rate, the test solution concentration for each treatment in g/100 mL was calculated (Table 3) as the test solutions were prepared in 100 mL aliquots. The required mass of each powdered floor treatment was weighed into a stomacher bag (Whirl-Pak, Madison, WI) and 100 mL of sterile DI water was dispensed. After dispensing, the contents were processed in a laboratory blender for 2 minutes to ensure complete mixing. For sharp crystalized treatment products, the mixture was hand mixed for 2 minutes to avoid breaking the stomacher bag. After diluting the dry treatments with water, they were used within 3 hours.

3.5 Verification of antimicrobial neutralization by Hi-Cap neutralization broth

To determine if the Hi-Cap Neutralization Broth (Hi-Cap NB) (World Bio-Products LLC, Bothell, WA) would effectively neutralize the test solutions after the 10 minute exposure time and reduce carry over antimicrobial activity that would lower recovery during enumeration, the neutralization confirmation assay from the EPA MLB SOP MB-20 Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilms was performed (EPA, 2017). Four milliliters of each test solution were dispensed into a 36 mL aliquot of Hi-Cap Neutralization broth in a 50 mL conical tube in triplicate for each of the three inoculum

cocktails. After briefly swirling and within 10 seconds of mixing the test solution and the Hi-Cap NB, 100 µl of the inoculum cocktail prepared as above and diluted to approximately 10^6 CFU/mL was pipetted into the mixture. The conical tube was vortexed on high for 10 seconds to mix. After holding the tubes at room temperature for 10 minutes, the conical tube was vortexed again on high for 10 seconds and was enumerated with 3M Aerobic Plate Count (APC) Petrifilm (3M Company, Maplewood, MN) and 9 mL Butterfield's (BF) dilution blanks for 10-fold serial dilutions. Culture titer control samples were prepared as above but using 40 mL BF buffer in triplicate for each pf the three culture cocktails. Neutralizer toxicity controls were also performed as above but with 40 mL of Hi-Cap NB to determine if the neutralizer itself inhibits recovery of the bacteria. According to the EPA protocol, if the recovery of the neutralizer toxicity samples was less than 50% of the BF culture titer controls, Hi-Cap NB would not be a suitable neutralizer (EPA, 2014). If the recovery from the neutralized test solution samples is within 50% of the BF titer control samples, 4 mL test solution to 36 mL Hi-Cap NB was used in the study. If the recovery was less than 50% of the BF culture titer control for any of the test solutions and culture cocktails, indicating incomplete neutralization, this part of the experiment was repeated as above but with 196 mL of Hi-Cap NB instead of 36 mL. If the 196 mL aliquot of neutralizer was found to have acceptable recovery per the specification above, those samples in the study were neutralized with 196 mL Hi-Cap NB while all other samples were neutralized with 36 mL Hi-Cap NB.

3.6 Carrier inoculation

To inoculate the carriers, approximately 70 carriers were placed into a specimen cup and covered with 100 mL sterile Synthetic Broth (Wright and Mundy broth, HIMEDIA, Mumbai, India) and inoculum so that the total inoculated level was approximately 1 x 10^7 CFU/mL. The

container was swirled briefly to eliminate bubbles or air pockets and the carriers were allowed to sit in the inoculum for 15 minutes. After 15 minutes the carriers were removed from the inoculum with sterilized 1 by 2 toothed forceps, tapping on the neck of the container to remove any hanging drops, and were transferred to sterile Petri dishes lined with sterilized Whatman filter paper #3 (Whatman Plc, GE Healthcare, Buckinghamshire, UK) while ensuring that the carriers did not touch. After the lid to the dish was replaced, the inoculated carriers were dried at 35°C for 40 minutes and were used within 2 hours after drying.

3.7 Determining the curcuminoid content of turmeric used in the experiment

To extract and quantify the curcuminoids from the turmeric powder (Swad, Burlington, MA), 120 mg commercial turmeric powder, in triplicate, was weighed and then suspended in separate tubes containing 5 mL of acetone (an approximate 1:1 ratio w/v) to prepare the stock solutions for analysis. The samples were stirred for 12 hours at ambient temperature. The turmeric powder suspension was then sonicated for 10 minutes in an ice bath to extract the curcuminoids. The resulting homogenate was centrifuged (model J2-21, Beckman, Fullerton, CA) for 20 minutes at 20°C at 10,000 x g. The supernatant was drawn off and then evaporated under reduced pressure. The resulting acetone extracted turmeric rhizome powder (AETRP) was stored under refrigeration at 4°C and quantified using HPLC.

The HPLC chromatographic method was adapted from the United States Pharmacopeia Forum (USP 40-NF 35). Approximately 5 mg of curcuminoid reference standard ASB-00003928 (99.7%) containing known concentrations of curcumin (CUR), desmethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) (ChromaDex, Irvine, CA) was weighed and diluted at a ratio of 1:10 with mobile phase (tetrahydrofuran:1 mg/mL of citric acid in water at a ratio of 4:6). Approximately 5 g of the AETRP test samples were weighed into 50 mL

volumetric flasks, dissolved in acetone, and then diluted at a ratio of 1:10 with mobile phase (tetrahydrofuran:1 mg/mL of citric acid in water at a ratio of 4:6). A 1 mL aliquot of each sample was transferred into clean conical microcentrifuge tubes and centrifuged at 10,000 x g for 5 minutes to spin down any undissolved material. The supernatant, containing the curcuminoids, was drawn off for analysis.

The reference standard and test curcuminoid supernatant samples were filtered through 0.22 µm membranes and then assayed under reverse phase chromatographic conditions on an Agilent 1200 series HPLC with DAD detector and Zorbax C18 (4.6 x 250 mm) 5µm column (Agilent, Santa Clara, CA). Peak intensities were monitored using a UV detector at 420 nm and samples were resolved at a constant flow rate of 1 mL/min at 40° C. The order of elution for the curcuminoids were CUR, DMC then BDMC. The amounts of the three curcuminoids present in the test samples were quantified by calculating the area of the peaks and comparing to the area of the peaks of the known concentrations from the standard.

3.8 Measuring the antimicrobial effect of commercially available floor treatments and turmeric

To measure the antimicrobial effect of the various treatments, for each test solution and sterile DI water control group, three carriers each that had been inoculated with the *Salmonella*, *Listeria*, or *E. coli* culture cocktails and dried following section 3.6 Carrier inoculation, were then placed into each of 9 separate 50 mL conical tubes. Four milliliters of room temperature test solution, or 4 mL sterile DI water for the control group, was added to each tube, completely covering the carrier. The conical tube was then gently swirled to eliminate any air bubbles and promote complete contact of all the carrier surfaces with the test solution. After an exposure time of 10 minutes, 36 mL of room temperature Hi-Cap NB was dispensed into the conical tube

to neutralize the contents and halt the antimicrobial effects of the treatment. For the samples with test solutions earlier identified as needing additional neutralizer, the entire contents of the conical tube were instead poured into a sterilized 250 mL jar containing 196 mL of the neutralizer. After the addition of neutralizer, each conical tube was vortexed at the highest setting for 30 seconds. The 250 mL jars were shaken vigorously or 30 seconds. Next, the containers and conical tubes were placed into an ultrasonic bath set to 37 kHz and sonicated on 100% power without sweep for 1 minute with the water level in the bath matching the water level in the containers. After initial sonication, all containers were vortexed or shaken again for 30 seconds and placed back in the ultrasonic bath for another minute at the previous settings. After the second sonication, the containers were vortexed or shaken again as above for 30 seconds and then the neutralized test solution was enumerated with 3M APC Petrifilm (3M, Maplewood, MN) with serial 10-fold dilutions performed with 9 mL Butterfield's buffer (BF) dilution blanks (Edge Biological, Memphis, TN). The average of the triplicate enumerations of $\log_{10}$ CFU/carrier were calculated for the treatments and controls for each repetition of the experiment. The mean $\log_{10}$ CFU/carrier reductions of the treatments versus sterile water controls of the three repetitions of the experiment were then calculated and analyzed.

3.9 Investigation of potential synergy between turmeric and a percarbonate based treatment

To investigate if turmeric rhizome powder in combination with a percarbonate based sanitizer would provide an additional antimicrobial effect over the percarbonate based sanitizer alone, turmeric and SPC based treatment 7 were tested as previously described alone and in combination at half the previous concentrations. The treatment concentrations were halved to

better resolve any additional $\log_{10}$ reduction due to synergism since SPC based treatment 7 at the recommended concentration resulted in very low recovery of *L. monocytogenes*.

3.10 Statistical analysis

Completely randomized design was used for the study and all antimicrobial treatments were tested in triplicate and the experiment was repeated three times. Data were analyzed with JMP Pro 14.1.0 for Macintosh. One-way analysis of variance (ANOVA) of results was performed. Mean $\log_{10}$ CFU/carrier reductions for each treatment were compared using Tukey's Honestly Significant Difference (HSD) test. Significant difference was determined at $P < 0.05$.

Chapter 4: Results and Discussion

4.1 Hi-Cap NB neutralization confirmation and toxicity results

HI-Cap NB Neutralization confirmation and toxicity results are presented in Tables 4 and 5. Hi-Cap NB was able to effectively neutralize, defined by >50% inoculum recovery, all treatments at 4 mL test solution to 36 mL neutralizing buffer except for SPC based treatment 7, SC and QAC based treatment 8, SPC and SC based treatment 11, SPC based treatment 15, and SPC based treatment 17 for the *Salmonella* cocktail inoculum and SPC based treatment 15 for the *L. monocytogenes* cocktail inoculum (Table 4). When these test solutions were retested at 4 mL test solution to 196 mL Hi-Cap NB, all had recovery greater than 95% (Table 5). These data compare to results from a study performed by World-Bioproducts, manufacturer of the Hi-Cap neutralizing buffer, where the only sanitizer it did not completely neutralize was a 1:100 dilution of Ecolab Tsunami 200, a 13% peroxyacetic acid (PAA), 1-5% H_2O_2, and 5-20% caprylic acid sanitizer possibly indicating a reduced capacity for neutralizing H_2O_2 containing compounds which included all the treatments that had <50% recovery at a 1:9 dilution. This same study also supported that Hi-Cap neutralizing buffer displayed better pH buffering capacity than Dey-Engley (D/E) broth among other common neutralizing broths (Ward, 2013). The neutralizer toxicity test inoculum percent recovery from Hi-Cap NB alone, to test if the neutralizing buffer itself may inhibit any of the inoculums, were all over 90% for each of the *Salmonella, E. coli*, and *L. monocytogenes* inoculum cocktails tested (Tables 4 and 5). The Hi-cap broth itself was also plated and had recovery of <1 CFU/mL. These results indicate that Hi-Cap NB was able to effectively neutralize the wide variety of antimicrobial treatments tested at the appropriate dilution factor without itself impairing recovery of the organisms according to the acceptance criteria in the EPA Standard Operating Procedure for Neutralization of Microbicidal Activity

using the OECD Quantitative Method for Evaluating Bactericidal Activity of Microbicides Used on Hard, Non-Porous Surfaces (EPA, 2014).

4.2 Curcuminoid determination

The mean total curcuminoid content of the turmeric rhizome powder treatment 18 used in in this study was found to be 2.70 g/100 g turmeric powder (2.7% w/w), with 1.76 g/100 g being curcumin (CUR), 0.63 g/100 g demethoxycurcumin (DMC), and 0.32 g/100 g bisdemethoxycurcumin (BDMC) (Table 8). These results are similar to another study that compared curcumin content of powdered turmeric that found for the 9 different brands of turmeric powder samples tested, curcumin ranged from 0.58% to 3.14% with an average of 1.58% (Tayyem et al., 2006).

From this data, the concentration of total curcuminoids in the aqueous turmeric test solution single treatment study was calculated to be 6.8 mg/mL with 4.4 mg/mL being curcumin, 1.6 mg/mL being demethoxycurcumin, and 0.8 mg/mL being bisdemethoxycurcumin. The concentrations of curcuminoids in the turmeric test solution in the synergy portion of the study were 3.4 mg/mL total curcuminoids, 2.2 mg/mL curcumin, 0.8 mg/mL demethoxycurcumin, and 0.4 mg/mL bisdemethoxycurcumin well over the calculated MIC in another study of 125 μg/mL for Gram-positive *L. monocytogenes* and *S. aureus* and 250 μg/mL for Gram-negative *Salmonella* Typhimurium and *E. coli* (Altunatmaz et al., 2016).

4.3 Measuring the antimicrobial effect of commercially available floor treatments and turmeric

Mean $\log_{10}$ CFU/carrier reductions versus sterile water control results for all three repetitions are presented in Table 6. The Log_{10} CFU/Carrier control enumerations for *Salmonella* were 6.30, 5.94, and 6.73 for each of the three repetitions performed on different days. *E. coli* control

recovery was 6.23, 5.92, and 6.18 Log_{10} CFU/Carrier. Control recovery for *L. monocytogenes* was 6.80, 6.74, and 7.37 Log_{10} CFU/Carrier for days 1, 2, and 3.

Four of the commercially available floor treatments containing sodium percarbonate/sodium carbonate, SPC based treatment 7, SC and QAC based treatment 8, SPC and SC based treatment 11, and SPC based treatment 17, displayed the greatest statistically different log reductions for all three organism cocktails, *Salmonella, Listeria, and E. coli*. Sodium percarbonate (SPC) based treatment 7, SC and QAC based treatment 8, SPC and SC based treatment 11, and SPC based treatment 17 all contained a maximum concentration between 70% and 100% of sodium percarbonate, sodium carbonate, or sodium carbonate peroxyhydrate, and had application rates that were 67.81 g/m^2, 61.59 g/m^2, 92.38 g/m^2, and 68.00 g/m^2 respectively. When SPC compounds are exposed to water or moisture, they decompose and release hydrogen peroxide, which is the active antimicrobial (McKillop & Sanderson, 1995). Other treatments that contained sodium percarbonate, SPC and QAC based treatment 3, SPC and QAC based treatment 4, SPC and QAC treatment 6, and SPC based treatment 9 had much lower minimum manufacturer recommended application rates, 13.02 g/m^2, 13.02 g/m^2, 11.29 g/m^2, and 4.88 g/m^2 respectively, as well as lower concentrations of SPC/SC in their composition with treatment 6 having a maximum of 30%, treatments 3 and 4 having a maximum of 55%, and SPC based treatment 9 not supplying the concentration of the active ingredient (Tables 2 and 3). These differences in application rates and concentrations of hydrogen peroxide forming ingredients in each treatment formulation may explain the differences observed in the mean log_{10} reductions similar to a previous study that found a 3% H_2O_2 solution reduced planktonic *L. monocytogenes* by 6.0 log_{10} CFU/mL and a 3.5% H_2O_2 solution reduced *L. monocytogenes* by 8.7 log_{10} CFU/mL after 10 minute contact times (Robbins et al., 2005). The data from the

Robbins study represent an additional 2.7 log reduction while only increasing the H_2O_2 solution concentration by 16%. Another experiment conducted with five constituent strain cocktails of *Salmonella* Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* inoculated on lettuce leaves and exposed to 2% H_2O_2 at 50°C for 90 seconds found reductions of 4.5, 4.7, and 2.7 $\log_{10}$ CFU per leaf, respectively, (Robbins et al., 2005). These two studies are consistent with the results in this study that indicate treatments having an application rate greater than 61.59 $g/m^2$of the SPC component were more effective than those containing less.

Although differences in application rates could be responsible for some of the differences in the observed reductions, two out of these previously mentioned top performers had no specific application rates other than instructions to apply uniformly, so the application rates were calculated for the study as previously mentioned, see Table 3. Only SPC based treatment 7 at 67.81 g/m^2 and SPC based treatment 17 at 68.00 g/m^2 of the top four treatments with the highest statistically significant log reductions for all three organisms had application rates specified by the manufacturer: For the other two, SC and QAC based treatment 8 had an experimentally derived application rate of 61.59 g/m^2 and SPC and SC based treatment 11 had a rate of 92.38 g/m^2. Despite this, the two treatments, treatments 7 and 17, with manufacturer recommended application rates near 68 g/m^2, out-performed by a statistically significant margin, SPC based treatment 15 in reducing *L. monocytogenes* even though treatment 15 had a nearly 60% higher application rate and is composed of <95% sodium percarbonate (Table 3).

Since QACs kill by disrupting cell membranes and Gram-negative bacteria like *Salmonella* and *E. coli* have an additional outer membrane versus Gram-positive bacteria like *L. monocytogenes*, QACs are generally better at killing Gram-positive bacteria (Minbiole et al., 2016). In the results of this study, however, none of the QAC containing treatments, except SC

and QAC based treatment 8 which also contained 85-95% sodium carbonate peroxyhydrate as discussed earlier, were in the top performing group against *Listeria*. If fact, the QAC containing treatments included in the study, besides treatment 8, were among the worst performers against *Listeria* and no QAC containing treatment, again besides treatment 8, showed a greater reduction versus sterile water against *Listeria* than for *Salmonella* or *E. coli* greater than 0.16 $\log_{10}$ CFU/carrier. The concentrations of quat in the treatments can be found in Table 2 and range from <0.2% QAC content for the worst performing commercial floor treatment, treatment 2, a sodium bicarbonate (SBC)/QAC, to <5% for SPC/QAC based treatment 6. One study found that *Salmonella typhimurium* ATCC 7823 was reduced <99.999% when exposed to 100 ppm QAC while *L. monocytogenes* ATCC 13932 was reduced by >99.999%, At 200 ppm QAC, the reductions for both organisms were >99.999% (Lopes, 1986). Another study on various sanitizers against *L. monocytogenes* found that the minimum inhibitory concentration (MIC) of H_2O_2 ranged from 9.4 μg/mL to 37.5 μg/mL while the MIC of two QAC products ranged from 0.77 μg/mL to 6.25 μg/mL for 19 different strains of *L. monocytogenes* indicating that *L. monocytogenes* may be more sensitive to QACs than H_2O_2 (Romanova et al., 2002). It is possible that although the concentrations of QAC present in the evaluated QAC based floor treatments are not sufficient to achieve similar log reductions to the higher concentration SPC treatments tested, they could provide a bacteriostatic effect when used in plant that this study was not able to detect. The data are even more interesting and warrants further study when compared to one researcher's findings that the antimicrobial activity of QACs against Gram-negative bacteria is typically 8 times less than against Gram-positive bacteria (Jennings et al., 2015).

Borax based treatment 16, which performed near the middle of all the treatments overall (Table 6), had mean $\log_{10}$ CFU/carrier reductions of 2.54, 2.07, and 2.16 for *Salmonella, E. coli,*

and L. monocytogenes respectively. A previous study on the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of borax found that for Gram-negatives *E. coli* ATCC 3528 and *Pseudomonas aeruginosa* ATCC 27853, both the MIC and MBC was 47.60 mg/mL for each and for Gram-positive *Staphylococcus aureus* ATCC 25923 and Gram-negative *Acinetobacter septicus* DSM 19415, both the MIC and MBC were 23.80 mg/mL (Yilmaz, 2012). Another study in soil found that while boric acid reduced the bacterial biomass of the soil sample, the samples treated with borax in a solution of 0.05 mg/mL showed increased bacterial biomass, both Gram-negative and Gram-positive bacteria relative to the control, consistent with the Yilmaz study which determined the MIC and MBC to be much higher (Vera et al., 2019; Yilmaz, 2012).

Urea was a component of three of the commercially available floor treatments (Table 2): treatments 10 and 12, which also included QAC, and treatment 14 which only listed urea as an ingredient. Treatments 10 and 12 both were among the statistically significant best performers against *Salmonella* but not for *E. coli* or *L. monocytogenes* while treatment 14 was not in any of the statistically different groups with the greatest log reductions among any of the three organisms tested. A previous study investigating at the effect of urea added to manure for the reduction of *Salmonella* in compost found that it took 10.2 days for a 2% (w/w) urea and manure mixture to achieve a five-$\log_{10}$ reduction of *Salmonella* (Ottoson et al., 2008). In another compost study, the addition of 3% (w/w) urea resulted in the reduction of fecal coliforms and *Salmonella* to non-detectable levels in 5 days (Vinnerås, 2007). These studies indicate that urea can reduce the levels of Salmonella and E. coli. Another study indicates urea denatures proteins via a combination of the chemical directly interacting with the proteins and also the indirect

interaction of urea with the solvent, so this could explain the antimicrobial activity observed. (Bennion & Daggett, 2003).

Turmeric powder did not produce statistically significant mean $\log_{10}$ CFU/carrier reductions against any of the *Salmonella*, *E. coli, or L. monocytogenes* cocktails. This result was not completely unexpected since curcumin (CUR), demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC), the main antimicrobial components curcuminoids of turmeric, are not very soluble in water at acidic or neutral pH (Amalraj et al., 2017). From the mean total curcuminoid content of the turmeric rhizome powder treatment 18 used in in this study (Table 8), the concentration of total curcuminoids in the aqueous turmeric test solution single treatment study was calculated to be 6.8 mg/mL with 4.4 mg/mL being curcumin, 1.6 mg/mL being demethoxycurcumin, and 0.8 mg/mL being bisdemethoxycurcumin, which are relatively high considering the MIC found in another study for curcumin to be 125 μg/mL for Gram-positive *L. monocytogenes* and *S. aureus* and 250 μg/mL for Gram-negative *Salmonella* Typhimurium and *E. coli*, but still appears that not enough curcumin was in solution to reduce the pathogens in this experiment (Altunatmaz et al., 2016). A more recent study found the MBC of a crude ethanolic turmeric extract to be 20 mg/mL for Gram-positive S. aureus and 80 mg/mL for Gram -negative P. aeruginosa and MIC to be 10 mg/mL and 40 mg/mL respectively, but the proportion of the curcuminoids in the crude extract could not be determined from the data in the paper (Suwal et al., 2021). In a previous study where turmeric oil extract was prepared by mixing 1 kg of turmeric powder with 5 liters of room temperature water and allowed to steep, similar to the experimental conditions in this experiment where 25 g turmeric powder was mixed with 100ml water at room temperature, 6 μl and 12 μl of turmeric oil placed on a filter paper disk produced a zone of inhibition for *E. coli* of 7 mm and 7 mm and *Salmonella typhi* of 7 mm and 10 mm

respectively while having no inhibition for *C. albicans* and *Staph. aureus* for either concentration (Gul & Bakht, 2015). In another study, the zones of inhibition for turmeric extract against *L. monocytogenes* and *E. coli* did not differ from the control at various concentrations from 5 mg/mL to 80 mg/mL (Zhang et al., 2009). In a meat model study, chicken breast that was processed into small pieces and inoculated with *E. coli* or *S. aureus* and then treated with 1% (w/w) turmeric powder and the held at 4°C for 48 hours showed no decrease in either *E. coli* or *S. aureus* (Lourenço et al., 2013).

Another study determined the MIC of curcumin solubilized in ethanol for methicillin sensitive *S. aureus* at 219 μg/mL, methicillin resistant *S. aureus* at 217 μg/mL, *Enterococcus faecalis* at 293 μg/mL, *Bacillus subtilis* at 129 μg/mL, *P. aeruginosa* at 175 μg/mL, *E. coli* at 163 μg/mL, and *Klebsiella pneumoniae* at 216 μg/mL (Gunes et al., 2016). In the same study, when 0.5%, 1%, and 2% (w/w) amounts of curcumin extract were incorporated into veal minced meat medium inoculated with either *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* Typhimurium or *S. aureus*, it was found that after 7 days, the 0.5% treatment reduced *L. monocytogenes* and *S. aureus* by about 2 $\log_{10}$ CFU/g and *E. coli* and *Salmonella* by about 1 $\log_{10}$ CFU/g, the 1% curcumin treatment reduced *L. monocytogenes*, *E. coli*, *Salmonella*, and *S. aureus* by approximately 2 $\log_{10}$ CFU/g, and the 2% treatment reduced *L. monocytogenes* and *S. aureus* but about 3 $\log_{10}$ CFU/g and *Salmonella* and *E. coli* by about 2 $\log_{10}$ CFU/g indicating that the turmeric group 18 might have performed better had the curcuminoids been solubilized into the solution (Altunatmaz et al., 2016). The curcuminoids were not extracted and solubilized from the turmeric powder for this study since the methods for the Gul study only used a room temperature water extraction for the production of turmeric oil that showed some reductions and the Lourenço study only used a 1% w/w concentration, much lower than in this study, of

powdered turmeric though did not see reductions. There might have been enough curcuminoids solubilized even under unfavorable conditions at a concentration of 25 g turmeric powder/100 mL water to provide some antimicrobial effect, but that does not appear to be the case. Of the commercially available floor treatments, those containing sodium percarbonate and with an application rate greater than 61.59 g/m^2 performed best against *Listeria*, *Salmonella,* and *E. coli* under the testing conditions and should be considered for use when microbiological testing indicate that the processing environment flooring could be a source of contamination. Under the conditions tested, turmeric powder did not appear to be effective as an antimicrobial floor treatment.

4.4 Investigation of potential synergy between turmeric powder and a percarbonate based treatment

SPC based treatment 7 was evaluated with turmeric rhizome powder treatment 18 to determine if there might be synergistic effects between lower levels of sodium percarbonate floor treatments and turmeric, but for the results for all three inoculum cocktails tested, treatment SPC based treatment 7 at half the concentration of the single treatment experiment, performed much better than when mixed with turmeric. The mean $\log_{10}$ reductions of SPC based treatment 7 at half the previous concentration alone were greater than when mixed with half the previous concentration of turmeric rhizome powder treatment 18 for the *Salmonella, E. coli, and L. monocytogenes* cocktail inoculated carriers indicating antagonism rather than synergism (Table 7). When turmeric rhizome powder treatment 18 and was combined with SPC based treatment 7, both at half their previous concentrations to the initial study, the mean $\log_{10}$ CFU/carrier *Salmonella* reduction decreased by 1.57 logs to 1.89 $\log_{10}$ CFU/carrier, the mean $\log_{10}$

CFU/carrier *E. coli* reduction decreased by 2 logs to $\log_{10}$ CFU/carrier, and the mean $\log_{10}$ CFU/carrier *L. monocytogenes* reduction decreased by 1.79 logs to 0.01 mean $\log_{10}$ CFU/carrier.

One possible explanation is that the antioxidant effects of turmeric or simply the organic load of the turmeric itself, reduced the antimicrobial action of SPC based treatment 7. Since the SPC creates an alkaline solution in water and curcumin (CUR), demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC), the main antimicrobial components curcuminoids of turmeric are more soluble in alkaline environments, there may have been more solubilized curcuminoids, but the turmeric may have consumed the H_2O_2 produced by the SPC (Amalraj et al., 2017). Another interesting result that at the manufacturer's recommended minimum usage rate, 67.81 g/m^2 SPC containing treatment 7 achieved a 4.88 log reduction for *L. monocytogenes,* but at half that minimum rate, 33.91 g/m^2, the treatment only achieved a 1.8 log reduction, a three log difference. Further investigation is needed to determine if the spice powder could be bacteriostatic beyond the ten-minute exposure time or if additional additives could solubilize active antimicrobial fractions to enhance efficacy.

Chapter 5: Conclusion

This thesis was intended to address a gap in research around antimicrobial strategies to reduce the pathogen loads on flooring despite all the evidence of high pathogen contamination rates in the many food processing environmental survey studies cited herein. Many studies discussed indicate the high risk of aerosolizing contamination from the floors and drains and having potentially pathogenic bacteria settle onto food contact surfaces several meters away during routine sanitation and the high risk of pathogens being vectored from the floor to food contact surfaces in a variety of other ways during food production. Prior to this thesis there were no published studies available to compare the effectiveness of such a wide variety of commercially available floor treatments and the natural antimicrobial turmeric against *Salmonella, E. coli,* and *L. monocytogenes.*

Several approaches to the examination of floor treatments in this thesis were novel, including making carriers out of actual flooring material to better reflect actual floor surfaces, using Hi-Cap neutralizing buffer to neutralize the wide variety of antimicrobial treatments, and including several commonly used floor treatments that are labeled for anti-slip applications and contain an antimicrobial ingredient, but are not EPA registered sanitizers.

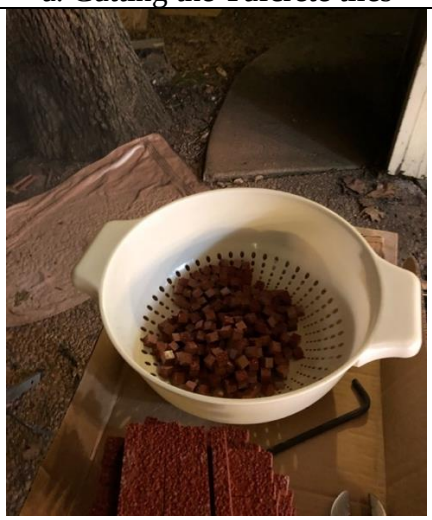
Of the commercially available floor treatments, those containing sodium percarbonate and with an application rate greater than 61.59 g/m^2 performed best against *Listeria*, *Salmonella,* and *E. coli* under the testing conditions and should be considered for use when microbiological testing indicate that the processing environment flooring could be a source of contamination. Under the conditions tested, turmeric powder did not appear to be effective as an antimicrobial floor treatment.

Further investigation is needed to determine if the turmeric spice powder could be bacteriostatic beyond the ten-minute exposure time or if additional additives could solubilize active antimicrobial fractions to enhance efficacy. Future directions include in-facility baseline floor and drain pathogen monitoring and microbiome analysis including cracks and damaged areas, prior to beginning to use the various high performing sodium percarbonate treatments and continuing sampling through the study period to determine if the results will translate from the model system to actual production facilities and see how the microbiome changes.

Tables and Figures

a. Cutting the Tufcrete tiles

b. Producing strips from the tiles



c. After cutting the strips into cubes

d. Inoculating and drying the carriers

Figure 1- Selected images from the Tufcrete carrier production and inoculation process
Photos by author

Table 1-Summary statistics for the total surface area of 50 randomly sampled Tufcrete polyurethane-concrete carriers

Mean	647.97 mm^2
Median	647.81 mm^2
Standard Deviation	17.78 mm^2
Skewness	-0.099
Range	75.51 mm^2
Minimum	612.84 mm^2
Maximum	688.34 mm^2
Standard Error	2.51
Confidence Level (95.0%)	5.05

The length, width, and height of each of the 50 randomly sampled carriers were measured with digital calipers and the total surface area was calculated for each.

Table 2-Floor treatment composition information obtained from manufacturers chemical labeling

Treatment Name	Composition Information
1. QAC Based Treatment	Alkyl Dimethyl Benzyl Ammonium Chloride: 0.479%, Octyl Decyl Dimethyl Ammonium Chloride: 0.359%, Didecyl Dimethyl Ammonium Chloride: 0.215%, Dioctyl Dimethyl Ammonium Chloride: 0.144%
2. SBC/QAC Based Treatment	Sodium Bicarbonate: >99.5%, Tetradecyl dimethyl benzyl ammonium chloride: <0.2%, Trade Secret 1: <0.007%, Trade Secret 2: <0.007%
3. SPC/QAC Based Treatment	Sodium carbonate peroxyhydrate: 45-55%, Trade Secret 1: 45-55%, Tetradecyl dimethyl benzyl ammonium chloride: 0.5-1.5%, Trade Secret 2: < 0.04%, Trade Secret 3: < 0.04%
4. SPC/QAC Based Treatment	Sodium carbonate peroxyhydrate: 45-55%, Trade Secret 1: 45-55%, Tetradecyl dimethyl benzyl ammonium chloride: 1-2%
5. QAC Based Treatment	Quaternary ammonium compound powder
6. SPC/QAC Based Treatment	Disodium carbonate, hydrogen peroxide: 10-20%, sodium carbonate: 5-10%, Tetrasodium ethylenediaminetetraacetate: 5-10%, Polyethylene glycol: <5% Benzyldimethyltetradecylammonium chloride: <5%
7. SPC Based Treatment	Sodium percarbonate: 90-100%
8. SC/QAC Based Treatment	Sodium carbonate: 85-95%, Quaternary ammonium compounds, benzyl-C12-18 alkydimethyl, chlorides: <2.5%
9. SPC Based Treatment	Sodium percarbonate
10. Urea/QAC Based Treatment	Urea and quaternary ammonium compounds
11. SPC/SC Based Treatment	Sodium percarbonate: 50-70%, Sodium carbonate: 40-50%, Pentasodium triphosphate: 1-5%, Sodium metasilicate: 1-5%, Ethoxylate alcohols: 0.1-1.0%
12. Urea/QAC Based Treatment	Urea: 97-99%, Cationic surfactants: 1-3%
13. SBC/QAC Based Treatment	Sodium Bicarbonate: 99-100%, Quaternary ammonium compounds, benzyl-C12-18 alkydimethyl, chlorides: 0.1-1.0%
14. Urea Based Treatment	Urea: 90-100%
15. SPC Based Treatment	Sodium Percarbonate: <95%
16. Borax Based Treatment	Sodium Tetraborate Decahydrate: <40%, Sodium Tetraborate Pentahydrate: <40%
17. SPC Based Treatment	Sodium carbonate peroxyhydrate: >85%
18. Turmeric	Powdered Turmeric Rhizome

Abbreviation SPC is sodium percarbonate, SC is sodium carbonate, QAC is quaternary ammonium compounds, and SBC is sodium bicarbonate

Table 3-Floor treatment application rates and calculated solution concentration for each treatment

Treatment	Application Rate (g/m^2)	Concentration of Test Solution (g/100 mL)
1. QAC Based Treatment	12.91	4.29
2. SBC/QAC Based Treatment	13.02	4.33
3. SPC/QAC Based Treatment	13.02	4.33
4. SPC/QAC Based Treatment	13.02	4.33
5. QAC Based Treatment	48.82	16.22
6. SPC/QAC Based Treatment	11.29	3.75
7. SPC Based Treatment	67.81	22.53
8. SC/QAC Based Treatment	61.59*	20.46
9. SPC Based Treatment	4.88	1.62
10. Urea/QAC Based Treatment	129.37*	42.98
11. SPC/SC Based Treatment	92.38*	30.69
12. Urea/QAC Based Treatment	84.73*	28.15
13. SBC/QAC Based Treatment	87.21*	28.97
14. Urea Based Treatment	148.80*	49.44
15. SPC Based Treatment	108.71*	36.12
16. Borax Based Treatment	136.81*	45.45
17. SPC Based Treatment	68.00	22.59
18. Turmeric	75.23*	24.99

Treatments marked with an asterisk (*) in the Application Rate column above did not have exact usage instructions from the manufacturer so the amount necessary to provide uniform coverage was calculated experimentally. Abbreviation SPC is sodium percarbonate, SC is sodium carbonate, QAC is quaternary ammonium compounds, and SBC is sodium bicarbonate

Table 4- Neutralizer Confirmation and Toxicity Test Results: Mean percent recovery of *Salmonella, E. coli, and L. monocytogenes* inoculum cocktails after 10-minute exposure to 4 mL aqueous solutions of treatments and 36 mL HiCap Neutralizing Buffer or 40 mL HiCap Neutralizing Buffer alone

Treatments	Percent (%) Recovery versus Test Culture Titer		
	Salmonella	***E. coli***	***L. monocytogenes***
1. QAC Based Treatment	104	120	98
2. SBC/QAC Based Treatment	116	106	70
3. SPC/QAC Based Treatment	93	101	97
4. SPC/QAC Based Treatment	107	100	88
5. QAC Based Treatment	94	108	97
6. SPC/QAC Based Treatment	78	101	112
7. SPC Based Treatment	27	78	77
8. SC/QAC Based Treatment	20	62	97
9. SPC Based Treatment	100	101	122
10. Urea/QAC Based Treatment	113	99	130
11. SPC/SC Based Treatment	10	75	89
12. Urea/QAC Based Treatment	89	101	102
13. SBC/QAC Based Treatment	70	98	85
14. Urea Based Treatment	85	100	101
15. SPC Based Treatment	27	101	29
16. Borax Based Treatment	92	89	81
17. SPC Based Treatment	37	60	52
18. Turmeric	83	101	120
Neutralizer Toxicity Test	415	100	91
Test Culture Titer (log_{10} CFU/mL)	4.87	4.83	5.07

Percent recovery values calculated versus inoculated Butterfield's Buffer from means of three repetitions. Results >50% and >100% were deemed acceptable per procedure in the EPA Neutralization procedure indicating acceptable antimicrobial neutralization (EPA, 2017). Results <50% were retested at 4 mL Test Solution to 196 mL Hi Cap Neutralizer. Abbreviation SPC is sodium percarbonate, SC is sodium carbonate, QAC is quaternary ammonium compounds, and SBC is sodium bicarbonate

Table 5- Neutralizer Confirmation and Toxicity Test Results: Mean percent recovery of *Salmonella, E. coli, and L. monocytogenes* inoculum cocktails after 10-minute exposure to 4 mL aqueous solutions of treatments and 196 mL Hi-Cap Neutralizing Buffer or 200 mL Hi-Cap Neutralizing Buffer alone

Treatments	Percent (%) Recovery versus Test Culture Titer		
	Salmonella	***E. coli***	***L. monocytogenes***
7. SPC Based Treatment	105	NT	NT
8. SC/QAC Based Treatment	111	NT	NT
11. SPC/SC Based Treatment	95	NT	NT
15. SPC Based Treatment	108	NT	97
17. SPC Based Treatment	104	NT	NT
18. Turmeric	99	101	109
Neutralizer Toxicity Test	103	95	96
Test Culture Titer (log_{10} CFU/mL)	4.59	4.60	4.57

Percent recovery values calculated versus the test culture titer (TCT) which was inoculated Butterfield's Buffer from means of three repetitions. Results >50% and >100% were deemed acceptable per procedure in the EPA Neutralization procedure indicating acceptable antimicrobial neutralization (EPA, 2017). Only treatment inoculum combinations that were <50% recovery at 4 mL test solution to 36 mL Hi-Cap NB were retested at 4 mL Test Solution to 196 mL Hi Cap Neutralizer except for turmeric, which was retested to improve plate readability. Abbreviation NT is not tested, SPC is sodium percarbonate, SC is sodium carbonate, and QAC is quaternary ammonium compounds

Table 6-Mean log_{10} reductions of *Salmonella, E. coli, and L. monocytogenes* cocktails on TufCrete flooring material carriers after 10-minute exposure to aqueous solutions of turmeric or other powdered floor treatments versus sterile water controls

Treatments	Mean Log_{10} (CFU/Carrier) Reductions		
	Salmonella	***E. coli***	***L. monocytogenes***
1. QAC Based Treatment	1.36 ± 0.58^{fgh}	1.35 ± 0.25^{def}	1.38 ± 0.24^{defg}
2. SBC/QAC Based Treatment	0.56 ± 0.40^{gh}	0.72 ± 0.23^{efg}	0.44 ± 0.38^{fg}
3. SPC/QAC Based Treatment	2.63 ± 0.40^{cdef}	2.50 ± 0.24^{bc}	1.37 ± 0.56^{defg}
4. SPC/QAC Based Treatment	2.74 ± 0.65^{bcdef}	2.00 ± 0.56^{cd}	1.43 ± 0.22^{defg}
5. QAC Based Treatment	0.46 ± 0.65^{gh}	0.52 ± 0.14^{fg}	0.58 ± 0.36^{fg}
6. SPC/QAC Based Treatment	1.87 ± 0.23^{efg}	1.62 ± 0.21^{cdef}	1.21 ± 0.58^{efg}
7. SPC Based Treatment	3.68 ± 0.51^{abc}	4.78 ± 0.14^{a}	4.88 ± 0.6^{a}
8. SC/QAC Based Treatment	4.08 ± 0.19^{ab}	4.78 ± 0.22^{a}	3.29 ± 1.54^{abcd}
9. SPC Based Treatment	2.18 ± 0.58^{def}	2.37 ± 0.32^{bcd}	0.84 ± 0.32^{efg}
10. Urea/QAC Based Treatment	3.17 ± 0.33^{abcde}	3.18 ± 0.44^{b}	2.41 ± 0.28^{cdef}
11. SPC/SC Based Treatment	4.29 ± 0.34^{a}	4.81 ± 0.16^{a}	3.57 ± 1.14^{abc}
12. Urea/QAC Based Treatment	2.99 ± 0.18^{abcde}	2.64 ± 0.48^{bc}	2.64 ± 0.56^{cde}
13. SBC/QAC Based Treatment	1.88 ± 0.35^{efg}	1.73 ± 0.58^{cde}	1.89 ± 0.31^{cdef}
14. Urea Based Treatment	2.79 ± 0.60^{bcdef}	2.65 ± 0.46^{bc}	1.88 ± 0.34^{cdef}
15. SPC Based Treatment	3.52 ± 0.53^{abcd}	4.78 ± 0.15^{a}	2.83 ± 0.48^{bcde}
16. Borax Based Treatment	2.54 ± 0.41^{cdef}	2.07 ± 0.52^{bcd}	2.16 ± 0.63^{cdef}
17. SPC Based Treatment	3.68 ± 0.79^{abc}	4.74 ± 0.13^{a}	4.78 ± 1.19^{ab}
18. Turmeric	0.004 ± 0.23^{h}	-0.16 ± 0.62^{g}	-0.16 ± 0.15^{g}
***P* value**	<0.0001	<0.0001	<0.0001

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column are significantly different ($P < 0.05$). Abbreviation SPC is sodium percarbonate, SC is sodium carbonate, QAC is quaternary ammonium compounds, SBC is sodium bicarbonate

Table 7-Log_{10} reductions of *Salmonella, E. coli, and L. monocytogenes* cocktails on TufCrete flooring material carriers after 10-minute exposure to aqueous solutions containing commercial turmeric powder and 7. SPC Based Treatment at 50% each of the previous concentrations alone and in combination

Treatments	Log_{10} (CFU/Carrier) Reductions		
	Salmonella	***E. coli***	***L. monocytogenes***
7. SPC Based Treatment	3.46±0.69[a]	3.71±0.60[a]	1.80±0.08[a]
7. SPC Based Treatment +18. Turmeric	1.89±0.24[b]	1.71±0.23[b]	0.01±0.06[b]
18. Turmeric	0.03±0.37[c]	-0.24±0.16[c]	-0.17±0.18[b]
***P* value**	0.0004	< 0.0001	< 0.0001

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column are significantly different ($P < 0.05$). Abbreviation SPC is sodium percarbonate

Table 8- HPLC assay quantitation of curcuminoids in turmeric rhizome powder samples

	Curcuminoid Mass (g) / 100 g Turmeric Powder			
	CUR	**DMC**	**BDMC**	**Total Curcuminoid Content**
18. Turmeric (Lot 06/29/18) - Rep 1	1.76	0.63	0.32	2.71
18. Turmeric (Lot 06/29/18) - Rep 2	1.74	0.62	0.31	2.67
18. Turmeric (Lot 06/29/18) - Rep 3	1.78	0.63	0.32	2.73
Mean Values	1.76	0.63	0.32	2.70

Turmeric tested in triplicate with means. Abbreviation CUR is curcumin, DMC is demethoxycurcumin, and BDMC is bisdemethoxycurcumin

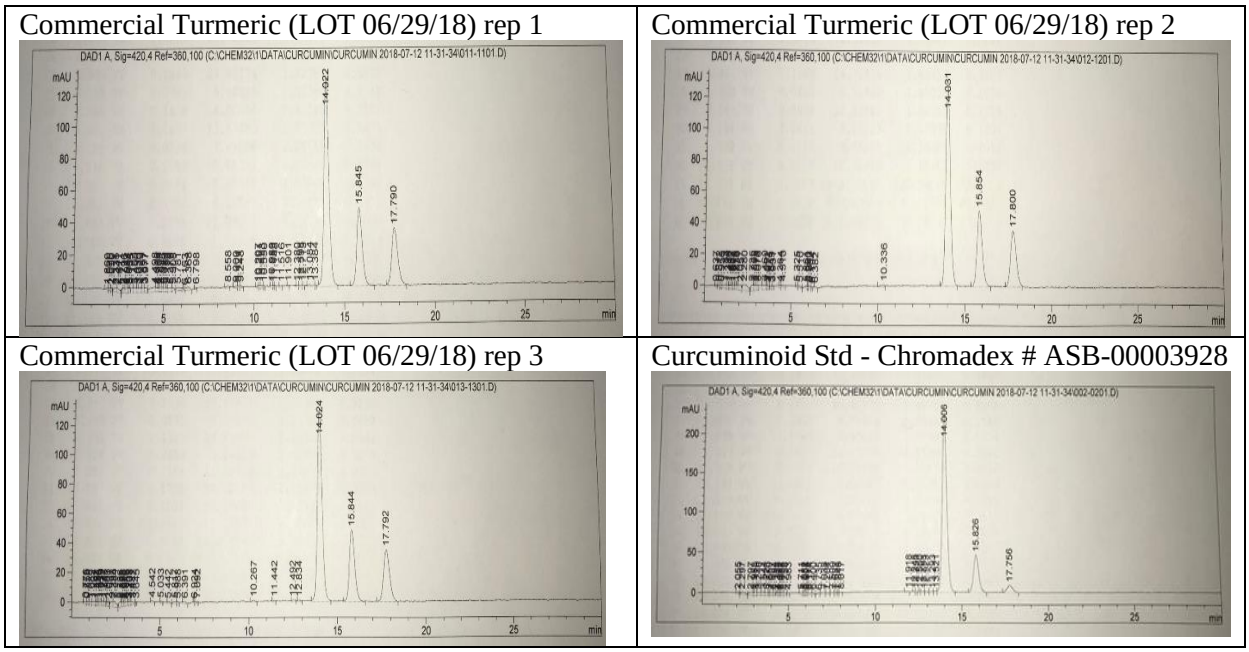


Figure. 2 HPLC chromatograms of commercial turmeric samples

Samples of commercial turmeric samples resolved on C18 reverse phase (4.6x250mm) column with tetrahydrofuran and 1mg/mL citric acid in water (4:6) ratio. Peak intensity was measured at 420nm. Three major curcuminoids separated at specific retention times in the order of elution with curcumin (CUR), demethoxycurcumin (DMC) and (bisdemethoxycurcumin) BDMC.

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