THESIS

SURVIVAL AND INACTIVATION OF *LISTERIA MONOCYTOGENES* BIOFILMS ON FOOD CONTACT SURFACES USING COMMERCIALLY AVAILABLE SANITIZERS AND HOUSEHOLD COMPOUNDS

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ABSTRACT OF THESIS

SURVIVAL AND INACTIVATION OF *LISTERIA MONOCYTOGENES* BIOFILMS ON FOOD CONTACT SURFACES USING COMMERCIALLY AVAILABLE SANITIZERS AND HOUSEHOLD COMPOUNDS

During recent years, *Listeria monocytogenes* has become a major concern to the food industry due to an apparent increase in the incidence of listeriosis. This pathogen attaches to and grows on different kinds of surfaces even at low temperatures forming biofilms and may persist on various food contact materials such as stainless steel, rubber, glass, polyethylene, and polypropylene. *L. monocytogenes* is capable of survival and growth in multispecies biofilms. The presence of *L. monocytogenes* on food contact surfaces is often considered as an important source of (re) contamination of foodstuffs and surfaces especially when this microorganism is present as a biofilm. Hence, proper cleaning and sanitation of food contact surfaces is important in reducing cross-contamination within the home.

The objective of the first study was to determine the survival and persistence of *L. monocytogenes* on high density polyethylene (HDPE) cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on polypropylene (PP) cutting boards and utensils at ambient (25°C) and refrigerator temperature (4°C) under simulated

conditions similar to food preparation and cleaning practices commonly employed in households and the food industry. Effectiveness of three commonly available sanitizers (one each lactic acid-, quaternary ammonium-, sodium hypochlorite-based) and three household compounds (one each sodium hypochlorite-, acetic acid-, and hydrogen peroxide-based) in reducing young and established biofilms of *L. monocytogenes* on HDPE and PP surfaces at ambient and refrigerator temperatures was also compared.

PP and rough and smooth surface HDPE coupons (2x5 cm) were inoculated (6.0-7.0 log CFU/cm^2) with the 5-strain composite of *L. monocytogenes* habituated in ham homogenate. HDPE coupons were incubated at 25°C and PP coupons at 25°C and 4°C for 8 h, and then washed with distilled water to remove loosely attached cells. In repeated 24 h cycles, coupons were bathed in 0.3 ml diluted broth (TSBYE), incubated for 8 h, rinsed with distilled water, and stored without liquid medium (starvation) for 16 h at 4 or 25°C. Sanitizer treatments were applied to coupons on days 0, 0.25, 7 and 14. Biofilm bacteria were removed from coupons by vortexing for 2 min and samples were spread-plated on PALCAM agar and tryptic soy agar with 0.6% yeast extract (TSAYE).

Multi-species biofilms of 7.0-7.5 log CFU/cm^2, containing 5.0-6.0 log CFU/cm^2 *L. monocytogenes*, developed during storage and survived for at least 14 d on all surfaces tested at 25°C, but not on polypropylene at 4°C. Biofilm survival and resistance was greater on rough than smooth HDPE surfaces. Routine food preparation with irregular cleaning may provide nutrients and moisture for biofilm formation containing *L. monocytogenes* and environmental microorganisms, and so sanitation should be performed soon, preferably within 6 h. All sanitizers were effective in reducing *L. monocytogenes*, and more effective on younger than older biofilms. Among the sanitizers

evaluated, the lactic acid- and quaternary ammonium-based sanitizers were the most effective against developed biofilms.

The objective of the second study was to determine the survival and persistence of *L. monocytogenes* on high density polyethylene (HDPE) cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on polypropylene (PP) cutting boards and utensils at ambient (25°C) and refrigerator temperature (4°C) under simulated conditions with and without daily exposure to nutrients, similar to food preparation and cleaning practices commonly employed in households and the food industry. The effectiveness of three commonly available sanitizers and three household compounds in reducing young and established biofilms of *L. monocytogenes* on HDPE and PP surfaces at ambient and refrigerator temperature was also compared. The study helped to understand the impact of nutrient versus without nutrient enrichment of *L. monocytogenes* biofilms on the efficacy of commercial and homemade sanitizers at 25°C and 4°C.

HDPE (rough and smooth surface) and PP (smooth) coupons (2x5 cm) were inoculated (6.0-7.0 log CFU/cm^2) with a 5-strain composite of *L. monocytogenes* in ham homogenate. HDPE coupons were stored at 25°C and PP coupons at 25°C or 4°C for up to 21 d. In repeated 24-h cycles, 0.3 ml diluted broth (TSBYE) was deposited on the inoculated surface of one-half of coupons to simulate nutrient-rich use, then rinsed with 10 ml distilled water 8 h later and stored 16 h (starvation); additional inoculated coupons were stored throughout without added broth. Sanitizer solutions (one each lactic acid-, quaternary ammonium-, acetic acid-, and hydrogen peroxide-based and two sodium hypochlorite-based) were applied to coupons at 0 h, 6 h, 24 h, 4 d, 7 d, 14 d and 21 d

storage. Coupons were analyzed for pathogen (PALCAM agar) and total microbial (tryptic soy agar with 0.6% yeast extract) counts.

Multi-species biofilms, containing 5.0-6.0 log CFU/cm^2 *L. monocytogenes*, developed and survived up to 21 d on all surfaces at 25°C, with survival greater on HDPE than on PP surfaces and on all coupons with repeated nutrient exposure. All products were effective against *L. monocytogenes* on coupons sanitized within 24 h (4° or 25°C). On established biofilms (4, 7, 14 or 21 d), all products were effective against *L. monocytogenes* on all coupons stored at 4°C and on coupons stored at 25°C without daily nutrient enrichment. However, at 25°C, all sanitizers were increasingly ineffective on coupons with established biofilms treated daily with nutrients (2-4 log CFU/cm^2 survival on HDPE surfaces sanitized on d 21). Sanitizer efficacies were higher against older biofilms on smooth surfaces versus those on rough surfaces. Among the sanitizers evaluated, the lactic acid-and quaternary ammonium-based sanitizers tended to be more effective than the other sanitizers. Repeated exposure of food contact surfaces to nutrients as during daily food preparation without regular cleaning and sanitizing increased the resistance of *L. monocytogenes* biofilms to sanitizers. To reduce such risk, sanitation should be performed immediately after each use at least or within 6 h after use to avoid biofilm formation on cutting boards.

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CHAPTER 1

INTRODUCTION

Listeria monocytogenes is a facultative intracellular organism. It is also psychrotropic as it can grow at refrigeration temperatures (>1°C) and in a wide range of pH (pH 4-9) (Moretro and Langsrud, 2004). *Listeria monocytogenes* is responsible for listeriosis with an overall mortality rate around 20–30%, and it can persist in food processing environments over many years, becoming an important source of food contamination (Purkrtova et al., 2010). The threat posed by *L. monocytogenes* is to some extent a function of its ability to grow over a broad temperature range (Beresford et al., 2001). *Listeria monocytogenes* has been shown to be able to form multi-layer biofilms (Chavant et al., 2002). Biofilms have gained increased interest in recent years, due in part to the emergence of *L .monocytogenes* as a foodborne pathogen (Somers and Wong, 2004). Biofilm formation creates major problems in the food industry because it may represent an important source of food contamination.

Biofilms can be defined simply and broadly as communities of microorganisms that are attached to a surface (O'Toole et al., 2000). Formation of microbial biofilms on food contact surfaces is a matter of huge concern in kitchen homes. Biofilm bacteria are difficult to remove, and even with routine cleaning, they may remain and survive on food contact surfaces. Failure to effectively remove bacteria from food contact surfaces can cause serious implications in the transmission of foodborne disease. Planktonic cells, in

contrast to biofilms, are freely and individually living in liquids and can easily be removed with sanitizers. According to Lomander et al. (2004), the formation of an exopolysaccharide (EPS) matrix surrounding the biofilm helps to protect the biofilm from attack by sanitizers, and supplies it with nutrients. *L. monocytogenes* adhering to surfaces in biofilms are less susceptible to sanitizers and disinfectants than planktonic cells (Frank and Koffi et al., 1990; Norwood and Gilmour, 2000; Stopforth et al., 2002). Biofilms can be difficult to control since they can form where water is plentiful and cleaning is not performed properly. Association of *L. monocytogenes* with surfaces has been studied mainly in the laboratory. Laboratory experiments have confirmed that *L. monocytogenes* adheres to rubber, glass, stainless steel and polymers (Mafu et al., 1990; Blackman and Frank, 1996; Beresford et al., 2001). Biofilm formation also increases with increased contact time of the cells with the surface and conditions that increase the rate of bacterial growth, such as nutrient level, pH level and temperature.

The importance of regular and frequent cleaning and sanitation to prevent biofilm formation is supported by laboratory studies. Since biofilm formation is a time dependent process, it is important to have immediate cleaning and sanitation after preparation, so that the bacteria do not have the chance to colonize the surface and form thick biofilms (Moretro and Langsrud, 2004). Studies have been conducted to evaluate the efficacy of various sanitizers against microbial biofilms in food processing environments (Somers and Wong, 2004; Pan et al., 2006), but little attention has been given to the efficacy of commonly available sanitizers for home use against *L. monocytogenes* biofilms on food contact surfaces of cutting boards.

The objectives of the studies undertaken in Chapters 3 and 4 were to evaluate the survival and persistence of *L. monocytogenes* on HDPE cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on PP cutting boards at ambient (25°C) and refrigerator temperatures (4°C) under laboratory conditions that mimic food preparation and cleaning practices commonly employed in households and the food service industry. Overall, the goal was to evaluate the resistance of *L. monocytogenes* biofilms to stresses under laboratory conditions that mimic food preparation and processing environments in home kitchens. The effectiveness of three commonly available sanitizers and three household compounds used against reducing young and established biofilms of *L. monocytogenes* on HDPE at ambient temperatures and PP surfaces at ambient and refrigerator temperature was also compared. The study in Chapter 4 helped to understand the impact of daily exposure of coupons containing *L. monocytogenes* biofilms to nutrients, as might occur in food preparation/ processing areas on the efficacy of commercial and homemade sanitizers at 25°C and 4°C. The objective of the study in Chapter 5 was to compare the efficacy of the two methods of sanitizer application used in Chapter 3 and 4 on minimizing the survival of *L. monocytogenes* on various surfaces.

CHAPTER 2

REVIEW OF LITERATURE

2.1. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive, non-spore-forming, facultative anaerobic rod. As psychrotropic it grows at refrigeration temperatures (>1°C) and in a wide pH range (pH 4-9) (Moretro and Langsrud, 2004). *L. monocytogenes* has been known to survive temperatures ranging from 1 to 45°C, but it grows best at 30°C (Pan et al., 2006). *L. monocytogenes* is a bacterium with a negative cell surface charge and is ubiquitous in nature and so commonly found in the food industries and domestic kitchens (Kalmokoff et al., 2001). This intracellular pathogen has been implicated within the past decade as the causative organism in several outbreaks of foodborne disease (Farber and Peterkin, 1991). *Listeria monocytogenes* is found in a variety of food products such as soft cheeses, dairy products, raw foods, ready to eat products, and equipment surfaces (Jeyasekaran and Karunasagar, 2000).

L. monocytogenes is considered to be one of the five major food-related microorganisms causing foodborne disease (Moretro and Langsrud, 2004). According to the European Centre for Disease Control and Prevention, listeriosis was the fifth most common zoonotic infection in Europe in 2006, while it accounts for approximately 28% of the deaths resulting from food-borne illnesses in the United States (Poimenidou et al., 2009).

When preparing food, this pathogen, if present, can contaminate cooking utensils, such as chopping boards and knives, the food-processing surfaces and the equipment used to clean surfaces, such as dish clothes (Teixeira et al., 2008).

2.2. Biofilm Formation

More than 60 years after the first report on biofilms, they are still a concern in a broad range of areas, and specifically in the food, environmental and biomedical fields (Simoes et al., 2010). Biofilms can be defined as communities of microorganisms attached to a surface (O'Toole et al., 2000). All surfaces, whether hydrophobic, hydrophilic, metal, and plastic and/ or glass, are sites where biofilms can develop (Deza et al., 2005).

Biofilms were first identified in the scientific community in 1702, when Van Leeuwenhoek described them as "animalcules;" however, it was not until Costerton defined them in 1978 that they became more widely studied (Donlan and Costerton, 2002). Costerton stated that biofilms are a community of microorganisms encased in an exopolysaccharide (EPS) matrix and are attached to each other or to a surface (Donlan and Costerton, 2002).

Biofilm formation consists of four steps: 1) conditioning of the surface by macromolecules, 2) initial adherence, 3) physical irreversible adherence that involves the production of exopolymers that fix the cells, and 4) growth of the microorganisms which form microcolonies, and congregation leading to establishment of the biofilm (Chavant et al., 2002). Multi-layers of bacterial cells entrapped within the EPS-containing matrices develop within the biofilm (Kumar and Anand, 1998). The extracellular polymeric

substances (EPS) of cells in suspension condition the surface properties of the microorganisms and hence their degree of adhesion to surfaces (Carpentier and Cerf, 1993). This protection helps the biofilm to resist antibacterial agents and allows the biofilms to survive on surfaces even after cleaning and sanitation (Deza et al., 2005). In simple words, when bacteria interact with a surface, the first phase is a reversible adhesion of bacteria to the surface, which takes place in a period of minutes to a few hours. Cells ultimately adhere irreversibly to the surface and start to multiply and produce extracellular compounds, forming microcolonies and subsequently thicker multilayer and multi-species biofilm (Moretro and Langsrud, 2004). The capacity of pathogens to adhere to surfaces has been well documented (Frank and Koffi, 1990)

Attachment of the bacteria to food contact surfaces or food products leads to serious hygienic problems. In food systems, the attachment of microorganisms leading to the formation of biofilms may be undesirable and also detrimental (Kumar and Anand, 1998). Biofilms have the ability to readily form because of the availability of water, suitable attachment surfaces, ample nutrients and raw materials, or the environment supplying the inocula (Gibson et al., 1999).

Improper cleaning and sanitizing of food contact surfaces may lead to contamination of the product that is physically touching the contaminated surface. Surfaces that are improperly cleaned and sanitized can lead to the transfer of microorganisms through the air, human contact, and contact with other equipment (Gibson et al., 1999). Biofilms are known to be resistant against cleaners and sanitizers and thus survive and grow on food contact surfaces after cleaning and sanitizing. This allows the biofilm to contaminate food products and further grow and cause illness once

ingested. The ability to resist chemical agents has resulted in the need for new and more effective sanitation methods which are important in processing environments.

L. monocytogenes has been shown to be able to form multi-layer biofilms (Chavant et al., 2002). *L. monocytogenes* has been shown to adhere to various surface materials normally in contact with foods such as stainless steel, rubber, glass, polyethylene, and polypropylene, and form biofilms (Chae et al., 2006). Biofilms have gained increased interest in recent years; due in part to the emergence of *L. monocytogenes* as a foodborne pathogen (Somers and Wong, 2004). Many studies have been carried out to determine the ability of *L. monocytogenes* to adhere to food-contact surfaces, all of which determined that the pathogen can attach to industry surfaces including plastic and stainless steel, but very little attention has been given to the efficiency of commonly available sanitizers for home use against *L. monocytogenes* biofilms on food contact surfaces. Proper surface selection can help in reducing the ability of microorganisms to form biofilms. Several studies have been carried out to determine the effect of sanitizers on biofilms in food industries (Norwood and Gilmour, 1999; Jessen and Lammert, 2003; Oulahal et al., 2008) but few have been carried out on food contact surfaces in kitchen homes.

Association of *L. monocytogenes* with food contact surfaces has been studied mainly in laboratories. Laboratory experiments have confirmed that *L. monocytogenes* adheres to rubber, glass, stainless steel and polymers (Mafu et al., 1990; Blackman and Frank, 1996; Beresford et al., 2001). In one study (Teixiera et al., 2008), the adhesion of *L. monocytogenes* ATCC 15313 to glass, granite, marble, polypropylene from a bowl, polypropylene from a cutting board and stainless steel was investigated. These materials

are commonly used in kitchens. In this study, the effect of surface hydrophobocity and roughness on the adhesion process was also analyzed. Hydrophobicity was evaluated through contact angle measurements and by using the approach of van Oss et al. (1987, 1988, 1997). Atomic force microscopy (AFM) was used to perform quantitative measurements of surface topography and roughness. The results showed that the highest adhesion of *L. monocytogenes* occurred to stainless steel, followed by glass, with adhesion being somewhat less than that of other materials studied. However, it was not possible to establish a correlation between surface hydrophobicity or roughness and the extent of adhesion of *L. monocytogenes*. Blackman and Frank (1996) tested the ability of *L. monocytogenes* to grow as a biofilm on various food contact surfaces including stainless steel, Teflon, nylon and polyester floor sealant. Biofilm formation was greatest on polyester and least on nylon.

Chavant et al. (2002) studied the ability of *L. monocytogenes* to form biofilms on stainless steel and polytetrafluoroethylene (PTFE) at 8, 20 and 37°C. The surfaces were cut into 3 X 1 cm coupons, sterilized with surfactant and rinsed with both tap water and demineralized water. The surfaces were then autoclaved. The coupons were kept in a petri dish containing 0.7 ml of *L. monocytogenes* bacterial suspension. Surfaces were tested at two and six hours and one, two, five and seven days. The coupons were washed with sterile tryptone salt (TS) to remove any remaining cells. Coupons were then placed in 5 ml of TS and sonicated for 3 minutes. The recovered cells were made into serial dilutions and plated on tryptic soy agar. Scanning electron microscope was used to observe the bacterial growth on surfaces. This study determined that the initial attachment of *L. monocytogenes* was greater on stainless steel irrespective of the

temperature, though at the low temperature, growth was slowed on both surfaces. For PTFE, 100% biofilm coverage was only seen in the samples that were incubated at 20°C. Biofilm formation occurred on all surfaces after two hours at both 20 and 37°C and detachment was seen on these surfaces after two days. This study showed that *L. monocytogenes* readily forms biofilms regardless of temperature, but the lower temperatures do slow the process and stainless steel showed a greater allowance for attachment.

2.3. Sanitation

Several studies have been carried out to determine the effect of sanitizers on biofilms in food industries but very few have been carried out on food contact surfaces in kitchen homes (Romanova et al., 2002; Lomander et al., 2004). However, in these studies it was also determined that the type of material, exposure time, temperature and the type of sanitizer, all had an effect on the reduction of pathogen. The importance of frequent cleaning and sanitation/disinfection to prevent biofilm formation is supported by laboratory studies. In one study, *Listeria* was allowed to adhere to stainless steel coupons and plastic coupons for two days at 30°C (Jeysekaran and Karunasagar, 2000). The coupons were rinsed in PBS and dipped in either hypochlorite solution of 100-200 ppm or a 10-20 ppm iodophor solution or a combination of both sanitizers (hypochlorite 10 ppm and iodophor 1 ppm) for five minutes. After treatment, the coupons were placed in a neutralizing solution for 30 seconds. For cell enumeration, the coupons were then swabbed and serial dilutions were plated on tryptic soy agar. Total reduction by either sanitizer was most effective on the stainless steel coupons. Reduction was seen on the

plastic coupons, but it was significantly less when compared with the stainless steel. Hypochlorite was more effective in reducing biofilm cells than iodophor on either material. The combination of chlorine and iodophor provided complete inactivation of biofilm cells. The above study shows that stainless steel and the combination of the sanitizers would help in total reduction of *L. monocytogenes* biofilms (Jeyasekaran and Karunasagar, 2000).

Yang et al. (2009) compared the effectiveness of 10 commercially available sanitizers against *L. monocytogenes* biofilms on high density polyethylene cutting boards. Rough and smooth high density polyethylene coupons (2 X 5 cm) were inoculated with a five strain composite of *L. monocytogenes* in ham homogenate, incubated at 24C with ≥90% relative humidity for up to 21 days. Each day, 0.3 ml of a 10-fold diluted tryptic soy broth containing 0.6% yeast extract was added to each coupon (simulating exposure to nutrients during food preparation), and 8 h later each coupon was rinsed with sterile distilled water. Coupons were subjected to sanitizer treatments at zero and six hour and on days seven, fourteen and twenty one. Eight quaternary ammonium compound (QAC)-based sanitizers, one of lactic acid-based sanitizer, and one sodium hypochlorite-based sanitizer were applied to individual coupons according to the manufacturers' instructions. At 0 and 6 h, nine of the sanitizers (all except QAC-based sanitizer 10 with pH 6.24) had reduced *L. monocytogenes* to <0.60 log CFU/cm^2. Statistical analysis of sanitizer efficacy data at a given time and on each surface (rough or smooth), showed that the lactic acid based sanitizer (pH 3.03), was the most effective, while sanitizer # 10 (i.e., QAC-based with pH 6.24), was the least effective. Sanitizer efficacies were greater against younger (7

days) than older (21 days) biofilms on smooth surfaces. For 7 and 14 day biofilms, sanitizer efficacies were higher on smooth than on rough surfaces.

In another study, Chavant et al. (2004) investigated the individual or combined effects of sanitizers on survival of planktonic or sessile *L. monocytogenes* LO28 cells at different phases of growth. Stainless steel coupons (3 X 1.5 cm) were placed into a petri dish containing 7 ml of bacterial suspension. The medium was renewed after 2 h, and then every 24 h. Treatments with sanitizers were made 6 h, 1 and 7 days after initial adhesion. The sanitizers tested included: (i) acetic acid (pH 5.0), (ii) NaOH (pH 12.0), (iii) 10% Na_2SO_4, (iv) 10% Na_2SO_4 and acetic acid (pH 5.0), (v) 10% Na_2SO_4 and NaOH (pH 12.0), (vi) a quaternary ammonium (20 ppm) and (vii) glyceryl monolaurate (75 ppm). The results of the study revealed a great efficacy of alkaline treatments on both sessile and planktonic cells with a slightly higher resistance of 6 h biofilms. Quaternary ammonium appeared very effective in killing more than 98% of cells, but a resistance to 7 day biofilms was observed. Other sanitizers did not succeed in inhibiting totally the pathogen but acted in a similar way on both sessile and planktonic cells. Based on the studies discussed in this section, proper sanitation of food contact surfaces within a short period of time is the only reliable method of control for *L. monocytogenes* biofilms.

CHAPTER 3

Survival and inactivation of *Listeria monocytogenes* biofilms on food contact surfaces treated with commercially available sanitizers and household compounds

ABSTRACT

The objective of this study was to determine survival and persistence of *L. monocytogenes* on high density polyethylene (HDPE) cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on polypropylene (PP) cutting boards at ambient (25°C) and refrigerator temperature (4°C) under simulated conditions similar to food preparation and cleaning practices commonly employed in households and the food service industry. The effectiveness of three commonly available sanitizers (one each lactic acid-, quaternary ammonium-, sodium hypochlorite-based) and three household compounds (one each sodium hypochlorite-, acetic acid-, and hydrogen peroxide-based) in reducing young and established biofilms of *L. monocytogenes* on HDPE and PP surfaces at ambient and refrigerator temperatures was also compared. PP and rough and smooth surface HDPE coupons (2×5 cm) were inoculated (6.0-7.0 log CFU/ cm^2) with a 5-strain composite of *L. monocytogenes* habituated in ham homogenate. HDPE coupons were incubated at 25°C and PP coupons at 25°C and 4°C

for 8 h, and then washed with distilled water to remove loosely attached cells. In repeated 24 h cycles, coupons were bathed in 0.3 ml diluted broth (TSBYE), incubated for 8 h, rinsed with distilled water, and stored without liquid medium (starvation) for 16 h at 4 or 25°C. Sanitizer treatments were applied to coupons on days 0, 0.25, 7 and 14. Biofilm bacteria were removed from coupons by vortexing for 2 min and samples were spread-plated on PALCAM agar and tryptic soy agar with 0.6% yeast extract (TSAYE). Multi-species biofilms of 7.0-7.5 log CFU/cm^2, containing 5.0-6.0 log CFU/ cm^2 *L. monocytogenes,* developed during storage and survived for at least 14 d on all surfaces tested at 25°C, but not on polypropylene at 4°C. Biofilm survival and resistance was greater on rough than smooth HDPE surfaces. All sanitizers were effective in reducing *L. monocytogenes*, and more effective on younger than older biofilms. Sanitation should be performed as soon as possible after each use or at least within 6 h after use in order to avoid biofilm formation on cutting boards and other food contact surfaces. Among sanitizers evaluated, the lactic acid- and quaternary ammonium-based were most effective against developed biofilms. Among the other sanitizers, sanitizer #3≥5=4>6 on 7 d total bacterial biofilms, with no difference in their effectiveness on 14 day biofilms. This suggests that in the absence of commercial sanitizers, readily available household products like distilled white vinegar and diluted chlorine bleach solution should be used.

3.1. INTRODUCTION

Listeria monocytogenes is a Gram-positive rod-shaped, facultative, anaerobic, intracellular bacterium that is widely distributed in nature, and is also frequently isolated in food processing environments (Ryser and Marth, 2007). It is the agent of listeriosis, a

serious infection caused by eating contaminated food. Listeriosis has been recognized as an important public health problem in the United States (Todar, 2004).

Food has been shown to be the primary mode of transmission of *L. monocytogenes,* which has been implicated in numerous foodborne disease outbreaks (Linnan et al., 1988; Farber and Peterkin, 1991; Schlech, 1992; Fretz et al., 2010). The threat posed by *L. monocytogenes* is to some extent a function of its ability to grow and survive over a broad range of temperatures. This is made possible by its ability to modify its membrane composition in order to maintain membrane fluidity (Jones et al., 1997).

L. monocytogenes cells adhere to food contact surfaces, including polyethylene and polypropylene, and if not properly cleaned, form biofilms which may be a major source of contamination. Cells in a biofilm are known to be more resistant to sanitizers than planktonic cells (Mafu et al., 1990; Stopforth et al., 2002) due to formation of an exopolysaccharide matrix that binds cells, surrounds the biofilm, and protects it from sanitizers (Lomander et al., 2004). If the matrix is not completely removed when sanitizing a surface, the pathogen will more readily reattach to the surface and a biofilm will form again (Gibson et al., 1999). Previous research demonstrated that cell attachment and biofilm formation by *L. monocytogenes* are influenced by several factors, including characteristics of strains, physical and chemical properties of the substrate for attachment, growth phase of the bacteria, temperature, growth media, and the presence of other microorganisms (Pan et al., 2006).

If present in food, *L. monocytogenes* has the potential to contaminate food contact surfaces in the home. Contaminated food contact surfaces may serve as sources for bacterial survival and multiplication, and thus, as sources of cross-contamination to other

foods. Reduction in the risk of cross-contamination reduces the potential for transmission of microbiological foodborne illness and this can be achieved by good cleaning practices, hygiene and use of separate surfaces and equipment for handling raw and cooked foods (Gough and Dodd, 1998). The safety of foods can be improved if the persistence and survival of foodborne pathogens, such as *L. monocytogenes,* are better understood when cleaning and sanitizing processing equipment. Thus, the potential for cross-contamination can be reduced if food contact surfaces are properly cleaned and sanitized.

Studies have been conducted to evaluate the efficacy of various sanitizers against microbial biofilms in food processing environments (Somers and Wong, 2004; Pan et al., 2006), but little attention has been given to the efficacy of commonly available sanitizers for home use against *L. monocytogenes* biofilms on food contact surfaces such as cutting boards. The objective of this study was to evaluate the survival and persistence of *L. monocytogenes* on HDPE cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on PP cutting boards and utensils at ambient (25°C) and refrigerator temperatures (4°C) under laboratory conditions that mimic food preparation and cleaning practices commonly employed in households and the food service industry. The effectiveness of three commonly available sanitizers and three household compounds used in reducing young and established biofilms of *L. monocytogenes* on HDPE and PP surfaces at ambient and refrigerator temperature, was also compared.

3.2. MATERIALS AND METHODS

3.2.1. Preparation of ham homogenate. Ten gram of ham samples (cured with water, sugar, salt, dextrose, sodium phosphate, honey, sodium erythorbate and sodium nitrite)

were mixed with 90 ml of sterile distilled water in a whirl-pak bag (Nasco, Fort Atkinson, WI) and homogenized (Masticator, IUL Instruments, Barcelona, Spain) at 6 strokes/second for 2 minutes. The suspension of the product was passed through cheese-cloth, autoclaved for 18 minutes at 121°C and cooled at ambient temperature (25°C) before storing at 4°C for use within 2 days. Ham homogenate is used as the suspending medium of *L. monocytogenes* to simulate contamination on cutting boards.

3.2.2. Bacterial strains and growth conditions. Five strains (Table 3.1) of human disease associated *L. monocytogenes* covering genetic diversity of ribotypes, serotypes, and lineages (Fugett et al., 2006) were used in the experiment. All strains were kept on slants at 4°C and were activated by three successive transfers in tryptic soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton Dickinson Co., Sparks, Md) at 30°C for 24 hours. For inoculum preparation, 24 hour cultures of each strain were centrifuged separately (Eppendorf model 5810 R, Brinkmann Instruments Inc., Westbury, NY) at 6000 rpm for 15 minutes at 4°C. The harvested cells were re-suspended in 10 ml of phosphate buffered saline (pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4.7H_2O$, 8 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water) and centrifuged as above. The harvested cells were re-suspended in 10 ml of ham homogenate (each culture separately) prepared from product of the same lot as that used in the study (but kept frozen during the study at - 20°C). Cell cultures suspended in ham homogenate were stored at 7°C for 48 hours to allow for acclimatization of the cells to a low temperature food environment. Equal volumes (10 ml) of cell suspensions for each of the 5 strains were then combined for use in the study (Figure 3.1). The 5 strain mixture was surface plated on TSAYE and

PALCAM agar (Difco) for determination of initial populations as well as for testing the purity of the inoculum.

3.2.3. Cutting board coupons. Rough and smooth surface high density polyethylene (HDPE) and smooth surface polypropylene (PP) materials were obtained from Fort Collins Plastics (Fort Collins, CO) for use in the studies. These materials are Food and Drug Administration (FDA) approved for use as food contact surfaces (USFDA reg. 21CFR177.1520 item 2.1). The HDPE materials were cut into 2x5 cm coupons (thickness = 2 mm for smooth and 6 mm for rough HDPE), soaked in 300 ppm sodium hypochlorite solution (5 ml of 6% sodium hypochlorite bleach combined with 950 ml distilled water) for 2 – 3 hours, air dried, and then autoclaved under the gravity cycle at 121°C for 20 minutes. The polypropylene sheets were cut into 2x5 cm coupons (thickness = 2mm), soaked in detergent solution (approximately 3 teaspoons of Dawn dishwashing liquid soap, Procter & Gamble to 1 gallon of tap water) for 2-3 hours, rinsed with clear distilled water, air dried, and then autoclaved under gravity cycle at 121°C for 20 minutes.

3.2.4. Inoculation of coupons and biofilm formation. The sterile coupons were first placed on a clean sterile tray. Inoculated ham homogenate (100 μl) was placed on each coupon and spread evenly with a 100 μl pipette, resulting in a final concentration of 10^6 – 10^7 CFU/cm^2. The trays were then placed into an ambient temperature incubator (25°C) with the humidity adjusted to 90% using a saturated K_2SO_4 solution. HDPE coupons with rough and smooth surfaces were incubated at 25°C whereas PP coupons were incubated at 25°C and 4°C. The relative humidity was measured two times per day with an Electronic Humidity Meter (Time – Faver, Temperature and Humidity Data Logger, Dickson Addison, IL) and the temperature was measured twice daily using an Easy Read

Thermometer (H.B, USA) (Appendix 3.1 and 3.2). After incubation for 8 h at 25°C or 4°C, coupons were removed from the incubators with forceps and 10 ml of sterile distilled water was pipetted continuously on the upper end of each coupon so that the whole outer surface of the coupon was washed slowly with flowing distilled water to remove loosely attached cells from the biofilm. Coupons were then subjected to up to 14 repeated 24-hr cycles, modified from Pan et al. (2006), to simulate general cutting board use and cleaning conditions in the home. On each day, 10-fold diluted TSBYE medium (0.3 ml) was added to each coupon. The coupons were then incubated for 8 h at 25°C (for HDPE coupons) and 25°C or 4°C (for PP coupons) to simulate exposure to water and nutrients during food preparation, after which the coupons were rinsed with 10 ml sterile distilled water and stored without liquid medium (starvation) for 16 h at 25°C or 4°C with 90% humidity (Figure 3.1).

3.2.5. Sanitizing methods. Three commercially available sanitizers and three household compounds were purchased from a local supermarket based on their commercial availability and intended usage on food contact surfaces in the home (Table 3.2). The three commercial sanitizers came in spray bottles, while the three household compounds came in regular bottles, one of which was supplied by the manufacturer as a concentrated liquid (Sanitizer #4 – sodium hypochlorite). This sanitizer was diluted with sterile distilled water to 300 ppm sodium hypochlorite in our laboratory on the day of use. All sanitizers varied in chemical composition and concentrations of active ingredients (Table 3.2).

3.2.6. Sanitizer treatment of biofilm cells. For each type of surface, 16 inoculated coupons were removed at 0 h (before incubation), 6 h, 168 h and 336 h incubation, and

not treated (control), treated with sterile distilled water or treated with one of the six sanitizers (Figure 3.2). Untreated coupons were directly placed into a Nalgene centrifuge tube (Nalge Nunc, Rochester, NY) containing 40 ml of D/E neutralizing broth (Difco), while other coupons were first rinsed with 10 ml of sterile distilled water to remove loosely attached bacterial cells, and then treated as follows.

The sterile distilled water treatment involved pipetting 2 ml of sterile distilled water onto the surface of the coupon and allowing it to stand for 10 min. The coupon was then raised with the help of forceps so that the water on the surface of the coupon dropped into a petri dish. The coupon was again rinsed with 10 ml distilled water and then placed into a centrifuge tube containing 40 ml of D/E neutralizing broth (Difco) and 10 glass beads of 4 mm diameter (Fischer Scientific, Houston, TX). Glass beads aid in the removal of attached cells from surfaces (Stopforth et al., 2002).

For evaluation of sanitizer treatments, each coupon was placed into a petri dish and subjected to sanitation according to manufacturers' instructions as follows: for sanitizers #1, 2, 3, available in spray bottles, coupons were wetted by spraying sanitizers with consistent pressure 5 times at an approximate angle of 60° to the surface from 15 cm away. As indicated, sanitizer #4 was diluted with sterile distilled water to 300 ppm active ingredient and then 2 ml of the prepared solution was used to treat the coupons as the water control treated coupons. The same procedure followed for sanitizers #5 and 6 that came in regular bottles. The coupons were then allowed to stand for 10 min for sanitizers #1, 2, 4, 5 and 6 and 2 minutes for sanitizer #3, according to manufacturers' instructions for each sanitizer; then rinsed again with 10 ml sterile distilled water.

After sanitation, each coupon was placed into an 85 ml Nalgene centrifuge tube (Nalge Nunc, Rochester, NY) containing 40 ml of D/E neutralizing broth and 10 glass beads. Biofilm bacteria were removed from the coupons by vortexing (Vortex-Genie 2, Scientific Industries, Inc, Bohemia, NY) for 2 min at the speed level of 10 (Figure 3.2). Samples were spread plated onto both TSAYE and PALCAM plates after 10-fold dilutions in 0.1% buffered peptone water (Difco). Total bacterial counts were counted after incubation at 25°C for 48 h whereas *L. monocytogenes* colonies were counted after incubation at 30°C for 48 h. The liquid left in the petri dish was analyzed in order to check the number of cells that had been removed just with plain distilled water.

3.2.7. Data analysis. All tests were performed in two independent replication trials with two samples evaluated for each replicate. Microbial counts were reported in terms of $\log_{10}$ CFU/cm^2; estimated reductions were analyzed statistically to compare sanitizer treatment effects. Data were analyzed using the Glimmix Procedure in SAS (SAS Institute Inc., Cary, NC). The Glimmix procedure helps to specify a generalized linear mixed model and to perform confirmatory inference. Descriptive statistics (means and standard deviations) were computed and analyses of variance were performed for statistical differences ($P<0.05$). Independent variables in the mixed models procedure were type of surface, type of sanitizer, media, time and their interactions. Random effects were replicate and replicate interactions with surface and sanitizer. The least significant difference procedure was used to perform mean separation.

3.3. RESULTS

3.3.1. Survival of *L. monocytogenes* and formation of biofilms on HDPE rough surfaces at 25°C. According to the Analysis of variance, the main effects of sanitizer treatment, time and media were all significant ($P<0.001$). Two way interactions of sanitizer treatment by time and media by time were also significant ($P<0.001$) (Appendix 3.3). For the control coupons (no treatment), total bacterial cells on HDPE rough surfaces as enumerated on TSAYE (6.63 log CFU/cm^2 at 0 h), did not vary ($P\geq0.05$) throughout the 14 d evaluation (7.03 log CFU/cm^2 at 14 d) (Table 3.3, Figure 3.3). In contrast, the total number of *L. monocytogenes* cells, as enumerated on PALCAM, decreased ($P<0.05$) from 6.56 log CFU/cm^2 at 0 h and 5.39 log CFU/cm^2 at 6 h to 2.58 and 3.26 log CFU/cm^2 at 7 d and 14 d, respectively.

Rinsing coupons with distilled water at 0 and 6 h resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* (2.29 to 3.31 log CFU/cm^2 reductions from control coupons). However, rinsing with distilled water on 7 and 14 d developed biofilms produced no further reductions ($P\geq0.05$) from control coupon counts, with 6.89 CFU/cm^2 total bacteria and 2.99 log CFU/cm^2 *L. monocytogenes* remaining on d 14 rinsed coupons (Table 3.3, Figure 3.3).

Sanitizer #1 effectively decreased total bacteria and *L. monocytogenes* counts on inoculated HDPE rough surfaces to below the detection limit (0.60 log CFU/cm^2) at 0 and 6 h exposure. Sanitizers #2 and 6 also were fairly effective in that they reduced total and *L. monocytogenes* cells to below the detection limit at 6 h of exposure. Sanitizers #3, 4 and 5 were somewhat less effective in that 2.00-2.20 log CFU/cm^2 total bacteria and 1.38-1.90 log CFU/cm^2 *L. monocytogenes* counts remained after sanitizer treatment at 0 h

and 0.78 to 1.01 log CFU/cm^2 total and <0.60-0.72 log CFU/cm^2 *L. monocytogenes* counts remained following sanitizer treatment at 6 h (Table 3.3, Figure 3.3).

Treatment of 7 and 14 d biofilms with sanitizers #1, 2 and 3 produced significant decrease ($P<0.05$) in total bacteria counts from control and water treated coupons, but still had 3.40-5.52 log CFU/cm^2 cells remaining. In addition, sanitizer #2 reduced ($P<0.05$) *L. monocytogenes* counts to below the detection limit on d 7 biofilms and to 1.3 log CFU/cm^2 on d 14 biofilms. For all other sanitizer treatments, no reductions ($P>0.05$) in total or *L. monocytogenes* cells were detected for d 7 or 14 biofilms from bacteria remaining on control or water treated HDPE rough surface coupons (5.52-6.29 CFU/cm^2 total and 1.81-2.98 CFU/cm^2 *L. monocytogenes* cells remaining) (Table 3.3, Figure 3.3).

3.3.2. Survival of *L. monocytogenes* and formation of biofilms on HDPE smooth surfaces at 25°C. According to the Analysis of variance, the main effects of sanitizer treatment, time and media were all significant ($P<0.001$). Two way interactions of sanitizer treatment by media, sanitizer treatment by time and media by time were also significant (Appendix 3.4). For the control coupons (no treatment), total bacterial cells on HDPE smooth surfaces, as enumerated on TSAYE (6.65 log CFU/cm^2 at 0 h), decreased ($P<0.05$) to 5.05 log CFU/cm^2 on d 7 biofilms, then increased ($P<0.05$) to 6.67 log CFU/cm^2 on d 14 biofilms (Table 3.4, Figure 3.4). In contrast, the total number of *L. monocytogenes* cells, as enumerated on PALCAM, decreased ($P<0.05$) from 6.53 log CFU/cm^2 at 0h to 2.65 and 2.99 log CFU/cm^2 at 7 and 14 d, respectively.

Rinsing coupons with distilled water at 0 and 6 h resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* counts (2.85 to 3.89 log CFU/cm^2 reductions on control coupons). However, rinsing with distilled water on 7 and 14 d

biofilms produced no further reductions (P>0.05) from control counts; 5.83 CFU/cm^2 total bacteria and 2.16 log CFU/cm^2 *L. monocytogenes* remained at d 14 (Table 3.4, Figure 3.4).

Treatment with sanitizers #2 and 4 effectively decreased total bacteria and *L. monocytogenes* cells on HDPE smooth surfaces to below the detection limit (0.60 log CFU/cm^2) at 0 and 6 h. All other sanitizer treatments also were fairly effective in that they reduced total and *L. monocytogenes* cells to below the detection limit at 6 h (Table 3.4, Figure 3.4).

On biofilms allowed to develop for 7 and 14 d, all the sanitizers produced significant decreases (P<0.05) in total bacteria counts from control and/or water treated coupons, but treated coupons still had 1.70 - 4.86 log CFU/cm^2 cells remaining. Sanitizers #1, 2, 4 and 5 were effective against *L. monocytogenes* in reducing cells (P<0.05) to below the detection limit on d 7 biofilms, whereas sanitizer #3 and 6 reduced *L. monocytogenes* counts to 1.12 log CFU/cm^2 and 0.96 log CFU/cm^2, respectively, on d 7 biofilms. On biofilms allowed to develop for 14 d, none of the sanitizers reduced *L. monocytogenes* counts to below detection limit and had 0.72 – 2.53 log CFU/cm^2 remaining (Table 3.4, Figure 3.4).

3.3.3. Survival of *L. monocytogenes* and formation of biofilms on PP surfaces at 25°C. According to the Analysis of variance, the main effects of sanitizer treatment, time and media were all significant (P<0.001). Two way interactions of sanitizer treatment by media, sanitizer treatment by time and media by time were also significant (Appendix 3.5). For the control coupons (no treatment), total bacterial cells on PP surfaces as enumerated on TSAYE (6.63 log CFU/cm^2 at 0h) did not vary (P≥0.05) throughout the

14 d evaluation (6.10 log CFU/cm^2 on d 14) (Table 3.5, Figure 3.5). In contrast, the total number of *L. monocytogenes* counts as enumerated on PALCAM, decreased ($P<0.05$) from 6.53 log CFU/cm^2 at 0 h and 5.24 log CFU/cm^2 at 6 h to 2.31 and 0.75 log CFU/cm^2 at 7 and 14 d, respectively.

Rinsing inoculated coupons with distilled water at 0 and 6 h exposure resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* (1.51 to 3.43 log CFU/cm^2 reductions from control coupons). However, rinsing with distilled water caused no reductions on control coupons ($P\geq0.05$) on 7 and 14 d developed biofilms (Table 3.5, Figure 3.5).

Treatment with sanitizers #1 and 2 effectively decreased total bacteria and *L. monocytogenes* counts on PP surfaces to below the detection limit (0.60 log CFU/cm^2) at 0 and 6 h of exposure. Sanitizers #3 and 4 also were fairly effective in that they reduced total bacteria to 0.68 log CFU/cm^2 and *L. monocytogenes* counts to below the detection limit at 6 h. Sanitizers #5 and 6 were somewhat less effective; 0.95-1.63 log CFU/cm^2 total bacteria and 1.06-2.31 log CFU/cm^2 *L. monocytogenes* remained at 0 h and 6 h (Table 3.5, Figure 3.5).

On biofilms allowed to develop for 7 and 14 d, all sanitizers except #6 on d 7 and #4 and 6 on d 14 produced significant decreases ($P<0.05$) in total bacteria counts from control and water treated coupons, but still had 2.46 - 4.49 log CFU/cm^2 cells remaining. In contrast, no further reductions ($P\geq0.05$) were seen in *L. monocytogenes* counts, with <0.60-2.04 log CFU/cm^2 cells remaining (Table 3.5, Figure 3.5).

3.3.4. Survival of *L. monocytogenes* and formation of biofilms on PP surfaces at 4°C.

According to the Analysis of variance, the main effects of sanitizer treatment and time

were all significant ($P<0.001$). The two way interaction of sanitizer treatment by time was also significant (Appendix 3.6). Biofilms did not develop well on PP surfaces at 4°C. For the control coupons (no treatment), total bacterial cells on PP surfaces incubated at 4°C, as enumerated on TSAYE, decreased ($P<0.05$) from 6.66 log CFU/cm^2 at 0 h and 6.40 log CFU/cm^2 at 6 h to 1.10 and < 0.60 log CFU/cm^2 at 7 and 14 d respectively (Table 3.6, Figure 3.6). Similarly, the total number of *L. monocytogenes* cells, as enumerated on PALCAM, decreased ($P<0.05$) from 6.59 log CFU/cm^2 at 0 h and 5.28 log CFU/cm^2 at 6 h to 1.10 and < 0.60 log CFU/cm^2 at 7 and 14 d, respectively.

Rinsing coupons with distilled water at 0 and 6 h resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* (2.16 to 3.13 log CFU/cm^2 reductions from control coupons). However, rinsing with distilled water produced no further reductions ($P>0.05$) in counts on 7 d developed biofilms, with counts remaining at 1.10 log CFU.cm^2 for both total bacteria and *L. monocytogenes*, respectively. During this period of time, the inoculated *L. monocytogenes* competed with environmental microorganisms for survival as multiple-species biofilms were formed. There was presence of bacterial contamination due to environmental contamination (Table 3.6, Figure 3.6).

Sanitizers #1 and 2 effectively decreased total bacteria and *L. monocytogenes* counts on PP coupons to below the detection limit (0.60 log CFU/cm^2) at 0 and 6 h. Sanitizers #3 and 4 also were fairly effective in that they reduced total and *L. monocytogenes* counts to <0.60 – 0.78 log CFU/cm^2 at 6 h. Sanitizers #5 and 6 were somewhat less effective in that 1.30 - 2.34 log CFU/cm^2 total bacteria and 0.89 -1.92 log CFU/cm^2 *L. monocytogenes* were remaining at 0 h and 0.68 to 0.81 log CFU/cm^2 total

and <0.60 log CFU/cm^2 *L. monocytogenes* were remaining at 6 h. All sanitizers effectively reduced total and *L. monocytogenes* counts to below the detection limit (<0.60 log CFU/cm^2) on d 7 biofilms (0.50 log CFU/cm^2 reduction from control levels). Day 14 biofilms did not survive on PP at 4°C (Table 3.6, Figure 3.6).

3.4. DISCUSSION

The objective of this study was to evaluate resistance of *L. monocytogenes* biofilms to stresses under laboratory conditions that mimic food preparation and processing environments in home or catering kitchens. There are two steps to kitchen hygiene; cleaning and sanitizing. The main purpose of cleaning is to remove all the residual materials that may interfere with the sanitation process (Pan et al., 2006). In this study, this was done by rinsing each coupon with sterile distilled water (room temperature) prior to water or sanitizer treatment. Sanitation can be done by dipping or spraying of cleaned surfaces with sanitizer (Pan et al., 2006). In this study, we used commercially available sanitizers and household compounds. The combined starvation, cleaning and sanitation conditions evaluated in this study demonstrate the ability of *L. monocytogenes* to accumulate as a biofilm on materials commonly found in home kitchens. Mafu et al. (1990), Helke et al. (1993), Mostellar and Bishop (1993), Rodriguez et al. (2008) and Yang et al. (2009) demonstrated the ability of this pathogen to adhere to various surfaces, including stainless steel, polyethylene, polypropylene, rubber and Teflon.

At 25°C, rinsing coupons with distilled water produced significant ($P<0.05$) reductions in total bacteria and *L. monocytogenes* on all surfaces at 0 and 6 h incubation,

but still allowed 2-4 log CFU/cm^2 to remain. All of the sanitizer treatments evaluated produced further reductions ($P<0.05$) in total and *L. monocytogenes* counts at 0 and 6 h, with higher reductions seen on PP and smooth HDPE that on rough surfaces. However, after 7 or 14 days of incubation, biofilms were established and their resistance had increased, making water ineffective in removing total bacterial flora and *L. monocytogenes* cells.

On d 7 and 14 biofilms, treatment with sanitizers #1, 2 and 3 for all 3 surface types and sanitizers # 4 and 5 for smooth surface types did produce significant reductions in total bacteria, but none of the sanitizers were totally effective in reducing bacterial levels to below the detection limit. For *L. monocytogenes* cells, treatment of d 7 biofilms with sanitizers #1, 2, 4 and 5 on smooth HDPE and sanitizer #2 of rough HDPE surfaces successfully reduced counts to below the detection limit (0.60 CFU/ cm^2). However, by d 14 none of the sanitizers, with the exception of sanitizer #4 on PP surfaces, were able to reduce *L. monocytogenes* cells to below the detection limit (Table 3.3, 3.4, 3.5 and Figure 3.3, 3.4 and 3.5). Sanitizer #4 (sodium hypochlorite) is the most widely used chlorine compound. Due to chlorine's high oxidizing reactivity, the activity of cellular proteins is destroyed (Lomander et al., 2004).

As a psychrotrophe, *L. monocytogenes* can grow at refrigeration temperatures (Oulahal et al., 2008). Biofilms did not develop well on polypropylene coupons incubated at 4°C; only 1.10 log CFU/cm^2 remained on control and water treated samples at 7 d after inoculation and <0.60 log CFU/cm^2 at 14 d (Table 3.6, Figure 3.6). Given the very low survival rate, all sanitizers effectively reduced total and *L. monocytogenes* cell counts on PP coupons incubated at 4°C for 7 and 14 d to below the detection limit (<0.60

log CFU/cm^2). The inoculated *L. monocytogenes* competed with environmental microorganisms for survival during this time interval of formation of multiple-species biofilms (Yang et al., 2009). It was observed that on d 7 and d 14, there was presence of bacterial contamination due to environmental organisms. The data suggest that during repeated introduction of food residues and moisture, as during daily food preparation and inadequate cleaning practices, cutting boards may allow development of multiple-species biofilms containing *L. monocytogenes* cells.

L. monocytogenes and other bacteria survived sanitizer treatments employed on 7 and/or 14 d following pathogen exposure at 25°C. According to Lomander et al. (2004) this may be due to the formation of the exopolysaccharide (EPS) matrix surrounding the biofilms that supplies it with nutrients and protects it from attack by sanitizers. Also in this study, ham homogenate was used as the suspending medium of *L. monocytogenes* to simulate contamination of cutting boards. According to Moore et al. (2007) it is believed that presence of macromolecular nutrients, like proteins, protects cells against dehydration, and as a result, the viability of cells in desiccating environments increases. Other factors contributing to the viability of *L. monocytogenes* on HDPE and PP surfaces may have been the temperature (25°C) and the high relative humidity (90%). A decrease in both total bacterial and *L. monocytogenes* cells was observed on PP coupons that were incubated at 4°C. Previous research demonstrated that cell attachment and biofilm formation by *L. monocytogenes* are influenced by several factors, including characteristics of strains, physical and chemical properties of the substrate for attachment, growth phase of the bacteria, temperature, growth media and the presence of other microorganisms (Mafu et al., 1991; Blackman and Frank, 1996; Wong, 1998; Norwood

and Gilmour, 2000; Chavant et al., 2004; Pan et al., 2006). It is possible that temperature (4°C), relative humidity, the ham homogenate nutrient source used, or a combination of these factors may have played an important role in not supporting the growth of the *L. monocytogenes* biofilm on polypropylene at 4°C. According to Palmer et al. (2007), there could be a number of other factors involved in bacterial cell attachment such as surface conditioning, mass transport, surface charge, hydrophobicity, surface roughness and surface micro-topography.

3.4.1. Comparison of survival rate of *L. monocytogenes* cells across HDPE rough and smooth surfaces and PP surface incubated at 25°C. Differences in surface properties between HDPE with rough surface, HDPE with smooth surface and PP may cause variation in the rate of biofilm maturation and thus resistance to sanitation. To assess differences in response of the three surfaces to sanitizer treatment at 25°C, total bacterial and *L. monocytogenes* counts for each surface type were averaged across water and all 6 sanitizer treatments by treatment time. As seen on Table 3.7 (Figure 3.7), the total bacterial flora and *L. monocytogenes,* counts as enumerated on TSAYE and PALCAM, respectively, were higher ($P<0.05$) on rough than smooth HDPE surfaces at each time except at 6 h. This indicates that *L. monocytogenes* cells were more resistant on porous surfaces (rough surface) than on non-porous surfaces. Bacterial survival on PP (smooth) surfaces were similar to HDPE smooth surfaces except for total bacterial counts on d 7, which were higher on PP surfaces.

Development of a biofilm is a result of both adherence and growth following adherence (Blackman and Frank, 1996). Moretro and Langsrud. (2004) showed that biofilm adheres to rough surfaces more strongly than smooth surfaces. Also, the high rate

of evaporation on smooth surfaces may have resulted in more injured cells and thus lower bacterial survival on the smooth surfaces (Yang et al., 2009).

3.4.2. Impact of storage temperature on survival of *L. monocytogenes* cells on polypropylene surfaces. To assess the impact of storage temperature on survival rates, total bacteria and *L. monocytogenes* counts for PP were averaged across water and all 6 sanitizer treatments by storage temperature (25 and 4°C). As seen in Table 3.8 (Figure 3.8), the total bacterial and *L. monocytogenes* cell counts for 7 and 14 d biofilms as enumerated on TSAYE and PALCAM, respectively, were higher ($P<0.05$) on PP incubated at 25°C than PP incubated at 4°C. Significant differences were observed on polypropylene surfaces at 25° and 4°C on established biofilms. This indicates that *L. monocytogenes* cells survived better and were more resistant at room temperature than at refrigerator temperature. According to Wong (1998), biofilm survival is affected by temperature, relative humidity and attachment surface, and one or multiple factors may have played an important role in reduced survival of *L. monocytogenes* on PP incubated at 4°C.

3.4.3. Comparison of the efficacy of the six sanitizers. To compare the overall effectiveness of the six sanitizers across HDPE rough and smooth surfaces and PP surface incubated at 25°C, the differences between distilled water treatment and after sanitizer treatment were calculated and these were then averaged. The total reduction of bacterial cells on food contact surfaces consisted of physical removal caused by washing with distilled water and chemical inactivation caused by the sanitizer (Log CFU/cm^2). Application of each sanitizer was according to the manufacturer's instructions printed on the bottles, which for most of them included three major steps: rinsing with water,

reaction with sanitizer for certain time intervals (10 sec to 10 min), and rinsing with water again (Table 3.2). According to data analysis, the most effective sanitizer is the one that causes the highest reduction. According to the study, two of the six sanitizers used in the study were sodium hypochlorite based but their ingredients and concentrations were different. Two sanitizers were lactic acid-based (pH 2.92) and quaternary ammonium-based (pH 10.12) and the remaining two were acetic acid (pH 3.26) and hydrogen peroxide (pH 4.72). As seen in Table.3.9 (Figure 3.9), for d 7 and 14 biofilms sanitizer #2 (i.e., lactic acid-based, pH 2.92) was found to be the most effective sanitizer on all three surfaces followed by sanitizer #1 (QAC-based). Chavant et al. (2004) also found sanitizers with higher pHs to be effective on biofilms. Among the other sanitizers, sanitizer #3≥5=4>6 on 7 d total bacterial biofilms, with no difference in their effectiveness on 14 day biofilms. This suggests that when commercial sanitizers are not available, home prepared sodium hypochlorite (sanitizer #4) and full strength vinegar (sanitizer #5) can be just as effective as commercially available sodium hypochlorite solutions (Table 3.9, Figure 3.9). No significant differences were observed between sanitizers at all times on *L. monocytogenes* biofilms. In the present study, the high efficacy of the lactic acid-based sanitizer may be explained by its lower pH of 2.92. According to Yang et al. (2009) lactic acid-based sanitizer was the most effective sanitizer and our study supports the same results. Between sanitizer #3 and 4 (since both are sodium hypochlorite-based), sanitizer #3 was found to be more effective on d 7 – total bacteria, but similar on other days. Sodium hypochlorite is the most widely used chlorine compounds. When added to water (such as in bleach), ionization takes place, and the hypochlorite ion establishes equilibrium with HOCl (Lomander et al., 2004). Due to

chlorine's high oxidizing reactivity, the activity of cellular proteins is destroyed (Lomander et al., 2004).

3.5. CONCLUSION

Experiments related to the attachment of microorganisms to various food contact surfaces in home kitchens must be carried out under conditions existing in those environments. Such studies will help us understand fully the interactions between biotic and abiotic entities during/ after food processing in home kitchens. They are also required to understand the impact of cleaning and sanitation from the microbiological viewpoint. It can be concluded that *L. monocytogenes* can survive on food contact surfaces, e.g. cutting boards, plastic microwaveable utensils and refrigerator shelves, etc., forming a biofilm, and such adherent cells may not be removed completely during the washing and sanitizing processes unless special attention is paid to the prompt removal of biofilms.

In this study, *L. monocytogenes* developed during storage and survived for at least 14 days on all surfaces tested at 25°C, but not on polypropylene at 4°C. At this stage there is no obvious explanation why *L. monocytogenes* biofilms did not develop on polypropylene incubated at 4°C, although temperature (4°C) and relative humidity (90%) could have a played a very important role. This is an area needing further research. Repeated daily food preparation without any cleaning and sanitation would result in providing nutrients and moisture for biofilm formation. *L. monocytogenes*, when given sufficient time, can accumulate on a variety of surfaces to levels which might lead to the spread of the pathogen throughout food preparation and handling.

All sanitizers tested were effective in reducing *L. monocytogenes*, and more effective on younger than older biofilms. Among sanitizers evaluated, the lactic acid-based (pH 2.92) and quaternary ammonium-based (pH 10.12) were most effective against developed biofilms aged 7 days and older. Since the effect of sanitizer decreases as the biofilms matures, sanitation should be performed as soon as possible after each use or at least within 6 h after use in order to avoid biofilm formation on cutting boards and other food contact surfaces. Biofilm survival was found to be greater on rough than smooth HDPE surfaces and so cutting boards with a smooth surface should be considered due to delay in biofilms maturation.

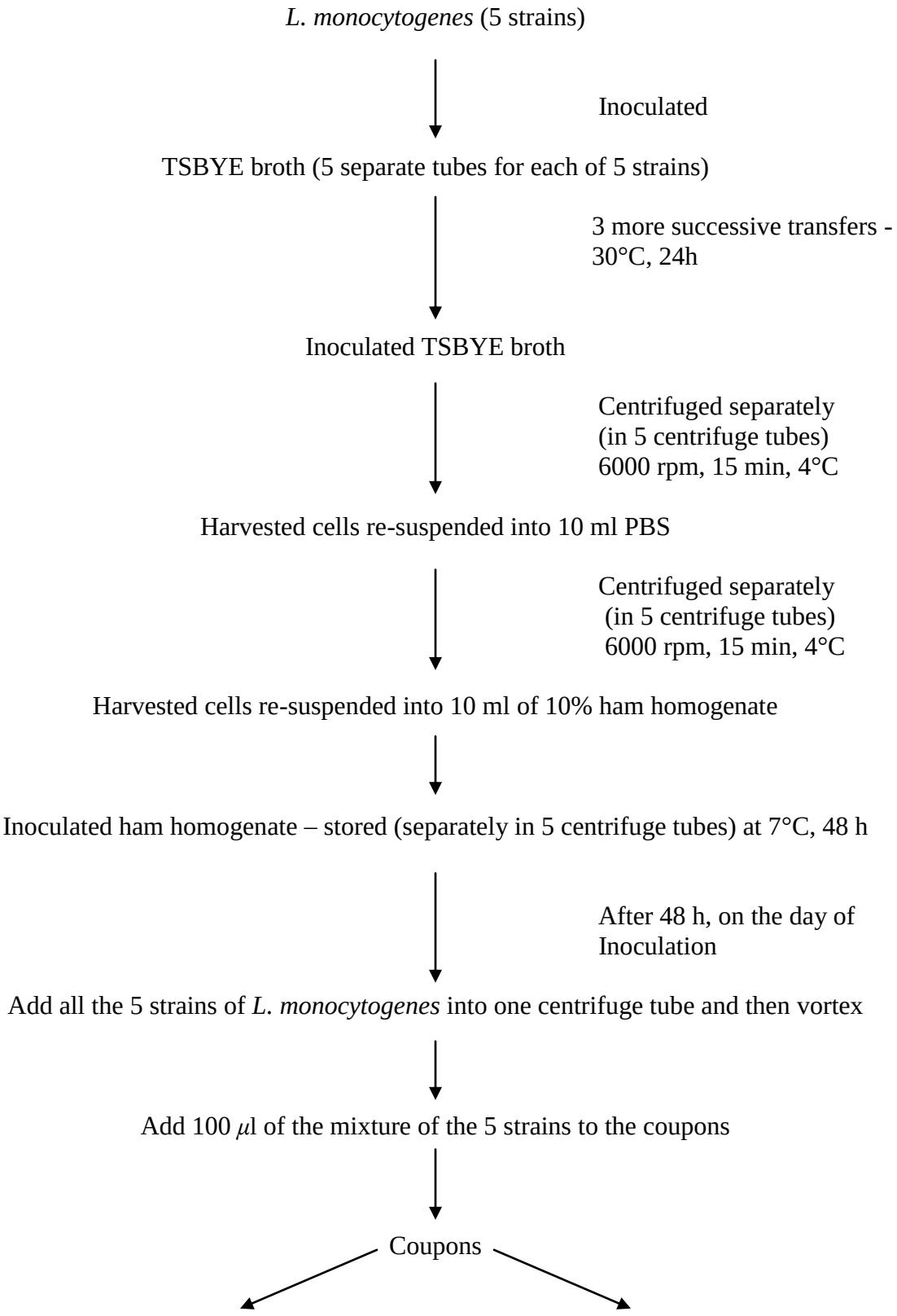
Table 3.1. *L. monocytogenes* strains used in the study (Fugett et al., 2006)

L. monocytogenes strain	Lineage	Serotype	Source
J1-177	I	1/2b	Human, sporadic
R2-499	II	1/2a	Human, epidemic, sliced turkey
N3-013	I	4b	Food, epidemic
N1-227	I	4b	Food, epidemic
C1-056	II	1/2a	Human, sporadic

Table 3.2. Sanitizers used for inactivating *Listeria monocytogenes* biofilms on rough and smooth high density polyethylene coupons and polypropylene coupons

Sanitizer #	**Active Ingredients and Concentration**	**pH**
1	Alkyl (67% C_{12}, 25% C_{14}, 7% C_{16}, 1% C_8-C_{10}-C_8) dimethyl benzyl ammonium chlorides (0.0860%) Alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium chlorides (0.0216%)	10.12
2	L-Lactic acid (0.18%)	2.92
3	Sodium hypochlorite (0.0095%) Available chlorine (0.009%)	6.55
4*	Sodium hypochlorite (6%) Other ingredients (94%) Yield 5.7% available Cl_2	6.22
5	Acetic acid (5%)	3.26
6	Hydrogen peroxide (3%)	4.72

* A fresh solution was prepared on the day of experiment.


L. monocytogenes (5 strains)
Inoculated
TSBYE broth (5 separate tubes for each of 5 strains)
3 more successive transfers - 30°C, 24h
Inoculated TSBYE broth
Centrifuged separately (in 5 centrifuge tubes) 6000 rpm, 15 min, 4°C
Harvested cells re-suspended into 10 ml PBS
Centrifuged separately (in 5 centrifuge tubes) 6000 rpm, 15 min, 4°C
Harvested cells re-suspended into 10 ml of 10% ham homogenate
Inoculated ham homogenate – stored (separately in 5 centrifuge tubes) at 7°C, 48 h
After 48 h, on the day of Inoculation
Add all the 5 strains of *L. monocytogenes* into one centrifuge tube and then vortex
Add 100 μl of the mixture of the 5 strains to the coupons
Coupons

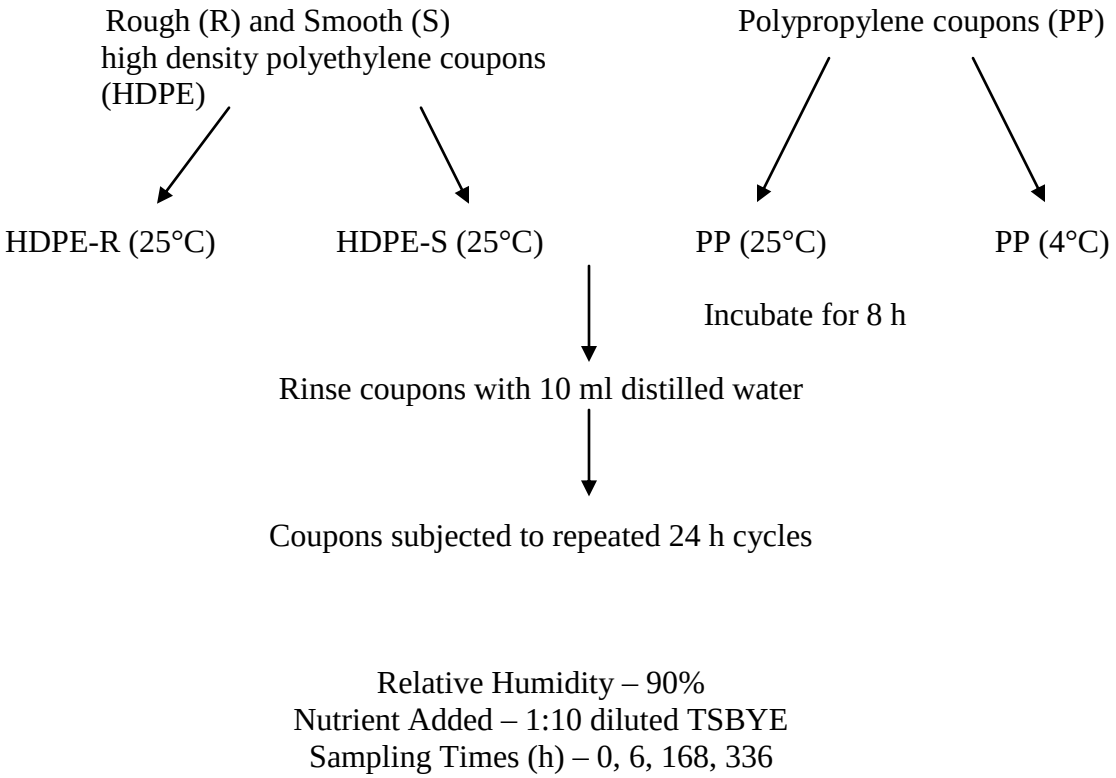


Figure 3.1. **Coupon preparation procedure**

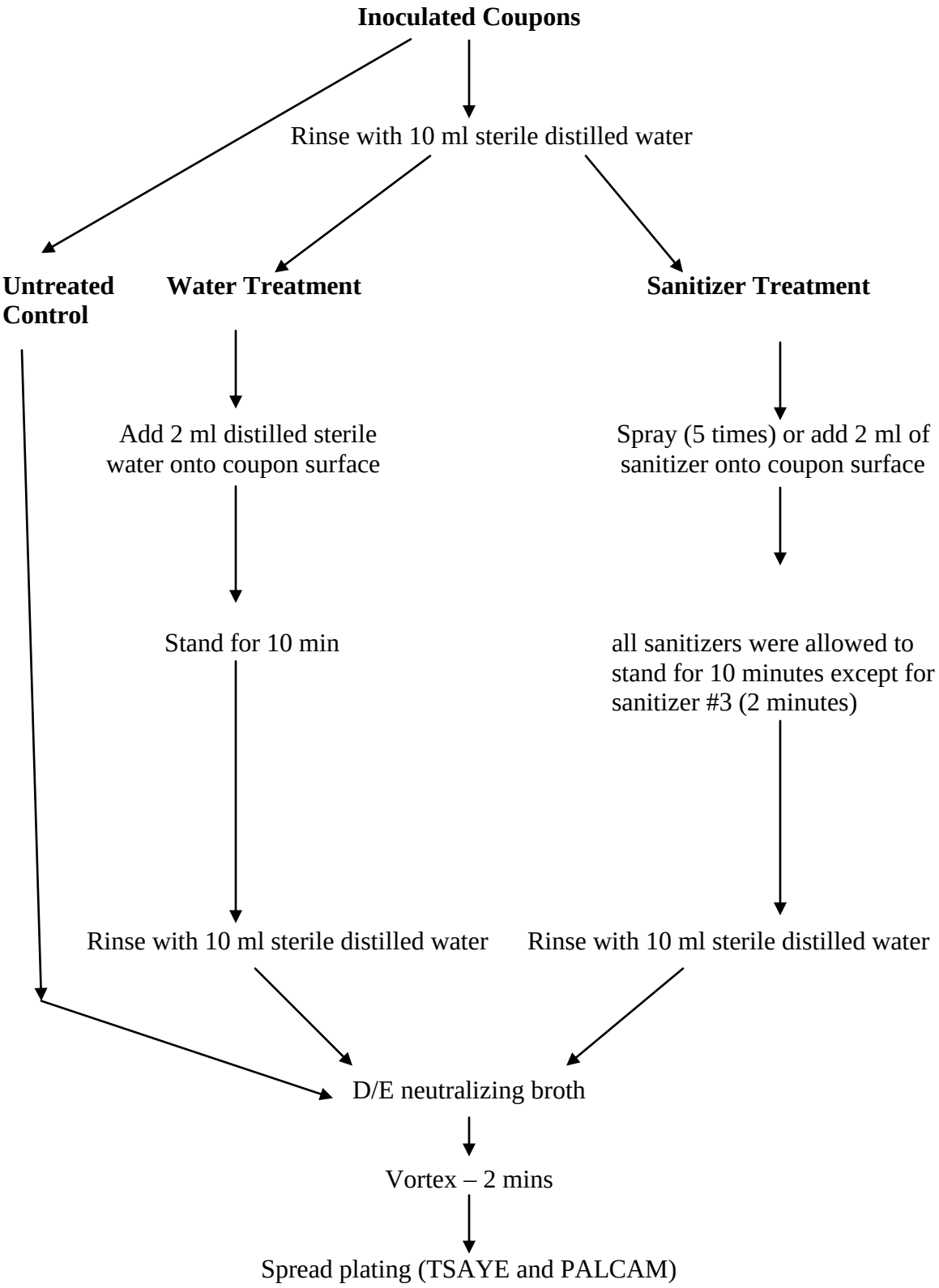
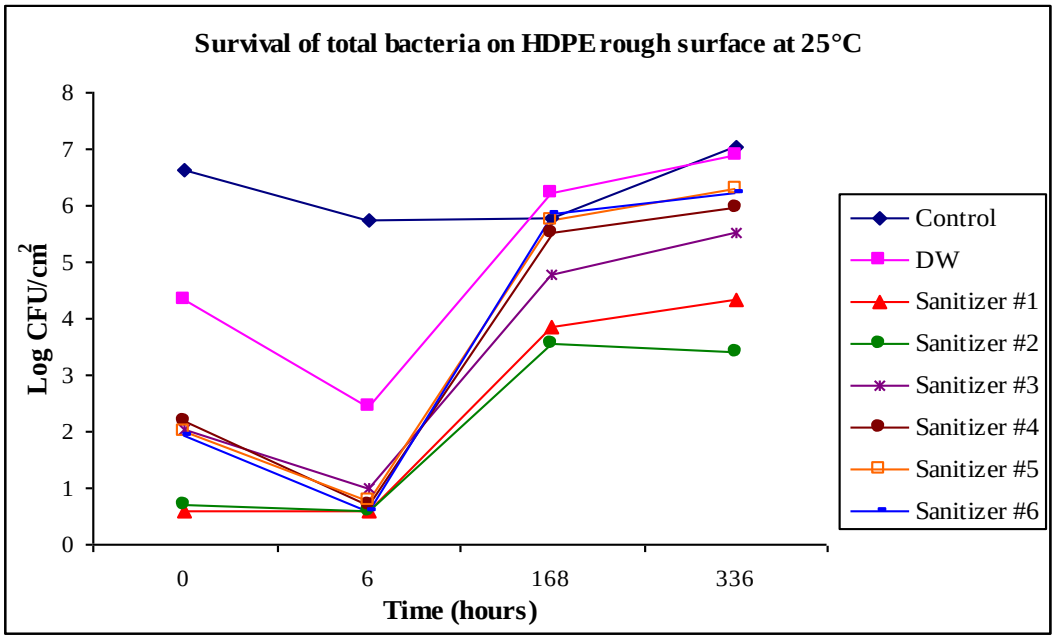


Figure 3.2. **Sanitizer treatment procedure**



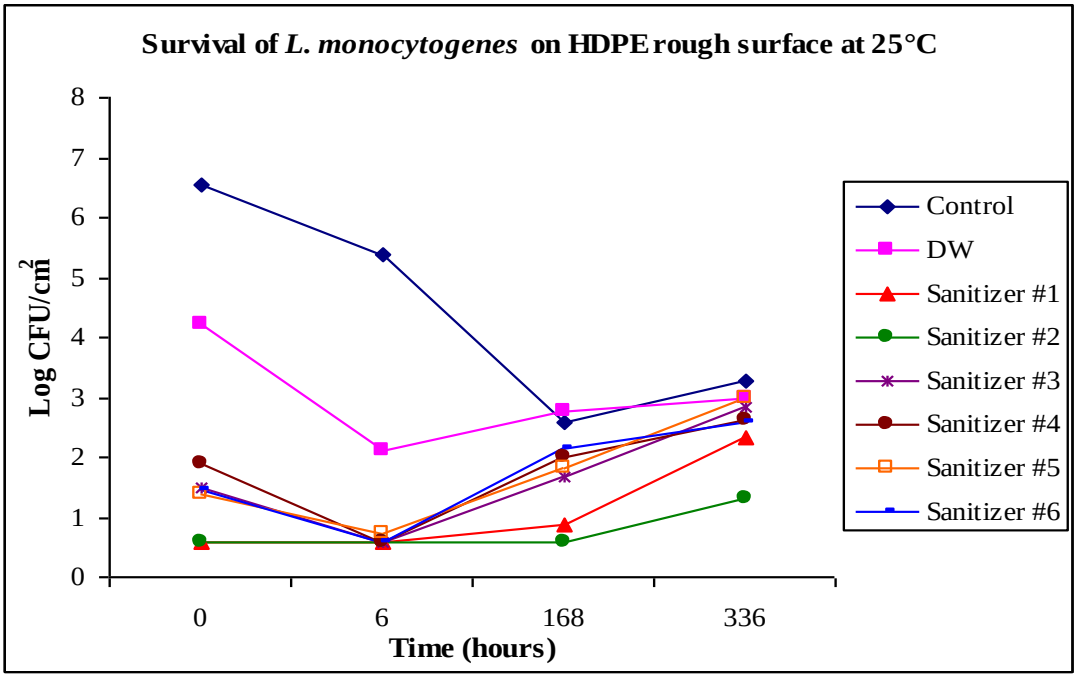
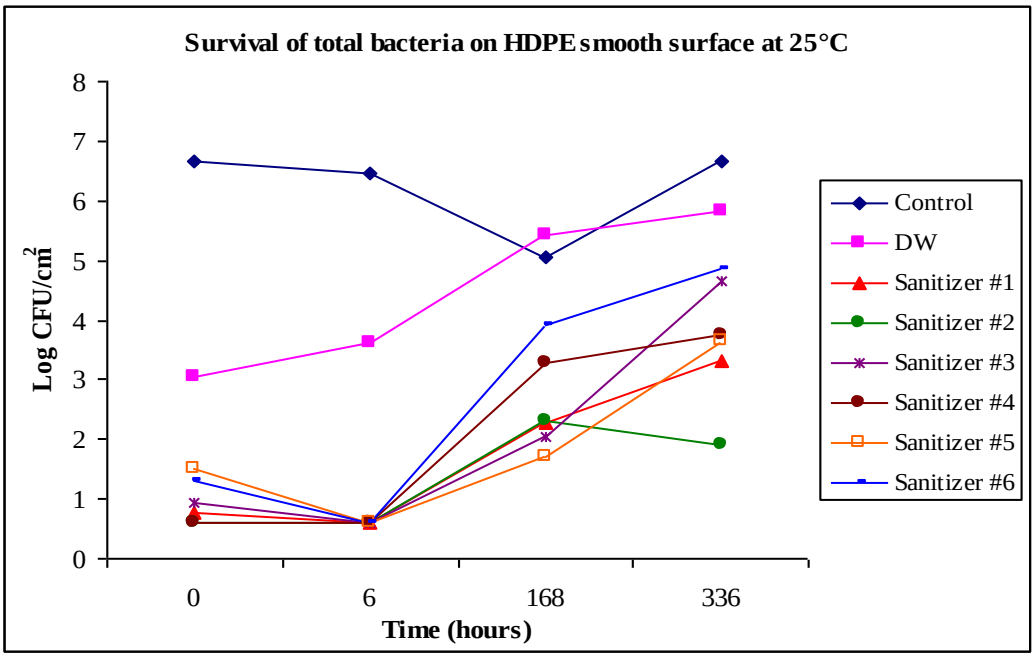


Figure 3.3. Data shown in Table 3.3. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



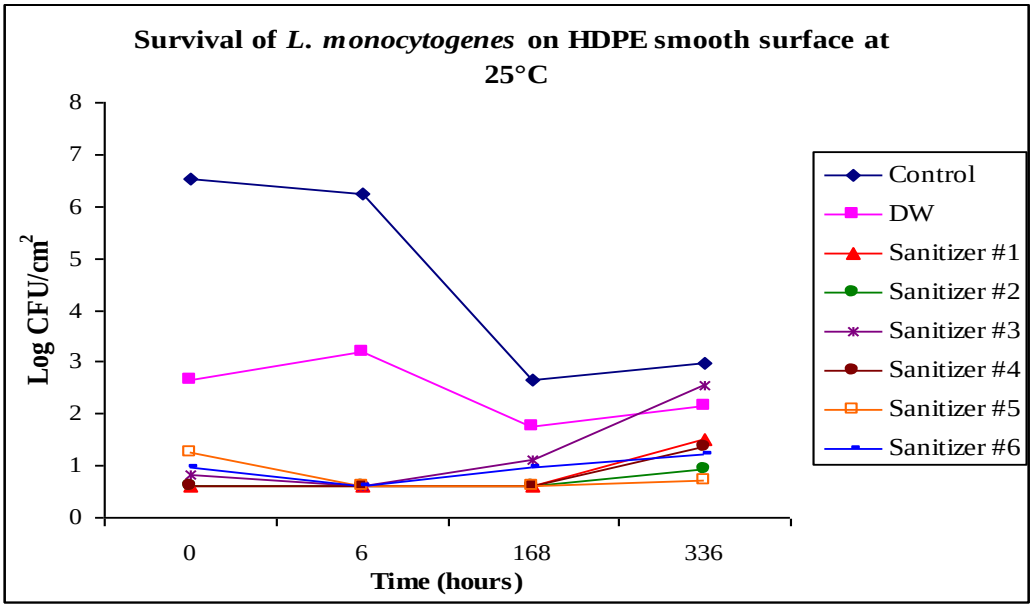
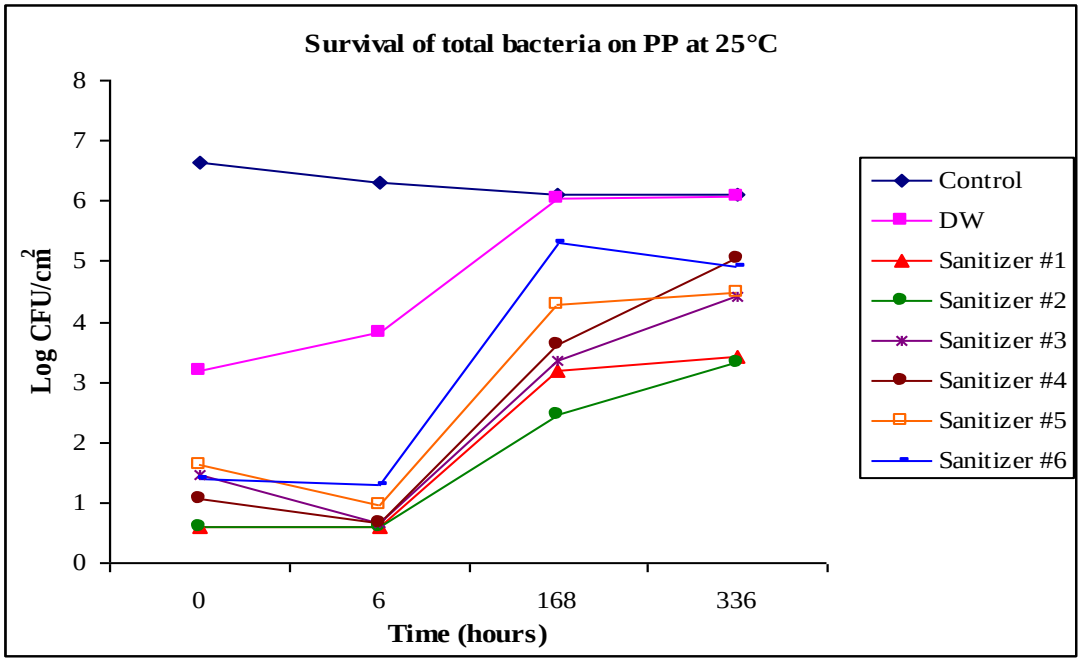


Figure 3.4. Data shown in Table 3.4. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



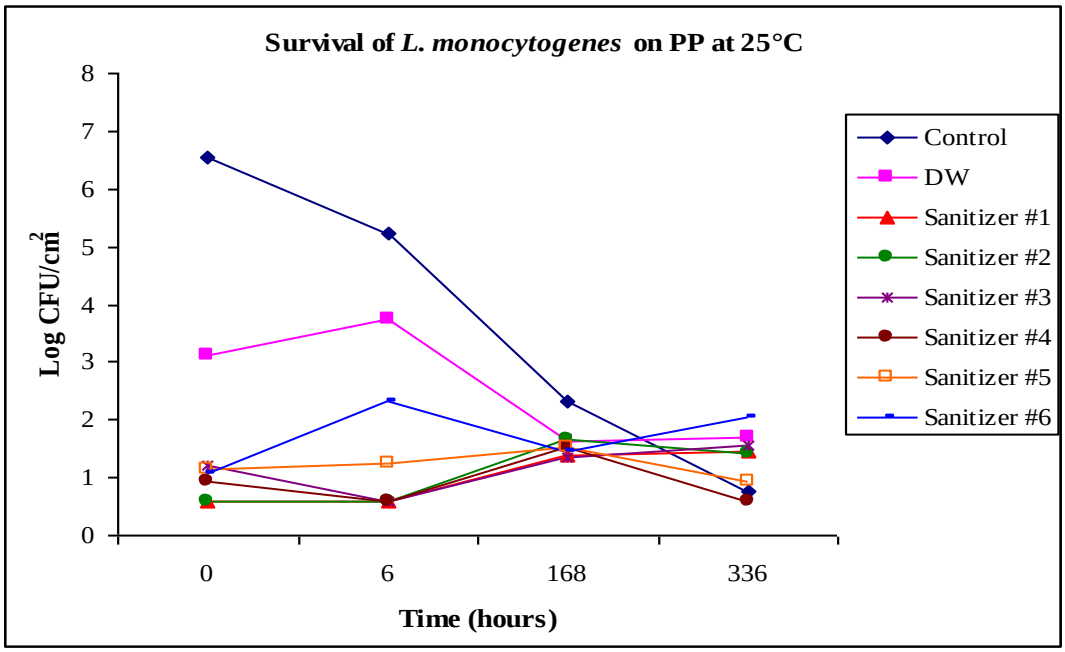
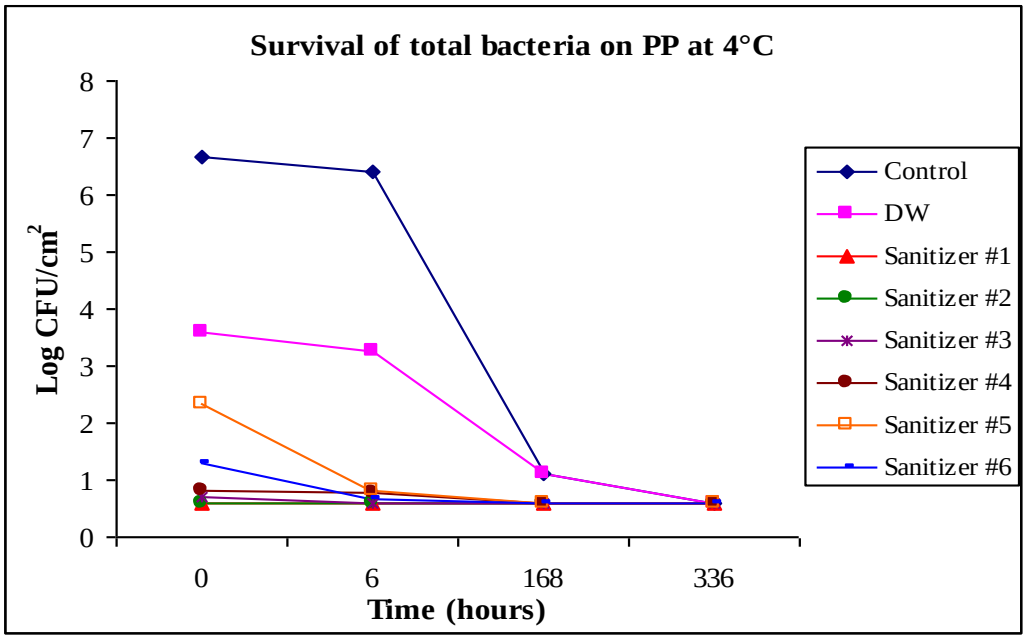


Figure 3.5. Data as shown in Table 3.5. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



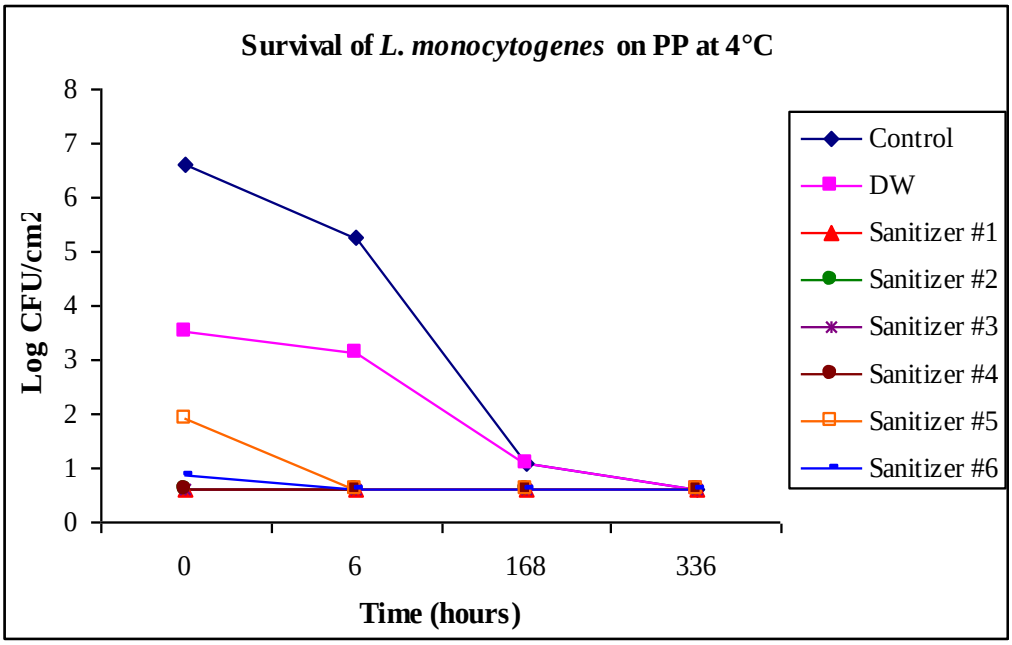
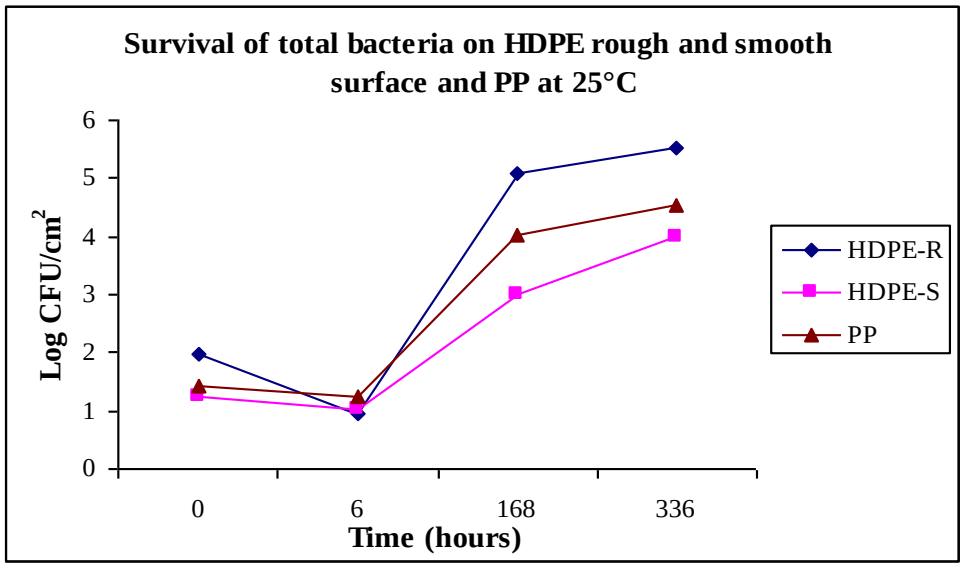


Figure 3.6. Data as shown in Table 3.6. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).



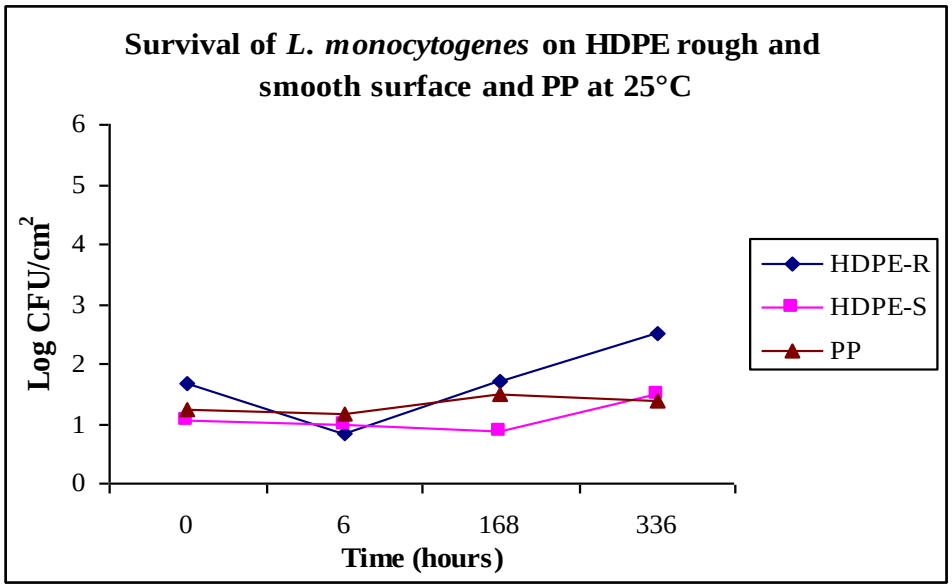
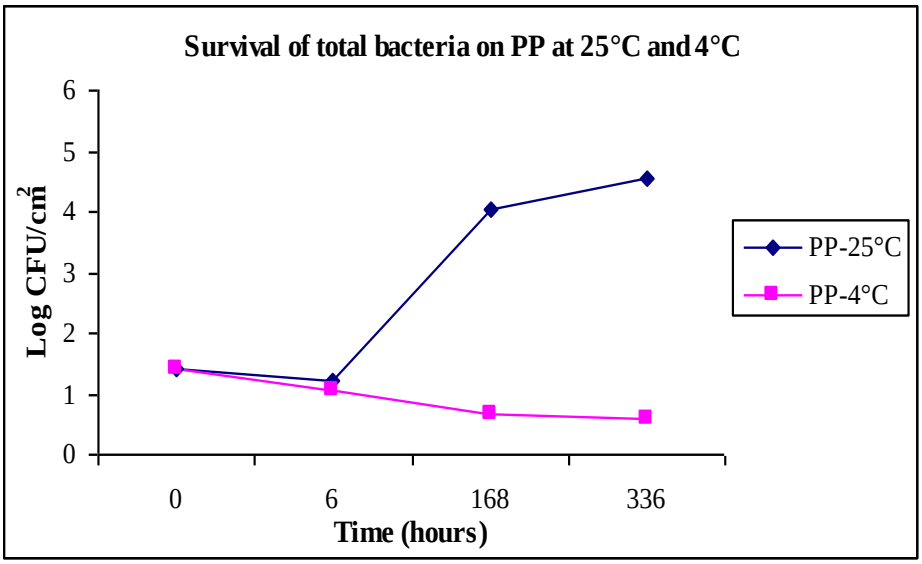


Figure 3.7. Data as shown in Table 3.7. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



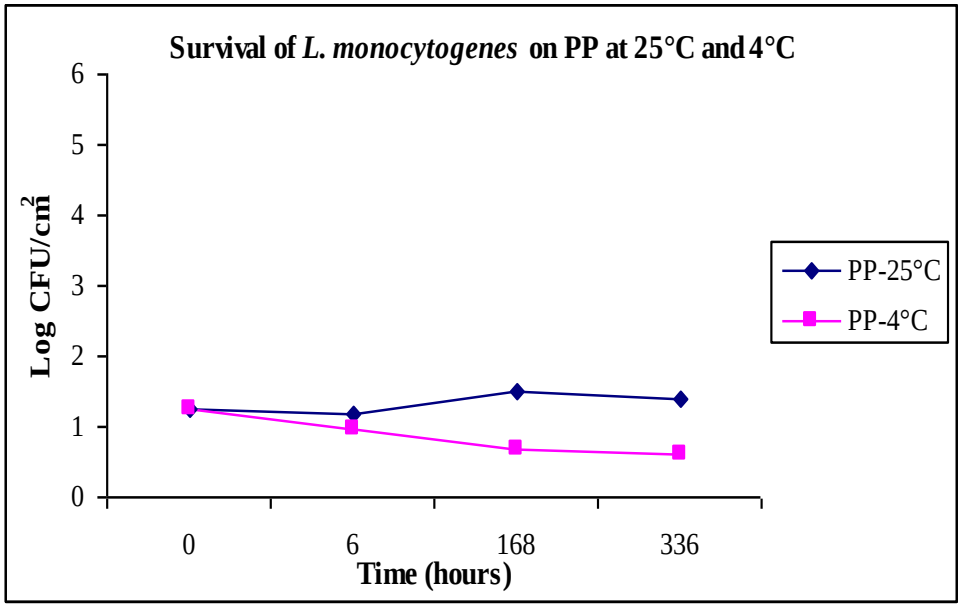
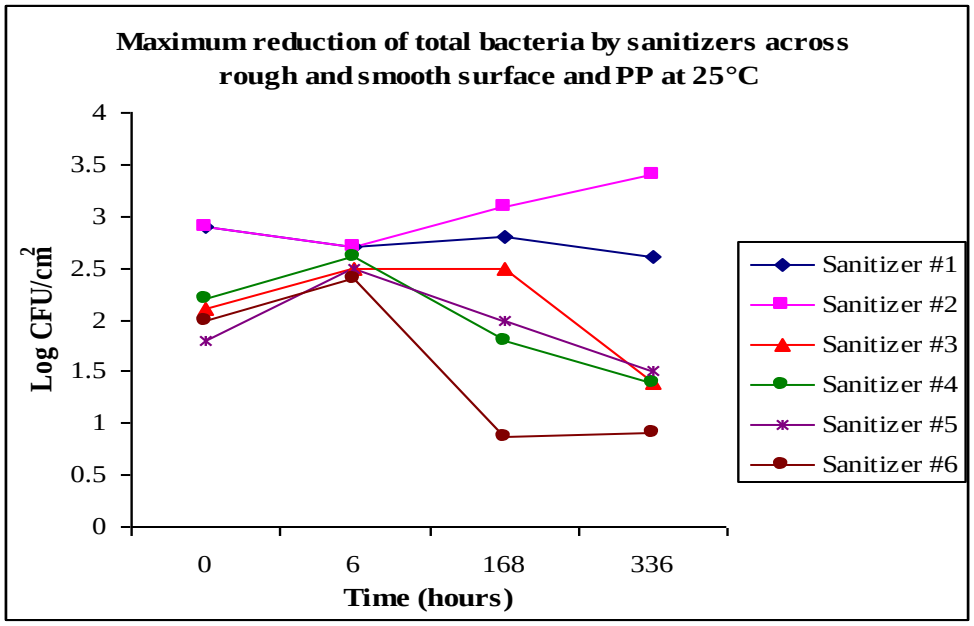


Figure 3.8. Data as shown in Table 3.8. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90%, 25°C and 4°C).



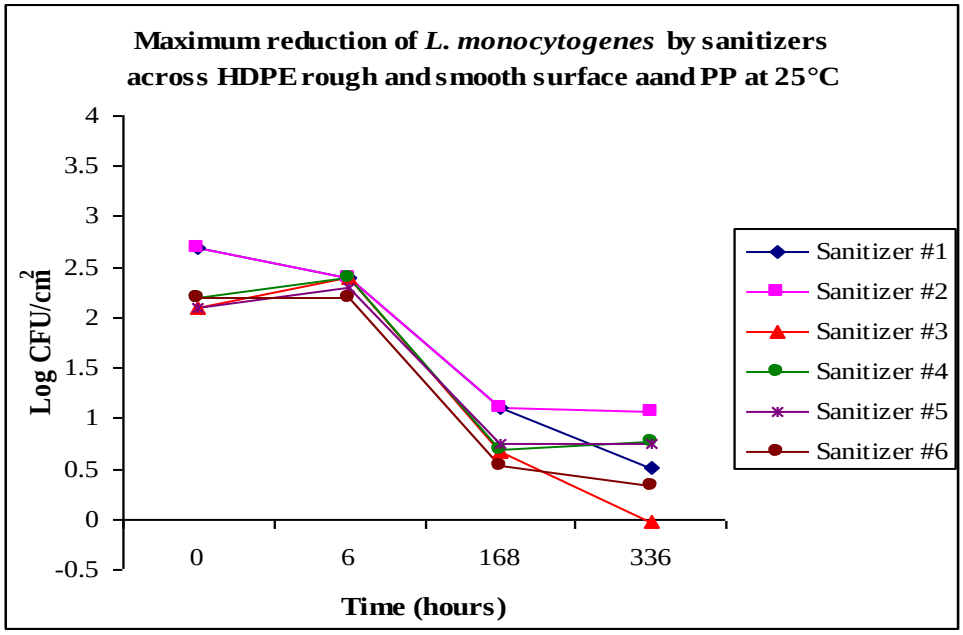


Figure 3.9. Data as shown in Table 3.9. Mean (Log CFU/cm^2) reduction (n = 12) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Table 3.3. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE				PALCAM			
	0 h	6 h	7 d	14 d	0 h	6 h	7 d	14 d
Control	6.63 (0.22) A^a	5.75 (0.56) A^a	5.79 (1.04) AC^a	7.03 (0.39) A^a	6.56 (0.28) A^a	5.39 (0.61) A^a	2.58 (1.13) A^b	3.26 (0.78) A^b
With Distilled Water	4.34 (0.30) B^a	2.44 (0.43) B^b	6.21 (0.25) A^c	6.89 (0.39) A^c	4.22 (0.38) B^a	2.12 (0.42) B^b	2.78 (1.38) A^b	2.99 (0.56) AB^{ab}
Sanitizer 1	< 0.60 C^a	< 0.60 C^a	3.87 (0.77) BD^b	4.33 (0.66) BC^b	< 0.60 C^a	< 0.60 C^a	0.87 (0.54) AB^a	2.33 (0.92) AB^b
Sanitizer 2	0.72 (0.24) CE^a	< 0.60 C^a	3.54 (0.95) BD^b	3.40 (0.89) B^b	< 0.60 C^a	< 0.60 C^a	< 0.60 B^a	1.30 (0.92) B^a
Sanitizer 3	2.02 (0.50) D^a	1.01 (0.81) C^a	4.79 (0.42) CD^b	5.52 (0.34) CD^b	1.50 (0.67) CD^{ab}	< 0.60 C^a	1.68 (1.40) AB^{ab}	2.84 (0.83) AB^b
Sanitizer 4	2.20 (1.40) D^a	0.72 (0.24) C^b	5.52 (0.26) AC^c	5.95 (0.44) AD^c	1.90 (1.39) D^{ab}	< 0.60 C^a	1.99 (1.62) AB^{ab}	2.62 (1.40) AB^b
Sanitizer 5	2.00 (1.25) D^a	0.78 (0.35) C^a	5.73 (0.45) AC^b	6.29 (0.12) AD^b	1.38 (0.90) CD^a	0.72 (0.24) C^a	1.81 (1.39) AB^{ab}	2.98 (0.22) AB^b
Sanitizer 6	1.94 (0.90) DE^a	< 0.60 C^a	5.85 (0.35) AC^b	6.21 (0.24) AD^b	1.45 (0.74) CD^{ab}	< 0.60 C^a	2.16 (1.79) AB^b	2.59 (0.91) AB^b

A–D, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-c, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 3.4. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE				PALCAM			
	0 h	6 h	7 d	14 d	0 h	6 h	7 d	14 d
Control	6.65 (0.27) A^a	6.45 (0.15) A^a	5.05 (1.26) AC^b	6.67 (0.51) A^a	6.53 (0.41) A^a	6.25 (0.14) A^a	2.65 (1.22) A^b	2.99 (0.34) A^b
With Distilled Water	3.05 (0.94) B^a	3.60 (0.00) B^a	5.43 (0.79) A^b	5.83 (0.77) AC^b	2.64 (1.24) B^{ab}	3.19 (0.18) B^a	1.77 (0.33) AB^b	2.16 (0.26) AB^{ab}
Sanitizer 1	0.78 (0.35) C^a	< 0.60 C^a	2.27 (1.45) BD^b	3.33 (0.75) B^b	< 0.60 C^a	< 0.60 C^a	< 0.60 B^a	1.51 (0.82) AB^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	2.31 (0.68) BD^b	1.92 (0.77) D^b	< 0.60 C^a	< 0.60 C^a	< 0.60 B^a	0.93 (0.65) BC^a
Sanitizer 3	0.95 (0.43) C^{ac}	< 0.60 C^a	2.05 (1.24) BD^c	4.65 (0.35) BC^b	0.83 (0.29) C^a	< 0.60 C^a	1.12 (1.04) AB^a	2.53 (0.70) AC^b
Sanitizer 4	< 0.60 C^a	< 0.60 C^a	3.27 (0.86) BE^b	3.74 (0.94) BE^b	< 0.60 C^a	< 0.60 C^a	< 0.60 B^a	1.35 (0.87) AB^a
Sanitizer 5	1.49 (0.87) C^a	< 0.60 C^a	1.70 (0.90) D^a	3.65 (1.80) BE^b	1.27 (0.44) C^a	< 0.60 C^a	< 0.60 B^a	0.72 (0.24) B^a
Sanitizer 6	1.32 (0.64) C^a	< 0.60 C^a	3.93 (0.53) CE^b	4.86 (0.99) CE^b	0.96 (0.43) C^a	< 0.60 C^a	0.96 (0.72) AB^a	1.23 (1.06) BC^a

A–E, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-c, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 3.5. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE				PALCAM			
	0 h	6 h	7 d	14 d	0 h	6 h	7 d	14 d
Control	6.63 (0.11) A^a	6.30 (0.15) A^a	6.11 (0.27) A^a	6.10 (0.38) A^a	6.53 (0.09) A^a	5.24 (1.13) A^a	2.31 (0.73) A^b	0.75 (0.30) A^c
With Distilled Water	3.20 (0.98) B^a	3.83 (1.55) B^a	6.03 (0.30) A^b	6.07 (0.03) A^b	3.10 (0.80) B^a	3.73 (1.75) B^a	1.63 (0.86) A^b	1.69 (1.56) A^b
Sanitizer 1	< 0.60 C^a	< 0.60 C^a	3.18 (0.82) BC^b	3.42 (0.26) B^b	< 0.60 C^a	< 0.60 C^a	1.38 (0.66) A^a	1.47 (1.00) A^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	2.46 (0.40) B^b	3.33 (0.18) B^b	< 0.60 C^a	< 0.60 C^a	1.65 (0.60) A^a	1.43 (0.95) A^a
Sanitizer 3	1.47 (1.11) C^a	0.68 (0.15) C^a	3.35 (0.63) BC^b	4.42 (0.67) BC^b	1.20 (1.20) C^a	< 0.60 C^a	1.36 (0.71) A^a	1.56 (1.12) A^a
Sanitizer 4	1.06 (0.38) C^a	0.68 (0.15) C^a	3.62 (0.91) BC^b	5.03 (0.73) AC^c	0.95 (0.43) C^a	< 0.60 C^a	1.52 (0.91) A^a	< 0.60 A^a
Sanitizer 5	1.63 (1.13) C^a	0.95 (0.40) C^a	4.29 (0.95) CD^b	4.49 (1.65) BC^b	1.15 (1.10) C^a	1.26 (0.85) C^a	1.52 (0.73) A^a	0.92 (0.63) A^a
Sanitizer 6	1.39 (0.92) C^a	1.31 (0.89) C^a	5.30 (0.59) AD^b	4.92 (0.61) AC^b	1.06 (0.72) C^a	2.31 (0.73) C^a	1.47 (1.10) A^a	2.04 (1.67) A^a

A–D, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-c, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 3.6. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Treatment	TSAYE				PALCAM			
	0 h	6 h	7 d	14 d	0 h	6 h	7 d	14 d
Control	6.66 (0.14) A^a	6.40 (0.27) A^a	1.10 (0.58) A^b	< 0.60 A^b	6.59 (0.18) A^a	5.28 (0.99) A^b	1.10 (0.58) A^c	< 0.60 A^c
With Distilled Water	3.58 (0.93) B^a	3.27 (0.97) B^a	1.10 (0.58) A^b	< 0.60 A^b	3.51 (0.87) B^a	3.12 (1.15) B^a	1.10 (0.58) A^b	< 0.60 A^b
Sanitizer 1	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 3	0.72 (0.24) C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 4	0.81 (0.42) C^a	0.78 (0.35) C^a	< 0.60 A^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 5	2.34 (0.52) BD^a	0.81 (0.42) C^b	< 0.60 A^b	< 0.60 A^b	1.92 (0.75) D^a	< 0.60 C^b	< 0.60 A^b	< 0.60 A^b
Sanitizer 6	1.30 (0.72) CD^a	0.68 (0.15) C^{ab}	< 0.60 A^b	< 0.60 A^b	0.89 (0.40) CD^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a

A–D, means within a column and within the same day of sanitizer treatment at 4°C are significantly different ($P < 0.05$)

a-c, means within a row and within each sanitizer is significantly different ($P < 0.05$)

Table 3.7. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Surfaces	TSAYE				PALCAM			
	Time							
	0 h	6 h	7 d	14 d	0 h	6 h	7 d	14 d
HDPE-R	1.97 (0.22) A	0.96 (0.12) A	5.07 (0.23) A	5.51 (0.23) A	1.66 (0.19) A	0.83 (0.13) A	1.70 (0.4) A	2.52 (0.2) A
HDPE-S	1.25 (0.22) B	1.03 (0.12) AB	2.99 (0.23) B	4.00 (0.23) B	1.07 (0.19) B	0.97 (0.13) A	0.89 (0.4) B	1.49 (0.2) B
PP	1.42 (0.22) B	1.23 (0.12) B	4.03 (0.23) C	4.53 (0.23) B	1.24 (0.19) AB	1.17 (0.13) A	1.50 (0.4) AB	1.39 (0.2) B

A–C, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P < 0.05$)

Table 3.8. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C and 4°C).

Surfaces	TSAYE				PALCAM			
	Time							
	0 h	6 h	7 d	14 d	0 h	6 h	7 d	14 d
PP - 25°C	1.42 (0.15) A	1.23 (0.19) A	4.03 (0.11) A	4.53 (0.08) A	1.24 (0.13) A	1.17 (0.17) A	1.50 (0.13) A	1.39 (0.28) A
PP - 4°C	1.42 (0.15) A	1.05 (0.19) A	0.67 (0.11) B	0.60 (0.08) B	1.24 (0.13) A	0.96 (0.17) A	0.67 (0.13) B	0.60 (0.28) B

A & B, means within a column and within the same day of sanitizer treatment on food contact surfaces stored at two different temperatures; 25°C and 4°C are significantly different ($P < 0.05$)

Table 3.9. Mean (Log CFU/cm^2) reduction (n = 12) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	TSAYE				PALCAM			
	0 h	6 h	7 d	14 d	0 h	6 h	7 d	14 d
Sanitizer #1	2.9 (0.53) A	2.7 (0.40) A	2.8 (0.33) A	2.6 (0.26) A	2.7 (0.6) A	2.4 (0.5) A	1.1 (0.6) A	0.51 (0.6) A
Sanitizer #2	2.9 (0.53) A	2.7 (0.40) A	3.1 (0.33) A	3.4 (0.26) B	2.7 (0.6) A	2.4 (0.5) A	1.1 (0.3) A	1.06 (0.6) A
Sanitizer #3	2.1 (0.53) AB	2.5 (0.40) A	2.5 (0.33) AC	1.4 (0.26) C	2.1 (0.6) A	2.4 (0.5) A	0.67 (0.3) A	-0.02 (0.6) A
Sanitizer #4	2.2 (0.53) AB	2.6 (0.40) A	1.8 (0.33) B	1.4 (0.26) C	2.2 (0.6) A	2.4 (0.5) A	0.69 (0.3) A	0.76 (0.6) A
Sanitizer #5	1.8 (0.53) B	2.5 (0.40) A	2.0 (0.33) BC	1.5 (0.26) C	2.1 (0.6) A	2.3 (0.5) A	0.75 (0.3) A	0.74 (0.6) A
Sanitizer #6	2.0 (0.53) AB	2.4 (0.40) A	0.86 (0.33) D	0.9 (0.26) C	2.2 (0.6) A	2.2 (0.5) A	0.53 (0.3) A	0.33 (0.6) A

A–D, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P < 0.05$)

CHAPTER 4

Efficacy of Commonly Available Sanitizers and Household Compounds against *Listeria monocytogenes* biofilms on Food Contact Surfaces With/Without Daily Exposure to Nutrients

ABSTRACT

Listeria monocytogenes cells may adhere to various food contact surfaces, including those in households, and, if not properly cleaned, form biofilms which can be resistant to sanitizers. This study is different from the previous study in two ways. Firstly, the sanitizer application methods are different and secondly, in this study half the food contact surfaces are treated with daily nutrients where as the rest are not. The objective of this study was to determine survival and persistence of *L. monocytogenes* on high density polyethylene (HDPE) cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on polypropylene (PP) cutting boards and utensils at ambient (25°C) and refrigerator temperature (4°C) under simulated conditions with and without daily exposure to nutrients as may happen under food preparation and cleaning practices commonly employed in households and the food industry. The effectiveness of three commonly available sanitizers and three household compounds in reducing young and established biofilms of *L. monocytogenes* on HDPE and PP surfaces at ambient and

refrigerator temperature was also compared. The study helped to understand the impact of daily exposure of *L. monocytogenes* biofilms to nutrients versus no exposure on the efficacy of commercial and homemade sanitizers at 25°C and 4°C. HDPE (rough and smooth surface) and PP (smooth) coupons (2x5 cm) were inoculated (6.0-7.0 log CFU/cm^2) with a 5-strain composite of *L. monocytogenes* in ham homogenate. HDPE coupons were stored at 25°C and PP coupons at 25°C or 4°C for up to 21 d. In repeated 24-h cycles, 0.3 ml diluted broth (TSBYE) was deposited on the inoculated surface of one-half of coupons to simulate nutrient-rich use, then rinsed with 10 ml distilled water 8 h later and stored 16 h (starvation); the other half of inoculated coupons were stored throughout without added broth. Sanitizer solutions (one each lactic acid-, quaternary ammonium-, acetic acid-, and hydrogen peroxide-based and two sodium hypochlorite-based) were applied to coupons at 0 h, 6 h, 24 h, 4 d, 7 d, 14 d and 21 d storage. Coupons were analyzed for pathogen (PALCAM agar) and total microbial (tryptic soy agar with 0.6% yeast extract) counts. Multi-species biofilms, containing 5.0-6.0 log CFU/cm^2 *L. monocytogenes*, developed and survived up to 21 d on all surfaces at 25°C, with survival greater on HDPE than PP surfaces and on coupons with daily nutrient exposure. All products were effective against *L. monocytogenes* on coupons sanitized within 24 h (4° or 25°C). On established biofilms (4, 7, 14 or 21 d), all products were effective against *L. monocytogenes* on all coupons stored at 4°C and on coupons stored at 25°C without daily nutrient enrichment i.e. sanitizers decreased total bacteria and *L. monocytogenes* cells to below detection limit. However, on coupons receiving daily nutrient enrichment and stored at 25°C, all products were increasingly ineffective as storage time increased (2-4 log CFU/cm^2 survival on HDPE surfaces sanitized on d 21). Repeated exposure of food

contact surfaces to nutrients such as during use or with no cleaning and sanitation increases the resistance of *L. monocytogenes* biofilms to sanitizers. To reduce such risk, consumers should regularly clean and sanitize after each use and may consider treating surfaces with household products such as vinegar when commercial sanitizers are not available.

4.1. INTRODUCTION

Poor sanitation of food contact surfaces and equipment has played an important role in foodborne disease outbreaks, especially those involving *L. monocytogenes. L. monocytogenes* is a foodborne pathogen which is widely distributed in the environment and also exhibits psychotropic growth (Kwang and Frank, 1994). Surfaces of equipment used for food handling and processing are recognized as sources of microbial contamination and recontamination, especially when improperly cleaned or sanitized.

Listeria monocytogenes cells adhere to food contact surfaces, including polyethylene and polypropylene, and if not properly cleaned, form biofilms which may be a major source of contamination (Blackman and Frank, 1996). Cutting boards, plastic microwaveable utensils, refrigerator shelves, etc. are made with polyethylene and polypropylene.

Biofilms of *Listeria* have been shown to be much more resistant to stress and to sanitizing agents than planktonic cells (Stopforth et al., 2002; Pan et al., 2006). The initial attachment of bacteria is critical for the formation of a bacterial biofilm as all other cells within a biofilm structure rely on the interaction between the surface and bacterial cell for their survival (Palmer et al., 2007). Cells in a biofilm are known to be more resistant to

sanitizers than planktonic cells due to the formation of an exopolysaccharide matrix that binds cells, surrounds the biofilm, and protects it from sanitizers (Lomander et al., 2004). If the matrix is not completely removed when sanitizing a surface, the pathogen will readily reattach to the surface and a biofilm will form again (Gibson et al., 1999). Studies have assessed the efficacy of various sanitizers against microbial biofilms in food processing environments (Jeyasekaran and Karunasagar, 2000; Pan et al., 2006; Yang et al., 2009), but little attention has focused on the efficacy of sanitizers designed for home use against *L. monocytogenes* biofilms in the presence or absence of daily exposure to nutrients.

The overall goal of this study was to evaluate the resistance of *L. monocytogenes* biofilms to stresses under laboratory conditions that mimic food preparation and processing environments in home kitchens. Specifically, the objectives of this study were to determine the survival and persistence of *L. monocytogenes* on HDPE cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on PP cutting boards and utensils at ambient (25°C) and refrigerator temperature (4°C) under simulated household conditions with and without daily exposure of nutrients as might occur during daily food preparation and cleaning practices in households and the food industry. The effectiveness of three commonly available sanitizers and three household compounds in reducing young and established biofilms of *L. monocytogenes* on HDPE and PP surfaces at room and refrigerator temperatures was also compared. The study helped to understand the impact of repeated nutrient enrichment of *L. monocytogenes* biofilms, as may occur in food processing and handling environments without daily sanitation, on the efficacy of commercial and homemade sanitizers at 25°C and 4°C.

4.2. MATERIALS AND METHODS

4.2.1. Preparation of ham homogenate. Ten gram of ham samples (cured with water, sugar, salt, dextrose, sodium phosphate, honey, sodium erythorbate and sodium nitrite) were mixed with 90 ml of sterile distilled water in a whirl-pak bag (Nasco, Fort Atkinson, WI) and homogenized (Masticator, IUL Instruments, Barcelona, Spain) at 6 strokes/second for 2 minutes. The suspension of the product was passed through cheese-cloth, autoclaved for 18 minutes at 121°C and cooled at ambient temperature (25°C) before storing at 4°C for use within 2 days. Ham homogenate is used as the suspending medium of *L. monocytogenes* to simulate contamination on cutting boards.

4.2.2. Bacterial strains and growth conditions. Five strains (Table 4.1) of human disease associated *L. monocytogenes* covering genetic diversity of ribotypes, serotypes, and lineages (Fugett et al., 2006) were used in the experiment. All strains were kept on slants at 4°C and were activated by three successive transfers in tryptic soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton Dickinson Co., Sparks, Md) at 30°C for 24 hours. For inoculum preparation, 24 hour cultures of each strain were centrifuged separately (Eppendorf model 5810 R, Brinkmann Instruments Inc., Westbury, NY) at 6000 rpm for 15 minutes at 4°C. The harvested cells were resuspended in 10 ml of phosphate buffered saline (pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4.7H_2O$, 8 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water) and centrifuged as above. The harvested cells were resuspended in 10 ml of ham homogenate (each culture separately) prepared from product of same lot as that used in the study (but kept frozen during the study at - 20°C). Cell cultures suspended in ham homogenate were stored at 7°C for 48 hours to allow for acclimatization of the cells to a low temperature food environment.

Equal volumes (10 ml) of cell suspensions of each of the 5 strains were then combined for use in the study (Figure 4.1). The 5 strain mixture was surface plated on TSAYE and PALCAM agar (Difco) for determination of initial populations as well as for testing the purity of the inoculum (Figure 4.1).

4.2.3. Cutting board coupons. High density polyethylene (HDPE) used for cutting boards with rough and smooth surfaces and polypropylene (PP) used for cutting boards, plastic utensils and as refrigerator materials were acquired (Fort Collins Plastics, Fort Collins, CO). These materials are Food and Drug Administration (FDA) approved for use on food contact surfaces (USFDA reg. 21CFR177.1520 item 2.1).The HDPE materials were cut into 2x5 cm coupons (thickness = 2 mm for HDPE smooth coupons and 6 mm for HDPE rough coupons), soaked in 300 ppm sodium hypochlorite solution (5 ml 6% sodium hypochlorite bleach into 950 ml distilled water) for 2-3 hours, air dried, and then autoclaved under the gravity cycle at 121°C for 20 minutes. The polypropylene sheets were cut into 2x5 cm coupons (thickness = 2 mm), soaked in a detergent solution (approximately 3 teaspoons of Dawn dishwashing liquid soap, Procter & Gamble to 1 gallon of tap water) for 2 – 3 hours, air dried, and then autoclaved under the gravity cycle at 121°C for 20 minutes.

4.2.4. Inoculation of coupons and biofilm formation. The sterile coupons were first placed on a clean sterile tray. Inoculated ham homogenate (100 µl) was placed on each coupon and spread evenly across the top surface of the coupon with the help of 100 µl pipette, resulting in a final concentration of $10^6 – 10^7$ CFU/cm^2. The trays were then placed into an ambient temperature incubator (25°C) with the humidity adjusted to 90% using a saturated K_2SO_4 solution. HDPE coupons with rough and smooth surfaces were

incubated at 25°C, whereas PP coupons were incubated at 25°C and 4°C. The relative humidity was measured two times per day with an Electronic Humidity Meter (Time – Faver, Temperature and Humidity Data Logger, Dickson Addison, IL) and the temperature was measured twice daily using an Easy Read Thermometer (H.B, USA) (Appendix 4.1, 4.2, 4.3 and 4.4). After incubation at 25°C and 4°C for 8 hours, half the coupons (of each type) were removed from the incubator with forceps and 10 ml of sterile distilled water was pipetted continuously at the upper end of the coupon so that the whole outer surface of the coupon was washed slowly with flowing distilled water to remove loosely attached cells from the biofilm. Coupons were then subjected to up to 21 repeated 24-hr cycles modified from Pan et al. (2006) to simulate general cutting board use and cleaning conditions in the home. On each day, 10-fold diluted TSBYE medium (0.3 ml) was added to half the coupons. The coupons were incubated for 8 hours at 25°C (for HDPE coupons) and 25°C & 4°C (for PP coupons) to simulate exposure to water and nutrients during food preparation, after which the coupons were rinsed with 10 ml sterile distilled water and stored without liquid medium (starvation) for 16 hours at 25°C and 4°C with 90% humidity. The remaining half of each type of coupons (not treated with nutrient to simulate daily use and cleaning of the cutting board) were incubated in respective incubators soon after inoculation with ham homogenate and removed only at the time of the scheduled sanitizer treatment, i.e., at 4 d, 7 d, 14 d and 21 d incubation (Figure 4.2).

4.2.5. Sanitizing methods. Three commercially available sanitizers and three household compounds were purchased from a local supermarket based on their commercial availability and intended usage on food contact surfaces in the home (Table 4.2). The

three commercial sanitizers came in spray bottles, while the three household compounds came in regular bottles, one of which was supplied by the manufacturer as a concentrated liquid (Sanitizer #4 – sodium hypochlorite). This sanitizer was diluted with sterile distilled water to 300 ppm sodium hypochlorite in our laboratory on the day of use. All sanitizers varied in chemical composition and concentrations of active ingredients (Table.4.2).

4.2.6. Sanitizer treatment of biofilm cells. For each type of surface, 16 inoculated coupons were removed at 0 h (before incubation), 6 h and 24 h incubation, and not treated (control), treated with sterile distilled water or treated with one of the six sanitizer treatments (Figure 4.3). At 96 h, 168 h, 336 h and 504 h of incubation (4, 7, 14 and 21 d respectively) 16 inoculated coupons of each surface type with and without daily nutrient exposure (HDPE with rough and smooth surfaces incubated at 25°C and PP incubated at 25°C and 4°C) were removed from the incubator and not treated (Control), treated with sterile distilled water or treated with one of the six sanitizer treatments (Figure 4.3). Untreated coupons (control) were directly placed into Nalgene centrifuge tube (Nalge Nunc, Rochester, NY) containing 40 ml of D/E neutralizing broth (Difco), while other coupons were first rinsed with 10 ml of sterile distilled water to remove loosely attached bacterial cells, then treated as follows.

For sterile distilled water treatment, 2 ml sterile distilled water was pipetted onto the surface of the coupon, allowed to stand for 10 min, and then tipped with the help of forceps so that water on the surface of the coupon dropped into a petri dish. The coupon was again rinsed with 10 ml distilled water, then placed into a centrifuge tube containing 40 ml D/E neutralizing broth (Difco) and 10 glass beads of 4 mm diameter (Fischer

Scientific, Houston, TX). Glass beads aid in the removal of attached cells from surfaces (Stopforth et al., 2002). The water left behind in the petri dish was poured into another centrifuge tube. This procedure was done to check the number of cells that were destroyed just with plain distilled water.

For evaluation of sanitizer treatment, each coupon was placed into a petri dish and subjected to sanitation according to manufacturers' instructions as follows: 10 ml of each sanitizer was first placed in an empty petri dish with the help of a pipette. The coupons were rinsed with 10 ml distilled water and placed into the petri dish containing the sanitizer such that the biofilm was in contact with the sanitizer. The coupons were allowed to stand for 10 minutes for sanitizers #1, 2, 4, 5, and 6 and 2 minutes for sanitizer #3, according to manufacturers' instruction for each sanitizer; and all the coupons were again rinsed with 10 ml sterile distilled water.

After sanitation, each coupon was placed into a 85 ml Nalgene centrifuge tube (Nalge Nunc, Rochester, NY) containing 40 ml of D/E neutralizing broth and 10 glass beads. Biofilm bacteria were removed from the coupons by vortexing (Vortex-Genie 2, Scientific Industries, Inc, Bohemia, NY) for 2 min at speed level of 10 (Figure 4.3). Samples were spread plated onto both TSAYE and PALCAM plates after 10-fold dilutions in 0.1% buffered peptone water (Difco). Total bacterial counts were counted after incubation at 25°C for 48 h, whereas *L. monocytogenes* colonies were counted after incubation at 30°C for 48 h.

One ml of the used sanitizer left in the petri dish after sanitizing the coupon was also added to 9 ml D/E neutralizing broth. These samples were also spread plated onto both TSAYE and PALCAM to check if *Listeria* survived in the sanitizer. It was found

that there was no survival of *Listeria* on PALCAM, but total bacterial colonies were found on TSAYE (Appendix 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11, and 4.12).

4.2.7. Data analysis. All tests were performed in two independent replication trials with two samples being evaluated per replicate. Microbial counts were reported in terms of $\log_{10}$ CFU/cm^2. Estimated reductions were analyzed statistically to compare sanitizer treatment effects. The chemical reductions due to sanitizers were calculated as the difference in cell numbers remaining after water treatment and those remaining after sanitizer treatment. Data were analyzed using the Glimmix Procedure in SAS (SAS Institute Inc., Cary, NC). The Glimmix procedure helps to specify a generalized linear mixed model and to perform confirmatory inference. Descriptive statistics (means and standard deviations) were computed and analyses of variance were performed for statistical differences ($P<0.05$). Independent variables in the mixed models procedure were type of surface, type of sanitizer, media, time and their interactions. Random effects were replicate and replicate interactions with surface and sanitizer. The least significant difference procedure was used to perform mean separation. Analysis of variance on the effects of nutrient, sanitizer treatment, media and storage time on high density polyethylene (HDPE) with rough and smooth surface incubated at 25°C and polypropylene (PP) surface incubated at 4 and 25°C is provided (Appendix 4.13, 4.14, 4.15 and 4.16).

4.3. RESULTS

4.3.1. Survival of *L. monocytogenes* and formation of biofilms on HDPE rough surfaces with daily exposure to nutrients at 25°C. For the control coupons (no

treatment), total bacterial cells on HDPE rough surfaces as enumerated on TSAYE (6.87 log CFU/cm^2 at 0 h), decreased (P<0.05) by 1.53 log CFU/cm^2 to 5.34 log CFU/cm^2 within 24 h, and then increased (P<0.05) to 7.77 log CFU/cm^2 by d 21 (Table 4.3, Figure 4.4). Similarly, the total number of *L. monocytogenes* cells, as enumerated on PALCAM (6.79 log CFU/cm^2 at 0 h), decreased (P<0.05) by 3.46 log CFU/cm^2 to 3.33 log CFU/cm^2 on d 4 and then increased (P<0.05) to 4.68 log CFU/cm^2 by d 21 (Table 4.4, Figure 4.4).

Rinsing coupons with distilled water resulted in significant (P<0.05) decreases in total bacteria and *L. monocytogenes* at 0 and 6 h (2.47 to 2.95 log CFU/cm^2 reductions from control coupons), but not thereafter, with 7.26 CFU/cm^2 total bacteria and 4.13 log CFU/cm^2 *L. monocytogenes* remaining at d 21 (Tables 4.3, 4.4 and Figure 4.4).

Sanitizer #2 effectively decreased total bacteria and *L. monocytogenes* counts on inoculated HDPE rough surfaces to below the detection limit (<0.60 log CFU/cm^2) at 0 and 6 h exposure. Even at 24 h, sanitizer #2 was fairly effective in that 0.99 log CFU/cm^2 total bacteria and 0.9 log CFU/cm^2 *L. monocytogenes* counts remained after sanitizer treatment. All other sanitizers tested were also fairly effective in that <0.60-1.14 log CFU/cm^2 total bacteria and *L. monocytogenes* counts remained after sanitizer treatment at 0 h, <0.60-2.28 log CFU/cm^2 total and <0.60-2.1 log CFU/cm^2 *L. monocytogenes* counts remained following sanitizer treatment at 6 h and <0.60-2.08 log CFU/cm^2 total and 0.72-1.96 log CFU/cm^2 *L. monocytogenes* counts remained following sanitizer treatment at 24 h (Tables 4.3, 4.4 and Figure 4.4).

Sanitizers# 1, 2 and 5 reduced (P<0.05) *L. monocytogenes* counts to below detection limit on d 4 biofilms, but not thereafter. On biofilms allowed to develop for 7-

21 d, all sanitizer treatments produced significant decreases ($P<0.05$) in counts from control and water treated coupons, but still had 4.32-6.49 log CFU/cm^2 total bacterial and 0.83-4.00 CFU/cm^2 *L. monocytogenes* cells remaining (Tables 4.3, 4.4 and Figure 4.4).

4.3.2. Survival of *L. monocytogenes* and formation of biofilms on HDPE smooth surfaces with daily exposure to nutrients at 25°C. For the control coupons (no treatment), total bacterial cells on HDPE smooth surfaces as enumerated on TSAYE decreased ($P<0.05$) from 6.80 log CFU/cm^2 at 0 h to 4.27 log CFU/cm^2 within 24 h, then increased ($P<0.05$) to 7.56 log CFU/cm^2 by d 21 (Table 4.5, Figure 4.5). Similarly, the total number of *L. monocytogenes* cells, as enumerated on PALCAM (6.75 log CFU/cm^2 at 0 h) decreased ($P<0.05$) by 3.71 log CFU/cm^2 within d 4, then increased ($P<0.05$) to 5.11 log CFU/cm^2 by d 21(Table 4.6, Figure 4.5).

Rinsing coupons with distilled water resulted in significant reductions ($P<0.05$) in total bacteria and *L. monocytogenes* at 0 and 6 h, but generally not thereafter. Coupons exposed daily to nutrients for 21 d had 7.41 CFU/cm^2 total bacteria and 4.45 log CFU/cm^2 *L. monocytogenes* remaining after washing with distilled water (Tables 4.5, 4.6 and Figure 4.5).

Sanitizer #1 and 2 effectively decreased total bacteria and sanitizers #1, 2 and 4 decreased *L. monocytogenes* counts on inoculated HDPE smooth surfaces to below the detection limit (<0.60 log CFU/cm^2) at 0 and 6 h exposure. At 24 h, sanitizers #2 and 5 were effective in decreasing the total bacterial and *L. monocytogenes* counts to <0.60 log CFU/cm^2. The other sanitizers were also fairly effective in that <1.0 log CFU/cm^2 total bacteria and *L. monocytogenes* counts remained after any sanitizer treatment at 24 h (Tables 4.5, 4.6 and Figure 4.5).

On biofilms allowed to develop for 4-21 d, all sanitizer treatments tended to produce significant decreases ($P<0.05$) in total bacteria and *L. monocytogenes* counts on control and water treated coupons, but still had 2.85-5.44 log CFU/cm^2 cells (total bacteria) and <0.60-3.91 log CFU/cm^2 cells (*L. monocytogenes*) remaining. Only on d 4 for sanitizers #2 and 6 and d 7 for sanitizer #5 were *L. monocytogenes* counts reduced to below the detection limit (Tables 4.5, 4.6 and Figure 4.5).

4.3.3. Survival of *L. monocytogenes* and formation of biofilms on PP surfaces with daily exposure to nutrients at 25°C. For the control coupons (no treatment), total bacterial cells on polypropylene surfaces as enumerated on TSAYE decreased ($P<0.05$) from 6.87 log CFU/cm^2 at 0 h to 3.34 log CFU/cm^2 on d 4, then increased ($P<0.05$) to 7.03 log CFU/cm^2 on d 21 (Table 4.7, Figure 4.6). Similarly, the total number of *L. monocytogenes* cells, as enumerated on PALCAM (6.83 log CFU/cm^2 at 0 h), decreased ($P<0.05$) by 3.72 log CFU/cm^2 by d 4 and then increased ($P>0.05$) by 0.67 log CFU/cm^2 to 3.78 log CFU/cm^2 on d 21 (Table 4.8, Figure 4.6).

Rinsing coupons with distilled water resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* at 0, 6 and 24 h (1.72 to 3.59 log CFU/cm^2 reductions from control coupons). However, rinsing with distilled water produced no reductions ($P\geq0.05$) in total bacteria from counts on control coupons on 4, 7, 14 and 21 d developed biofilms, with 6.34 CFU/cm^2 total bacteria remaining on d 21. In contrast, rinsing with distilled water did result in significant reductions in *L. monocytogenes* cells on d 21 with 2.23 log CFU/cm^2 cells remaining (Tables 4.7, 4.8 and Figure 4.6).

Sanitizers #2 and 5 effectively decreased total bacteria and *L. monocytogenes* counts on inoculated PP surfaces to below the detection limit (<0.60 log CFU/cm^2) at 0 h,

6 h and 24 h exposure. Counts (total bacteria and *L. monocytogenes*) for PP surfaces treated with sanitizer #3 at 0, 6 and 24 h ranged from 0.72-1.17 log CFU/cm^2. The remaining sanitizers decreased total bacteria and *L. monocytogenes* counts to near or below detection limit (≤0.95-0.60 log CFU/cm^2) at 0, 6 and 24 h (Tables 4.7, 4.8 and Figure 4.6).

On biofilms allowed to develop for 4-21 d, treatment with all sanitizers produced significant decreases ($P<0.05$) in total bacteria counts on control and water treated coupons, but still had 1.40-4.20 log CFU/cm^2 cells remaining. All sanitizers also produced significant decreases ($P<0.05$) in *L. monocytogenes* counts on d 4-21 biofilms, with each sanitizer except #4 reducing *L. monocytogenes* counts to below the detection limit (<0.60 log CFU/cm^2) on one or more of the days evaluated (Tables 4.7, 4.8 and Figure 4.6)

4.3.4. Survival of *L. monocytogenes* and formation of biofilms on PP surfaces with daily exposure to nutrients at 4°C. For the control coupons (no treatment), total bacterial cells on polypropylene surfaces as enumerated on TSAYE (6.74 log CFU/cm^2 at 0 h), decreased ($P<0.05$) to <0.60 log CFU/cm^2 by d 4 and then increased ($P<0.05$) by 2.62 log CFU/cm^2 to 3.22 log CFU/cm^2 by d 21 (Table 4.9, Figure 4.7). Similarly, the total number of *L. monocytogenes* cells, as enumerated on PALCAM (6.72 log CFU/cm^2 at 0 h), decreased ($P<0.05$) to <0.60 log CFU/cm^2 by d 4, then increased slightly ($P>0.05$) to 1.45 log CFU/cm^2 on d 21 (Table 4.10, Figure 4.7).

Rinsing coupons with distilled water resulted in significant ($P<0.05$) decrease in total bacteria and *L. monocytogenes* at 0, 6 and 24 h (2.79 to 3.40 log CFU/cm^2 reductions from control coupons). However, rinsing with distilled water produced no

reductions (P≥0.05) in counts from control coupons on 14 and 21d developed biofilms, with 2.66 CFU/cm^2 total bacteria and 0.90 log CFU/cm^2 *L. monocytogenes* remaining at d 21 (Tables 4.9, 4.10 and Figure 4.7).

Sanitizer #2 effectively decreased total bacteria on inoculated PP surfaces to below the detection limit (<0.60 log CFU/cm^2) at 0, 6 and 24 h exposure whereas sanitizers #1 and 2 decreased *L. monocytogenes* counts to below the detection limit (<0.60 log CFU/cm^2) at 0, 6 and 24 h exposure. Sanitizers #3, 4, 5 and 6 were fairly effective, in that <0.60-1.02 log CFU/cm^2 total bacteria and <0.60–0.92 log CFU/cm^2 *L. monocytogenes* counts remained after sanitizer treatment at 0, 6 and 24 h (Tables 4.9, 4.10 and Figure 4.7).

On biofilms allowed to develop on PP for 7, 14 and 21 days at 4°C, only sanitizers #2 and 4 on d 7 reduced total bacteria counts to below the detection limit (<0.60 log CFU/cm^2). All sanitizers except #5, produced significant (P<0.05) reductions in total bacterial counts on d 21 biofilms, but PP still had 1.18-1.40 log CFU/cm^2 cells remaining. *L. monocytogenes* biofilms did not survive well on PP at 4°C, and *L. monocytogenes* counts for all the sanitized surfaces were below the detection limit (<0.60 log CFU/cm^2) on d 4 to 21 (Tables 4.9, 4.10 and Figure 4.7).

4.3.5. Survival of *L. monocytogenes* and formation of biofilms on HDPE rough surfaces without daily exposure to nutrients at 25°C. Without daily exposure to nutrients, total bacterial cells on HDPE rough surfaces (no treatment) as enumerated on TSAYE (5.34 log CFU/cm^2 at 24 h), increased slightly to 5.54 log CFU/cm^2 on d 4, then steadily decreased (P<0.05) to 0.68 log CFU/cm^2 on d 21 (Table 4.11, Figure 4.8). *L.*

monocytogenes counts, as enumerated on PALCAM, decreased ($P<0.05$) from 5.25 log CFU/cm^2 at 24 h to <0.60 log CFU/cm^2 at 14 d and 21 d (Table 4.12, Figure 4.8).

Rinsing coupons with distilled water resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* counts at d 4 and 7 (1.29-2.94 log CFU/cm^2 reductions from control coupons respectively). However, rinsing with distilled water produced no further reductions ($P\leq0.05$) in counts from control coupons on 14 and 21 d developed biofilms (Tables 4.11, 4.12 and Figure 4.8).

On biofilms allowed to develop for 4 and 7 d without daily nutrient exposure, all sanitizer treatments also produced significant decreases ($P<0.05$) in total bacteria and *L. monocytogenes* counts, with <0.60-1.86 log CFU/cm^2 counts remaining. On biofilms allowed to develop for 14 and 21 days, sanitizers #1, 2, 3, 4 and 5 effectively reduced total bacterial counts to below or near the detection limit (<0.60-0.75 log CFU/cm^2). Without nutrient exposure, *L. monocytogenes* biofilms did not survive for 14 and 21 d on HDPE rough surfaces (Tables 4.11, 4.12 and Figure 4.8).

4.3.6. Survival of *L. monocytogenes* and formation of biofilms on HDPE smooth surfaces without daily exposure to nutrients at 25°C. Without daily exposure to nutrients, total bacterial cells on HDPE smooth surfaces (no treatment) as enumerated on TSAYE (4.27 log CFU/cm^2 at 24 h), increased ($P<0.05$) to 5.58 log CFU/cm^2 by d 7, then decreased ($P<0.05$) to <0.60 log CFU/cm^2 by d 21 (Table 4.13, Figure 4.9). *L. monocytogenes* counts followed a similar pattern (Table 4.14, Figure 4.9).

Rinsing coupons with distilled water resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* at d 4, 7 and 14, but still allowed 2.35-4.48 log

CFU/cm^2 total bacteria and 1.98-4.35 log CFU/cm^2 *L. monocytogenes* to remain (Tables 4.13, 4.14 and Figure 4.9).

Without daily exposure to nutrients, all sanitizers except #1 on d 4, #3 on d 7 and 14, and #6 on d 14 effectively decreased total bacteria on inoculated HDPE smooth surfaces to below the detection limit (<0.60 log CFU/cm^2) on d 4, 7, 14 and 21. Likewise, except for sanitizer #3 on d 7, all sanitizers decreased *L. monocytogenes* counts to below the detection limit (<0.60 log CFU/cm^2) on d 4, 7, 14 and 21 day biofilms (Tables 4.13, 4.14 and Figure 4.9).

4.3.7. Survival of *L. monocytogenes* and formation of biofilms on polypropylene surfaces without daily exposure to nutrients at 25°C. Without daily exposure to nutrients, total bacterial cells on polypropylene surfaces (no treatment) as enumerated on TSAYE (4.35 log CFU/cm^2 at 24 h) increased (P<0.05) to 5.80 log CFU/cm^2 by d 4, then decreased (P<0.05) to <0.60 log CFU/cm^2 by d 21 (Table 4.15, Figure 4.10). *L. monocytogenes* cells, as enumerated on PALCAM, followed a similar pattern (Table 4.16, Figure 4.10).

Rinsing coupons with distilled water resulted in significant (P<0.05) decreases in total bacteria and *L. monocytogenes* at 4 and 7 d (1.06 to 3.59 log CFU/cm^2 reductions from control coupons) (Tables 4.15, 4.16 and Figure 4.10).

All sanitizers produced significant reductions (P<0.05) in total bacteria and *L. monocytogenes* counts from water treated PP surfaces at 4 and 7 d. Sanitizers #1, 2 and 6 consistently reduced total bacteria and all sanitizers decreased *L. monocytogenes* counts on inoculated PP surfaces to below the detection limit (<0.60 log CFU/cm^2) on 4, 7, 14

and 21 d biofilms. Biofilms did not survive well at 25°C on 14 and 21 d PP inoculated surfaces with no exposure to nutrients (Tables 4.15, 4.16 and Figure 4.10).

4.3.8. Survival of *L. monocytogenes* and formation of biofilms on polypropylene surfaces without daily exposure to nutrients at 4°C. Without daily exposure to nutrients, total bacterial cells on polypropylene surfaces (no treatment) as enumerated on TSAYE (4.16 log CFU/cm^2 at 24 h) increased ($P<0.05$) to 5.26 log CFU/cm^2 by d 4, then decreased ($P<0.05$) to 0.72 log CFU/cm^2 by d 21 (Table.4.17, Figure 4.11). *L. monocytogenes* cells, as enumerated on PALCAM, followed a similar pattern (Table.4.18, Figure 4.11).

Rinsing coupons with distilled water resulted in significant ($P<0.05$) decreases in total bacteria and *L. monocytogenes* at 4, 7 and 14 d (0.94-3.73 log CFU/cm^2 reductions). Without daily nutrient exposure, bacteria did not survive well on 21 d developed biofilms, with 0.72 log CFU/cm^2 total bacteria and <0.60 log CFU/cm^2 *L. monocytogenes* remaining.

Without daily nutrient exposure, all sanitizers, except #1 on d 7 and #3 on d 14, reduced total bacteria and *L. monocytogenes* counts to below detection limit.

4.4. DISCUSSION

Biofilms have gained increased interest in recent years, due to the emergence of *L. monocytogenes* as a foodborne pathogen. It is well know that bacteria can attach and grow on various surfaces utilized in the food processing industry and home kitchens (Lomander et al., 2004). Improperly cleaned surfaces promote soil buildup, and, in the presence of water, contribute to the development of bacterial biofilms which may contain

pathogenic microorganisms (Chmielewski and Frank, 2003). The objective of this study was to evaluate the resistance of *L. monocytogenes* biofilms to stresses under laboratory conditions that mimic food preparation and processing environments in home kitchens.

Sanitation of food preparation surfaces, including cutting boards and cutting board materials, is critical for the control of microbial contamination of foods and is a significant concern during food preparation (Abrishami et al., 1994). Sanitation can be done by dipping or spraying cleaned surfaces with a sanitizer (Pan et al., 2006).

On all three surface types (HDPE coupon with rough and smooth surface and PP surface) incubated at 25°C, rinsing coupons with distilled water produced significant ($P<0.05$) reductions in total bacteria and *L. monocytogenes* at 0 and 6 h incubation, but still allowed 3.01-4.57 log CFU/cm^2 to remain. All of the sanitizer treatments evaluated produced further reductions ($P<0.05$) in total and *L. monocytogenes* counts at 0 and 6 h, with sanitizer #2 consistently reducing counts to below the detection limit (<0.60 log CFU/cm^2) (Tables 4.3- 4.10; Figure 4.4, 4.5, 4.6 and 4.7).

Rinsing coupons with distilled water produced significant ($P<0.05$) reductions in total bacteria and *L. monocytogenes* on most surfaces at 24 h incubation, but still allowed 2.45-4.42 log CFU/cm^2 to remain. All of the sanitizer treatments evaluated produced further reductions ($P<0.05$) in total and *L. monocytogenes* counts at 24 h, with sanitizers #1, 2 and 5 tending to be more effective than sanitizers #3, 4 and 6, especially on HDPE rough surfaces (Tables 4.3- 4.8; Figure 4.4, 4.5 and 4.6).

After 4 days of incubation, however, biofilms were well established on coupons exposed daily to nutrients, making water ineffective in removing total bacterial flora and *L. monocytogenes* cells. According to Moretro and Langsrud. (2004), cells ultimately

adhere irreversibly to the surface and start to multiply and produce extracellular compounds, forming micro colonies and subsequently thicker multilayer and multi-species biofilms. Treatment of these biofilms with sanitizers did produce significant reductions in total bacteria, but none of the sanitizers were totally effective in reducing total bacterial levels to below the detection limit. Sanitizers were somewhat better at reducing *L. monocytogenes* cells on established biofilms, with sanitizers #1, 2 and 5 tending to be more effective than sanitizers #3, 4 and 6. Overall, this shows that established *L. monocytogenes* biofilms are highly resistant to sanitizers commonly used in the kitchen. This resistance varied with the type of surface to which the cells adhered (Table 4.3- 4.10; Figure 4.4, 4.5, 4.6 and 4.7).

Joseph et al. (2001) noted that the efficiency of biofilm formation as well as resistance to treatment with sanitizers varies depending on the type of surface. On HDPE rough and smooth surfaces, sanitizers #1, 2 and 5 reduced *L. monocytogenes* on d 4 and 7 biofilms whereas all sanitizers were effective against *L. monocytogenes* d 4 and 7 biofilms on PP surfaces (Tables 4.3–4.8; Figure 4.4, 4.5 and 4.6). None of the sanitizers were effective against total bacterial flora on d 14 and 21 HDPE surface biofilms. In contrast, sanitizer #2 effectively reduced *L. monocytogenes* cells to below the detection limit on PP surface d 21 biofilms (Tables 4.7, 4.8; Figure 4.6). The time available for biofilm formation will depend on the frequency of cleaning regimes (Gibson et al., 1999).

Polypropylene is becoming more popular in the industry for the construction of tanks, pipeworks, accessories and cutting surfaces (Oulahal et al., 2008). *L. monocytogenes* is a psychrotrophe and can grow at refrigeration temperature. Biofilms did not survive and grow well on polypropylene coupons incubated at 4°C. Total

bacterial cells declined to below the detection limit on d 4 control samples, then increased to 3.22 log CFU/cm^2 by d 21. *L. monocytogenes* cells were below the detection limit on d 4, 7 and 14, then increased to 1.45 log CFU/cm^2 on d 21 (Tables 4.9, 4.10; Figure 4.7). Given the very low survival rate, all sanitizers were effective in reducing total and *L. monocytogenes* cell counts on PP coupons incubated at 4°C for 7, 14 and 21 d. The inoculated *L. monocytogenes* competed with environmental microorganisms for survival during this time interval of formation of multiple-species biofilms (Yang et al., 2009). *L. monocytogenes* samples from the surfaces usually contain other microorganisms as well. Biofilms are usually multi-species communities, where the different species are integrated in a complex structure (Moretro and Langsrud, 2004). It was observed that on d 7, 14 and 21, there was presence of bacterial contamination due to environmental organisms.

Total bacterial counts and *L. monocytogenes* survived sanitizer treatments employed on 4, 7, 14 and/or 21 d following pathogen exposure to 25°C. According to Lomander et al. (2004) this should be due to the formation of an exopolysaccharide (EPS) matrix surrounding the biofilms that supplies it with nutrients and protects it from attack by sanitizers. According to Moore et al. (2007) it is believed that the presence of macromolecular nutrients, like proteins, protects cells against dehydration, and as a result, the viability of cells in desiccating environments increases. Other factors contributing to the viability of *L. monocytogenes* on HDPE and PP surfaces may have been the temperature (25°C) and the high relative humidity (90%). A decrease in both total bacterial and *L. monocytogenes* cells was observed on PP coupons that were incubated at 4°C. Previous research demonstrated that cell attachment and biofilm

formation by *L. monocytogenes* are influenced by several factors, including characteristics of strains, physical and chemical properties of the substrate for attachment, growth phase of the bacteria, temperature, growth media and the presence of other microorganisms (Mafu et al., 1991; Blackman and Frank, 1996; Wong, 1998; Norwood and Gilmour, 2000; Chavant et al., 2004; Pan et al., 2006). It is possible that the temperature (4°C), relative humidity, the ham homogenate nutrient source used, or a combination of these factors may have played an important role in not supporting the growth of the *L. monocytogenes* biofilm on polypropylene at 4°C. According to Palmer et al. (2007), along with the above factors, there could be a number of other factors involved in bacterial cell attachment such as surface conditioning, mass transport, surface charge, hydrophobicity, surface roughness and surface micro-topography.

Food contact surfaces without daily exposure to nutrients showed decreased biofilm resistance. Treatment with sanitizers did produce significant reductions in total bacteria, and most of the sanitizers were totally effective in reducing bacterial levels to below the detection limit. According to Gibson et al (1999), conditions that favor attachment and biofilm formation in food processing environments include flowing water, suitable attachment surfaces, ample nutrients and raw materials, or the environment supplying the inocula. Since the food contact surfaces were not being treated daily with nutrients, *L. monocytogenes* biofilm that developed were less resistant to sanitizers. All sanitizers were effective in reducing total bacterial cells from HDPE rough and smooth surfaces and PP coupons incubated at 25°C at almost all times. On HDPE rough surfaces, sanitizers #1, 4, 5 and 6 and on HDPE smooth surfaces, all the sanitizers' successfully reduced *L. monocytogenes* cells to below the detection limit

(<0.60 log CFU/cm^2) on d 4 biofilms. All the sanitizers were effective on d 14 and 21 HDPE surface biofilms. Likewise, all the sanitizers effectively reduced *L. monocytogenes* cells to below the detection limit on PP surface at almost all times.

In the absence of daily exposure to nutrients, biofilms also did not develop well on PP coupons incubated at 4°C; total bacterial cells decreased from 5.26 log CFU/cm^2 on d 4 to 0.72 log CFU/cm^2 by d 21 and *L. monocytogenes* cells decreased from 5.86 log CFU/cm^2 to <0.60 log CFU/cm^2 by d 21. All the sanitizers effectively reduced *L. monocytogenes* cells to below the detection limit (<0.60 log CFU/cm^2) on PP surfaces at all times.

4.4.1. Impact of daily nutrient exposure on HDPE rough and smooth surfaces and PP surface incubated at 25°C. To assess differences in response of the three surfaces to sanitizer treatment at 25°C, total bacterial and *L. monocytogenes* counts for each surface type were averaged across water and all 6 sanitizer treatments by treatment time. As can be seen in Table 4.19 (Figure 4.12), total bacterial counts tended to be higher on HDPE than PP surfaces and higher on HDPE rough than smooth surfaces. Significant differences ($P<0.05$) were observed between HDPE rough and smooth surfaces at 24 h and on d 21-old biofilms and between HDPE rough surfaces and PP at all times except 0 and 6 h for survival of total bacterial cells. *L. monocytogenes* counts followed a similar pattern though fewer differences were noted between rough and smooth HDPE surfaces (Table 4.20, Figure 4.12). This indicates that *L. monocytogenes* cells were more resistant on porous surfaces (rough surface) than on non-porous surfaces. According to Silva et al. (2008), surface roughness influences bacterial adhesion, and higher the surface roughness, higher the significant effect on cell retention. The high porosity of rough

surfaces provides a larger surface area for bacterial attachment than smooth surfaces, and so, biofilm maturation might be faster on rough compared to smooth surfaces (Yang et al., 2009). Surface properties such as hydrophobicity, electrical charge, roughness and porosity are determinant in the adhesion process (Lima et al., 2004). Development of a biofilm is a result of both adherence and growth following adherence (Blackman and Frank, 1996).

4.4.2. Impact of storage temperature on polypropylene surfaces exposed daily to nutrients. To assess the impact of storage temperature on survival, total bacteria and *L. monocytogenes* counts for PP were averaged across water and all 6 sanitizer treatments by storage temperature (25 and 4°C). As seen in Tables 4.21, 4.22 and Figure 4.13, the total bacterial and *L. monocytogenes* counts, as enumerated on TSAYE and PALCAM respectively, were higher on PP incubated at 25°C than at 4°C, but significantly higher on d 4, 7, 14 and 21 biofilms. This indicates that *L. monocytogenes* cells survived better and were more resistant at ambient temperature than at refrigerator temperature. According to Wong (1998), temperature is one of the many factors that affect biofilm.

4.4.3. Comparison of the efficacy of the six sanitizers across HDPE rough and smooth surfaces and PP surface exposed to daily nutrients. To compare the overall effectiveness of the six sanitizers across HDPE rough and smooth surfaces and PP surface incubated at 25°C, the differences between distilled water treatment and after sanitizer treatment were calculated and these were then averaged. The total reduction of bacterial cells on food contact surfaces consisted of physical removal caused by washing with distilled water and chemical inactivation caused by the sanitizer (Log CFU/cm^2). Application of each sanitizer was according to the manufacturer's instructions printed on

the bottles, which for most of them included three major steps: rinsing with water, reaction with sanitizer for certain time intervals (10 sec to 10 min), and rinsing with water again (Tables 4.23, 4.24 and Figure 4.14). Two of the six sanitizers used in the study were sodium hypochlorite based but their ingredients and concentrations were different (Table 4.2). The other two sanitizers were lactic acid-based (pH 2.92) and quaternary ammonium-based (pH 10.12) and the remaining two were acetic acid (pH 3.26) and hydrogen peroxide (pH 4.72). All the sanitizers were found to be equally effective within 24 h. On d 21 when the biofilm development was greatest, sanitizer #2 (i.e., lactic acid-based, pH 2.92) was found to be the most effective on all three surfaces followed by sanitizer #5 (acetic acid) and #1 (QAC-based). Acetic acid (vinegar) is a readily available household product used widely in food industry as acidulate and in general household cleaning. In the absence of commercial sanitizers, vinegar can be used for sanitizing food contact surfaces. Sanitizers #3 and 6 were less effective on d 21 (Tables 4.23, 4.24 and Figure 4.14). Sanitizer # 6 is hydrogen peroxide and it is also a readily available sanitizer. Quaternary ammonium-based sanitizer by its higher pH of 10.12 was found to be effective; according to Chavant et al. (2004) increasing pH reveals a great efficacy on biofilm. In the present study, high efficacy of lactic acid-based sanitizer may be explained by its lower pH of 2.92. According to Yang et al. (2009) lactic acid-based sanitizer was the most effective sanitizer and our study supports the same results.

Sanitizer #3 was a commercial spray product (pH 6.55) and sanitizer #4 was prepared on day of use by combining household chlorine bleach (5.7% available chlorine) with distilled water to make a 298.5 ppm chlorine bleach solution (pH 6.22). Upon dissolution of bleach in water, ionization takes place, and the hypochlorite ion

establishes equilibrium with HOCl (Lomander et al., 2004). Due to chlorine's high oxidizing reactivity, the activity of cellular proteins is destroyed (Lomander et al., 2004). While differences in effectiveness were seen between sanitizers #3 and #4 at times throughout the study, when averaged across all three types of surfaces, except for d 21 biofilms where sanitizer #3 was more effective ($P<0.05$) than sanitizer #4 (home prepared), no significant differences were seen in the overall effectiveness of the two types of sanitizers (Tables 4.23, 4.24 and Figure 4.14).

4.4.4. Impact of no exposure to nutrients across HDPE rough and smooth surfaces and PP surface incubated at 25°C. To assess differences in response of the three surfaces to sanitizer treatment at 25°C, total bacterial and *L. monocytogenes* counts for each surface type were averaged across water and all 6 sanitizer treatments by treatment time. As can be seen in Tables 4.25, 4.26 and Figure 4.15, both total bacterial and *L. monocytogenes* counts tended to be higher on HDPE rough surfaces than on HDPE smooth or PP surfaces except for d 7 when counts were highest on HDPE smooth surface. This indicates that *L. monocytogenes* cells tended to be more resistant on porous surfaces (rough surface) than on non- porous surfaces. According to Teixeira et al. (2008) surface roughness impedes hygiene and cleaning procedures. The high porosity of rough surfaces provides a larger surface area for bacterial attachment than smooth surfaces, and so, biofilm maturation might be faster on rough compared to smooth surfaces (Yang et al., 2009).

4.4.5. Impact of storage temperature on polypropylene surfaces not exposed to daily nutrients. To assess the impact of storage temperature on survival rates, total bacteria and *L. monocytogenes* counts for PP were averaged across water and all 6 sanitizer

treatments by storage temperature (25 and 4°C). As seen in Tables 4.27, 4.28 and Figure 4.16, the total bacterial cell and *L. monocytogenes* cell counts as enumerated on TSAYE and PALCAM, respectively, tended to be higher on PP incubated at 25°C than at 4°C. Significant differences ($P<0.05$) were observed between PP (25°C) and PP (4°C) at d 4 and d 7-old biofilm for survival of total bacterial cells, whereas no significant differences ($P>0.05$) were found on *L. monocytogenes* counts on PP surfaces incubated at 25 and 4°C. This indicates that *L. monocytogenes* cells survived better and were more resistant at room temperature than at refrigerator temperature. According to Wong (1998), biofilm survival is affected by temperature, relative humidity and attachment surface and one or multiple factors may have played an important role in reduced survival of *L. monocytogenes* on PP incubated at 4°C.

4.4.6. Comparison of the efficacy of the six sanitizers across HDPE rough and smooth surfaces and PP surface not exposed to daily nutrients. To compare the overall effectiveness of the six sanitizers across HDPE rough and smooth surfaces and PP surface incubated at 25°C, the differences between distilled water treatment and after sanitizer treatment were calculated and these were then averaged. The total reduction of bacterial cells on food contact surfaces consisted of physical removal caused by washing with distilled water and chemical inactivation caused by the sanitizer (Log CFU/cm^2). Application of each sanitizer was according to the manufacturer's instructions printed on the bottles, which for most of them included three major steps: rinsing with water, reaction with sanitizer for certain time intervals (10 sec to 10 min), and rinsing with water again (Tables 4.29, 4.30, Figure 4.17). Two of the six sanitizers used in the study were sodium hypochlorite based but their ingredients and concentrations were different (Table

4.2). The other two sanitizers were lactic acid-based (pH 2.92) and quaternary ammonium-based (pH 10.12) and the remaining two were acetic acid (pH 3.26) and hydrogen peroxide (pH 4.72). According to data analysis, at all times all the sanitizers were found to be equally effective except on d 7, where sanitizer #3 was less effective ($P<0.05$) than all other sanitizers on total bacterial cells.

4.4.7. Impact of daily nutrient exposure vs. no exposure on survival of *L. monocytogenes* on HDPE rough surface incubated at 25°C. On d 4, 7, 14 and 21, survival rates for total bacteria (Table 4.31) and *Listeria monocytogenes* (Table 4.32) were generally higher ($P<0.05$) for control, water treated, and all sanitizer treated coupons with daily exposure to nutrients compared to no exposure except on d 4 control coupons where *L. monocytogenes* counts were found to be higher on coupons not treated with daily nutrients (Figure 4.18). In fact, without daily nutrient exposure, *Listeria monocytogenes* did not survive on 14 and 21 d sanitizer treated coupons. *L. monocytogenes* produces biofilms on surfaces in the presence of complex growth nutrients (Blackman and Frank, 1996). This indicates that presence of nutrients results in an increase in the survival of *L. monocytogenes* and formation of biofilm on HDPE rough surface.

4.4.8. Impact of daily nutrient exposure vs. no exposure on survival of *L. monocytogenes* on HDPE smooth surface incubated at 25°C. On d 4, 7, 14 and 21, survival rates for total bacteria (Table 4.33) and *Listeria monocytogenes* (Table 4.34) were generally higher ($P<0.05$) for control, water treated, and all sanitizer treated coupons with daily exposure to nutrients compared to no exposure except on d 4 & 7 control coupons where *L. monocytogenes* counts were found to be significantly higher on

coupons not treated with daily nutrients (Figure 4.19). Without daily nutrient exposure, *Listeria monocytogenes* did not survive on 14 and 21 d sanitizer treated coupons. For *L. monocytogenes* cells, significant differences were mainly observed on established biofilms (d 14 and 21). This indicates that the presence of nutrients results in an increase in the survival of *L. monocytogenes* and formation of biofilm on HDPE smooth surface. *L. monocytogenes* produces biofilms on surfaces in the presence of complex growth nutrients (Blackman and Frank, 1996). According to Moretro and Langsrud. (2004) nutrient limitation has been shown to decrease *L. monocytogenes* adhesion and biofilm formation.

4.4.9. Impact of daily nutrient exposure vs. no exposure on survival of *L. monocytogenes* on polypropylene surface incubated at 25°C. On d 4, 7, 14 and 21, survival rates for total bacteria (Table 4.35) and *Listeria monocytogenes* (Table 4.36) were generally higher ($P<0.05$) for control, water treated, and sanitized coupons with daily exposure to nutrients compared to no exposure except on d 4 control and water treated coupons where *L. monocytogenes* counts were found to be significantly higher on coupons not treated with daily nutrients (Figure 4.20). Without daily nutrient exposure, *Listeria monocytogenes* did not survive past d 7. This indicates that presence of nutrients results in an increase in the survival of *L. monocytogenes* and formation of biofilm on polypropylene surface incubated at 25°C. According to Moretro and Langsrud. (2004) nutrient limitation has been shown to decrease *L. monocytogenes* adhesion and biofilm formation.

4.4.10. Impact of daily nutrient exposure vs. no exposure on survival of *L. monocytogenes* on polypropylene surface incubated at 4°C. On d 4, 7 and 14, survival

rates for total bacteria (Table 4.37) and *Listeria monocytogenes* (Table 4.38) were significantly higher ($P<0.05$) for control coupons without daily exposure to nutrients compared to those with daily exposure, whereas on d 21, control coupons that were daily treated with nutrients had significantly higher survival than those with no exposure (Figure 4.21). *Listeria monocytogenes* did not survive from d 4 for sanitizer treated coupons with or without daily exposure to nutrients. It is unclear why *L. monocytogenes* biofilms developed better on 4°C PP control coupons not receiving daily exposure to nutrients than on coupons treated with daily nutrients. It is possible, that temperature (4°C), relative humidity (90%), the ham homogenate used or a combination of these factors may have played an important role in not supporting growth of the *L. monocytogenes* biofilms on polypropylene at 4°C. According to Palmer et al. (2007), these and many other factors impact bacterial cell attachment, including surface conditioning, mass transport, surface charge, hydrophobicity, surface roughness and surface micro-topography.

4.5. CONCLUSION

Based on the above results, it can be concluded that *L. monocytogenes* can survive on food contact surfaces, e.g. cutting boards, plastic microwaveable utensils and refrigerator shelves etc., forming a biofilm and such adherent cells may not be removed during the washing and sanitizing processes.

Under the conditions of this study, *L. monocytogenes* biofilms developed during storage and survived for at least 21 d on all surfaces tested at 25°C and 4°C, but not after d 14 on coupons that were not subjected daily to nutrients. All sanitizers were effective in

reducing *L. monocytogenes*, and more effective on younger than older biofilms. Among sanitizers evaluated, lactic acid-based (pH 2.92), 5% acetic acid-based (pH 3.26) and quaternary ammonium-based (pH 10.12) sanitizers were most effective against biofilms aged 7 days and older. Biofilm survival was greater on rough than smooth HDPE surfaces; the use of cutting boards with a smooth surface is recommended. Repeated exposure of food contact surfaces to nutrients as during use with no cleaning or sanitation increased the resistance of *L. monocytogenes* biofilms to sanitizers.

L. monocytogenes, when given sufficient time, can accumulate on a variety of surfaces to levels which might lead to the spread of the pathogen throughout food preparation. Repeated introduction of food residues and moisture during daily food preparation with inadequate cleaning practices may allow for the development of multi-species biofilms containing *L. monocytogenes* cells on cutting boards and other food preparation surfaces. In the absence of commercial sanitizers, readily available household products like distilled white vinegar and a diluted chlorine bleach solution should be used. Hence, to avoid the entrance and dispersion of bacteria in general and of *L. monocytogenes*, it is recommended that daily hygienic procedures using appropriate detergents and sanitizers be used.

Table 4.1. *L. monocytogenes* strains used in the study (Fugett et al., 2006)

L. monocytogenes strain	Lineage	Serotype	Source
J1-177	I	1/2b	Human, sporadic
R2-499	II	1/2a	Human, epidemic, sliced turkey
N3-013	I	4b	Food, epidemic
N1-227	I	4b	Food, epidemic
C1-056	II	1/2a	Human, sporadic

Table 4.2. Sanitizers used for inactivating *Listeria monocytogenes* biofilms on rough and smooth high density polyethylene coupons and polypropylene coupons

Sanitizer #	**Active Ingredients and Concentration**	**pH**
1	Alkyl (67% C_{12}, 25% C_{14}, 7% C_{16}, 1% C_8-C_{10}-C_8) dimethyl benzyl ammonium chlorides (0.0860%) Alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium chlorides (0.0216%)	10.12
2	L-Lactic acid (0.18%)	2.92
3	Sodium hypochlorite (0.0095%) Available chlorine (0.009%)	6.55
4*	Sodium hypochlorite (6%) Other ingredients (94%) Yield 5.7% available Cl_2	6.22
5	Acetic acid (5%)	3.26
6	Hydrogen peroxide (3%)	4.72

* A fresh solution was prepared on the day of experiment.

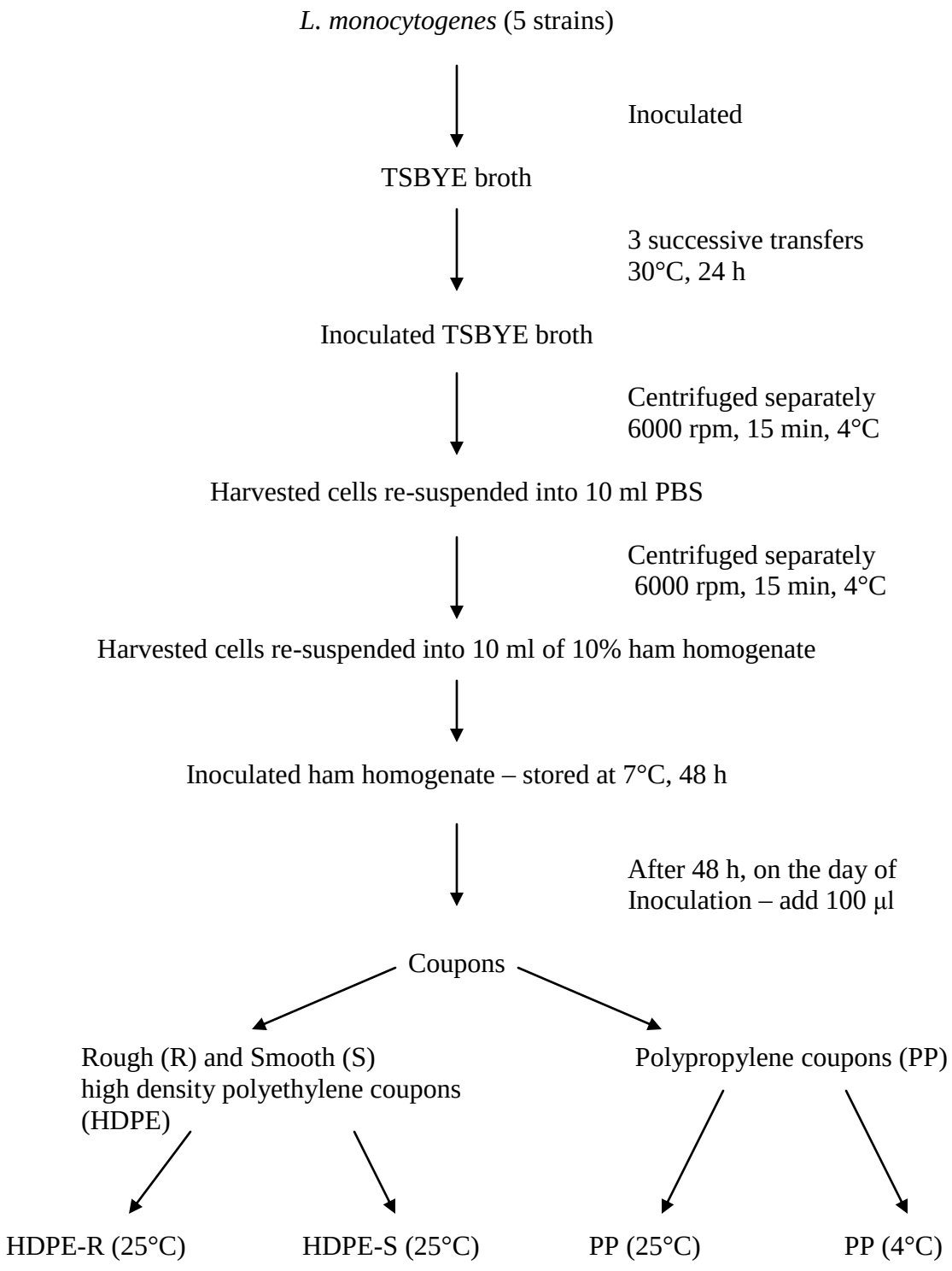
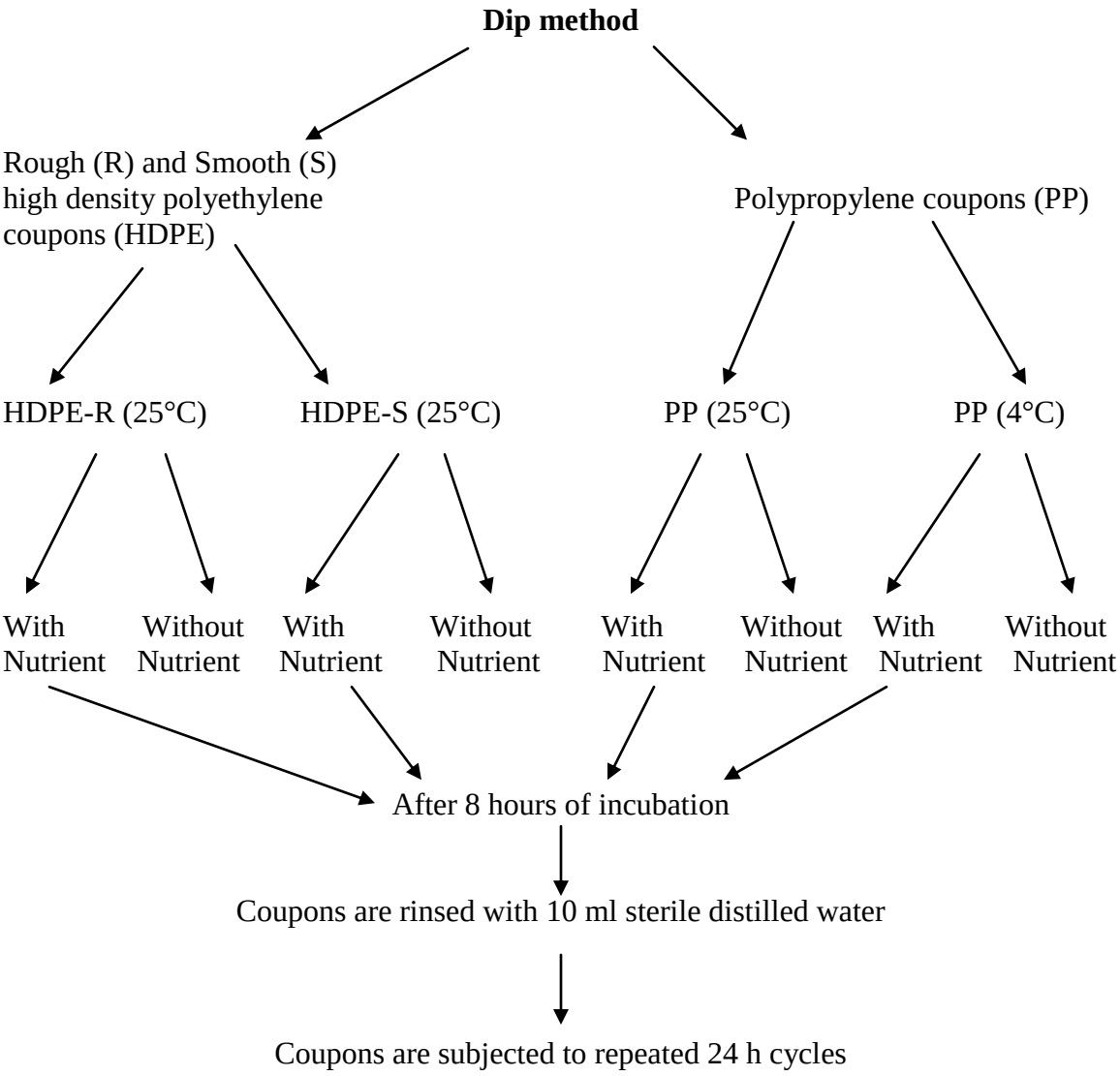


Figure.4.1. **Coupon preparation flow chart**



Relative Humidity = 90%
With Nutrient = 1:10 diluted TSBYE (sampling days: 0 h, 6 h, 24 h, 96 h, 168 h, 336 h, 504 h)
Without Nutrient = 1:10 diluted nutrient is not added (sampling days: 0 h, 6 h, 24 h, 96 h, 168 h, 336 h, 504 h)

Figure 4.2. **Experimental design**

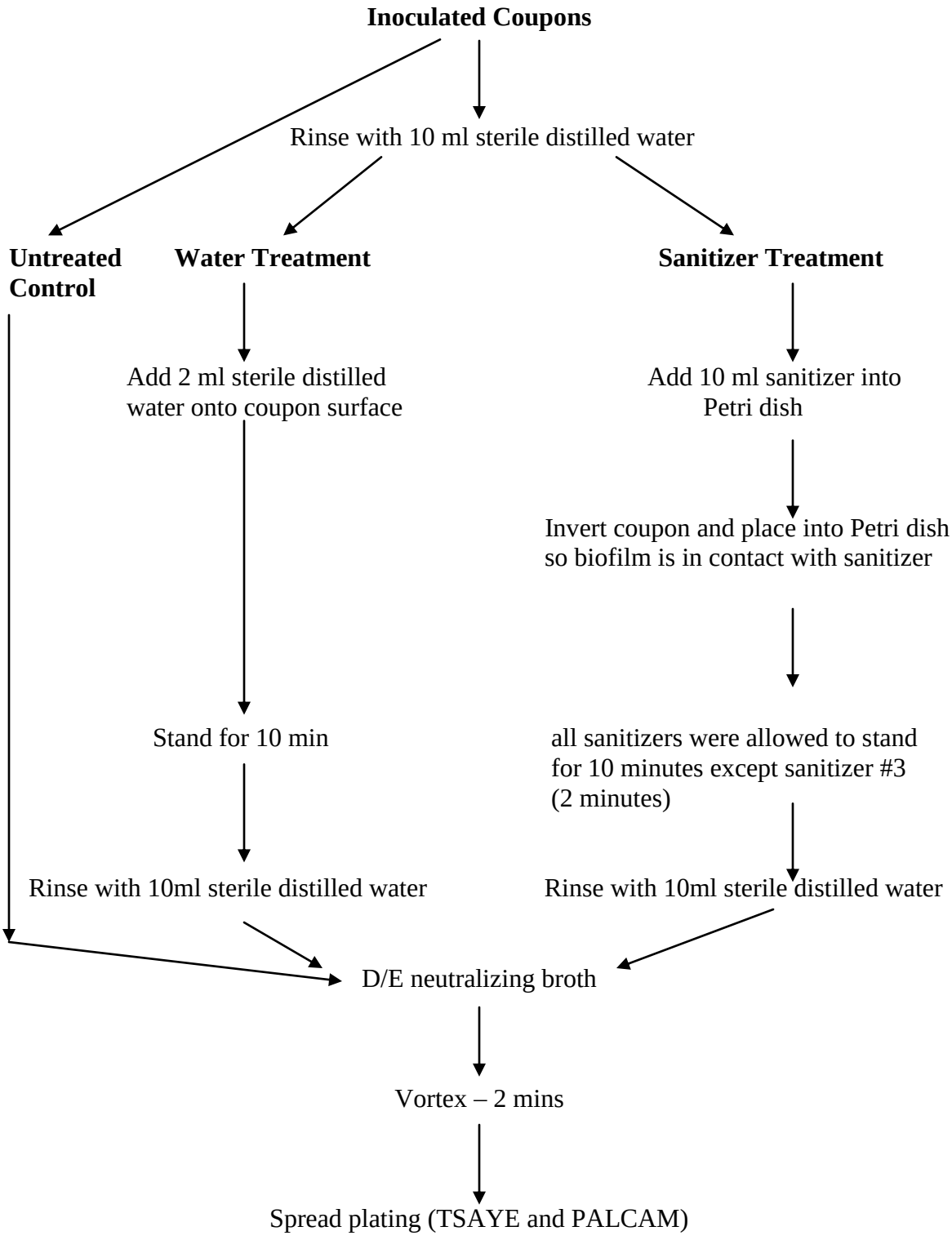
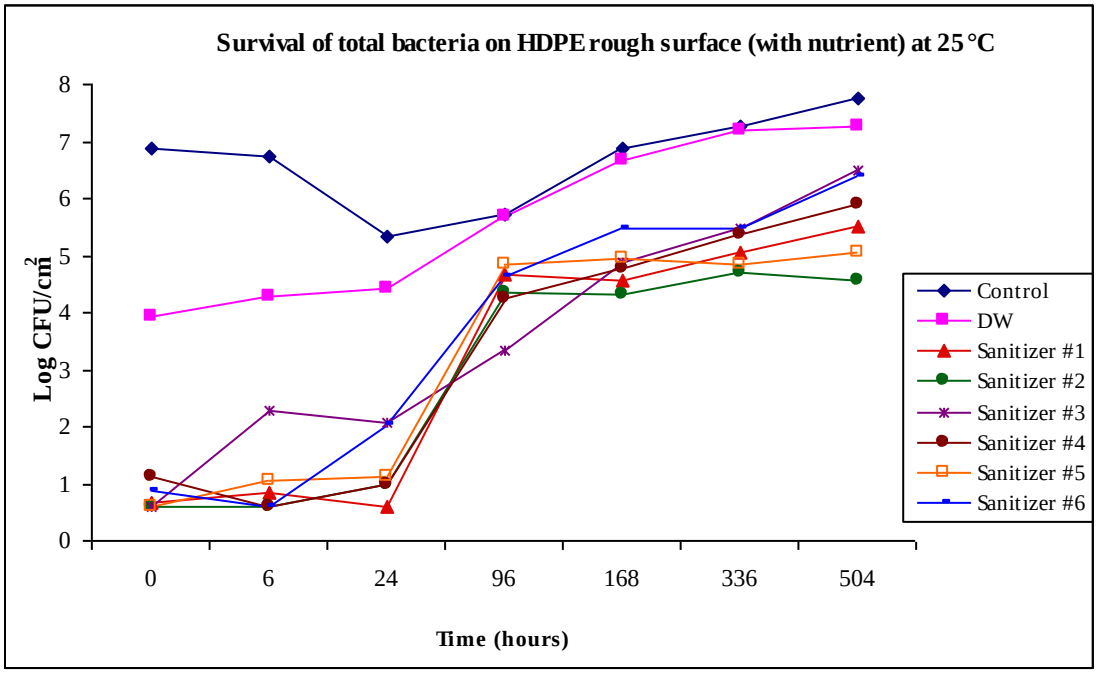


Figure 4.3. **Sanitizer treatment flow chart**



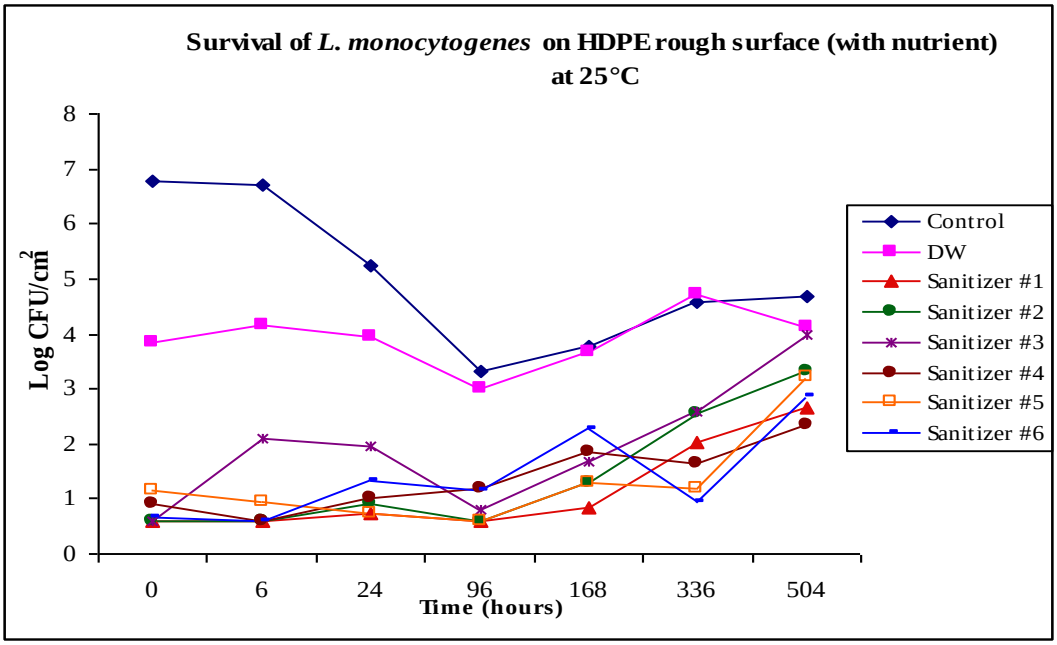
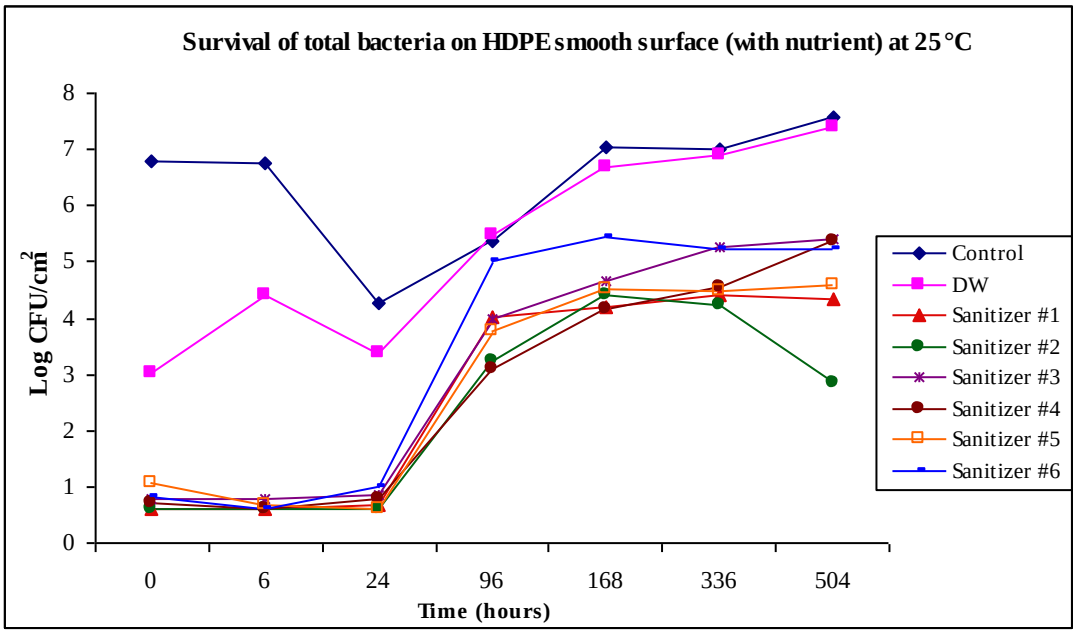


Figure 4.4. Data shown in Tables 4.3 and 4.4. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



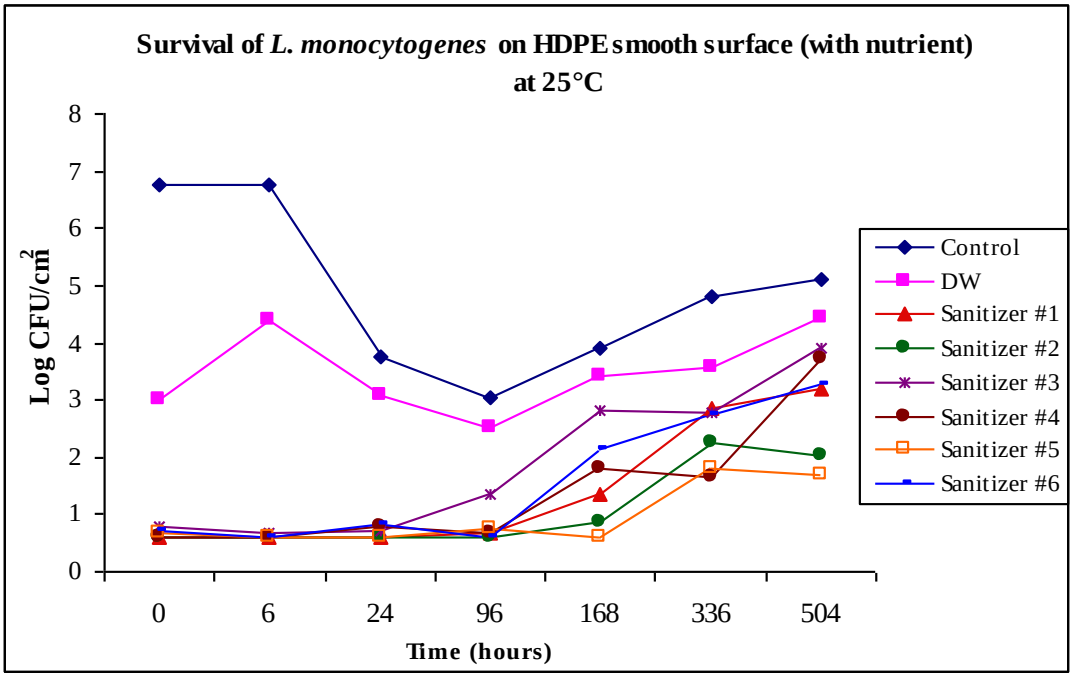
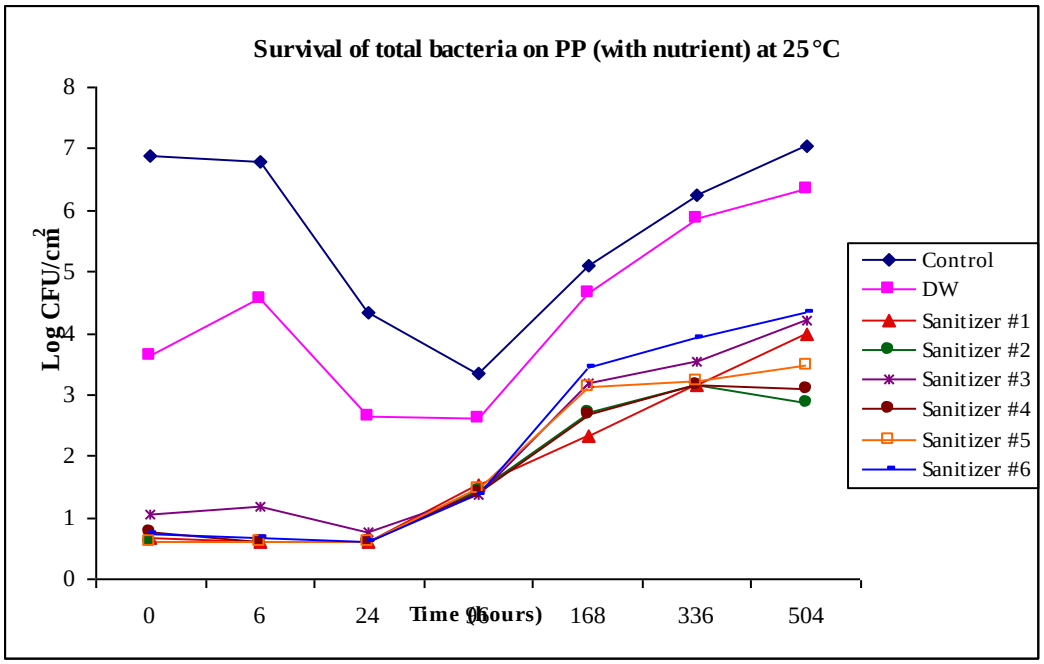


Figure 4.5. Data shown in Tables 4.5 and 4.6. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



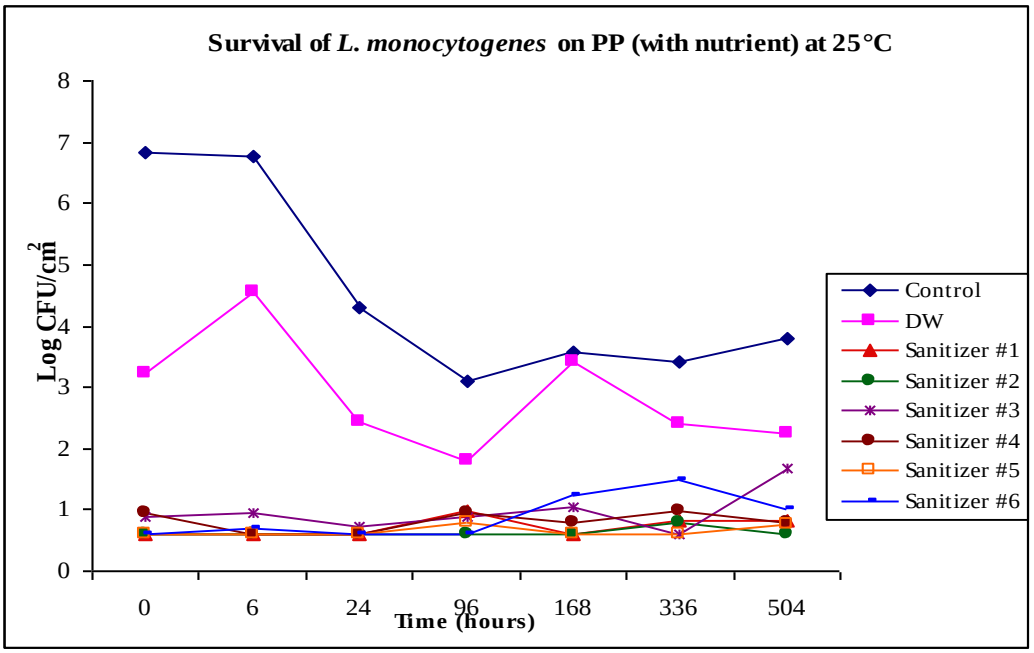
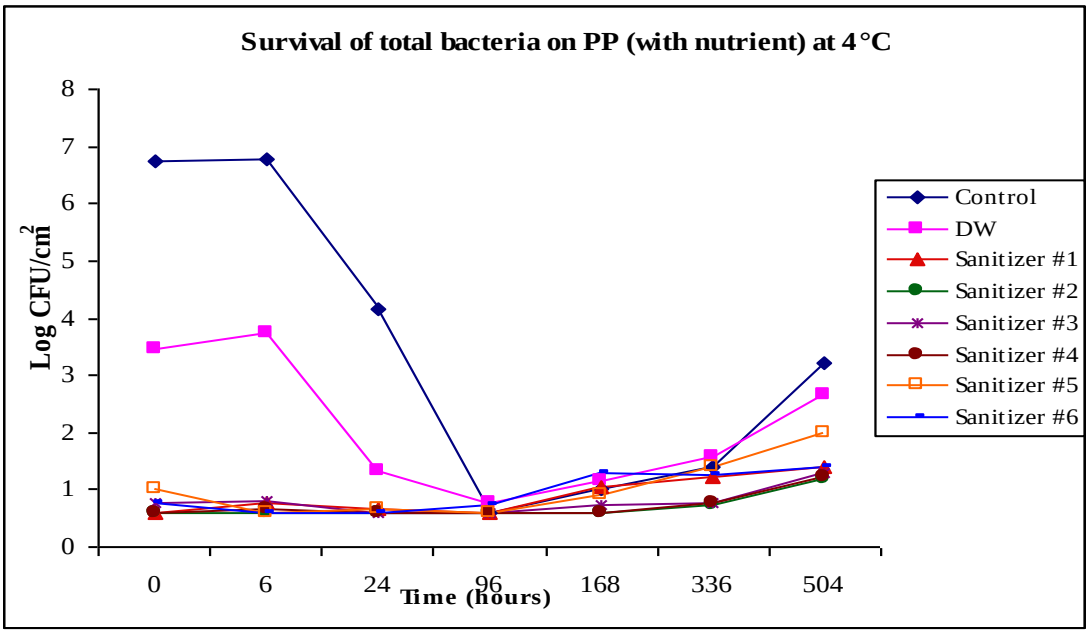


Figure 4.6. Data shown in Tables 4.7 and 4.8. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



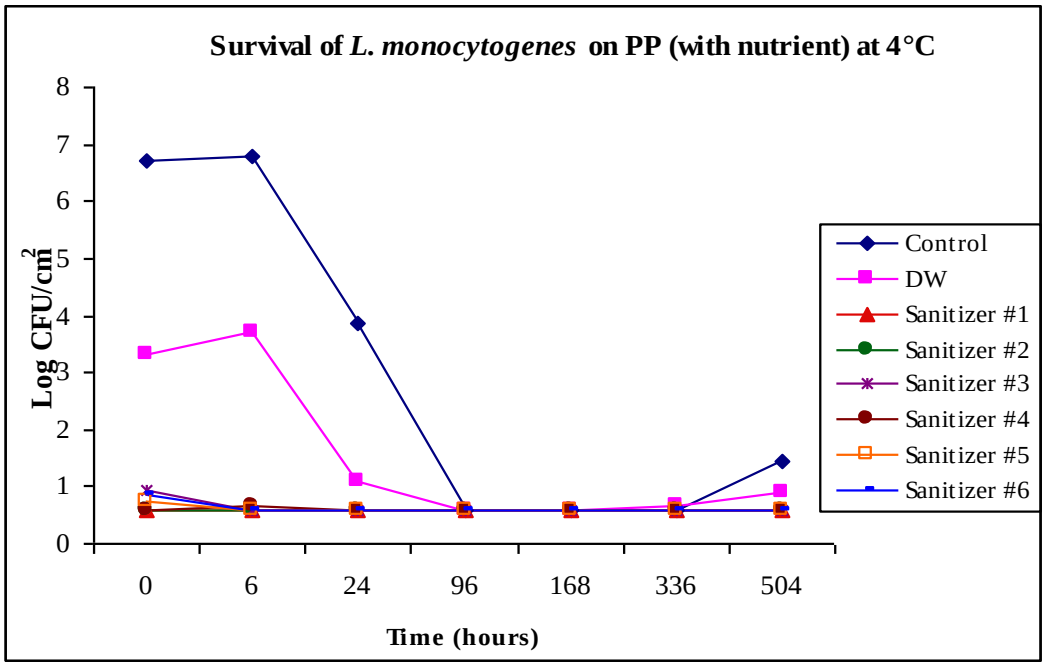
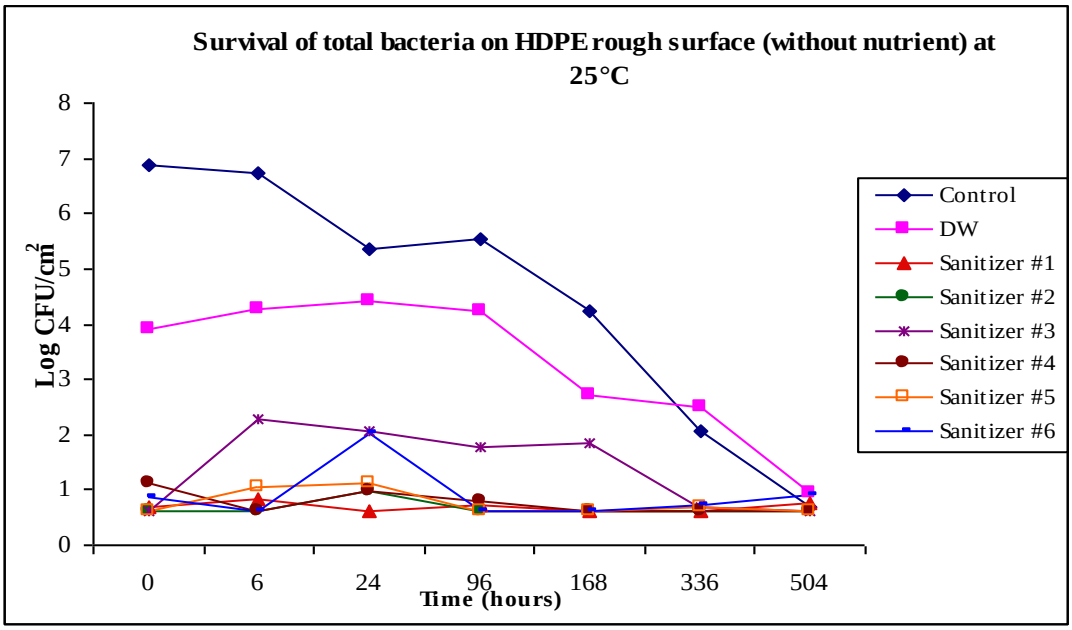


Figure 4.7. Data shown in Tables 4.9 and 4.10. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).



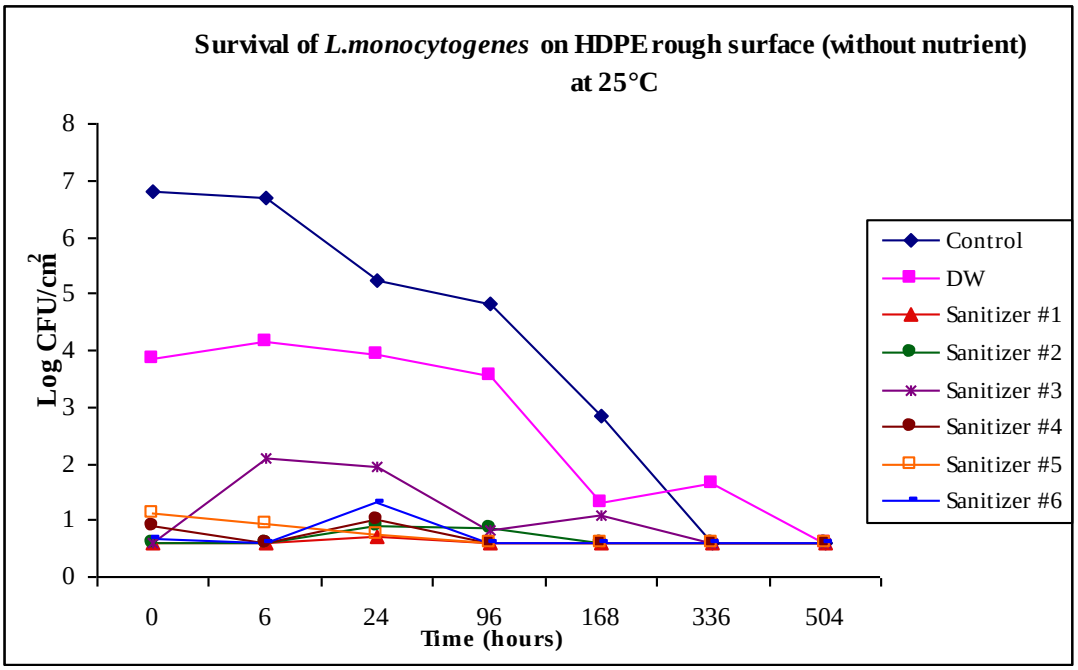
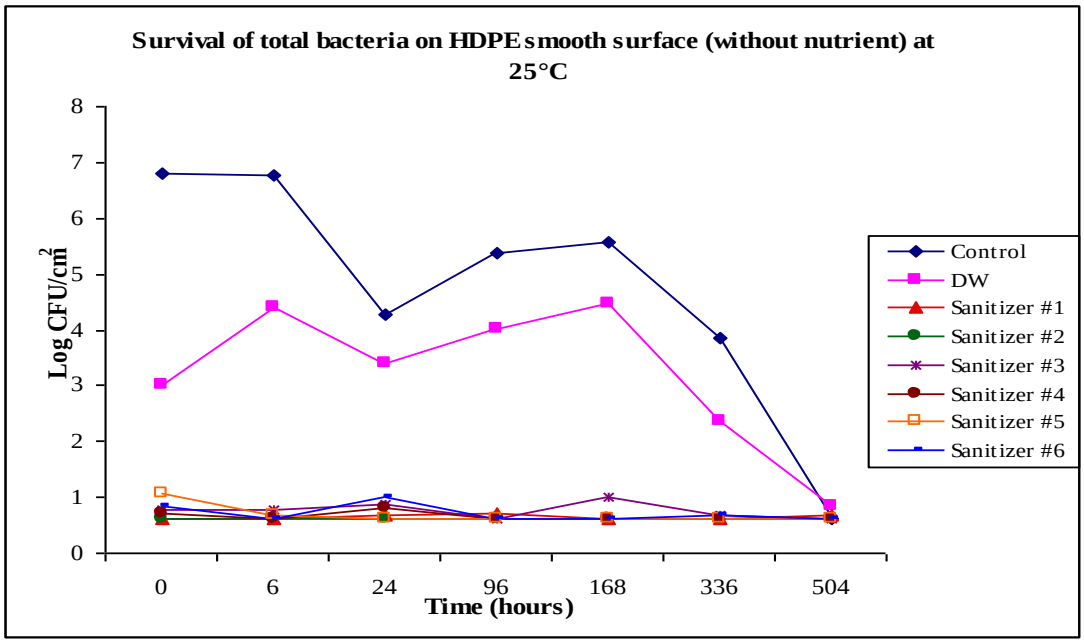


Figure 4.8. Data shown in Tables 4.11 and 4.12. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



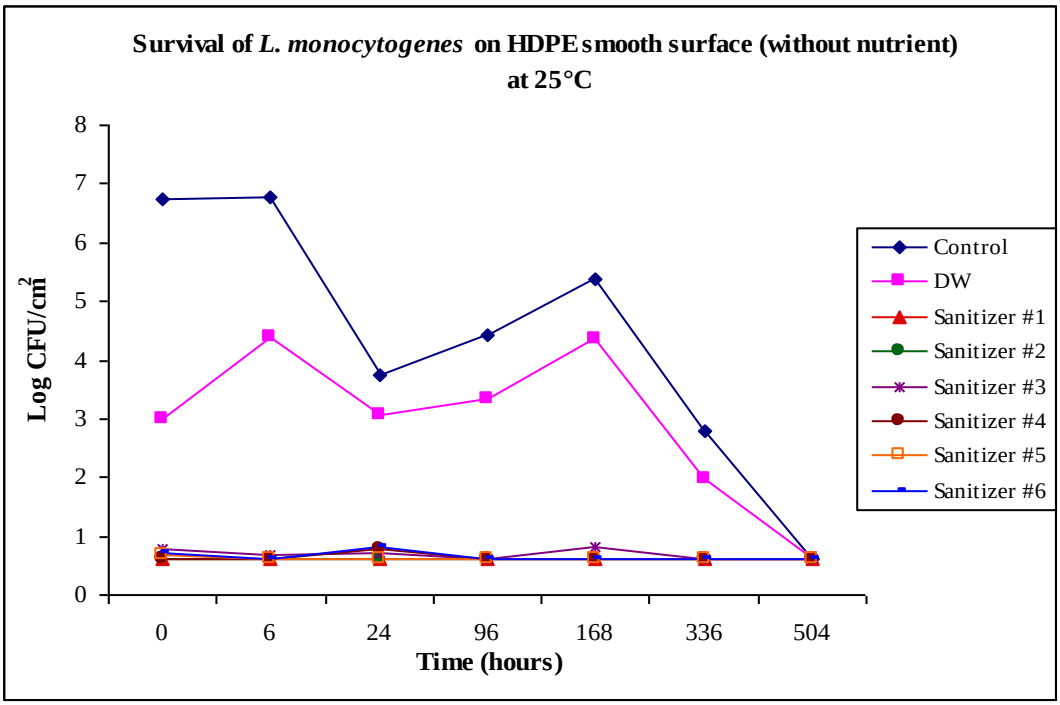
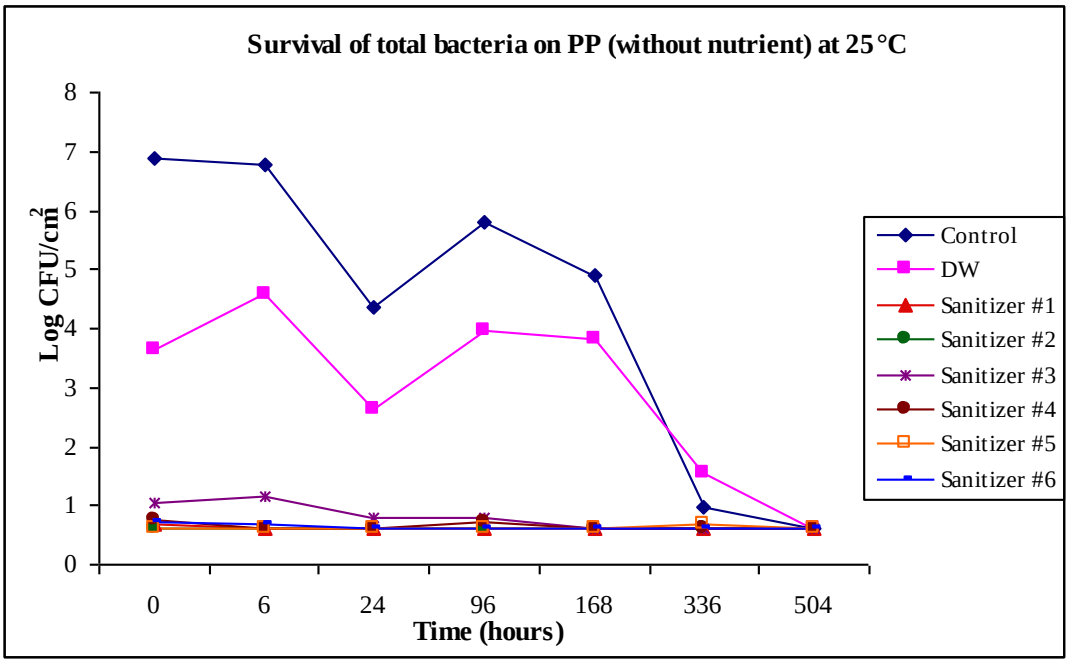


Figure 4.9. Data shown in Tables 4.13 and 4.14. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



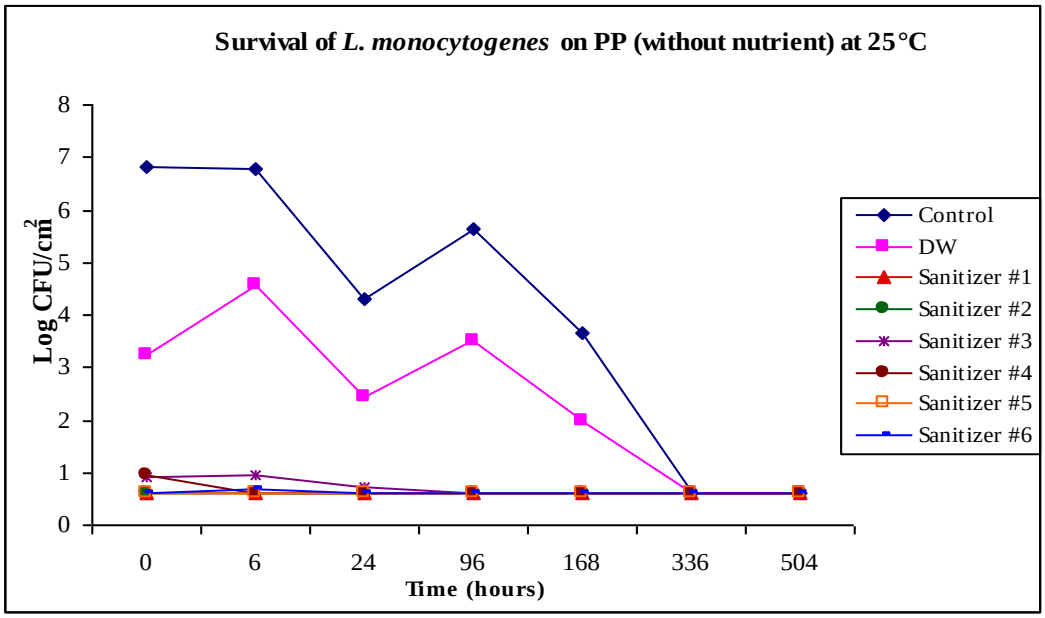
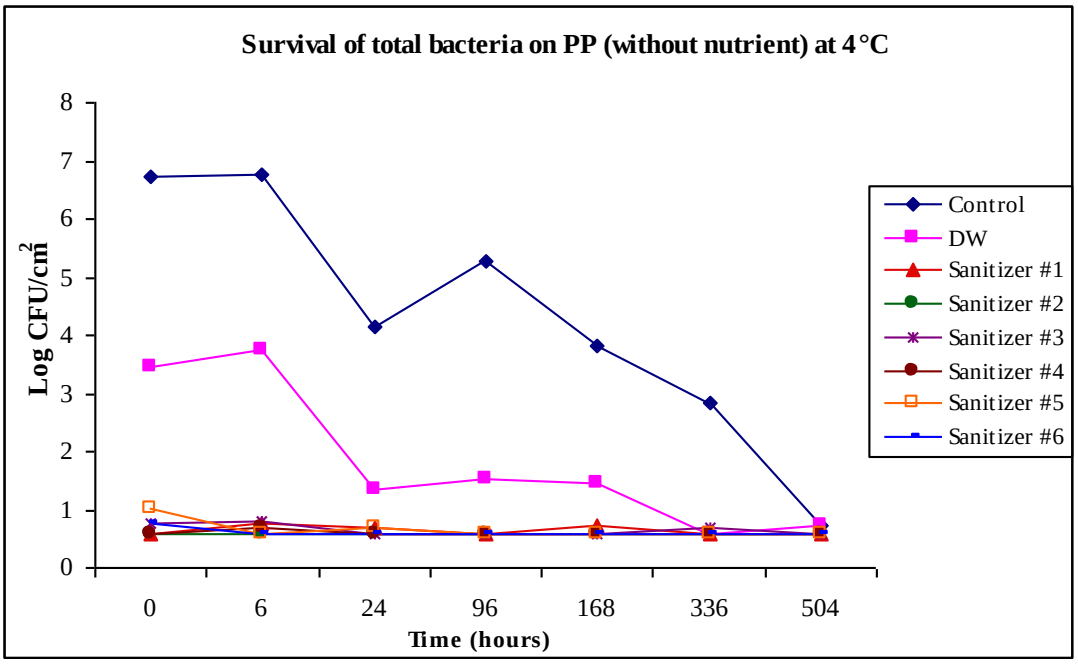


Figure 4.10. Data shown in Tables 4.15 and 4.16. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



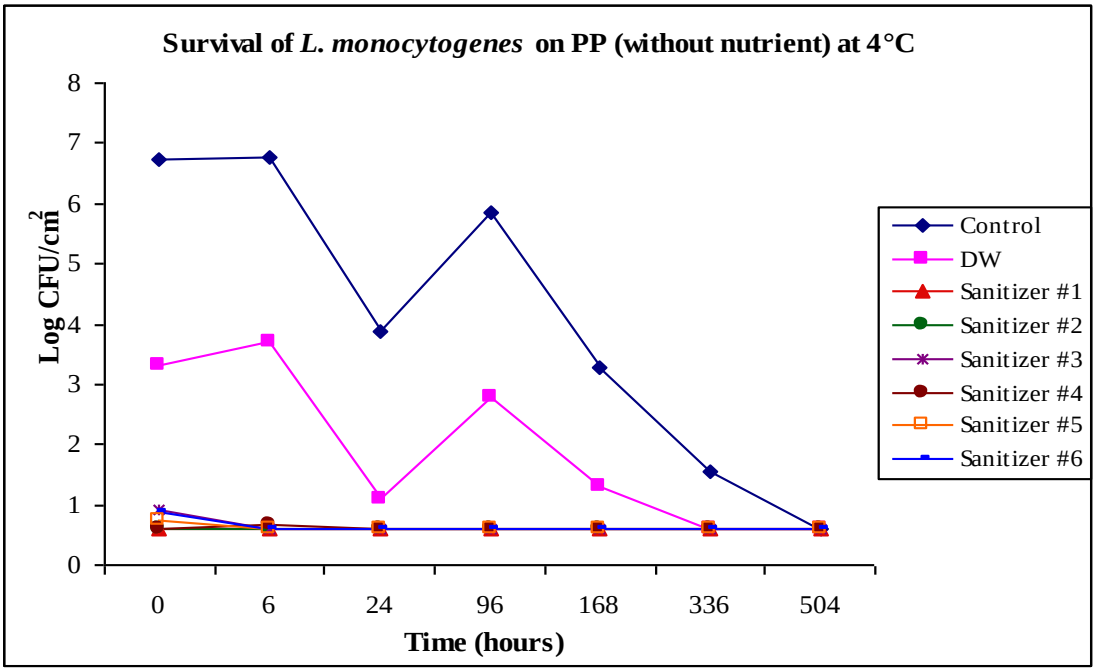
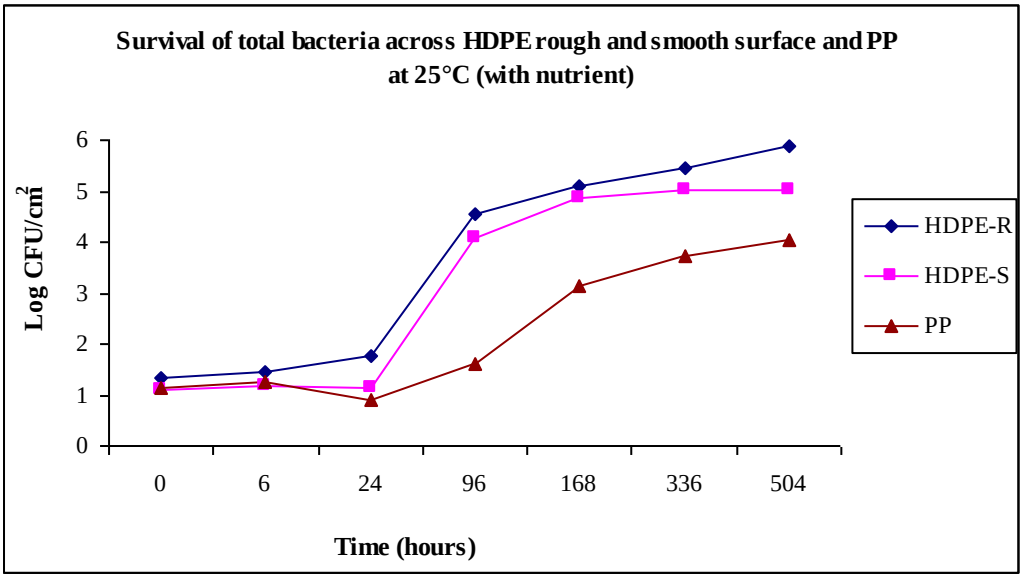


Figure 4.11. Data shown in Tables 4.17 and 4.18. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).



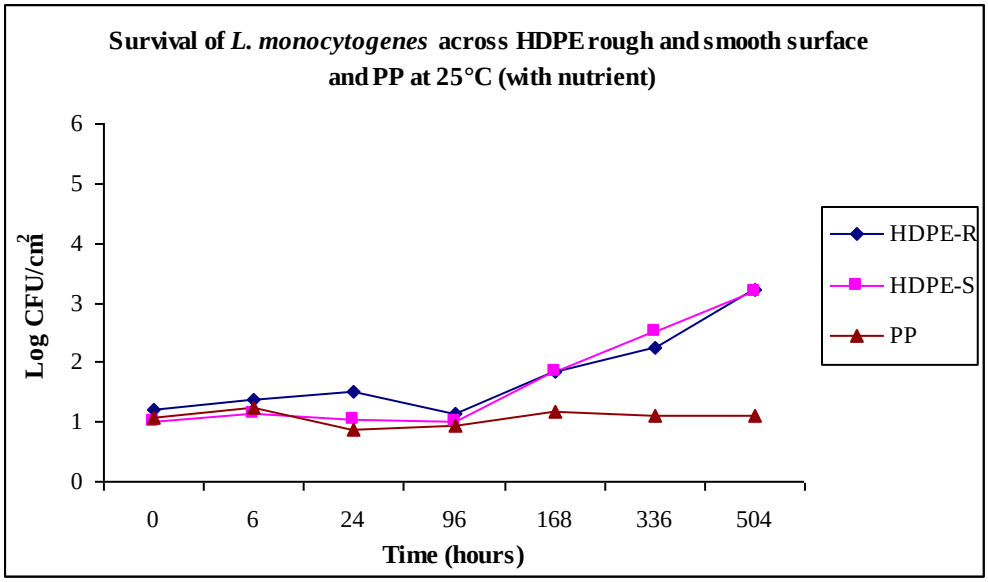


Figure 4.12. Data as shown in Tables 4.19 and 4.20. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C), subjected to daily nutrient exposure and treated with water or sanitizers.

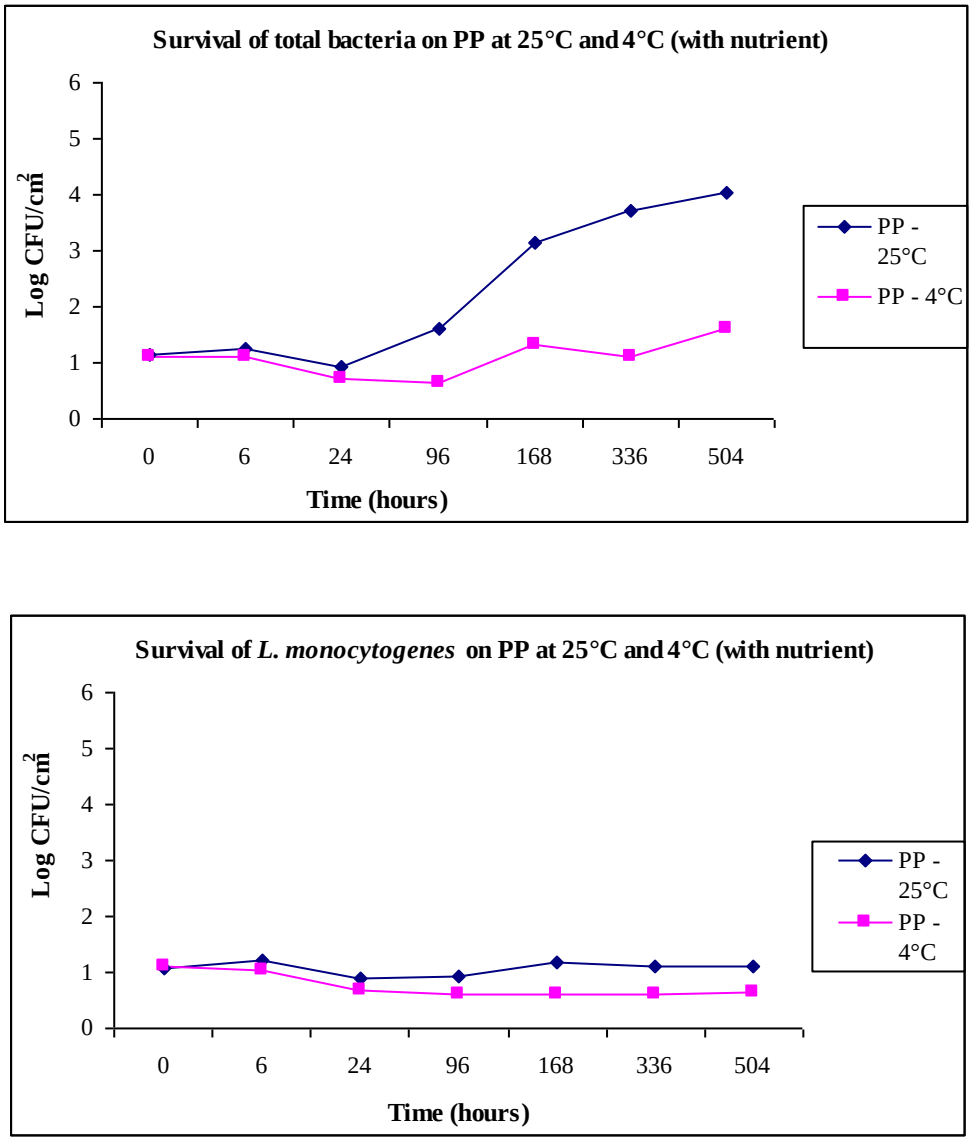
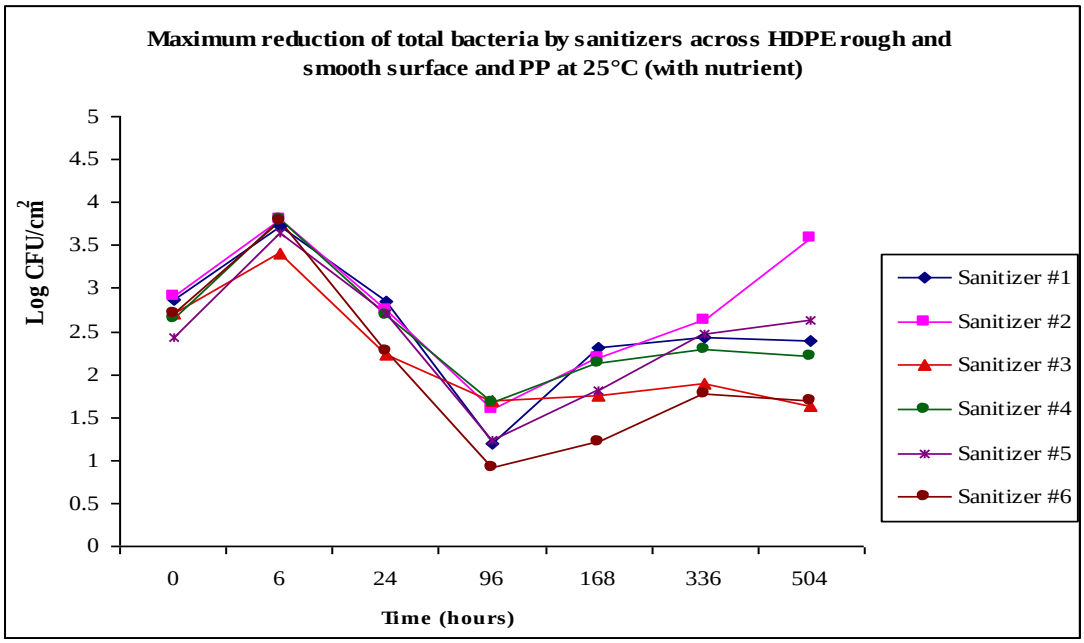


Figure 4.13. Data as shown in Tables 4.21 and 4.22. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90%, 25°C and 4°C), subjected to daily nutrient exposure and treated with water or sanitizers.



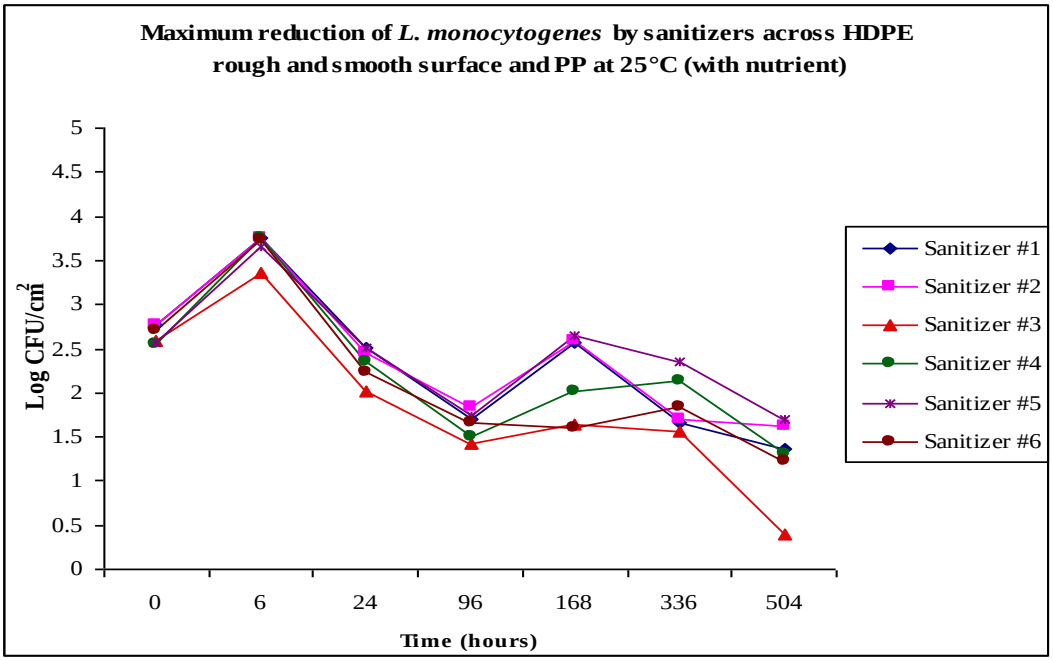
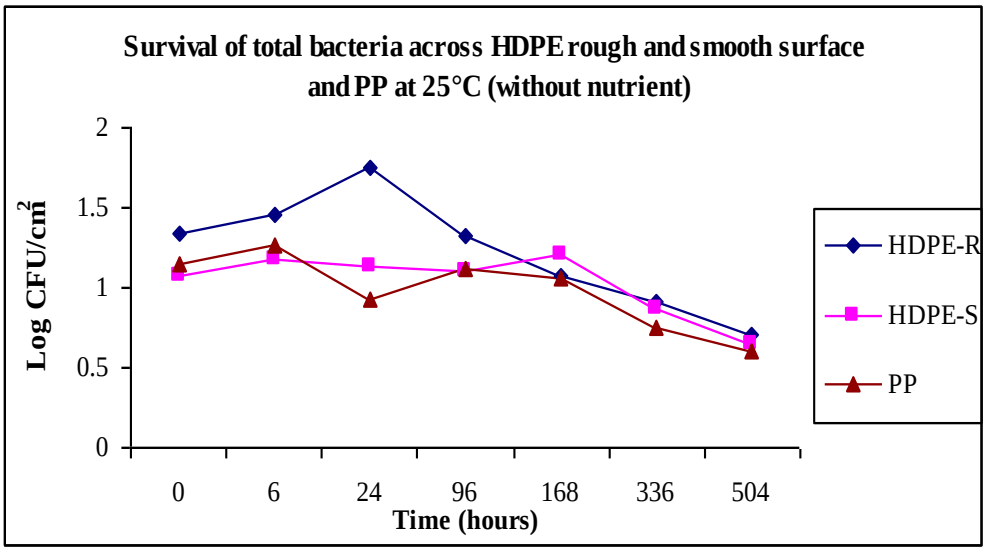


Figure 4.14. Data as shown in Tables 4.23 and 4.24. Mean (Log CFU/cm^2) reduction (n = 12) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to daily nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



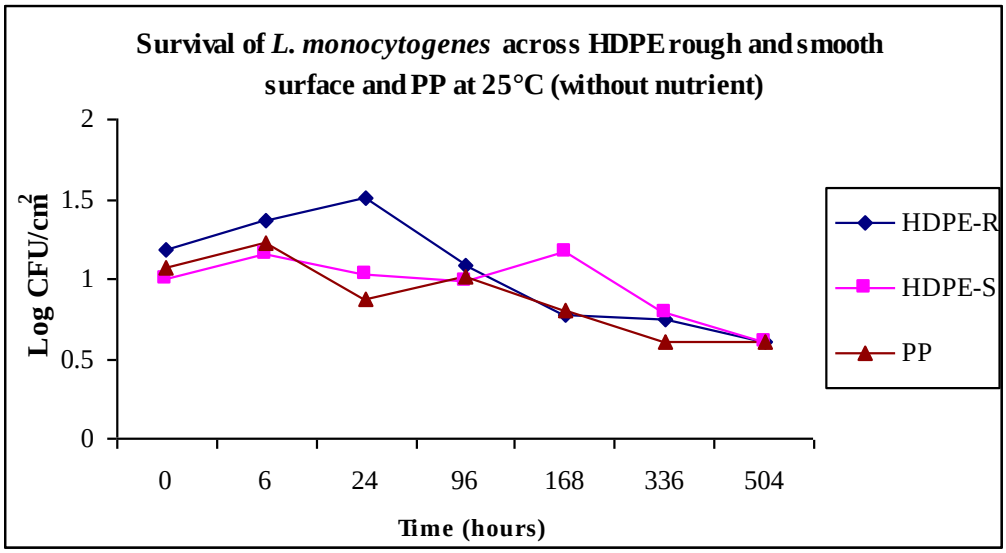
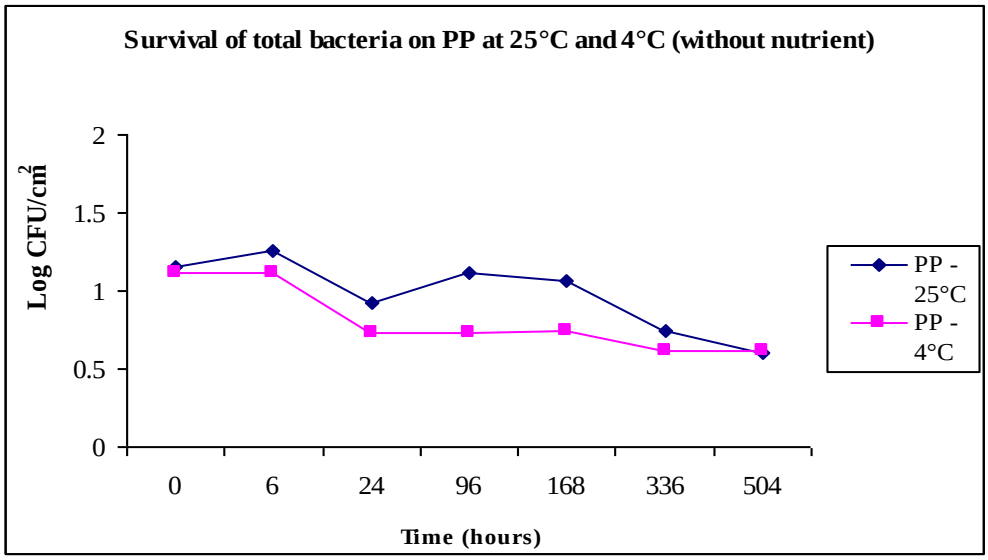


Figure 4.15. Data as shown in Tables 4.25 and 4.26. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to ham homogenate inoculated with 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C), not subjected to daily nutrient exposure but treated with water or sanitizers.



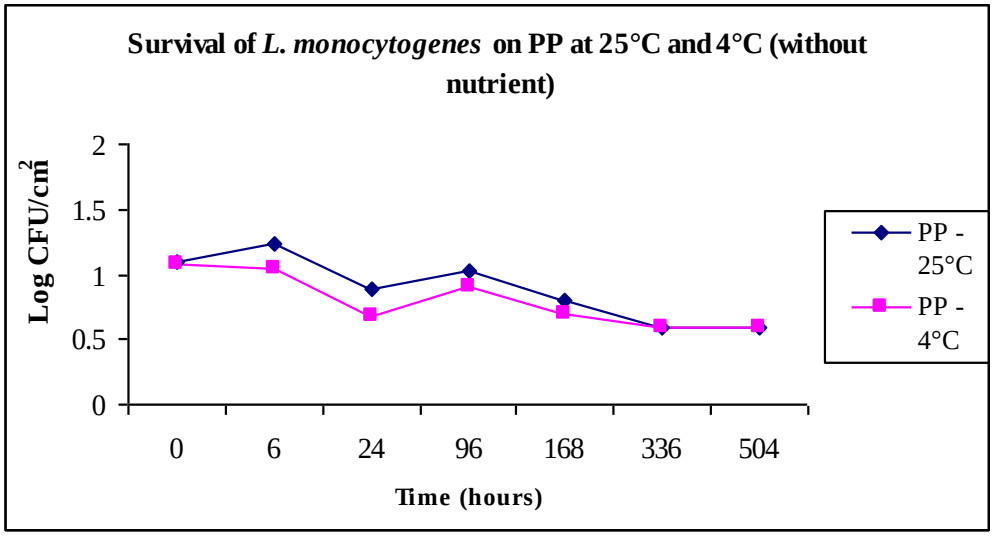
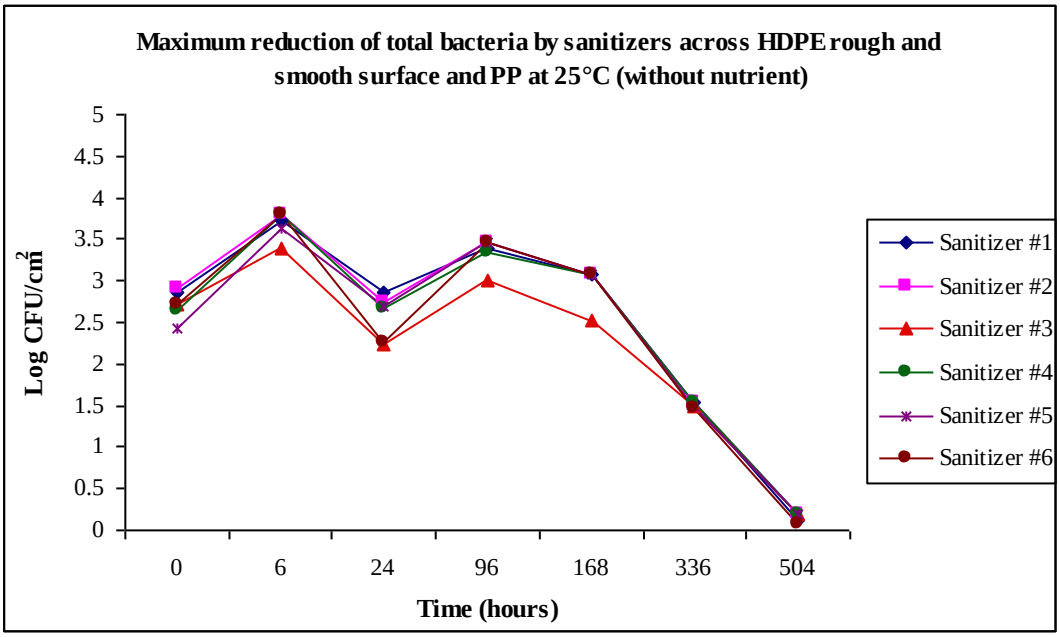


Figure 4.16. Data as shown in Tables 4.27 and 4.28. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces exposed to ham homogenate inoculated with 5-strain *L. monocytogenes* mixture (RH: 90%, 25°C and 4°C), not subjected to daily nutrient exposure but treated with water or sanitizers.



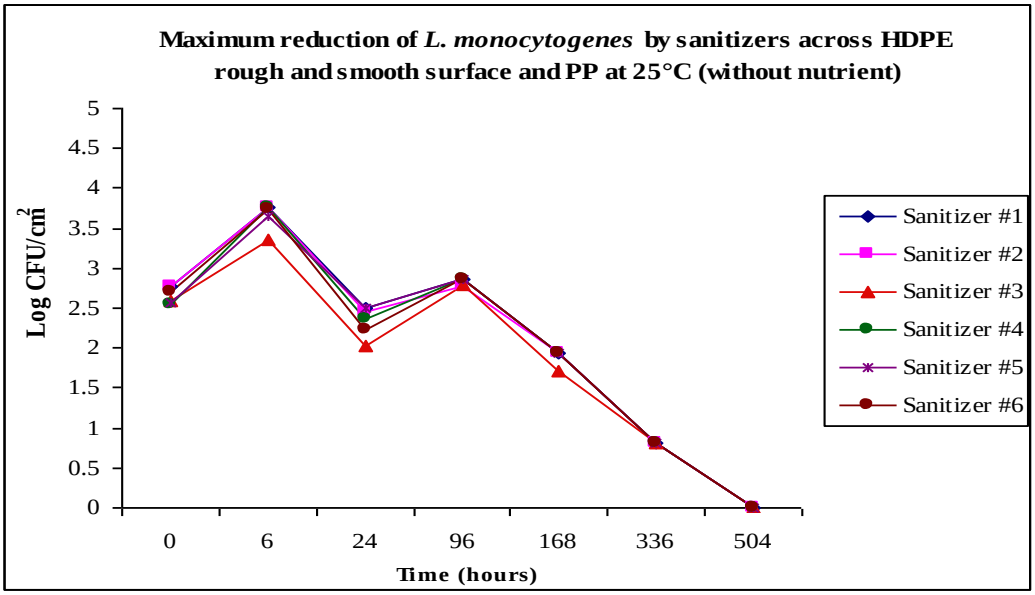
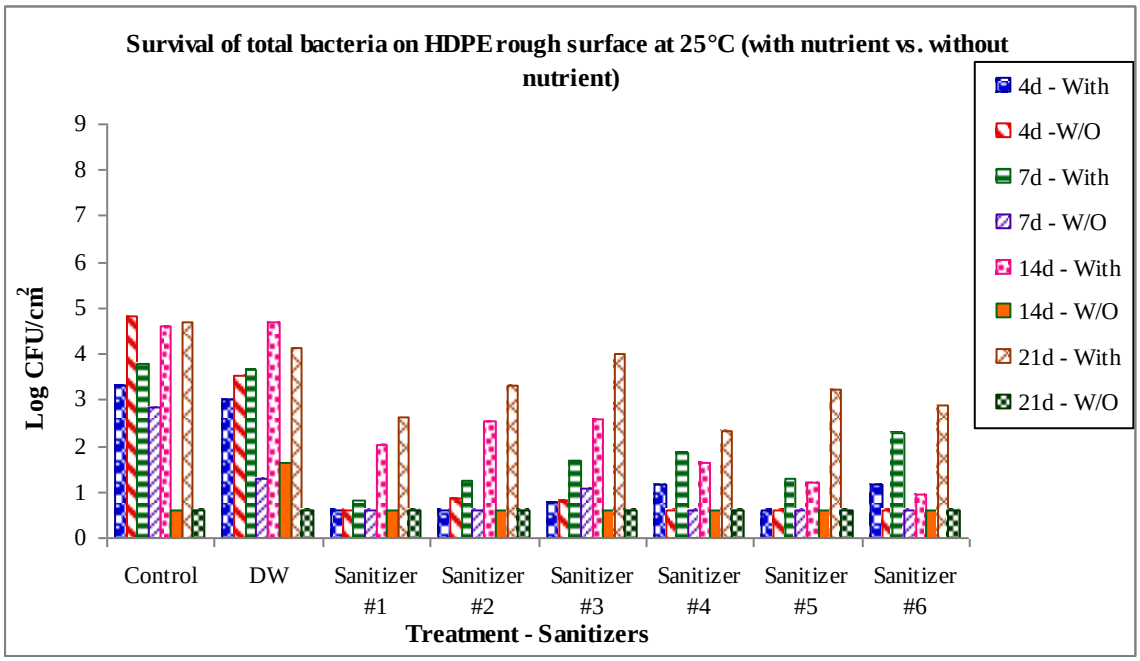


Figure 4.17. Data as shown in Tables 4.29 and 4.30. Mean (Log CFU/cm^2) reduction (n = 12) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces not exposed to daily nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



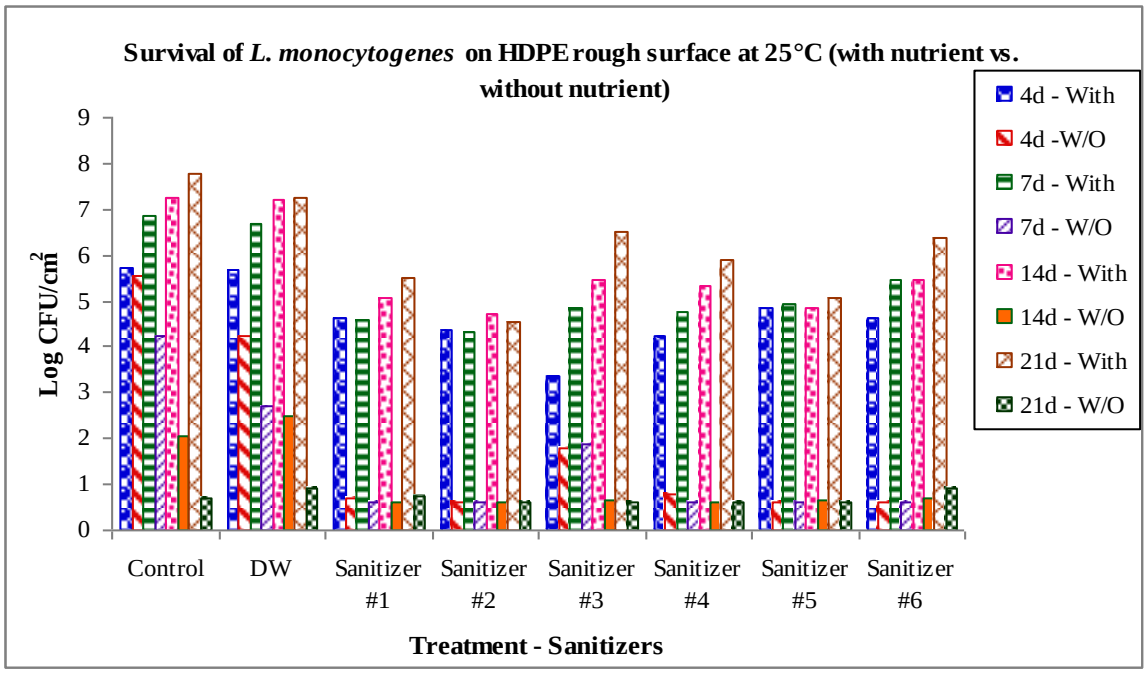
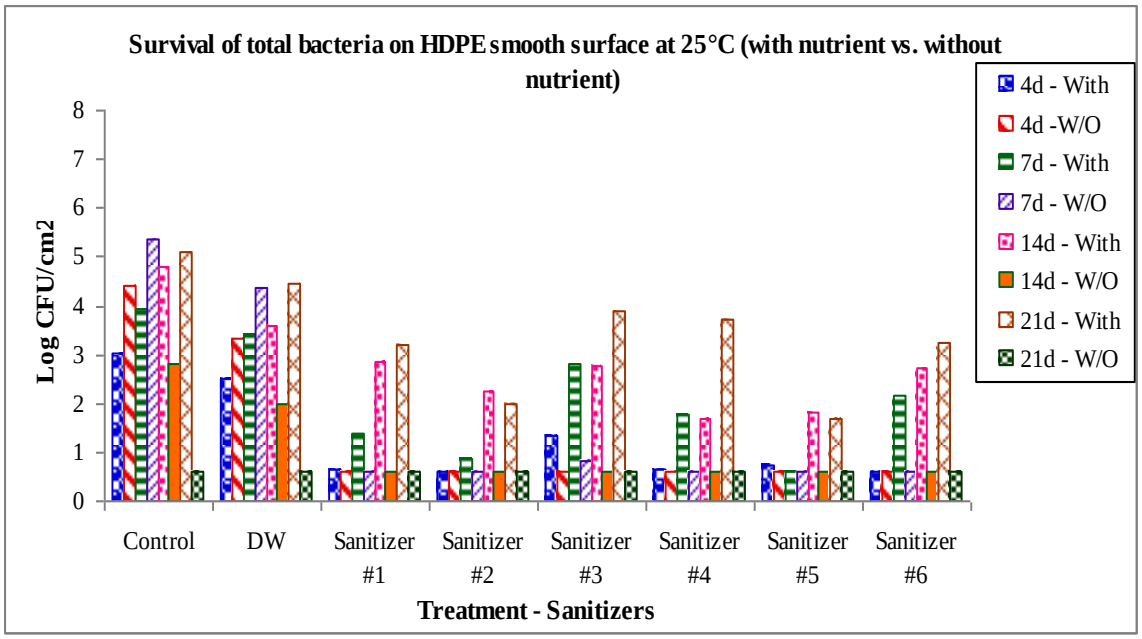


Figure 4.18. Data shown in Tables 4.31 and 4.32. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces (Comparison between coupons with daily exposure to nutrients and without daily exposure to nutrients), subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



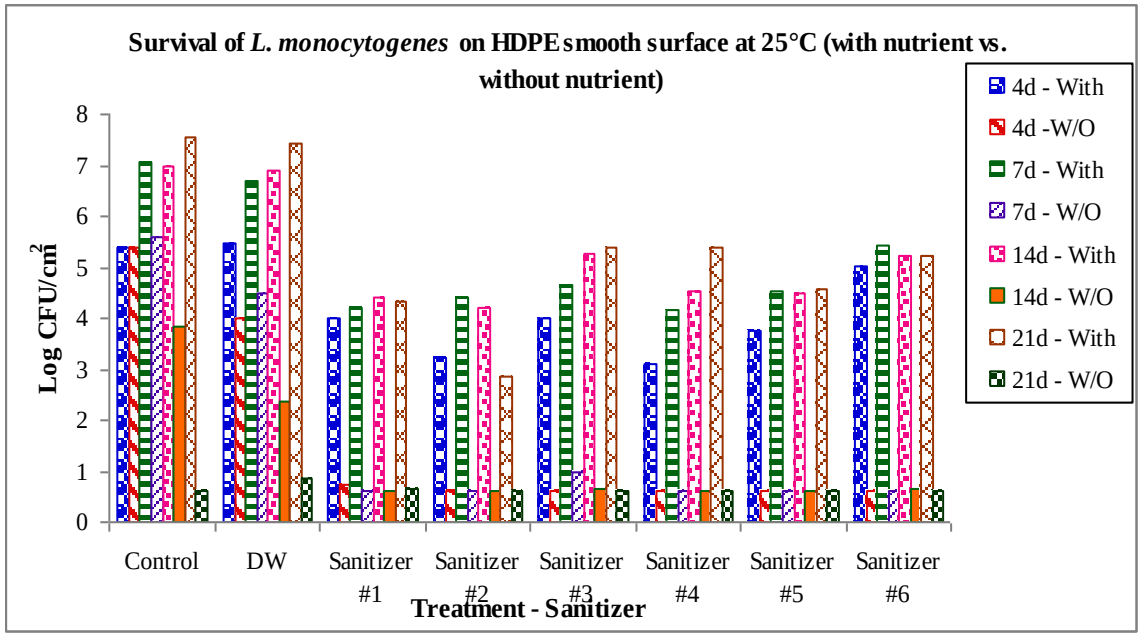
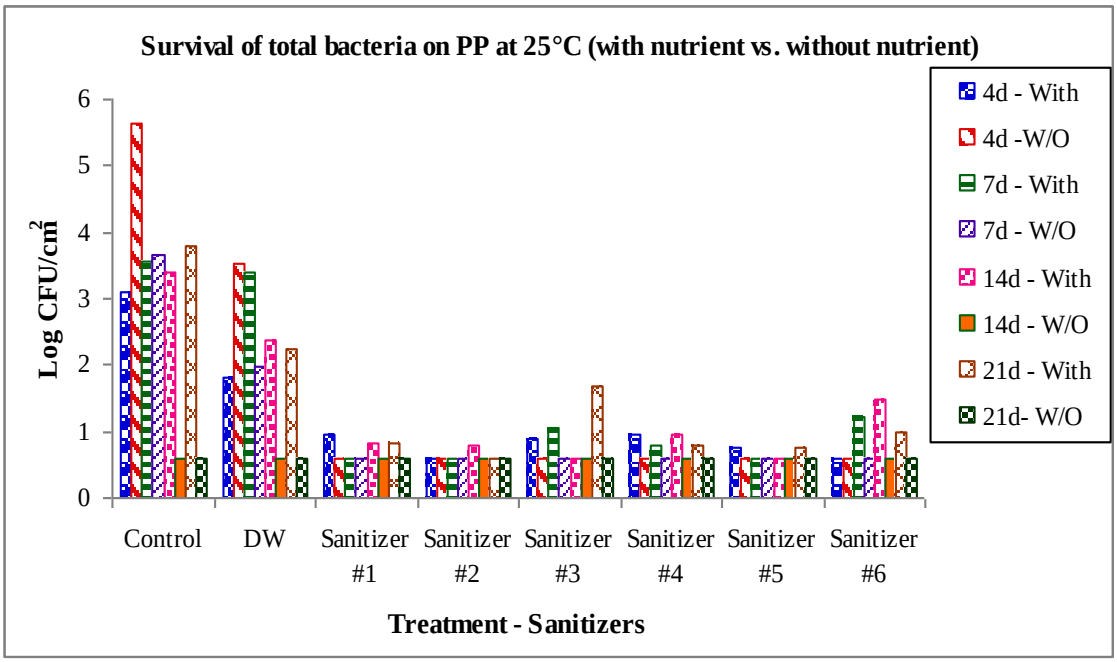


Figure 4.19. Data shown in Tables 4.33 and 4.34. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces (Comparison between coupons with daily exposure to nutrients and without daily exposure to nutrients), subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



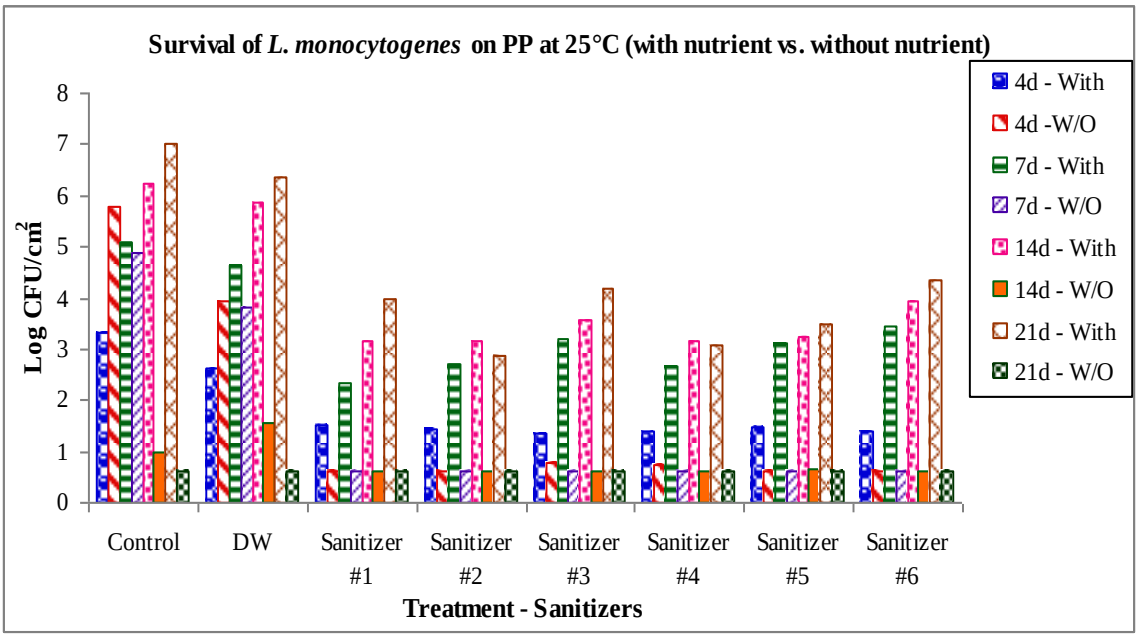
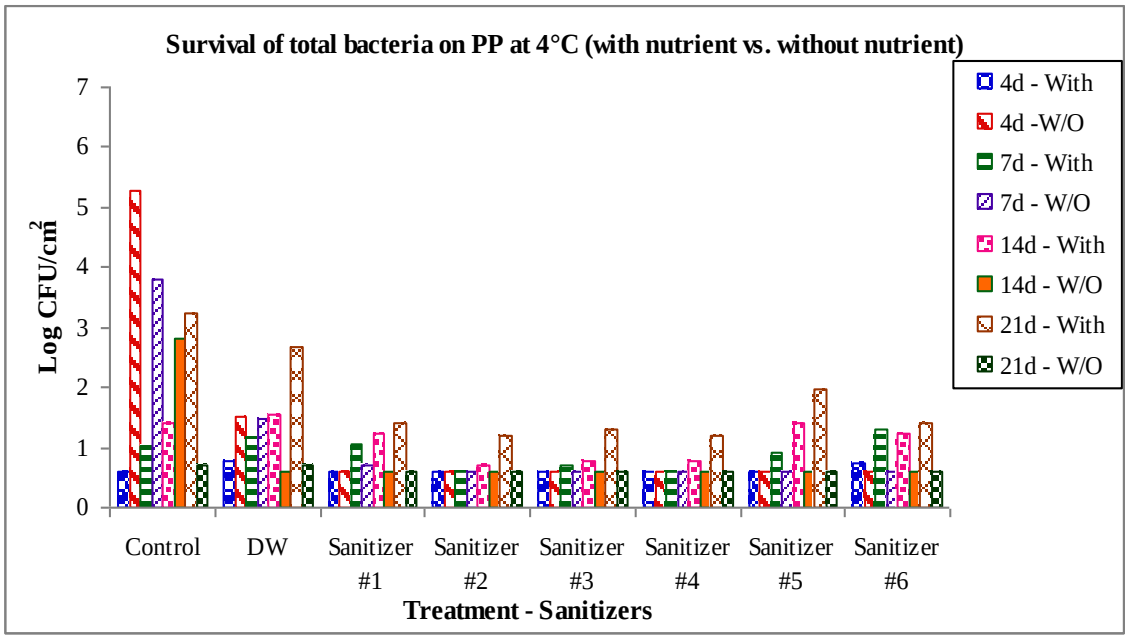


Figure 4.20. Data shown in Tables 4.35 and 4.36. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces (Comparison between coupons with daily exposure to nutrients and without daily exposure to nutrients), subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



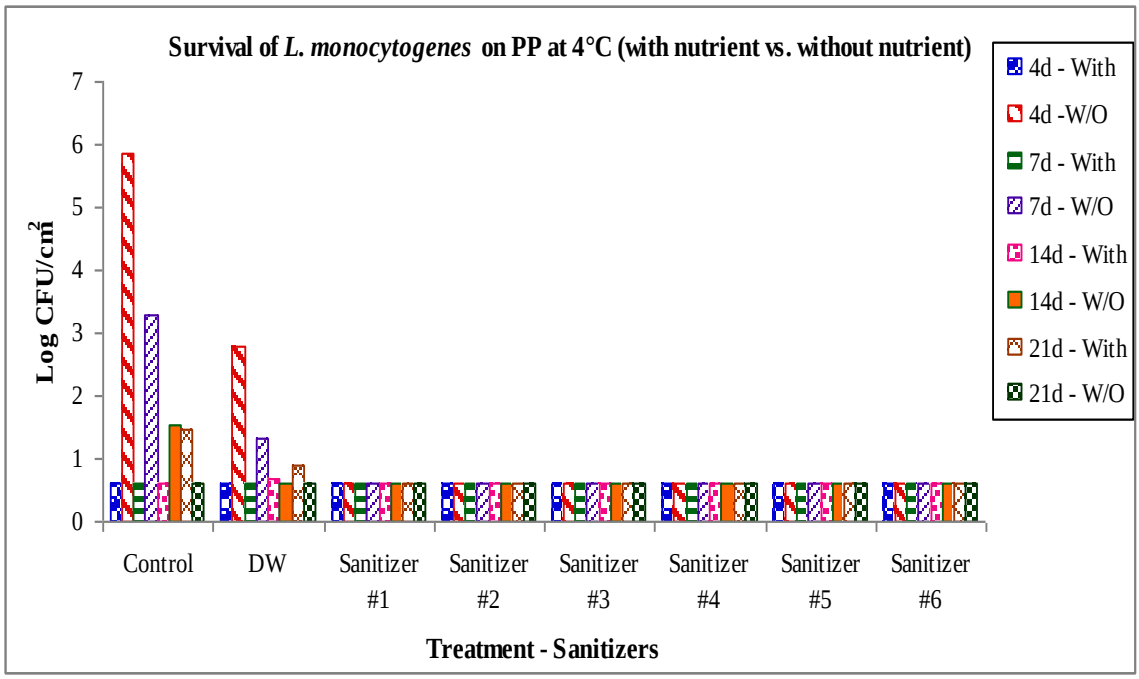


Figure4.21. Data shown in Tables 4.37 and 4.38. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces (Comparison between coupons with daily exposure to nutrients and without daily exposure to nutrients), subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Table 4.3. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) rough surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.87 (0.09) B^{a}	6.74 (0.25) B^{ac}	5.34 (0.57) A^{b}	5.73 (0.77) A^{bc}	6.88 (0.34) A^{a}	7.26 (0.09) A^{a}	7.77 (0.20) A^{a}
With Distilled Water	3.92 (0.38) A^{a}	4.27 (0.75) A^{a}	4.42 (0.13) A^{a}	5.70 (0.91) A^{c}	6.68 (0.29) A^{bc}	7.20 (0.11) A^{b}	7.26 (0.30) AC^{b}
Sanitizer 1	0.68 (0.15) C^{a}	0.84 (0.48) C^{a}	< 0.60 B^{a}	4.65 (0.11) AB^{b}	4.57 (0.40) BC^{b}	5.06 (0.51) B^{b}	5.50 (0.63) BF^{b}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	0.99 (0.47) BD^{a}	4.36 (0.68) AB^{b}	4.32 (0.43) B^{b}	4.71 (0.61) B^{b}	4.55 (0.87) D^{b}
Sanitizer 3	< 0.60 C^{a}	2.28 (1.30) D^{bd}	2.08 (0.99) C^{b}	3.35 (1.21) B^{d}	4.88 (0.40) BC^{e}	5.46 (0.65) B^{ce}	6.49 (0.49) CE^{c}
Sanitizer 4	1.14 (0.62) C^{a}	< 0.60 C^{a}	0.98 (0.57) BD^{a}	4.24 (0.84) AB^{c}	4.76 (0.44) BC^{dc}	5.36 (0.28) B^{bd}	5.89 (0.33) EF^{b}
Sanitizer 5	< 0.60 C^{a}	1.06 (0.91) C^{a}	1.12 (0.39) BC^{a}	4.85 (0.08) AB^{b}	4.93 (0.18) BC^{b}	4.83 (0.62) B^{b}	5.05 (0.92) BD^{b}
Sanitizer 6	0.87 (0.37) C^{a}	< 0.60 C^{a}	2.03 (1.48) CD^{b}	4.64 (1.04) AB^{d}	5.46 (0.70) C^{cd}	5.48 (0.55) B^{cd}	6.37 (0.31) E^{c}

A–F, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-e, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 4.4. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.79 (0.12) B^{a}	6.70 (0.30) B^{a}	5.25 (0.60) B^{b}	3.33 (2.17) A^{c}	3.78 (0.68) A^{cd}	4.59 (0.27) A^{bd}	4.68 (0.57) A^{bd}
With Distilled Water	3.85 (0.34) A^{ab}	4.16 (0.85) A^{a}	3.94 (0.92) A^{ab}	3.02 (1.77) A^{b}	3.67 (0.83) A^{ab}	4.71 (0.45) A^{a}	4.13 (0.33) AC^{a}
Sanitizer 1	< 0.60 C^{a}	< 0.60 C^{a}	0.72 (0.24) C^{a}	< 0.60 B^{a}	0.83 (0.45) B^{a}	2.02 (1.03) BC^{b}	2.64 (1.41) B^{b}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	0.90 (0.35) C^{a}	< 0.60 B^{a}	1.28 (0.83) BD^{a}	2.54 (0.36) B^{b}	3.33 (0.93) BC^{b}
Sanitizer 3	< 0.60 C^{a}	2.10 (0.94) D^{b}	1.96 (0.91) D^{b}	0.80 (0.24) B^{a}	1.68 (0.29) BC^{ab}	2.59 (1.37) B^{b}	4.00 (0.44) ACD^{c}
Sanitizer 4	0.90 (0.35) CD^{ac}	< 0.6 C^{a}	1.01 (0.54) CD^{ac}	1.18 (0.67) B^{ac}	1.85 (1.07) CD^{bc}	1.65 (0.82) BC^{ab}	2.34 (1.31) B^{b}
Sanitizer 5	1.14 (0.73) D^{a}	0.95 (0.70) C^{a}	0.75 (0.30) C^{a}	< 0.60 B^{a}	1.31 (0.88) BD^{a}	1.19 (1.18) C^{a}	3.23 (0.66) BC^{b}
Sanitizer 6	0.68 (0.15) CD^{a}	< 0.60 C^{a}	1.32 (0.83) CD^{ac}	1.15 (0.65) B^{a}	2.28 (1.31) C^{bc}	0.93 (0.65) C^{a}	2.87 (0.43) BD^{b}

A–D, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-d, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 4.5. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) smooth surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.80 (0.10) B^{a}	6.77 (0.20) B^{a}	4.27 (0.57) A^{b}	5.37 (0.72) A^{c}	7.05 (0.29) A^{a}	6.99 (0.28) A^{a}	7.56 (0.27) A^{a}
With Distilled Water	3.01 (0.24) A^{a}	4.41 (0.28) A^{bd}	3.39 (0.35) A^{ab}	5.47 (1.00) A^{d}	6.69 (0.42) A^{c}	6.91 (0.28) A^{c}	7.41 (0.45) A^{c}
Sanitizer 1	< 0.60 C^{a}	< 0.60 C^{a}	0.68 (0.15) B^{a}	4.01 (1.28) AB^{b}	4.19 (0.54) B^{b}	4.40 (0.38) BC^{b}	4.33 (0.47) B^{b}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	3.22 (1.63) B^{bc}	4.41 (0.76) B^{d}	4.22 (0.50) B^{bd}	2.85 (0.72) C^{c}
Sanitizer 3	0.78 (0.35) C^{a}	0.78 (0.35) C^{a}	0.87 (0.54) B^{a}	4.00 (0.10) AB^{c}	4.66 (0.81) BC^{bc}	5.27 (0.33) C^{b}	5.40 (0.08) D^{c}
Sanitizer 4	0.72 (0.24) C^{a}	< 0.60 C^{a}	0.80 (0.24) B^{a}	3.09 (1.71) B^{c}	4.17 (0.69) B^{cd}	4.54 (0.78) BC^{bd}	5.37 (0.45) DE^{b}
Sanitizer 5	1.07 (0.33) C^{a}	0.68 (0.15) C^{a}	< 0.60 B^{a}	3.76 (1.23) BC^{b}	4.53 (0.54) BC^{b}	4.48 (0.40) BC^{b}	4.59 (0.45) BE^{b}
Sanitizer 6	0.83 (0.29) C^{a}	< 0.60 C^{a}	0.99 (0.40) B^{a}	5.01 (0.31) AC^{b}	5.44 (0.70) C^{b}	5.22 (0.78) C^{b}	5.21 (0.39) DE^{b}

A–E, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-d, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 4.6. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.75 (0.14) B^{a}	6.76 (0.23) B^{a}	3.74 (1.19) A^{bd}	3.04 (0.30) AC^{d}	3.92 (0.61) A^{bd}	4.79 (0.50) B^{bc}	5.11 (0.85) A^{c}
With Distilled Water	3.01 (0.16) A^{a}	4.39 (0.25) A^{b}	3.08 (0.44) A^{a}	2.51 (1.31) AC^{a}	3.40 (0.94) AC^{ab}	3.58 (1.28) A^{ab}	4.45 (0.27) AC^{b}
Sanitizer 1	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	0.68 (0.15) BD^{a}	1.37 (0.63) BDF^{a}	2.84 (0.83) AD^{b}	3.21 (0.78) B^{b}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 BD^{a}	0.85 (0.50) BF^{a}	2.26 (0.59) CD^{b}	2.01 (1.39) D^{b}
Sanitizer 3	0.80 (0.39) C^{a}	0.68 (0.15) C^{a}	0.72 (0.24) B^{a}	1.34 (0.91) CD^{a}	2.82 (0.39) CE^{bc}	2.77 (1.15) ADE^{b}	3.91 (0.12) BC^{c}
Sanitizer 4	< 0.60 C^{a}	< 0.60 C^{a}	0.78 (0.35) B^{ac}	0.68 (0.15) BD^{a}	1.79 (1.37) BD^{bc}	1.67 (1.33) CE^{ac}	3.73 (0.38) BC^{b}
Sanitizer 5	0.68 (0.15) C^{ac}	< 0.60 C^{a}	< 0.60 B^{a}	0.75 (0.30) BD^{ab}	< 0.60 F^{a}	1.82 (1.03) CD^{b}	1.70 (1.42) D^{bc}
Sanitizer 6	0.72 (0.24) C^{a}	< 0.60 C^{a}	0.81 (0.42) B^{a}	< 0.60 BD^{a}	2.15 (1.21) DE^{c}	2.74 (0.30) ADE^{bc}	3.25 (0.86) B^{b}

A–F, means within a column and within the same day of sanitizer treatment at 25°C are significantly different (P<0.05)

a-d, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.7. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.87 (0.12) B^a	6.79 (0.11) B^a	4.35 (0.47) B^b	3.34 (1.37) A^c	5.10 (0.95) A^d	6.24 (0.88) A^a	7.03 (0.68) A^a
With Distilled Water	3.63 (0.42) A^a	4.57 (0.47) A^b	2.63 (0.96) A^c	2.62 (1.37) AB^c	4.64 (0.68) A^b	5.86 (0.86) A^d	6.34 (0.35) A^d
Sanitizer 1	0.68 (0.15) C^a	< 0.60 C^a	< 0.60 C^a	1.52 (1.06) B^c	2.32 (0.08) B^c	3.16 (0.32) B^b	3.97 (0.35) BE^b
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	1.43 (0.96) B^c	2.70 (0.61) BC^b	3.15 (0.44) B^b	2.86 (0.57) CD^b
Sanitizer 3	1.05 (0.54) C^a	1.17 (0.66) C^a	0.78 (0.35) C^a	1.36 (0.90) B^a	3.19 (0.49) BC^c	3.55 (0.07) B^{bc}	4.20 (0.05) BE^b
Sanitizer 4	0.75 (0.30) C^a	< 0.60 C^a	< 0.60 C^a	1.40 (0.97) B^a	2.68 (0.74) BC^b	3.17 (0.92) B^b	3.08 (0.67) CD^b
Sanitizer 5	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	1.48 (0.77) B^c	3.11 (0.19) BC^b	3.23 (0.89) B^b	3.47 (0.54) BD^b
Sanitizer 6	0.72 (0.24) C^a	0.68 (0.15) C^a	< 0.60 C^a	1.38 (0.90) B^a	3.43 (0.16) C^c	3.92 (0.06) B^{bc}	4.34 (0.03) E^b

A–E, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-d, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 4.8. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.83 (0.18) B^{a}	6.77 (0.13) B^{a}	4.31 (0.47) B^{b}	3.11 (0.49) A^{c}	3.57 (0.75) A^{bc}	3.41 (0.31) A^{c}	3.78 (1.10) B^{bc}
With Distilled Water	3.24 (0.17) A^{ad}	4.56 (0.53) A^{b}	2.45 (1.23) A^{ac}	1.81 (0.56) AB^{c}	3.41 (0.46) A^{d}	2.39 (0.56) AC^{c}	2.23 (0.38) A^{c}
Sanitizer 1	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	0.97 (0.74) B^{a}	< 0.60 B^{a}	0.83 (0.45) B^{a}	0.83 (0.29) C^{a}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 B^{a}	0.78 (0.35) B^{a}	< 0.60 C^{a}
Sanitizer 3	0.90 (0.43) C^{ab}	0.94 (0.49) C^{ab}	0.72 (0.24) C^{a}	0.90 (0.25) B^{ab}	1.05 (0.36) B^{ab}	< 0.60 B^{a}	1.67 (0.19) AC^{b}
Sanitizer 4	0.95 (0.40) C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	0.95 (0.70) B^{a}	0.80 (0.39) B^{a}	0.97 (0.56) B^{a}	0.78 (0.35) C^{a}
Sanitizer 5	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	0.78 (0.35) B^{a}	< 0.60 B^{a}	< 0.60 B^{a}	0.75 (0.30) C^{a}
Sanitizer 6	< 0.60 C^{a}	0.68 (0.15) C^{ab}	< 0.60 C^{a}	< 0.60 B^{a}	1.23 (0.73) B^{ab}	1.49 (0.30) BC^{b}	1.00 (0.49) C^{ab}

A–C, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-d, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 4.9. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.74 (0.26) B^{a}	6.77 (0.12) B^{a}	4.16 (0.75) B^{b}	< 0.60 A^{e}	1.01 (0.54) A^{ce}	1.41 (0.60) A^{c}	3.22 (0.06) A^{d}
With Distilled Water	3.47 (0.24) A^{a}	3.73 (0.77) A^{a}	1.34 (0.74) AC^{b}	0.78 (0.35) A^{d}	1.15 (0.41) A^{bd}	1.56 (0.73) A^{b}	2.66 (0.74) AC^{c}
Sanitizer 1	< 0.60 C^{a}	0.78 (0.35) C^{ac}	0.68 (0.15) AC^{a}	< 0.60 A^{a}	1.06 (0.38) A^{ad}	1.23 (0.29) A^{cd}	1.39 (0.82) BD^{bd}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	0.72 (0.24) A^{ab}	1.18 (0.56) BD^{b}
Sanitizer 3	0.78 (0.35) C^{a}	0.80 (0.24) C^{ab}	< 0.60 C^{a}	< 0.60 A^{a}	0.72 (0.24) A^{a}	0.78 (0.35) A^{a}	1.31 (0.82) BD^{b}
Sanitizer 4	< 0.60 C^{a}	0.68 (0.15) C^{a}	< 0.60 C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	0.78 (0.35) A^{ab}	1.21 (0.55) BD^{b}
Sanitizer 5	1.02 (0.29) C^{ac}	< 0.60 C^{a}	0.68 (0.15) AC^{a}	< 0.60 A^{a}	0.92 (0.23) A^{ac}	1.40 (0.14) A^{c}	1.98 (0.31) CD^{b}
Sanitizer 6	0.78 (0.35) C^{ac}	< 0.60 C^{a}	< 0.60 C^{a}	0.75 (0.17) A^{ad}	1.30 (0.16) A^{cb}	1.25 (0.31) A^{cbd}	1.40 (0.64) BD^{b}

A–D, means within a column and within the same day of sanitizer treatment at 4°C are significantly different (P<0.05)

a-e, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.10. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, daily exposed to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.72 (0.35) B^{a}	6.78 (0.12) B^{a}	3.88 (0.71) B^{b}	< 0.60 A^{c}	< 0.60 A^{c}	< 0.60 A^{c}	1.45 (0.59) A^{d}
With Distilled Water	3.32 (0.38) A^{a}	3.71 (0.76) A^{a}	1.09 (0.50) A^{b}	< 0.60 A^{b}	< 0.60 A^{b}	0.68 (0.15) A^{b}	0.90 (0.25) AB^{b}
Sanitizer 1	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 B^{a}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 B^{a}
Sanitizer 3	0.92 (0.45) C^{a}	< 0.60 C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 B^{a}
Sanitizer 4	< 0.60 C^{a}	0.68 (0.15) C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 B^{a}
Sanitizer 5	0.75 (0.17) C^{a}	< 0.60 C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 B^{a}
Sanitizer 6	0.87 (0.37) C^{a}	< 0.60 C^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 A^{a}	< 0.60 B^{a}

A–C, means within a column and within the same day of sanitizer treatment at 4°C are significantly different (P<0.05)

a-d, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.11. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) rough surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.87 (0.09) B^a	6.74 (0.25) B^a	5.34 (0.57) A^b	5.54 (0.37) B^b	4.23 (0.70) B^e	2.06 (1.21) A^c	0.68 (0.15) AB^d
With Distilled Water	3.92 (0.38) A^a	4.27 (0.75) A^a	4.42 (0.13) A^a	4.23 (0.36) A^a	2.71 (0.41) A^b	2.51 (1.49) A^b	0.93 (0.38) A^c
Sanitizer 1	0.68 (0.15) C^a	0.84 (0.48) C^a	< 0.60 B^a	0.72 (0.24) C^a	< 0.60 C^a	< 0.60 B^a	0.75 (0.30) AB^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	0.99 (0.47) BD^a	< 0.60 C^a	< 0.60 C^a	< 0.60 B^a	< 0.60 B^a
Sanitizer 3	< 0.60 C^a	2.28 (1.30) D^b	2.08 (0.99) C^b	1.78 (0.84) D^b	1.86 (0.24) D^b	0.68 (0.15) B^a	< 0.60 B^a
Sanitizer 4	1.14 (0.62) C^a	< 0.60 C^a	0.98 (0.57) BD^a	0.80 (0.39) C^a	< 0.60 C^a	< 0.60 B^a	< 0.60 B^a
Sanitizer 5	< 0.60 C^a	1.60 (0.91) C^{ab}	1.12 (0.39) BC^{ab}	< 0.60 C^b	< 0.60 C^b	0.68 (0.15) B^{ab}	< 0.60 B^b
Sanitizer 6	0.87 (0.37) C^a	< 0.60 C^a	2.03 (1.48) CD^b	< 0.60 C^a	< 0.60 C^a	0.72 (0.24) B^a	0.92 (0.45) A^a

A–D, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-d, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 4.12. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.79 (0.12) B^a	6.70 (0.30) B^a	5.25 (0.60) B^b	4.83 (1.08) B^b	2.85 (1.94) B^d	< 0.60 A^c	< 0.60 A^c
With Distilled Water	3.85 (0.34) A^a	4.16 (0.85) A^a	3.94 (0.92) A^a	3.54 (0.48) A^a	1.31 (0.82) A^{bc}	1.63 (1.28) A^b	< 0.60 A^c
Sanitizer 1	< 0.60 C^a	< 0.60 C^a	0.72 (0.24) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	0.90 (0.35) C^a	0.87 (0.54) C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 3	< 0.60 C^a	2.10 (0.94) D^b	1.96 (0.91) D^{bc}	0.83 (0.45) C^a	1.10 (0.58) AC^{ac}	< 0.60 A^a	< 0.60 A^a
Sanitizer 4	0.90 (0.35) CD^a	< 0.6 C^a	1.01 (0.54) CD^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 5	1.14 (0.73) D^a	0.95 (0.70) C^a	0.75 (0.30) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 6	0.68 (0.15) CD^a	< 0.60 C^a	1.32 (0.83) CD^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a

A–D, means within a column and within the same day of sanitizer treatment at 25°C are significantly different (P<0.05)

a-d, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.13. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) smooth surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.80 (0.10) B^{a}	6.77 (0.20) B^{a}	4.27 (0.57) A^{b}	5.37 (1.32) B^{d}	5.58 (0.16) B^{d}	3.84 (2.21) B^{b}	< 0.60 A^{c}
With Distilled Water	3.01 (0.24) A^{ad}	4.41 (0.28) A^{b}	3.39 (0.35) A^{ae}	4.02 (0.54) A^{be}	4.48 (0.15) A^{b}	2.35 (1.48) A^{d}	0.85 (0.50) A^{c}
Sanitizer 1	< 0.60 C^{a}	< 0.60 C^{a}	0.68 (0.15) B^{a}	0.72 (0.24) C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	0.68 (0.15) A^{a}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 A^{a}
Sanitizer 3	0.78 (0.35) C^{a}	0.78 (0.35) C^{a}	0.87 (0.54) B^{a}	< 0.60 C^{a}	1.00 (0.62) D^{a}	0.68 (0.15) C^{a}	< 0.60 A^{a}
Sanitizer 4	0.72 (0.24) C^{a}	< 0.60 C^{a}	0.80 (0.24) B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 A^{a}
Sanitizer 5	1.07 (0.33) C^{a}	0.68 (0.15) C^{a}	< 0.60 B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 A^{a}
Sanitizer 6	0.83 (0.29) C^{a}	< 0.60 C^{a}	0.99 (0.40) B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	0.68 (0.15) C^{a}	< 0.60 A^{a}

A–D, means within a column and within the same day of sanitizer treatment at 25°C are significantly different (P<0.05)

a-e, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.14. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.75 (0.14) B^{a}	6.76 (0.23) B^{a}	3.74 (1.19) A^{bd}	4.44 (2.58) B^{de}	5.37 (0.19) B^{e}	2.79 (2.52) A^{b}	< 0.60 A^{c}
With Distilled Water	3.01 (0.16) A^{a}	4.39 (0.25) A^{b}	3.08 (0.44) A^{a}	3.35 (0.28) A^{a}	4.35 (0.15) A^{b}	1.98 (1.60) AB^{c}	< 0.60 A^{d}
Sanitizer 1	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 A^{a}
Sanitizer 2	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 A^{a}
Sanitizer 3	0.80 (0.39) C^{a}	0.68 (0.15) C^{a}	0.72 (0.24) B^{a}	< 0.60 C^{a}	0.83 (0.29) C^{a}	< 0.60 B^{a}	< 0.60 A^{a}
Sanitizer 4	< 0.60 C^{a}	< 0.60 C^{a}	0.78 (0.35) B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 A^{a}
Sanitizer 5	0.68 (0.15) C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 A^{a}
Sanitizer 6	0.72 (0.24) C^{a}	< 0.60 C^{a}	0.81 (0.42) B^{a}	< 0.60 C^{a}	< 0.60 C^{a}	< 0.60 B^{a}	< 0.60 A^{a}

A–C, means within a column and within the same day of sanitizer treatment at 25°C are significantly different ($P<0.05$)

a-d, means within a row and within each sanitizer is significantly different ($P<0.05$)

Table 4.15. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.87 (0.12) B^a	6.79 (0.11) B^a	4.35 (0.47) B^b	5.80 (0.07) B^d	4.89 (0.27) B^e	0.99 (0.77) A^c	< 0.60 A^c
With Distilled Water	3.63 (0.42) A^a	4.57 (0.47) A^b	2.63 (0.96) A^c	3.95 (0.42) A^a	3.83 (0.23) A^a	1.55 (0.77) A^d	< 0.60 A^e
Sanitizer 1	0.68 (0.15) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 3	1.05 (0.54) C^a	1.17 (0.66) C^a	0.78 (0.35) C^{ab}	0.78 (0.35) C^{ab}	< 0.60 C^b	< 0.60 A^b	< 0.60 A^b
Sanitizer 4	0.75 (0.30) C^a	< 0.60 C^a	< 0.60 C^a	0.72 (0.24) C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 5	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	0.68 (0.15) A^a	< 0.60 A^a
Sanitizer 6	0.72 (0.24) C^a	0.68 (0.15) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a

A–C, means within a column and within the same day of sanitizer treatment at 25°C are significantly different (P<0.05)

a-e, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.16. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.83 (0.18) B^a	6.77 (0.13) B^a	4.31 (0.47) B^b	5.64 (0.28) B^d	3.67 (0.06) B^e	< 0.60 A^c	< 0.60 A^c
With Distilled Water	3.24 (0.17) A^a	4.56 (0.53) A^b	2.45 (1.23) A^c	3.52 (0.55) A^a	1.99 (1.00) A^e	< 0.60 A^d	< 0.60 A^d
Sanitizer 1	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 3	0.90 (0.43) C^a	0.94 (0.49) C^a	0.72 (0.24) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 4	0.95 (0.40) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 5	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 6	< 0.60 C^a	0.68 (0.15) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a

A–C, means within a column and within the same day of sanitizer treatment at 25°C are significantly different (P<0.05)

a-f, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.17. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Treatment	TSAYE						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.74 (0.26) B^a	6.77 (0.12) B^a	4.16 (0.75) B^b	5.26 (0.97) B^e	3.81 (0.15) B^b	2.83 (0.56) B^c	0.72 (0.24) A^d
With Distilled Water	3.47 (0.24) A^a	3.73 (0.77) A^a	1.34 (0.74) A^b	1.53 (0.73) A^b	1.46 (0.64) A^b	< 0.60 A^c	0.72 (0.24) A^c
Sanitizer 1	< 0.60 C^a	0.78 (0.35) C^a	0.68 (0.15) AC^a	< 0.60 C^a	0.72 (0.24) C^a	< 0.60 C^a	< 0.60 A^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a
Sanitizer 3	0.78 (0.35) C^a	0.80 (0.24) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	0.68 (0.15) C^a	< 0.60 A^a
Sanitizer 4	< 0.60 C^a	0.68 (0.15) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a
Sanitizer 5	1.02 (0.29) C^a	< 0.60 C^a	0.68 (0.15) AC^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a
Sanitizer 6	0.78 (0.35) C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a

A–C, means within a column and within the same day of sanitizer treatment at 4°C are significantly different (P<0.05)

a-d, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.18. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* population as enumerated on PALCAM on polypropylene (PP) surfaces, without daily exposure to nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Treatment	PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Control	6.72 (0.35) B^a	6.78 (0.12) B^a	3.88 (0.71) B^b	5.86 (0.08) B^e	3.27 (0.52) B^f	1.54 (0.70) B^c	< 0.60 A^d
With Distilled Water	3.32 (0.38) A^a	3.71 (0.76) A^a	1.09 (0.50) A^{bd}	2.79 (0.99) A^c	1.31 (0.59) A^d	< 0.60 A^b	< 0.60 A^b
Sanitizer 1	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 2	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 3	0.92 (0.45) C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 4	< 0.60 C^a	0.68 (0.15) C^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 5	0.75 (0.17) C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a
Sanitizer 6	0.87 (0.37) C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 C^a	< 0.60 C^a	< 0.60 A^a	< 0.60 A^a

A–C, means within a column and within the same day of sanitizer treatment at 4°C are significantly different (P<0.05)

a-f, means within a row and within each sanitizer is significantly different (P<0.05)

Table 4.19. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C), subjected to daily nutrient exposure and treated with water or sanitizers.

Surfaces	**TSAYE** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
HDPE-R	1.34 (0.07) A	1.46 (0.12) A	1.75 (0.12) A	4.54 (0.53) A	5.09 (0.12) A	5.44 (0.24) A	5.87 (0.21) A
HDPE-S	1.09 (0.07) A	1.18 (0.12) A	1.13 (0.12) B	4.08 (0.53) A	4.87 (0.12) A	5.01 (0.24) A	5.03 (0.21) B
PP	1.15 (0.07) A	1.26 (0.12) A	0.92 (0.12) B	1.60 (0.53) B	3.15 (0.12) B	3.72 (0.24) B	4.04 (0.21) C

A–C, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.20. Mean (Log CFU/cm^2) survival (n = 28) of *L. monocytogenes* population as enumerated on PALCAM on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C), subjected to daily nutrient exposure and treated with water or sanitizers.

Surfaces	**PALCAM** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
HDPE-R	1.19 (0.06) A	1.37 (0.09) A	1.51 (0.12) A	1.14 (0.26) A	1.84 (0.11) A	2.23 (0.24) A	3.22 (0.21) A
HDPE-S	1.00 (0.06) A	1.15 (0.09) A	1.03 (0.12) B	1.02 (0.26) A	1.85 (0.11) A	2.52 (0.24) A	3.18 (0.21) A
PP	1.07 (0.06) A	1.23 (0.09) A	0.88 (0.12) B	0.94 (0.26) A	1.18 (0.11) B	1.09 (0.24) B	1.12 (0.21) B

A & B, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.21. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C and 4°C), subjected to daily nutrient exposure and treated with water or sanitizers.

Surfaces	**TSAYE** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
PP – 25°C	1.15 (0.06) A	1.26 (0.06) A	0.92 (0.08) A	1.60 (0.43) A	3.15 (0.11) A	3.72 (0.25) A	4.04 (0.15) A
PP - 4°C	1.12 (0.06) A	1.11 (0.06) A	0.73 (0.08) A	0.65 (0.43) B	1.31 (0.11) B	1.10 (0.25) B	1.59 (0.15) B

A & B, means within a column and within the same day of sanitizer treatment on food contact surface (PP) stored at two temperatures 25°C and 4°C are significantly different ($P<0.05$)

Table 4.22. Mean (Log CFU/cm^2) survival (n = 28) of *L. monocytogenes* populations as enumerated on PALCAM on polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C and 4°C), subjected to daily nutrient exposure and treated with water or sanitizers.

Surfaces	**PALCAM** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
PP – 25°C	1.07 (0.06) A	1.23 (0.08) A	0.88 (0.09) A	0.94 (0.07) A	1.18 (0.08) A	1.09 (0.04) A	1.12 (0.08) A
PP - 4°C	1.09 (0.06) A	1.05 (0.08) A	0.67 (0.09) A	0.60 (0.07) B	0.60 (0.08) B	0.61 (0.04) B	0.64 (0.08) B

A & B, means within a column and within the same day of sanitizer treatment on food contact surface (PP) stored at two temperatures 25°C and 4°C are significantly different ($P<0.05$)

Table 4.23. Mean (Log CFU/cm^2) reduction (n = 12) of total bacterial populations as enumerated on TSAYE with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to daily nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**TSAYE**						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Sanitizer #1	2.86 (0.24) A	3.73 (0.25) A	2.85 (0.39) A	1.20 (0.46) A	2.31 (0.27) A	2.44 (0.61) A	2.39 (0.19) A
Sanitizer #2	2.91 (0.24) A	3.81 (0.25) A	2.74 (0.39) A	1.59 (0.46) A	2.19 (0.27) A	2.62 (0.61) A	3.58 (0.19) B
Sanitizer #3	2.71 (0.24) A	3.40 (0.25) A	2.24 (0.39) A	1.69 (0.46) A	1.76 (0.27) AB	1.89 (0.61) A	1.63 (0.19) C
Sanitizer #4	2.64 (0.24) A	3.81 (0.25) A	2.68 (0.39) A	1.68 (0.46) A	2.13 (0.27) A	2.30 (0.61) A	2.22 (0.19) AC
Sanitizer #5	2.43 (0.24) A	3.64 (0.25) A	2.70 (0.39) A	1.23 (0.46) A	1.81 (0.27) AB	2.47 (0.61) A	2.63 (0.19) A
Sanitizer #6	2.71 (0.24) A	3.79 (0.25) A	2.27 (0.39) A	0.92 (0.46) A	1.22 (0.27) B	1.78 (0.61) A	1.69 (0.19) C

A–C, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.24. Mean (Log CFU/cm^2) reduction (n = 12) of *L. monocytogenes* populations as enumerated on PALCAM with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to daily nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM**						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Sanitizer #1	2.76 (0.18) A	3.76 (0.29) A	2.51 (0.39) A	1.69 (0.34) A	2.56 (0.24) ABC	1.66 (0.53) A	1.37 (0.18) A
Sanitizer #2	2.76 (0.18) A	3.76 (0.29) A	2.45 (0.39) A	1.84 (0.34) A	2.58 (0.24) AC	1.70 (0.53) A	1.62 (0.18) A
Sanitizer #3	2.59 (0.18) A	3.35 (0.29) A	2.02 (0.39) A	1.43 (0.34) A	1.64 (0.24) AB	1.57 (0.53) A	0.40 (0.18) B
Sanitizer #4	2.55 (0.18) A	3.76 (0.29) A	2.36 (0.39) A	1.51 (0.34) A	2.01 (0.24) ABC	2.13 (0.53) A	1.31 (0.18) A
Sanitizer #5	2.56 (0.18) A	3.65 (0.29) A	2.51 (0.39) A	1.73 (0.34) A	2.65 (0.24) C	2.35 (0.53) A	1.70 (0.18) A
Sanitizer #6	2.70 (0.18) A	3.74 (0.29) A	2.24 (0.39) A	1.66 (0.34) A	1.61 (0.24) B	1.84 (0.53) A	1.23 (0.18) A

A–C, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.25. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C), not subjected to daily nutrient exposure but treated with water or sanitizers.

Surfaces	**TSAYE** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
HDPE-R	1.34 (0.07) A	1.46 (0.12) A	1.75 (0.12) A	1.33 (0.07) A	1.08 (0.04) A	0.91 (0.24) A	0.71 (0.05) A
HDPE-S	1.08 (0.07) A	1.18 (0.12) A	1.13 (0.12) B	1.10 (0.07) A	1.21 (0.04) B	0.87 (0.24) A	0.65 (0.05) A
PP	1.15 (0.07) A	1.26 (0.12) A	0.92 (0.12) B	1.12 (0.07) A	1.06 (0.04) A	0.75 (0.24) A	0.60 (0.05) A

A & B, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.26. Mean (Log CFU/cm^2) survival (n = 28) of *L. monocytogenes* populations as enumerated on PALCAM on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C), not subjected to daily nutrient exposure but treated with water or sanitizers.

Surfaces	**PALCAM** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
HDPE-R	1.19 (0.06) A	1.37 (0.09) A	1.51 (0.12) A	1.09 (0.12) A	0.77 (0.07) A	0.75 (0.25) A	0.60 (0.04) A
HDPE-S	1.00 (0.06) A	1.15 (0.09) A	1.03 (0.12) B	0.99 (0.12) A	1.17 (0.07) B	0.79 (0.25) A	0.60 (0.04) A
PP	1.07 (0.06) A	1.23 (0.09) A	0.88 (0.12) B	1.02 (0.12) A	0.80 (0.07) A	0.60 (0.25) A	0.60 (0.04) A

A & B, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.27. Mean (Log CFU/cm^2) survival (n = 28) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces exposed to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90%, 25°C and 4°C), not subjected to daily nutrient exposure but treated with water or sanitizers.

Surfaces	**TSAYE** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
PP – 25°C	1.15 (0.06) A	1.26 (0.06) A	0.92 (0.08) A	1.12 (0.09) A	1.06 (0.05) A	0.75 (0.05) A	0.60 (0.02) A
PP - 4°C	1.12 (0.06) A	1.11 (0.06) A	0.73 (0.08) A	0.73 (0.09) B	0.74 (0.05) B	0.61 (0.05) A	0.62 (0.02) A

A & B, means within a column and within the same day of sanitizer treatment on the food contact surface stored at two temperatures 25°C and 4°C are significantly different ($P<0.05$)

Table 4.28. Mean (Log CFU/cm^2) survival (n = 28) of *L. monocytogenes* populations as enumerated on PALCAM on polypropylene (PP) surfaces exposed ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90%, 25°C and 4°C), not subjected to daily nutrient exposure but treated with water or sanitizers.

Surfaces	**PALCAM** Time						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
PP – 25°C	1.09 (0.06) A	1.23 (0.08) A	0.88 (0.09) A	1.02 (0.07) A	0.80 (0.08) A	0.60 (0.01) A	0.60 (0.01) A
PP - 4°C	1.07 (0.06) A	1.05 (0.08) A	0.67 (0.09) A	0.91 (0.07) A	0.70 (0.08) A	0.60 (0.01) A	0.60 (0.01) A

A, means within a column and within the same day of sanitizer treatment on the food contact surface stored at two temperatures 25°C and 4°C are significantly different ($P<0.05$)

Table 4.29. Mean (Log CFU/cm^2) reduction (n = 12) of total bacterial populations as enumerated on TSAYE with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces not exposed to daily nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**TSAYE**						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Sanitizer #1	2.86 (0.24) A	3.73 (0.25) A	2.85 (0.39) A	3.38 (0.46) A	3.07 (0.27) A	1.53 (0.61) A	0.11 (0.19) A
Sanitizer #2	2.91 (0.24) A	3.81 (0.25) A	2.74 (0.39) A	3.46 (0.46) A	3.07 (0.27) A	1.53 (0.61) A	0.19 (0.19) A
Sanitizer #3	2.71 (0.24) A	3.40 (0.25) A	2.24 (0.39) A	3.01 (0.46) A	2.52 (0.27) B	1.48 (0.61) A	0.19 (0.19) A
Sanitizer #4	2.64 (0.24) A	3.81 (0.25) A	2.68 (0.39) A	3.35 (0.46) A	3.07 (0.27) A	1.53 (0.61) A	0.19 (0.19) A
Sanitizer #5	2.43 (0.24) A	3.64 (0.25) A	2.70 (0.39) A	3.46 (0.46) A	3.07 (0.27) A	1.48 (0.61) A	0.19 (0.19) A
Sanitizer #6	2.71 (0.24) A	3.79 (0.25) A	2.27 (0.39) A	3.46 (0.46) A	3.07 (0.27) A	1.47 (0.61) A	0.08 (0.19) A

A, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.30. Mean (Log CFU/cm^2) reduction (n = 12) of *L. monocytogenes* populations as enumerated on PALCAM with the help of sanitizers on high density polyethylene (HDPE) rough surface, high density polyethylene (HDPE) smooth surface and polypropylene (PP) surfaces not exposed to daily nutrients subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM**						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Sanitizer #1	2.76 (0.18) A	3.76 (0.29) A	2.51 (0.39) A	2.86 (0.34) A	1.94 (0.24) A	0.80 (0.53) A	$-335*10^{-18}$ (0.18) A
Sanitizer #2	2.76 (0.18) A	3.76 (0.29) A	2.45 (0.39) A	2.77 (0.34) A	1.94 (0.24) A	0.80 (0.53) A	$2.39*10^{-16}$ (0.18) A
Sanitizer #3	2.59 (0.18) A	3.35 (0.29) A	2.02 (0.39) A	2.79 (0.34) A	1.71 (0.24) A	0.80 (0.53) A	$-102*10^{-19}$ (0.18) A
Sanitizer #4	2.55 (0.18) A	3.76 (0.29) A	2.36 (0.39) A	2.86 (0.34) A	1.94 (0.24) A	0.80 (0.53) A	$-446*10^{-18}$ (0.18) A
Sanitizer #5	2.56 (0.18) A	3.65 (0.29) A	2.51 (0.39) A	2.86 (0.34) A	1.94 (0.24) A	0.80 (0.53) A	$9.94*10^{-17}$ (0.18) A
Sanitizer #6	2.70 (0.18) A	3.74 (0.29) A	2.24 (0.39) A	2.86 (0.34) A	1.94 (0.24) A	0.80 (0.53) A	$-102*10^{-16}$ (0.18) A

A, means within a column and within the same day of sanitizer treatment on three food contact surfaces stored at 25°C are significantly different ($P<0.05$)

Table 4.31. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) rough surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**TSAYE** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	5.72 (0.39) a	5.54 (0.39) a	6.87 (0.39) a	4.23 (0.39) b	7.26 (0.39) a	2.06 (0.39) b	7.76 (0.39) a	0.68 (0.39) b
D/W	5.70 (0.39) a	4.23 (0.39) a	6.67 (0.39) a	2.71 (0.39) b	7.19 (0.39) a	2.51 (0.39) b	7.26 (0.39) a	0.93 (0.39) b
Sanitizer #1	4.65 (0.39) a	0.72 (0.39) b	4.57 (0.39) a	<0.60 (0.39) b	5.06 (0.39) a	<0.60 (0.39) b	5.50 (0.39) a	0.75 (0.39) b
Sanitizer #2	4.36 (0.39) a	<0.60 (0.39) b	4.32 (0.39) a	<0.60 (0.39) b	4.71 (0.39) a	<0.60 (0.39) b	4.55 (0.39) a	<0.60 (0.39) b
Sanitizer #3	3.35 (0.39) a	1.78 (0.39) b	4.87 (0.39) a	1.86 (0.39) b	5.46 (0.39) a	0.67 (0.39) b	6.49 (0.39) a	<0.60 (0.39) b
Sanitizer #4	4.23 (0.39) a	0.80 (0.39) b	4.76 (0.39) a	<0.60 (0.39) b	5.35 (0.39) a	<0.60 (0.39) b	5.89 (0.39) a	<0.60 (0.39) b
Sanitizer #5	4.84 (0.39) a	<0.60 (0.39) b	4.93 (0.39) a	<0.60 (0.39) b	4.83 (0.39) a	0.67 (0.39) b	5.05 (0.39) a	<0.60 (0.39) b
Sanitizer #6	4.63 (0.39) a	<0.60 (0.39) b	5.46 (0.39) a	<0.60 (0.39) b	5.47 (0.39) a	0.72 (0.39) b	6.37 (0.39) a	0.92 (0.39) b

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different (P<0.05)

Table 4.32. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* populations as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	3.33 (0.39) a	4.83 (0.39) b	3.78 (0.39) a	2.85 (0.39) a	4.59 (0.39) a	<0.60 (0.39) b	4.68(0.39) a	<0.60 (0.39) b
D/W	3.01 (0.39) a	3.54 (0.39) a	3.67 (0.39) a	1.31 (0.39) b	4.71 (0.39) a	1.63 (0.39) b	4.13 (0.39) a	<0.60 (0.39) b
Sanitizer #1	<0.60 (0.39) a	<0.60 (0.39) a	0.82 (0.39) a	<0.60 (0.39) a	2.02 (0.39) a	<0.60 (0.39) b	2.64 (0.39) a	<0.60 (0.39) b
Sanitizer #2	<0.60 (0.39) a	0.87 (0.39) a	1.27 (0.39) a	<0.60 (0.39) a	2.54 (0.39) a	<0.60 (0.39) b	3.33 (0.39) a	<0.60 (0.39) b
Sanitizer #3	0.79 (0.39) a	0.83 (0.39) a	1.68 (0.39) a	1.09 (0.39) a	2.59 (0.39) a	<0.60 (0.39) b	3.99 (0.39) a	<0.60 (0.39) b
Sanitizer #4	1.18 (0.39) a	<0.60 (0.39) a	1.85 (0.39) a	<0.60 (0.39) b	1.64 (0.39) a	<0.60 (0.39) a	2.34 (0.39) a	<0.60 (0.39) b
Sanitizer #5	<0.60 (0.39) a	<0.60 (0.39) a	1.31 (0.39) a	<0.60 (0.39) a	1.19 (0.39) a	<0.60 (0.39) a	3.23 (0.39) a	<0.60 (0.39) b
Sanitizer #6	1.15 (0.39) a	<0.60 (0.39) a	2.27 (0.39) a	<0.60 (0.39) b	0.93 (0.39) a	<0.60 (0.39) a	2.87 (0.39) a	<0.60 (0.39) b

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different ($P<0.05$)

Table 4.33. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) smooth surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**TSAYE** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	5.37 (0.45) a	5.37 (0.45) a	7.05 (0.45) a	5.58 (0.45) b	6.99 (0.45) a	3.84 (0.45) b	7.56 (0.45) a	<0.60 (0.45) b
D/W	5.47 (0.45) a	4.02 (0.45) b	6.70 (0.45) a	4.48 (0.45) b	6.91 (0.45) a	2.35 (0.45) b	7.41 (0.45) a	0.85 (0.45) b
Sanitizer #1	4.01 (0.45) a	0.72 (0.45) b	4.19 (0.45) a	<0.60 (0.45) b	4.40 (0.45) a	<0.60 (0.45) b	4.33 (0.45) a	0.67 (0.45) b
Sanitizer #2	3.22 (0.45) a	<0.60 (0.45) b	4.41 (0.45) a	<0.60 (0.45) b	4.22 (0.45) a	<0.60 (0.45) b	2.85 (0.45) a	<0.60 (0.45) b
Sanitizer #3	3.99 (0.45) a	<0.60 (0.45) b	4.65 (0.45) a	1.00 (0.45) b	5.26 (0.45) a	0.67 (0.45) b	5.40 (0.45) a	<0.60 (0.45) b
Sanitizer #4	3.09 (0.45) a	<0.60 (0.45) b	4.17 (0.45) a	<0.60 (0.45) b	4.54 (0.45) a	<0.60 (0.45) b	5.37 (0.45) a	<0.60 (0.45) b
Sanitizer #5	3.76 (0.45) a	<0.60 (0.45) b	4.53 (0.45) a	<0.60 (0.45) b	4.48 (0.45) a	<0.60 (0.45) b	4.58 (0.45) a	<0.60 (0.45) b
Sanitizer #6	5.01 (0.45) a	<0.60 (0.45) b	5.44 (0.45) a	<0.60 (0.45) b	5.21 (0.45) a	0.67 (0.45) b	5.21 (0.45) a	<0.60 (0.45) b

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different (P<0.05)

Table 4.34. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* populations as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	3.04 (0.45) a	4.44 (0.45) b	3.92 (0.45) a	5.37 (0.45) b	4.79 (0.45) a	2.79 (0.45) b	5.11 (0.45) a	<0.60 (0.45) b
D/W	2.51 (0.45) a	3.35 (0.45) a	3.40 (0.45) a	4.35 (0.45) a	3.58 (0.45) a	1.98 (0.45) b	4.45 (0.45) a	<0.60 (0.45) b
Sanitizer #1	0.67 (0.45) a	<0.60 (0.45) a	1.37 (0.45) a	<0.60 (0.45) a	2.84 (0.45) a	<0.60 (0.45) b	3.21 (0.45) a	<0.60 (0.45) b
Sanitizer #2	<0.60 (0.45) a	<0.60 (0.45) a	0.85 (0.45) a	<0.60 (0.45) a	2.26 (0.45) a	<0.60 (0.45) b	2.01 (0.45) a	<0.60 (0.45) b
Sanitizer #3	1.34 (0.45) a	<0.60 (0.45) a	2.82 (0.45) a	0.83 (0.45) b	2.77 (0.45) a	<0.60 (0.45) b	3.91 (0.45) a	<0.60 (0.45) b
Sanitizer #4	0.67 (0.45) a	<0.60 (0.45) a	1.79 (0.45) a	<0.60 (0.45) a	1.67 (0.45) a	<0.60 (0.45) a	3.73 (0.45) a	<0.60 (0.45) b
Sanitizer #5	0.75 (0.45) a	<0.60 (0.45) a	<0.60 (0.45) a	<0.60 (0.45) a	1.82 (0.45) a	<0.60 (0.45) a	1.69 (0.45) a	<0.60 (0.45) a
Sanitizer #6	<0.60 (0.45) a	<0.60 (0.45) a	2.15 (0.45) a	<0.60 (0.45) b	2.74 (0.45) a	<0.60 (0.45) b	3.25 (0.45) a	<0.60 (0.45) b

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different (P<0.05)

Table 4.35. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**TSAYE** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	3.34 (0.32) a	5.80 (0.32) b	5.09 (0.32) a	4.89 (0.32) a	6.24 (0.32) a	0.98 (0.32) b	7.03 (0.32) a	<0.60 (0.32) b
D/W	2.62 (0.32) a	3.95 (0.32) b	4.64 (0.32) a	3.83 (0.32) a	5.86 (0.32) a	1.55 (0.32) b	6.34 (0.32) a	<0.60 (0.32) b
Sanitizer #1	1.52 (0.32) a	<0.60 (0.32) b	2.32 (0.32) a	<0.60 (0.32) b	3.16 (0.32) a	<0.60 (0.32) b	3.97 (0.32) a	<0.60 (0.32) b
Sanitizer #2	1.43 (0.32) a	<0.60 (0.32) a	2.70 (0.32) a	<0.60 (0.32) b	3.15 (0.32) a	<0.60 (0.32) b	2.86 (0.32) a	<0.60 (0.32) b
Sanitizer #3	1.36 (0.32) a	0.78 (0.32) a	3.19 (0.32) a	<0.60 (0.32) b	3.55 (0.32) a	<0.60 (0.32) b	4.19 (0.32) a	<0.60 (0.32) b
Sanitizer #4	1.40 (0.32) a	0.72 (0.32) a	2.68 (0.32) a	<0.60 (0.32) b	3.17 (0.32) a	<0.60 (0.32) b	3.08 (0.32) a	<0.60 (0.32) b
Sanitizer #5	1.49 (0.32) a	<0.60 (0.32) a	3.11 (0.32) a	<0.60 (0.32) b	3.23 (0.32) a	0.67 (0.32) b	3.47 (0.32) a	<0.60 (0.32) b
Sanitizer #6	1.38 (0.32) a	<0.60 (0.32) a	3.43 (0.32) a	<0.60 (0.32) b	3.92 (0.32) a	<0.60 (0.32) b	4.34 (0.32) a	<0.60 (0.32) b

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different (P<0.05)

Table 4.36. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* populations as enumerated on PALCAM on polypropylene (PP) surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	3.11 (0.32) a	5.64 (0.32) b	3.57 (0.32) a	3.67 (0.32) a	3.41 (0.32) a	<0.60 (0.32) b	3.78 (0.32) a	<0.60 (0.32) b
D/W	1.81 (0.32) a	3.52 (0.32) b	3.41 (0.32) a	1.99 (0.32) b	2.39 (0.32) a	<0.60 (0.32) b	2.23 (0.32) a	<0.60 (0.32) b
Sanitizer #1	0.97 (0.32) a	<0.60 (0.32) a	<0.60 (0.32) a	<0.60 (0.32) b	0.83 (0.32) a	<0.60 (0.32) b	0.83 (0.32) a	<0.60 (0.32) b
Sanitizer #2	<0.60 (0.32) a	<0.60 (0.32) a	<0.60 (0.32) a	<0.60 (0.32) b	0.78 (0.32) a	<0.60 (0.32) b	<0.60 (0.32) a	<0.60 (0.32) b
Sanitizer #3	0.90 (0.32) a	<0.60 (0.32) a	1.05 (0.32) a	<0.60 (0.32) b	<0.60 (0.32) a	<0.60 (0.32) b	1.67 (0.32) a	<0.60 (0.32) b
Sanitizer #4	0.95 (0.32) a	<0.60 (0.32) a	0.80 (0.32) a	<0.60 (0.32) b	0.97 (0.32) a	<0.60 (0.32) b	0.78 (0.32) a	<0.60 (0.32) b
Sanitizer #5	0.78 (0.32) a	<0.60 (0.32) a	<0.60 (0.32) a	<0.60 (0.32) b	<0.60 (0.32) a	<0.60 (0.32) b	0.75 (0.32) a	<0.60 (0.32) b
Sanitizer #6	<0.60 (0.32) a	<0.60 (0.32) a	1.23 (0.32) a	<0.60 (0.32) b	1.49 (0.32) a	<0.60 (0.32) b	1.00 (0.32) a	<0.60 (0.32) b

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different (P<0.05)

Table 4.37. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Sanitizers	**TSAYE** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	<0.60 (0.19) a	5.26 (0.19) b	1.01 (0.19) a	3.81 (0.19) b	1.40 (0.19) a	2.83 (0.19) b	3.22 (0.19) a	0.72 (0.19) b
D/W	0.77 (0.19) a	1.53 (0.19) b	0.15 (0.19) a	1.46 (0.19) a	1.56 (0.19) a	<0.60 (0.19) b	2.66 (0.19) a	0.72 (0.19) b
Sanitizer #1	<0.60 (0.19) a	<0.60 (0.19) a	1.06 (0.19) a	0.72 (0.19) a	1.23 (0.19) a	<0.60 (0.19) b	1.39 (0.19) a	<0.60 (0.19) b
Sanitizer #2	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	0.72 (0.19) a	<0.60 (0.19) a	1.18 (0.19) a	<0.60 (0.19) b
Sanitizer #3	<0.60 (0.19) a	<0.60 (0.19) a	0.72 (0.19) a	<0.60 (0.19) a	0.77 (0.19) a	0.67 (0.19) a	1.31 (0.19) a	<0.60 (0.19) b
Sanitizer #4	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	0.77 (0.19) a	<0.60 (0.19) a	1.21 (0.19) a	<0.60 (0.19) b
Sanitizer #5	<0.60 (0.19) a	<0.60 (0.19) a	0.92 (0.19) a	<0.60 (0.19) a	1.39 (0.19) a	<0.60 (0.19) b	1.98 (0.19) a	<0.60 (0.19) b
Sanitizer #6	0.75 (0.19) a	<0.60 (0.19) a	1.30 (0.19) a	<0.60 (0.19) b	1.24 (0.19) a	<0.60 (0.19) b	1.40 (0.19) a	<0.60 (0.19) b

a & b, means within a row and within the same day of sanitizer treatment at 4°C is significantly different (P<0.05)

Table 4.38. Mean (Log CFU/cm^2) survival (n = 4) of *L. monocytogenes* populations as enumerated on PALCAM on polypropylene (PP) surfaces, with daily exposure to nutrients compared to those not treated with nutrients and also subjected to general cutting board use and cleaning conditions in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Sanitizers	**PALCAM** Time (d)							
	4 d - With	**4 d – W/O**	**7 d - With**	**7 d – W/O**	**14 d - With**	**14 d – W/O**	**21 d – With**	**21 d – W/O**
Control	<0.60 (0.19) a	5.86 (0.19) b	<0.60 (0.19) a	3.27 (0.19) b	<0.60 (0.19) a	1.54 (0.19) b	1.45 (0.19) a	<0.60 (0.19) b
D/W	<0.60 (0.19) a	2.79 (0.19) b	<0.60 (0.19) a	1.31 (0.19) b	0.67 (0.19) a	<0.60 (0.19) a	0.90 (0.19) a	<0.60 (0.19) a
Sanitizer #1	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a
Sanitizer #2	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a
Sanitizer #3	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a
Sanitizer #4	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a
Sanitizer #5	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a
Sanitizer #6	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a	<0.60 (0.19) a

a & b, means within a row and within the same day of sanitizer treatment at 4°C is significantly different ($P<0.05$)

CHAPTER 5

Impact of sanitizer application method on survival of *L. monocytogenes* biofilms

ABSTRACT

The objective of this study was to understand the impact of sanitizer application methods on survival of *L. monocytogenes* biofilms on high density polyethylene (HDPE) cutting boards with rough and smooth surfaces at ambient temperature (25°C), and on polypropylene (PP) cutting boards at ambient (25°C) and refrigerator temperature (4°C). The first sanitizer application method (A) consisted of either wetting food contact surface with the help of sanitizer or depositing 2 ml of sanitizer on to the coupon surface; whereas, the second sanitizer application method (B) consisted of wetting the food contact surface by dipping it into the sanitizer such that the biofilm touched the sanitizer. Both methods were effective in decreasing total bacterial and *L. monocytogenes* biofilms when food contact surfaces were cleaned within 6 h.

5.1. INTRODUCTION

Microbial biofilms are attracting attention of scientists in different areas such as the medical field, aquatic environment, food processing industries (Joseph et al., 2001). A biofilm consists of surface-colonizing microbes and associated polymers. The conditions

in home kitchen environments often favor microbial attachment and biofilm formation. These conditions include flowing water, suitable attachment surfaces, ample nutrients and ample sources of bacteria from raw foods or the environment (Gibson et al., 1999). Biofilms are considered detrimental and undesirable as they have the capacity to attach and colonize on the surface of most materials.

The foodborne pathogen *Listeria monocytogenes* is of particular concern because of its ability to grow at refrigeration temperatures (Norwood and Gilmour, 1999). Food has been shown to be the primary mode of transmission of *L. monocytogenes*. If present in food, *L. monocytogenes* has the potential to contaminate food contact surfaces in the home. Contaminated food contact surfaces may serve as sources for bacterial survival and multiplication, and thus, as sources of cross-contamination to other foods. Listeria attaches to numerous surfaces, including stainless steel, glass, wood, porcelain, iron, plastic, polyester, propylene, and rubber (Blackman and Frank, 1996). Attachment and biofilm formation of *L. monocytogenes* on solid surfaces occurs in the following sequence: cell-deposition on the surface, adhesion to the surface, surface colonization, biofilm formation and biofilm development (Chavant et al., 2002). Adhesion of *Listeria* to surfaces has been attributed to hydrophilic interactions, presence of flagella, fibrils and synthesis of exopolysachharides (Ryser and Marth, 2007).

Cells in a biofilm are known to be more resistant to sanitizers than planktonic cells (Frank and Koffi, 1990; Mafu et al., 1990; Stopforth et al., 2002) due to formation of an exopolysaccharide matrix that binds cells, surrounds the biofilm, and protects it from sanitizers (Lomander et al., 2004). The time available for biofilm formation depends on the frequency of cleaning and sanitation. If the matrix is not completely removed

when sanitizing a surface, the pathogen will more readily reattach to the surface and a biofilm will form again, even if the previous pathogens were reduced below detection limit (Gibson et al., 1999).

Biofilm bacteria are difficult to remove, and even with cleaning practices routinely used, they may remain and survive on food contact surfaces. A study was carried out to evaluate the survival and inactivation *L. monocytogenes* biofilms on food contact surfaces using commercially available sanitizers and household compounds by spraying or wetting (method A) compared to immersion of the surface containing the biofilm in the sanitizing solution (method B). The objective of this study was to understand the impact of sanitizer application method on survival of *L. monocytogenes* biofilms on HDPE rough and smooth surfaces and PP surface.

5.2. MATERIALS AND METHODS

5.2.1. Preparation of ham homogenate. Ten gram of ham samples were mixed with 90 ml of sterile distilled water in a whirl-pak bag (Nasco, Fort Atkinson, WI) and homogenized (Masticator, IUL Instruments, Barcelona, Spain). The suspension of the product was passed through cheese-cloth, autoclaved and cooled at ambient temperature (25°C) before storing at 4°C for use within 2 days.

5.2.2. Bacterial strains and growth conditions. Five strains (Table 5.1) of *L. monocytogenes* were activated by three successive transfers in tryptic soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton Dickinson Co., Sparks, Md) at 30°C for 24 hours. For inoculum preparation, 24 hour cultures of each strain were centrifuged separately (Eppendorf model 5810 R, Brinkmann Instruments Inc., Westbury,

NY) at 6000 rpm for 15 minutes at 4°C. The harvested cells were resuspended in 10 ml of phosphate buffered saline and centrifuged as above. The harvested cells were resuspended in 10 ml of ham homogenate (each culture separately) prepared from product of same lot as that used in the study (but kept frozen during the study at - 20°C). Cell cultures suspended in ham homogenate were stored at 7°C for 48 hours to allow for acclimatization of the cells to a low temperature food environment. Equal volumes (10 ml) of cell suspensions of each of the 5 strains were then combined for use in the study (Figure 4.1). The 5 strain mixture was surface plated on TSAYE and PALCAM agar (Difco) for determination of initial populations as well as for testing the purity of the inoculum (Figure 4.1).

5.2.3. Cutting board coupons. Rough and smooth surface high density polyethylene (HDPE) and smooth surface polypropylene (PP) materials were obtained from Fort Collins Plastics (Fort Collins, CO) for use in the studies. These materials are Food and Drug Administration (FDA) approved for use as food contact surfaces (USFDA reg. 21CFR177.1520 item 2.1).Coupons were cleaned and autoclaved before use.

5.2.4. Inoculation of coupons and biofilm formation. Inoculated ham homogenate was deposited on to each coupon surface. HDPE (rough and smooth) coupons were incubated at 25°C whereas PP surfaces were incubated at 25 and 4°C. All the coupons were exposed to daily nutrient (diluted TSBYE medium).

5.2.5. Sanitizing methods. Three commercially available sanitizers and three household compounds were purchased from a local supermarket based on their commercial availability and intended usage on food contact surfaces in the home (Table 5.2). The three commercial sanitizers came in spray bottles, while the three household compounds

came in regular bottles, one of which was supplied by the manufacturer as a concentrated liquid (Sanitizer #4 – sodium hypochlorite). This sanitizer was diluted with sterile distilled water to 300 ppm sodium hypochlorite in our laboratory on the day of use. All sanitizers varied in chemical composition and concentrations of active ingredients.

5.2.6. Method A. For each type of surface, 16 inoculated coupons were removed at 0 h (before incubation), 6 h, 168 h and 336 h incubation, and not treated (control), treated with sterile distilled water or treated with one of the six sanitizers. Untreated coupons were directly placed into a centrifuge tube containing 40 ml of D/E neutralizing broth (Difco), while all other coupons were first rinsed with 10 ml of sterile distilled water to remove loosely attached bacterial cells, then treated as follows.

The sterile distilled water treatment involved pipetting 2 ml of sterile distilled water onto the surface of the coupon and allowing it to stand for 10 min. The coupon was rinsed with 10 ml distilled water and then placed into a centrifuge tube containing D/E neutralizing broth.

For treatment with sanitizers #1, 2, 3, which came in spray bottles, coupons were wetted by spraying sanitizers with consistent pressure 5 times at an approximate angle of 60° to the surface from 15 cm away. For sanitizers #4, 5 and 6, available in regular bottles, coupons were wetted by placing 2 ml of sanitizer on each coupon. The coupons were then allowed to stand for 10 min for sanitizers #1, 2, 4, 5 and 6 and 2 minutes for sanitizer #3. All the coupons were rinsed again with 10 ml sterile distilled water. After sanitation, each coupon was placed into an 85 ml centrifuge tube containing 40 ml of D/E neutralizing broth. (Please see Chapter 3 for a more detailed explanation).

5.2.7. Method B. For each type of surface, 16 inoculated coupons were removed at 0 h (before incubation), 6 h, 168 h and 336 h incubation, and not treated (Control), treated with sterile distilled water or treated with one of the six sanitizers. Untreated coupons were directly placed into a centrifuge tube containing 40 ml of D/E neutralizing broth (Difco), while other coupons were first rinsed with 10 ml of sterile distilled water.

The sterile distilled water treatment involved pipetting 2 ml of sterile distilled water onto the surface of the coupon and allowing it to stand for 10 min. The coupon was rinsed with 10 ml distilled water and then placed into a centrifuge tube containing D/E neutralizing broth.

For evaluation of the sanitizer treatments, 10 ml of each sanitizer was first placed in an empty petri dish with the help of a pipette. The coupons were rinsed with 10 ml distilled water and then placed into the petri dish containing the sanitizer such that the biofilm was in contact with the sanitizer. The coupons were allowed to stand for 10 minutes for sanitizers #1, 2, 4, 5, and 6 and 2 minutes for sanitizer #3. All the coupons were again rinsed with 10 ml sterile distilled water. After sanitation, each coupon was placed into an 85 ml centrifuge tube containing 40 ml of D/E neutralizing broth and 10 glass beads. (Please see Chapter 4 for a more detailed explanation).

5.2.8. Data analysis. All tests were performed in two independent replication trials with two samples being evaluated for each replicate. Microbial counts were reported in terms of $\log_{10}$ CFU/cm^2. Estimated reductions were analyzed statistically to compare sanitizer treatment effects. Data were analyzed using the Glimmix Procedure in SAS (SAS Institute Inc., Cary, NC). The Glimmix procedure helps to specify a generalized linear mixed model and to perform confirmatory inference. Descriptive statistics (means and

standard deviations) were computed and analyses of variance were performed for statistical differences ($P<0.05$). Independent variables in the mixed models procedure were type of surface, type of sanitizer method, media, time and their interactions. Random effects were replicate and replicate interactions with surface and sanitizer. The least significant difference procedure was used to perform mean separation.

5.3. RESULTS

5.3.1. Impact of sanitizer application method on *L. monocytogenes* biofilms on HDPE rough surfaces at 25°C. Without sanitizer treatment, total bacterial cells, as enumerated on TSAYE, and *L. monocytogenes* cells, as enumerated on PALCAM, survived on young and established biofilms (Table 5.3, 5.4 and Figure 5.1).

For the coupons treated with sanitizers, with the exception of sanitizer #3, both application methods were equally effective on young biofilms (0 and 6 h) ($P>0.05$). On d 7 total bacterial and *L. monocytogenes* biofilms, no significant differences ($P>0.05$) in effectiveness were observed between the direct application and dip method. However, on 14 d sanitizer #2 was significantly more effective ($P<0.05$) when applied by the method A and sanitizer #5 was more effective ($P<0.05$) when applied by method B (Table 5.3, 5.4 and Figure 5.1).

5.3.2. Impact of sanitizer application method on *L. monocytogenes* biofilms on HDPE smooth surfaces at 25°C. Without sanitizer treatment, total bacterial cells, as enumerated on TSAYE, and *L. monocytogenes* cells, as enumerated on PALCAM, survived well on young and established biofilms (Table 5.5, 5.6 and Figure 5.2).

For the coupons treated with sanitizers, both application methods were equally effective in young biofilms (0 and 6 h) (P>0.05). However, on d 7 and 14 biofilms all of the sanitizers tested tended to be more effective when applied using method A than method B (Table 5.5, 5.6 and Figure 5.2).

5.3.3. Impact of sanitizer application method on *L. monocytogenes* biofilms on polypropylene at 25°C. Without sanitizer treatment, total bacterial cells, as enumerated on TSAYE, and *L. monocytogenes* cells, as enumerated on PALCAM, survived well on young and biofilms (Table 5.7, 5.8 and Figure 5.3).

For the coupons treated with sanitizers, both application methods were equally effective in young biofilms (0 and 6 h) (P>0.05). On 7 d, sanitizers #5 and 6 and on d 14, sanitizers #4 and 5 were found to be more effective (P<0.05) on total bacterial biofilms when applied by the method B than method A. On d 7 and 14 established *L. monocytogenes* biofilms, no significant differences (P>0.05) were observed between the two methods of sanitizer application (Table 5.7, 5.8 and Figure 5.3).

5.3.4. Impact of sanitizer application method on *L. monocytogenes* biofilms on polypropylene at 4°C. Without sanitizer treatment, total bacterial cells, as enumerated on TSAYE, and *L. monocytogenes* cells, as enumerated on PALCAM, survived well on young biofilms whereas fewer survived on the established biofilms (7 and 14 d) (Table 5.9, 5.10 and Figure 5.4).

For the coupons treated with sanitizers, both application methods were equally effective in young biofilms (0 and 6 h) (P>0.05). On d 7 and 14 total bacterial biofilms, sanitizers #5 and 6 were found to be more effective (P<0.05) when applied by the method A than method B. On d 7 and 14 established *L. monocytogenes* biofilms, no significant

differences (P>0.05) were observed between either method of sanitizer application (Table 5.9, 5.10 and Figure 5.4).

5.4. DISCUSSION

L. monocytogenes has been shown to exist predominantly attached to surfaces in contact with liquids (Palmer et al., 2007). Any food residues left on food contact surfaces may provide a niche in which microorganisms can rapidly grow (Jun et al., 2010) Microorganisms on wet surfaces have been found to coalesce and grow, resulting in a complex biofilm. *L. monocytogenes* is known to adhere to and grow on different kinds of surfaces at different temperatures. *L. monocytogenes* biofilms can tolerate anaerobic conditions and a wide range of pH (Aarnisalo et al., 2007) and thus the bacterium is difficult to eradicate. Biofilm formation in kitchens on food contact surfaces has become a concern because sessile bacteria within biofilms are highly resistant to common cleaners and sanitizers, compared to bacteria in planktonic state (Chae et al., 2006).

Both sanitizer application methods were effective in reducing total bacterial and *L. monocytogenes* cells at 0 h and 6 h. Significant differences were observed on established biofilms but the differences were inconsistent. The differences appeared to be somewhat associated with density. PP surfaces were less dense (light weighted) than the HDPE rough and smooth surfaces and so the PP surfaces were floating on the sanitizers. None of the sanitizers worked that well on the HDPE rough surfaces for established biofilms. It is probable that the abrasive surface (HDPE rough surface) formed tiny air bubbles when dipped which would have resulted in parts of the surface not being covered with the liquid. An even distribution of direct application method would have ensured

that the tiny cavities of the abrasive HDPE rough surface were exposed to the sanitizer. For HDPE smooth surface, direct application method was generally better than the dip method on established biofilms. For PP surfaces (at 25°C), dip method was somewhat better than the direct application method. The differences seen overall between PP and HDPE smooth surfaces could be due to the differences in the density or probably the surfaces could either be hydrophobic or hydrophilic in nature. Additional work is required to be done in this area.

5.5. CONCLUSION

Based on the above results, it can be concluded that *L. monocytogenes* can survive on food contact surfaces, e.g. cutting boards, forming a biofilm which overtime becomes increasingly resistant to sanitizer treatment. Both application methods were effective in decreasing total bacterial and *L. monocytogenes* biofilms when food contact surfaces were cleaned within 6 h. Since the effectiveness of a sanitizer decreases as the biofilms matures, sanitation should be performed as soon as possible after each use or at least within 6 h after use in order to avoid biofilm formation on cutting boards and other food contact surfaces. In practical use, this could be accomplished through the use of a 3 compartment sink installed in kitchens in which food contact surfaces such as knives, peelers, small cutting boards and food containers could be washed, rinsed then sanitized (3^{rd} compartment) using the dip method. In contrast, slicers, countertops and large cutting boards could be sanitized by the direct application method. Also, in a three compartment sink, small utensils could be immersed completely in the sanitizing solution whereas the less dense utensils would float and so for such utensils direct application method works

better. Both methods worked well because the surfaces were constantly exposed to sanitizers. Under the conditions of this study, listeria did not survive at 4°C on developed biofilms.

Experiments related to the method of sanitation of various food contact surfaces in home kitchens must be carried out under commonly existing conditions. Additional studies are needed to understand fully the efficiency of both the methods and also to test the strength of various sanitizers at different concentrations.

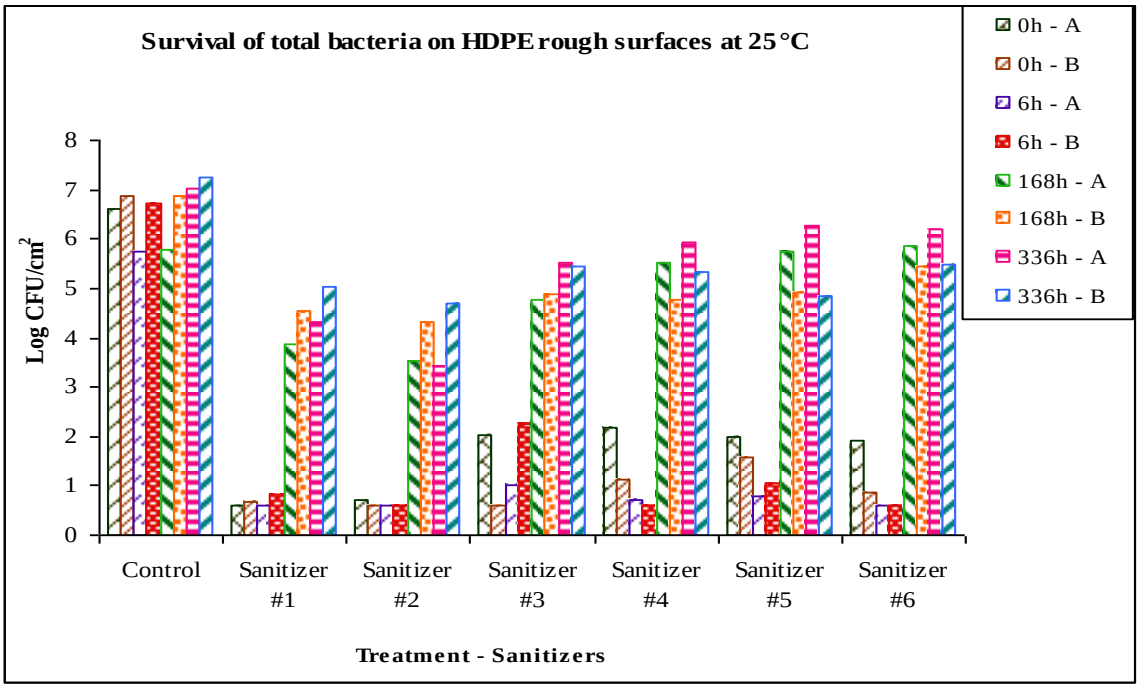
Table 5.1. *L. monocytogenes* strains used in the study (Fugett et al., 2006)

L. monocytogenes strain	Lineage	Serotype	Source
J1-177	I	1/2b	Human, sporadic
R2-499	II	1/2a	Human, epidemic, sliced turkey
N3-013	I	4b	Food, epidemic
N1-227	I	4b	Food, epidemic
C1-056	II	1/2a	Human, sporadic

Table 5.2. Sanitizers used for inactivating *Listeria monocytogenes* biofilms on rough and smooth high density polyethylene coupons and polypropylene coupons

Sanitizer #	**Active Ingredients and Concentration**	**pH**
1	Alkyl (67% C_{12}, 25% C_{14}, 7% C_{16}, 1% C_8-C_{10}-C_8) dimethyl benzyl ammonium chlorides (0.0860%) Alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium chlorides (0.0216%)	10.12
2	L-Lactic acid (0.18%)	2.92
3	Sodium hypochlorite (0.0095%) Available chlorine (0.009%)	6.55
4*	Sodium hypochlorite (6%) Other ingredients (94%) Yield 5.7% available Cl_2	6.22
5	Acetic acid (5%)	3.26
6	Hydrogen peroxide (3%)	4.72

* A fresh solution was prepared on the day of experiment.



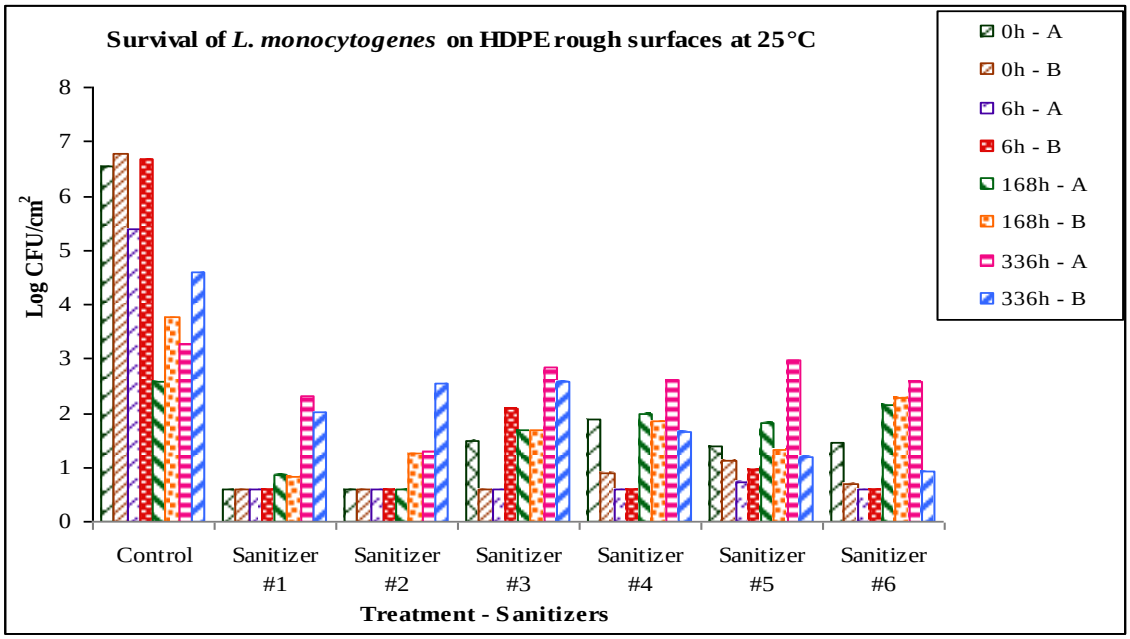
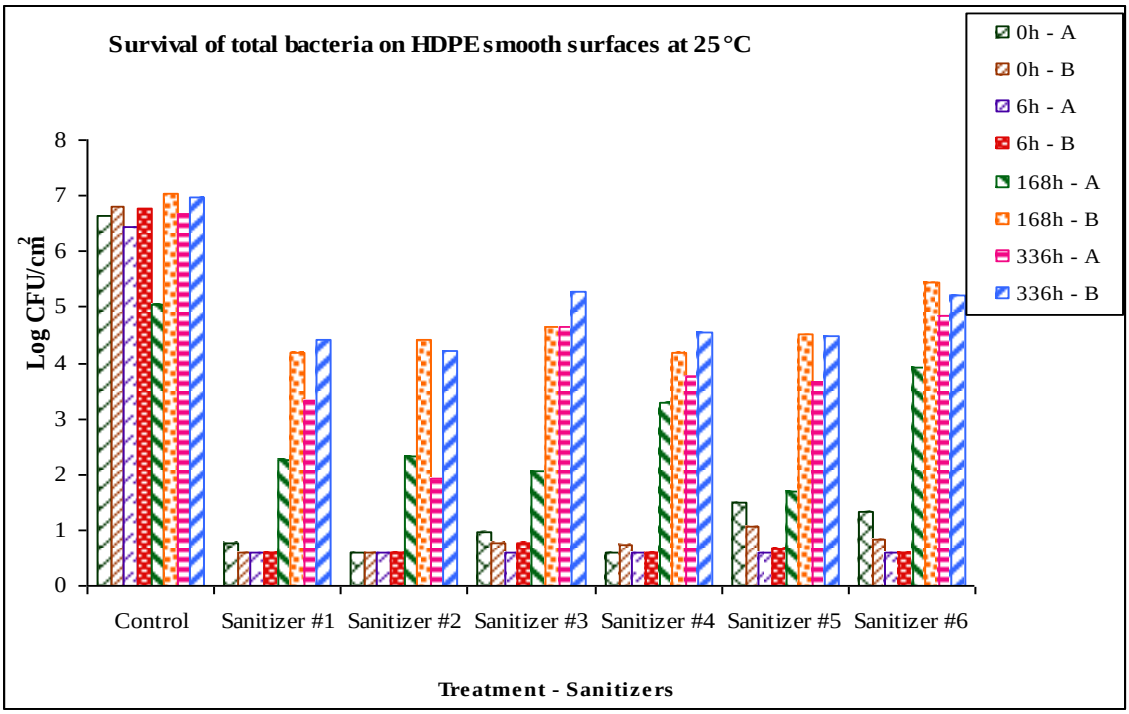


Figure 5.3. Data shown in Tables 5.3 and 5.4. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations and *L. monocytogenes* as enumerated on TSAYE and PALCAM respectively on high density polyethylene (HDPE) rough surface with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



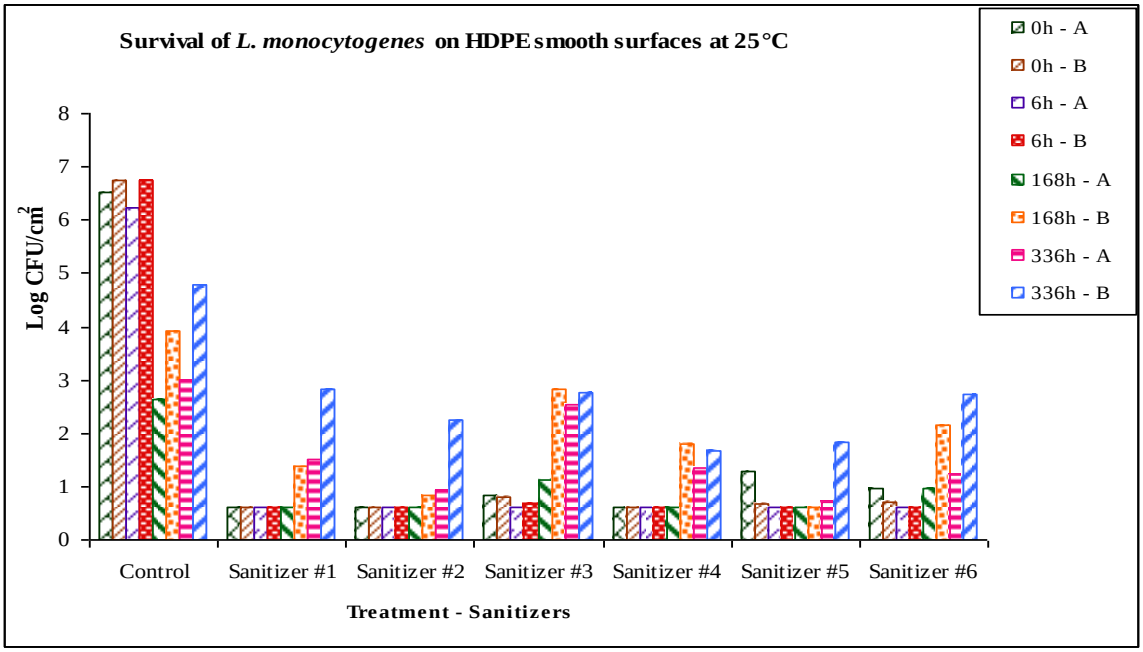
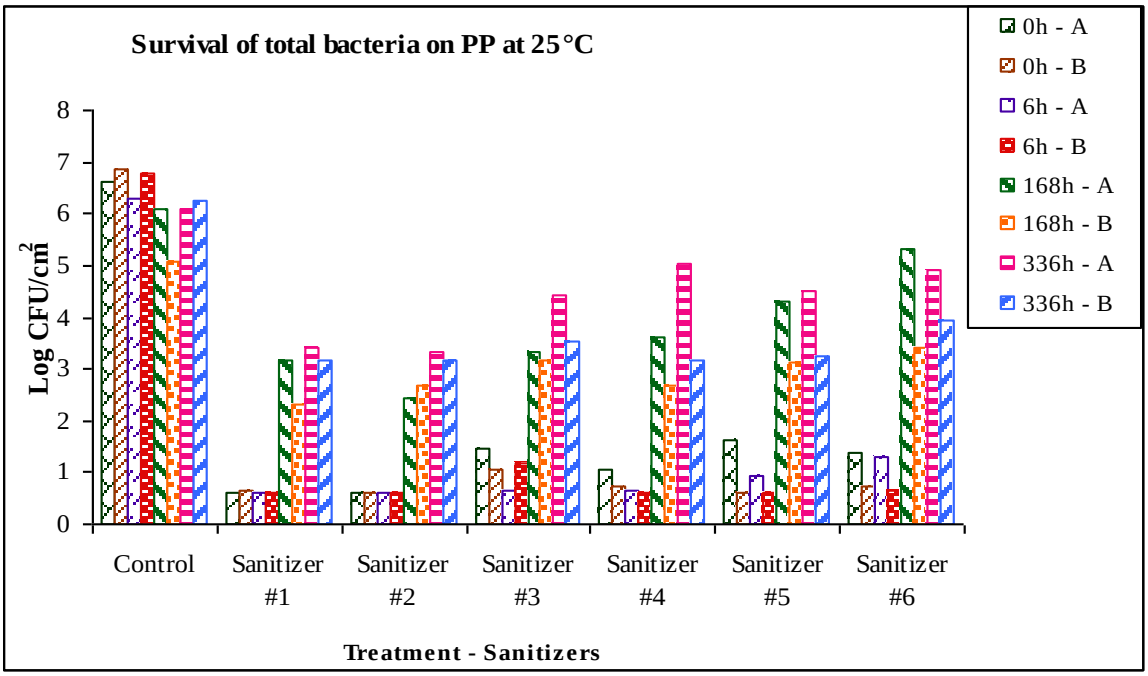


Figure 5.4. Data shown in Tables 5.5 and 5.6. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations and *L. monocytogenes* as enumerated on TSAYE and PALCAM respectively on high density polyethylene (HDPE) smooth surface with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



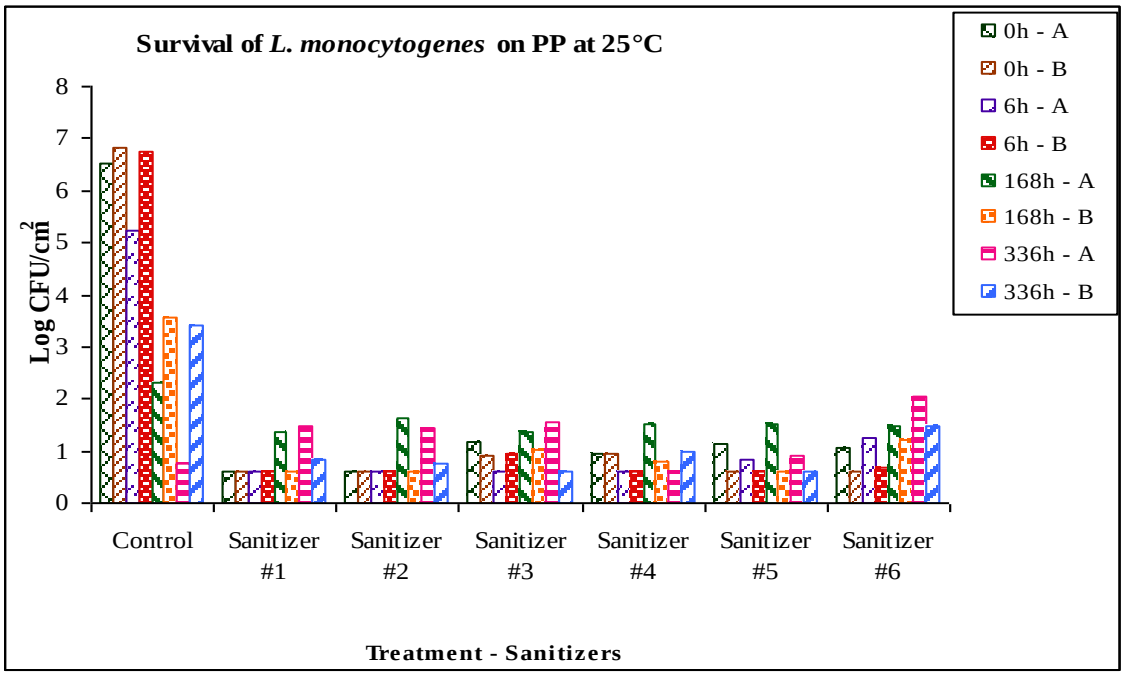
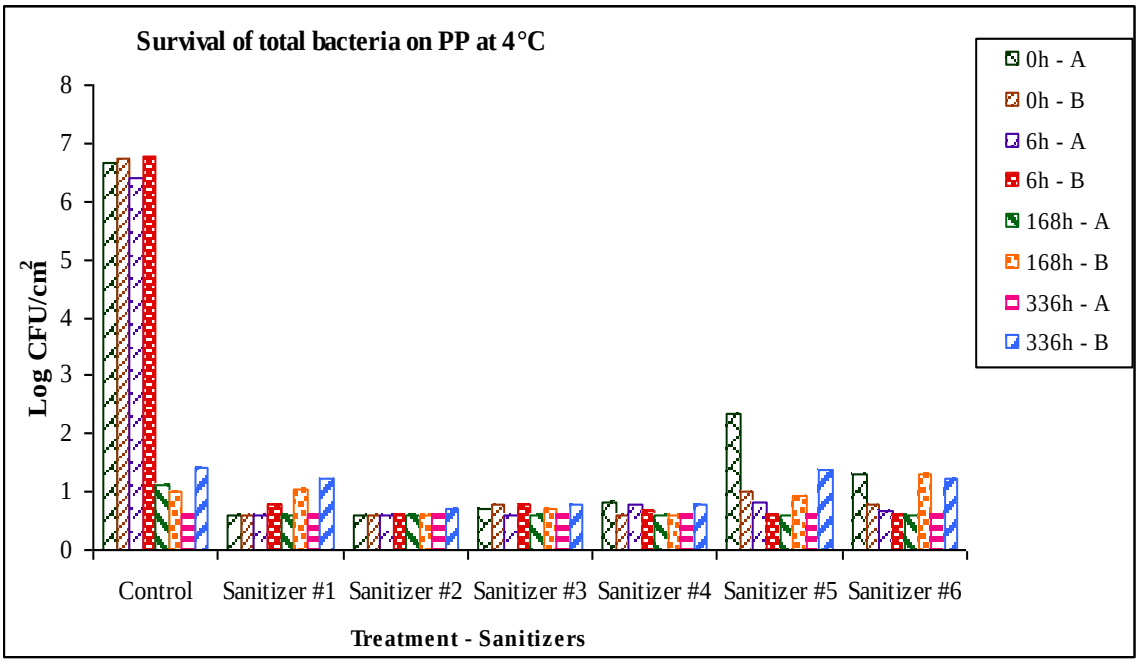


Figure 5.5. Data shown in Tables 5.7 and 5.8. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations and *L. monocytogenes* as enumerated on TSAYE and PALCAM respectively on polypropylene (PP) surface with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).



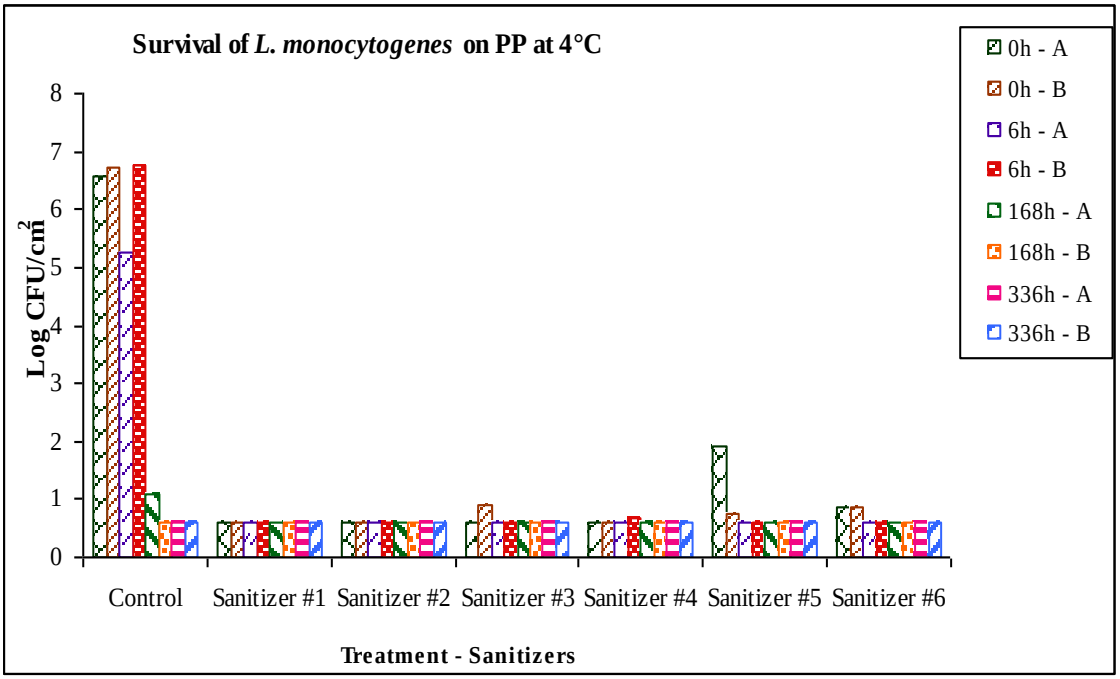


Figure 5.6. Data shown in Tables 5.9 and 5.10. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations and *L. monocytogenes* as enumerated on TSAYE and PALCAM respectively on polypropylene (PP) surface with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Table 5.3. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) rough surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	TSAYE Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.62 (0.46) a	6.86 (0.46) a	5.75 (0.46) a	6.74 (0.46) a	5.79 (0.46) a	6.87 (0.46) a	7.02 (0.46) a	7.26 (0.46) a
Sanitizer #1	0.60 (0.46) a	0.67 (0.46) a	0.60 (0.46) a	0.84 (0.46) a	3.86 (0.46) a	4.56 (0.46) a	4.32 (0.46) a	5.05 (0.46) a
Sanitizer #2	0.72 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	3.53 (0.46) a	4.31 (0.46) a	3.40 (0.46) a	4.70 (0.46) b
Sanitizer #3	2.02 (0.46) a	0.60 (0.46) b	1.01 (0.46) a	2.27 (0.46) b	4.78 (0.46) a	4.87 (0.46) a	5.51 (0.46) a	5.46 (0.46) a
Sanitizer #4	2.19 (0.46) a	1.14 (0.46) a	0.72 (0.46) a	0.60 (0.46) a	5.52 (0.46) a	4.76 (0.46) a	5.95 (0.46) a	5.35 (0.46) a
Sanitizer #5	1.99 (0.46) a	1.59 (0.46) a	0.77 (0.46) a	1.05 (0.46) a	5.73 (0.46) a	4.92 (0.46) a	6.29 (0.46) a	4.83 (0.46) b
Sanitizer #6	1.93 (0.46) a	0.87 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	5.85 (0.46) a	5.46 (0.46) a	6.21 (0.46) a	5.47 (0.46) a

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different ($P < 0.05$)

Table 5.4. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on PALCAM on high density polyethylene (HDPE) rough surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM** Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.56 (0.46) a	6.78 (0.46) a	5.39 (0.46) a	6.69 (0.46) b	2.58 (0.46) a	3.78 (0.46) a	3.26 (0.46) a	4.58 (0.46) b
Sanitizer #1	0.60 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	0.87 (0.46) a	0.83 (0.46) a	2.33 (0.46) a	2.02 (0.46) a
Sanitizer #2	0.60 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	1.27 (0.46) a	1.29 (0.46) a	2.53 (0.46) b
Sanitizer #3	1.50 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	2.09 (0.46) b	1.68 (0.46) a	1.68 (0.46) a	2.83 (0.46) a	2.59 (0.46) a
Sanitizer #4	1.89 (0.46) a	0.89 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	1.98 (0.46) a	1.84 (0.46) a	2.61 (0.46) a	1.65 (0.46) a
Sanitizer #5	1.38 (0.46) a	1.13 (0.46) a	0.72 (0.46) a	0.95 (0.46) a	1.81 (0.46) a	1.31 (0.46) a	2.98 (0.46) a	1.19 (0.46) b
Sanitizer #6	1.45 (0.46) a	0.68 (0.46) a	0.60 (0.46) a	0.60 (0.46) a	2.15 (0.46) a	2.27 (0.46) a	2.58 (0.46) a	0.92 (0.46) b

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different ($P < 0.05$)

Table 5.5. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on high density polyethylene (HDPE) smooth surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**TSAYE** Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.65 (0.36) a	6.80 (0.36) a	6.45 (0.36) a	6.77 (0.36) a	5.04 (0.36) a	7.05 (0.36) b	6.67 (0.36) a	6.98 (0.36) a
Sanitizer #1	0.77 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	2.27 (0.36) a	4.19 (0.36) b	3.33 (0.36) a	4.40 (0.36) b
Sanitizer #2	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	2.31 (0.36) a	4.41 (0.36) b	1.92 (0.36) a	4.22 (0.36) b
Sanitizer #3	0.95 (0.36) a	0.77 (0.36) a	0.60 (0.36) a	0.77 (0.36) a	2.05 (0.36) a	4.66 (0.36) b	4.65 (0.36) a	5.27 (0.36) a
Sanitizer #4	0.60 (0.36) a	0.72 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	3.26 (0.36) a	4.17 (0.36) a	3.74 (0.36) a	4.54 (0.36) a
Sanitizer #5	1.48 (0.36) a	1.06 (0.36) a	0.60 (0.36) a	0.67 (0.36) a	1.70 (0.36) a	4.53 (0.36) b	3.65 (0.36) a	4.48 (0.36) a
Sanitizer #6	1.32 (0.36) a	0.83 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	3.93 (0.36) a	5.44 (0.36) b	4.86 (0.36) a	5.22 (0.36) a

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different ($P < 0.05$)

Table 5.6. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on PALCAM on high density polyethylene (HDPE) smooth surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM** Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.53 (0.36) a	6.75 (0.36) a	6.24 (0.36) a	6.75 (0.36) a	2.64 (0.36) a	3.92 (0.36) b	2.99 (0.36) a	4.79 (0.36) b
Sanitizer #1	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	1.37 (0.36) a	1.51 (0.36) a	2.84 (0.36) b
Sanitizer #2	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.85 (0.36) a	0.93 (0.36) a	2.26 (0.36) b
Sanitizer #3	0.83 (0.36) a	0.79 (0.36) a	0.60 (0.36) a	0.67 (0.36) a	1.12 (0.36) a	2.82 (0.36) b	2.53 (0.36) a	2.77 (0.36) a
Sanitizer #4	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	1.79 (0.36) b	1.35 (0.36) a	1.67 (0.36) a
Sanitizer #5	1.27 (0.36) a	0.67 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.72 (0.36) a	1.82 (0.36) b
Sanitizer #6	0.96 (0.36) a	0.72 (0.36) a	0.60 (0.36) a	0.60 (0.36) a	0.96 (0.36) a	2.15 (0.36) b	1.23 (0.36) a	2.74 (0.36) b

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different ($P < 0.05$)

Table 5.7. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	TSAYE Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.63 (0.41) a	6.86 (0.41) a	6.30 (0.41) a	6.79 (0.41) a	6.11 (0.41) a	5.09 (0.41) a	6.10 (0.41) a	6.24 (0.41) a
Sanitizer #1	0.60 (0.41) a	0.67 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	3.17 (0.41) a	2.32 (0.41) a	3.42 (0.41) a	3.16 (0.41) a
Sanitizer #2	0.60 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	2.45 (0.41) a	2.70 (0.41) a	3.33 (0.41) a	3.15 (0.41) a
Sanitizer #3	1.47 (0.41) a	1.04 (0.41) a	0.67 (0.41) a	1.16 (0.41) a	3.35 (0.41) a	3.18 (0.41) a	4.41 (0.41) a	3.55 (0.41) a
Sanitizer #4	1.06 (0.41) a	0.75 (0.41) a	0.67 (0.41) a	0.60 (0.41) a	3.62 (0.41) a	2.68 (0.41) a	5.03 (0.41) a	3.16 (0.41) b
Sanitizer #5	1.63 (0.41) a	0.60 (0.41) a	0.94 (0.41) a	0.60 (0.41) a	4.29 (0.41) a	3.11 (0.41) b	4.49 (0.41) a	3.23 (0.41) b
Sanitizer #6	1.38 (0.41) a	0.72 (0.41) a	1.31 (0.41) a	0.67 (0.41) a	5.30 (0.41) a	3.42 (0.41) b	4.92 (0.41) a	3.92 (0.41) a

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different ($P < 0.05$)

Table 5.8. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on PALCAM on polypropylene (PP) surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 25°C).

Sanitizers	**PALCAM** Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.53 (0.41) a	6.83 (0.41) a	5.24 (0.41) a	6.76 (0.41) b	2.30 (0.41) a	3.57 (0.41) b	0.75 (0.41) a	3.41 (0.41) b
Sanitizer #1	0.60 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	1.38 (0.41) a	0.60 (0.41) a	1.47 (0.41) a	0.82 (0.41) a
Sanitizer #2	0.60 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	1.64 (0.41) a	0.60 (0.41) a	1.43 (0.41) a	0.77 (0.41) a
Sanitizer #3	1.19 (0.41) a	0.90 (0.41) a	0.60 (0.41) a	0.93 (0.41) a	1.36 (0.41) a	1.04 (0.41) a	1.56 (0.41) a	0.60 (0.41) a
Sanitizer #4	0.94 (0.41) a	0.94 (0.41) a	0.60 (0.41) a	0.60 (0.41) a	1.52 (0.41) a	0.80 (0.41) a	0.60 (0.41) a	0.97 (0.41) a
Sanitizer #5	1.15 (0.41) a	0.60 (0.41) a	0.83 (0.41) a	0.60 (0.41) a	1.52 (0.41) a	0.60 (0.41) a	0.92 (0.41) a	0.60 (0.41) a
Sanitizer #6	1.06 (0.41) a	0.60 (0.41) a	1.26 (0.41) a	0.67 (0.41) a	1.47 (0.41) a	1.22 (0.41) a	2.04 (0.41) a	1.49 (0.41) a

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 25°C is significantly different ($P < 0.05$)

Table 5.9. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on TSAYE on polypropylene (PP) surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Sanitizers	TSAYE Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.65 (0.21) a	6.74 (0.21) a	6.40 (0.21) a	6.77 (0.21) a	1.10 (0.21) a	1.01 (0.21) a	0.60 (0.21) a	1.41 (0.21) b
Sanitizer #1	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.77 (0.21) a	0.60 (0.21) a	1.06 (0.21) a	0.60 (0.21) a	1.23 (0.21) b
Sanitizer #2	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.72 (0.21) a
Sanitizer #3	0.72 (0.21) a	0.77 (0.21) a	0.60 (0.21) a	0.79 (0.21) a	0.60 (0.21) a	0.72 (0.21) a	0.60 (0.21) a	0.78 (0.21) a
Sanitizer #4	0.81 (0.21) a	0.60 (0.21) a	0.77 (0.21) a	0.67 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.77 (0.21) a
Sanitizer #5	2.33 (0.21) a	1.02 (0.21) a	0.81 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.92 (0.21) b	0.60 (0.21) a	1.39 (0.21) b
Sanitizer #6	1.29 (0.21) a	0.77 (0.21) a	0.67 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	1.30 (0.21) b	0.60 (0.21) a	1.24 (0.21) b

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 4°C is significantly different ($P < 0.05$)

Table 5.10. Mean (Log CFU/cm^2) survival (n = 4) of total bacterial populations as enumerated on PALCAM on polypropylene (PP) surfaces with an exposure to nutrients, subjected to general cutting board use and cleaning conditions (comparing method A and method B) in home after exposure to ham homogenate inoculated with a 5-strain *L. monocytogenes* mixture (RH: 90% and 4°C).

Sanitizers	**PALCAM** Time (h)							
	0 h – A	**0 h – B**	**6 h – A**	**6 h – B**	**168 h – A**	**168 h – B**	**336 h – A**	**336 h – B**
Control	6.59 (0.21) a	6.72 (0.21) a	5.27 (0.21) a	6.77 (0.21) b	1.10 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a
Sanitizer #1	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a
Sanitizer #2	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a
Sanitizer #3	0.60 (0.21) a	0.92 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a
Sanitizer #4	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.67 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a
Sanitizer #5	1.92 (0.21) a	0.75 (0.21) b	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a
Sanitizer #6	0.88 (0.21) a	0.87 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60 (0.21) a	0.60(0.21) a	0.60 (0.21) a

A & B – Sanitizer application methods

a & b, means within a row and within the same day of sanitizer treatment at 4°C is significantly different ($P < 0.05$)

SUMMARY

Listeria monocytogenes occurs widely in environment and has been isolated from a range of sources, including vegetables, raw fish and animal products, processed foods and soil. This bacterium is the causative agent of listeriosis. *L. monocytogenes* attaches to and grows on different kinds of surfaces forming biofilms. Microorganisms attached to a surface are an important potential source of contamination for any food material coming into contact with that surface. Due to the ubiquitous nature and hardy growth characteristics of the bacterium, *L. monocytogenes* is able to contaminate and thrive in kitchen homes. *L. monocytogenes* cells rapidly attach and form biofilms on food contact surfaces such as plastic, polypropylene, rubber, stainless steel and glass. Biofilm formation has become a concern because sessile bacteria within biofilms are highly resistant to common household cleaners and sanitizers, compared to bacteria in planktonic state.

The results of the first study demonstrated that *L. monocytogenes* can survive on food contact surfaces, e.g., cutting boards, plastic utensils and refrigerator shelves, forming biofilms that if not promptly and properly cleaned and sanitized, became increasingly resistant to sanitizer treatment. Multi-species biofilms containing high levels of *L. monocytogenes* developed and survived for up to 14 days on high density polyethylene and polypropylene surfaces at ambient temperature (25°C). All six sanitizers tested (application by spraying or wetting) were effective at 0 and 6 h, but

increasingly ineffective on established biofilms. Among the sanitizers evaluated, the lactic acid-based sanitizer (pH 2.92) and quaternary ammonium-based (pH 10.12) were most effective against developed biofilms. Since the effect of a sanitizer decreases as the biofilms matures, sanitation should be performed as soon as possible after each use or at least within 6 h after use in order to avoid biofilm formation on cutting boards and other food contact surfaces. Biofilm survival was found to be greater on rough than smooth HDPE surfaces and so cutting boards with a smooth surface should be more considered due to delay in the biofilm maturation.

The results in the second study showed that *L. monocytogenes* biofilms developed during storage and survived for at least 21 d on all surfaces at 25°C and 4°C with daily exposure to nutrients, but not after d 14 on coupons that were not subjected daily to nutrients. All sanitizers (applied by dipping the coupon in the sanitizer) were effective in reducing *L. monocytogenes*, but more effective on younger than older biofilms. Among the sanitizers evaluated, the lactic acid-based (pH 2.92) sanitizer was the most effective overall on day 21 biofilms, followed by the 5% acetic acid-based (pH 3.26), quaternary ammonium-based (pH 10.12) and sodium hypochlorite based (pH 6.22) sanitizers. In the absence of commercial sanitizers, readily available household products like distilled white vinegar and a diluted chlorine bleach solution should be used. Sanitation of cutting boards should be performed with selected sanitizers after each use, or at least daily, in order to achieve maximum efficacy. The results also demonstrated that repeated exposure of food contact surfaces to nutrients as during use with no cleaning or sanitation increases the resistance of *L. monocytogenes* biofilms to sanitizers. At this stage there is no obvious explanation why *L. monocytogenes* biofilms did not develop on polypropylene incubated

at 4°C with daily nutrient enrichment, but did on coupons that did not receive daily enrichment, although temperature (4°C), relative humidity (90%) and differences in competing inhibition could have a played a very important role.

A comparison of the two methods of sanitizer application showed that both methods were effective when food contact surfaces were cleaned within 6 h. With both application methods, the effectiveness of sanitizers decreased as the biofilm matured, so sanitation should be performed as soon as possible (preferably within 6 h after use) to avoid biofilm formation. In practical use, this could be achieved by using a three compartment sink installed in kitchens in which food contact surfaces such as knives, peelers, small cutting boards and food containers could be washed, rinsed and then sanitized (3^{rd} compartment) using the dip method. In contrast, slicers, countertops and large cutting boards could be sanitized by the direct application method.

Experiments related to the attachment of microorganisms to various food contact surfaces in home kitchens must be carried out under conditions existing in those environments. Such studies will help to understand fully the interactions between biotic and abiotic entities during/ after food processing in home kitchens. They are also required to understand the impact of cleaning and sanitation from the microbiological viewpoint.

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APPENDIX

Appendix 3.1. Temperature and relative humidity record table - HDPE with rough and smooth surface at 25°C

Date	Temperature (°C)	Relative Humidity (%)
12/03/2007	25.5	94.7
	25.5	94.0
	26.0	81.1
12/04/2007	26.5	91.7
	27.0	94
12/05/2007	27.0	93.6
	27.0	94.9
12/06/2007	26.5	95.2
	26.5	97.2
12/07/2007	26.5	95.6
	27.0	93.6
12/08/2007	26.0	97.6
	25.5	98.7
12/09/2007	25.0	96.8
	25.0	98.4
12/10/2007	25.0	97.8
	25.5	96.6
12/11/2007	25.0	97.1
	24.0	98.5
12/12/2007	25.0	97.6
	24.0	96.3
12/13/2007	25.0	97.8
	24.0	96.1
12/14/2007	25.0	97.8
	24.0	96.5
12/15/2007	24.0	97.3
	26.0	98.0

12/16/2007	25.5	98.5
	25.5	95.6
12/17/2007	24.0	99.7

Appendix 3.2. Temperature and relative humidity record table – PP surface at 25°C and 4°C

Date	Temperature (°C) – PP, 25°C	Relative Humidity (%)	Temperature (°C) – PP, 4°C	Relative Humidity (%)
12/07/2007	26.5	95.6	4.0	95.6
	27.0	96.1	4.5	96.1
	27.0	93.6	4.5	93.6
12/08/2007	26.0	97.6	4.0	97.6
	25.5	98.7	4.0	98.7
12/09/2007	25.0	96.8	4.0	96.8
	25.0	98.4	4.0	98.4
12/10/2007	25.0	97.8	4.5	97.8
	25.5	96.6	4.5	96.6
12/11/2007	25.0	97.1	4.0	97.1
	24.0	98.5	4.0	98.5
12/12/2007	25.0	97.6	5.0	97.6
	24.0	98.5	4.5	98.5
12/13/2007	25.0	97.8	4.5	97.8
	24.0	96.1	4.0	96.1
12/14/2007	25.0	97.8	4.5	97.8
	24.0	96.5	3.0	96.5
12/15/2007	24.0	97.3	4.5	97.3
	26.0	92.8	4.0	92.8
12/16/2007	25.5	98.5	3.5	98.5
	25.5	95.6	3.5	95.6
12/17/2007	24.0	99.7	4.0	99.7
	25.0	98.6	3.5	98.6
12/18/2007	25.0	96.7	4.5	96.7
	25.5	94.2	4.5	94.2

12/19/2007	25.0	95.5	4.5	95.5
	25.5	90.1	4.0	90.1
12/20/2007	25.5	92.2	4.5	92.2
	24.0	94.1	4.5	94.1
12/21/2007	25.5	96.8	4.0	96.8

Appendix 3.3. Analysis of variance on the effects of sanitizer treatment, media and storage time on high density polyethylene (HDPE) coupon with rough surface incubated at 25°C

Effect	Num DF	Den DF	F Value	Pr>F
Sanitizer treatment	7	63	51.91	<.0001
Media	1	63	192.06	<.0001
Time	3	63	90.14	<.0001
Sanitizer treatment*Media	7	63	0.70	0.6715
Sanitizer treatment*Time	21	63	6.42	<.0001
Media*Time	3	63	49.08	<.0001
Sanitizer treatment*Media*Time	21	63	0.28	0.9991

Appendix 3.4. Analysis of variance on the effects of sanitizer treatment, media and storage time on high density polyethylene (HDPE) coupon with smooth surface incubated at 25°C

Effect	Num DF	Den DF	F Value	Pr>F
Sanitizer treatment	7	63	101.07	<.0001
Media	1	63	141.75	<.0001
Time	3	63	31.32	<.0001
Sanitizer treatment*Media	7	63	2.65	0.0181
Sanitizer treatment*Time	21	63	7.21	<.0001
Media*Time	3	63	39.34	<.0001
Sanitizer treatment*Media*Time	21	63	0.91	0.5768

Appendix 3.5. Analysis of variance on the effects of sanitizer treatment, media and storage time on polypropylene (PP) surface incubated at 25°C

Effect	Num DF	Den DF	F Value	Pr>F
Sanitizer treatment	7	63	54.46	<.0001
Media	1	63	179.69	<.0001
Time	3	63	28.37	<.0001
Sanitizer treatment*Media	7	63	3.45	0.0035
Sanitizer treatment*Time	21	63	6.95	<.0001
Media*Time	3	63	48.81	<.0001
Sanitizer treatment*Media*Time	21	63	1.15	0.3214

Appendix 3.6. Analysis of variance on the effects of sanitizer treatment, media and storage time on polypropylene (PP) surface incubated at 4°C

Effect	Num DF	Den DF	F Value	Pr>F
Sanitizer treatment	7	63	161.62	<.0001
Media	1	63	2.57	0.1138
Time	3	63	131.22	<.0001
Sanitizer treatment*Media	7	63	0.35	0.9249
Sanitizer treatment*Time	21	63	45.50	<.0001
Media*Time	3	63	0.89	0.4489
Sanitizer treatment*Media*Time	21	63	0.39	0.9903

Appendix 4.1.Temperature and relative humidity record table - HDPE with rough and smooth surface at 25°C (1st replicate)

Date	Temperature (°C)	Relative Humidity (%)
01/14/2008	25.0	87.8
	26.0	90.5
	25.5	91.0
01/15/2008	26.0	95.5
	26.0	91.8
01/16/2008	24.0	98.4
	26.0	92.7
01/17/2008	26.0	98.1
	25.5	94.8
01/18/2008	25.5	98.2
	26.5	94.0
01/19/2008	26.0	97.7
	26.5	95.9
01/20/2008	26.0	98.0
	24.0	92.5
01/21/2008	25.5	92.7
	26.0	91.1
01/22/2008	25.0	97.4
	25.5	94.2
01/23/2008	25.5	97.7
	26.5	94.1
01/24/2008	25.5	97.8
	26.0	96.5
01/25/2008	26.0	98.2
	26.5	94.6
01/26/2008	26.0	96.4
	26.0	95.9

01/27/2008	26.0	96.1
	26.0	95.4
01/28/2008	26.5	96.1
	27.0	89.1
01/29/2008	26.5	95.4
	26.5	94.7
01/30/2008	27.0	95.3
	27.0	89.9
01/31/2008	26.5	95.1
	27.0	92.4
02/01/2008	26.5	95.2
	25.5	94.1
02/02/2008	25.5	96.6
	25.5	95.5
02/03/2008	25.5	96.7
	26.0	96.2
02/04/2008	25.5	96.5

Appendix 4.2.Temperature and relative humidity record table – PP surface at 25°C and 4°C (1st replicate)

Date	Temperature (°C) – PP, 25°C	Relative Humidity (%)	Temperature (°C) – PP, 4°C	Relative Humidity (%)
04/15/2008	27.5	92.3	4.0	83.4
	27.0	93.6	4.0	80.5
	27.0	89.6	4.5	81.6
04/16/2008	27.0	93.1	3.0	83.4
	27.0	92.0	4.5	80.6
04/17/2008	28.0	91.2	4.5	100.0
	27.5	03.5	4.5	100.0
04/18/2008	27.0	94.6	1.5	75.0
	26.5	95.2	5.0	81.0
04/19/2008	27.5	94.1	2.0	75.1
	26.5	87.6	3.0	80.9
04/20/2008	27.0	94.0	4.0	100.0
	26.5	88.3	3.5	100.0
04/21/2008	27.5	93.4	4.0	100.0
	26.5	94.9	3.0	83.3
04/22/2008	27.0	94.2	4.5	100.0
	27.0	85.7	4.0	100.0
04/23/2008	27.0	93.4	4.0	85.3
	26.0	95.4	4.5	86.5
04/24/2008	27.0	93.3	4.0	100.0
	27.0	93.1	5.5	86.6
04/25/2008	27.0	94.1	3.0	98.5
	27.0	93.2	4.0	100.0
04/26/2008	26.5	93.5	3.5	100.0
	26.0	94.5	4.5	100.0
04/27/2008	26.5	95.2	4.5	100.0

	26.0	94.9	3.5	87.8
04/28/2008	26.5	94.5	3.5	81.6
	26.5	93.0	2.0	98.2
04/29/2008	27.0	92.8	3.0	98.4
	27.0	79.3	3.5	94.0
04/30/2008	27.5	87.9	3.5	100.0
	27.0	86.5	4.0	94.2
05/01/2008	27.5	87.1	3.5	80.0
	27.0	89.1	5.5	100.0
05/02/2008	27.0	89.7	3.9	96.7
	27.0	92.3	4.5	84.8
05/03/2008	27.0	91.7	3.5	82.8
	26.5	90.9	4.0	89.9
05/04/2008	25.5	93.3	4.0	87.8
	26.0	92.0	4.5	90.2
05/05/2008	26.0	94.6	3.5	91.4
	26.5	91.9	3.5	89.5
05/06/2008	26.0	92.2	3.5	91.1

Appendix 4.3.Temperature and relative humidity record table - HDPE with rough and smooth surface (2nd replicate)

Date	Temperature (°C)	Relative Humidity (%)
03/19/2008	26.5	92.3
	26.5	94.7
03/20/2008	26.5	95.2
	26.5	94.2
03/21/2008	26.5	95.9
	26.0	96.5
03/22/2008	26.5	95.5
	26.0	96.0
03/23/2008	26.0	96.7
	26.0	91.6
03/24/2008	26.0	95.1
	26.0	96.4
03/25/2008	26.5	96.4
	26.0	96.2
03/26/2008	26.5	96.6
	26.5	89.6
03/27/2008	26.5	92.7
	26.5	91.6
03/28/2008	26.5	95.8
	26.5	95.4
03/29/2008	26.5	96.5
	25.5	97.1
03/30/2008	26.5	96.6
	25.5	96.3
03/31/2008	26.0	96.5
	25.5	96.7
04/01/2008	26.5	96.5

	26.0	93.6
04/02/2008	26.0	61.7
	25.5	59.4
04/03/2008	26.5	89.3
	25.5	80.0
04/04/2008	26.5	89.2
	26.0	86.0
04/05/2008	26.5	90.9
	26.0	79.3
04/06/2008	26.5	88.3
	26.0	93.6
04/07/2008	26.5	93.7
	26.5	89.6
04/08/2008	26.5	95.3
	26.5	94.5
04/09/2008	26.5	94.9

Appendix 4.4.Temperature and relative humidity record table – PP surface at 25°C and 4°C (2nd replicate)

Date	Temperature (°C) – PP, 25°C	Relative Humidity (%)	Temperature (°C) – PP, 4°C	Relative Humidity (%)
05/12/2008	27.5	93.5	5.0	85.4
	27.5	89.5	3.5	100.0
05/13/2008	28.0	93.6	4.5	84.8
	27.5	92.9	4.0	89.6
05/14/2008	27.5	92.3	4.0	83.4
	27.5	94.3	3.0	91.3
05/15/2008	27.5	95.1	4.0	100.0
	27.0	95.5	4.5	100.0
05/16/2008	27.5	95.2	4.0	100.0
	27.0	92.2	5.5	100.0
05/17/2008	27.5	94.2	4.0	88.1
	26.5	95.2	4.0	100.0
05/18/2008	27.5	94.8	3.0	93.7
	27.5	93.3	3.0	87.8
05/19/2008	28.0	94.2	4.0	89.1
	26.5	89.9	4.0	91.0
05/20/2008	28.0	92.4	4.5	100.0
	27.5	90.0	4.5	89.1
05/21/2008	28.0	92.3	4.0	89.9
	27.0	91.6	4.5	92.1
05/22/2008	28.0	92.6	3.5	100.0
	26.5	94.6	5.5	100.0
05/23/2008	27.0	94.1	4.5	88.6
	27.5	91.6	5.0	100.0
05/24/2008	28.0	93.9	3.0	94.8
	27.0	84.9	4.0	100.0

05/25/2008	27.5	94.9	3.0	96.6
	27.5	94.2	3.0	88.1
05/26/2008	26.5	95.2	4.5	100.0
	27.5	86.2	4.0	100.0
05/27/2008	27.5	93.7	3.0	81.1
	27.0	94.6	3.5	97.6
05/28/2008	27.0	92.9	4.5	100.0
	27.0	94.8	4.5	87.2
05/29/2008	26.5	94.6	3.0	94.2
	27.5	92.8	3.0	96.0
05/30/2008	27.5	94.7	5.5	90.4
	27.0	92.2	3.5	84.8
05/31/2008	28.0	93.4	3.5	100.0
	27.0	94.3	3.5	85.8
06/01/2008	28.0	93.6	4.5	87.6
	27.5	91.1	5.0	90.9
06/02/2008	27.0	95.2	5.0	91.3

Appendix 4.5. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat high density polyethylene (HDPE) rough surfaces (with daily exposure to nutrients), RH: 90% and 25°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0h	6h	24h	96h	168h	336h	504h	0h	6h	24h	96h	168h	336h	504h
Sanitizer 1	2.40 (0.47)	2.00	2.00	3.28 (0.38)	3.26 (1.10)	3.83 (0.37)	3.75 (0.05)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.00	2.00	2.00	3.64 (0.80)	3.14 (1.15)	3.51 (0.33)	3.48 (0.03)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.15 (0.30)	2.08 (0.15)	2.30 (0.60)	2.82 (1.16)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.08 (0.15)	2.00	2.00	3.07 (0.71)	3.11 (0.54)	2.59 (0.16)	3.66 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.25 (0.50)	2.00	2.21 (0.42)	3.19 (0.36)	3.14 (0.96)	2.15 (0.21)	3.76 (0.21)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.00 (0.75)	3.16 (0.81)	3.03 (0.16)	2.89 (0.12)	3.74	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.6. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat high density polyethylene (HDPE) smooth surfaces (with daily exposure to nutrients), RH: 90% and 25°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0h	6h	24h	96h	168h	336h	504h	0h	6h	24h	96h	168h	336h	504h
Sanitizer 1	2.00	2.00	2.00	3.21 (0.61)	3.26 (0.66)	3.29 (0.78)	2.63 (0.46)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.00	2.00	2.00	3.21 (1.25)	3.19 (0.67)	3.40 (0.20)	2.60 (0.46)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.00	2.08 (0.15)	2.33 (0.47)	2.50 (0.42)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.00	2.15 (0.30)	2.00	2.72 (0.72)	3.00 (0.72)	2.15 (0.17)	3.37 (0.16)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.25 (0.50)	2.00	2.00	2.97 (0.77)	2.65 (0.60)	2.99 (0.54)	3.74 (0.58)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.24 (0.48)	2.95 (0.49)	3.53 (0.35)	2.48 (0.40)	3.56 (0.67)	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.7. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat polypropylene (PP) (with daily exposure to nutrients), RH: 90% and 25°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0h	6h	24h	96h	168h	336h	504h	0h	6h	24h	96h	168h	336h	504h
Sanitizer 1	2.08 (0.15)	2.00	2.00	2.17 (0.35)	2.40 (0.32)	2.68 (0.48)	3.21 (0.42)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.00	2.00	2.00	2.12 (0.24)	2.36 (0.43)	2.35 (0.43)	2.61 (0.72)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.23 (0.29)	2.00	2.00	2.17 (0.35)	2.50 (0.59)	2.37 (0.43)	2.71 (0.88)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.00	2.00	2.08 (0.15)	2.00	2.60 (0.69)	2.75 (0.62)	2.37 (0.30)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.00	2.19 (0.39)	2.00	2.70 (0.51)	2.72 (0.51)	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.8. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat polypropylene (PP) (with daily exposure to nutrients), RH: 90% and 4°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0h	6h	24h	96h	168h	336h	504h	0h	6h	24h	96h	168h	336h	504h
Sanitizer 1	2.00	2.00	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.15 (0.30)	2.00	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.00	2.00	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.08 (0.15)	2.00	2.00	2.00	2.08 (0.15)	2.71 (0.53)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.00	2.00	2.00	2.66 (0.45)	2.12 (0.24)	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.9. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat high density polyethylene (HDPE) rough surfaces (without daily exposure to nutrients), RH: 90% and 25°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Sanitizer 1	2.40 (0.47)	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.00	2.00	2.00	2.00	2.12 (0.24)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.25 (0.50)	2.00	2.21 (0.42)	2.00	2.29 (0.59)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.10. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat high density polyethylene (HDPE) smooth surfaces (without daily exposure to nutrients), RH: 90% and 25°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Sanitizer 1	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.00	2.15 (0.30)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.25 (0.50)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.24 (0.48)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.11. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat polypropylene (PP) surfaces (without daily exposure to nutrients), RH: 90% and 25°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504h	0 h	6 h	24 h	96 h	168h	336h	504h
Sanitizer 1	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.23 (0.29)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.12. Mean (Log CFU/cm2) survival (n = 4) of total bacterial population as enumerated on TSAYE and *L. monocytogenes* population as enumerated on PALCAM in sanitizers used to treat polypropylene (PP) surfaces (without daily exposure to nutrients), RH: 90% and 4°C. (2.00 Log CFU/cm^2 = Lowest detection limit)

Treatment	TSAYE							PALCAM						
	0 h	6 h	24 h	96 h	168 h	336 h	504 h	0 h	6 h	24 h	96 h	168 h	336 h	504 h
Sanitizer 1	2.00	2.00	2.00	2.00	2.08 (0.15)	2.00	2.15 (0.30)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 2	2.15 (0.30)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 3	2.00	2.00	2.00	2.00	2.00	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 4	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 5	2.08 (0.15)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sanitizer 6	2.00	2.00	2.35	2.08 (0.15)	2.35 (0.40)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Appendix 4.13. Analysis of variance on the effects of nutrient, sanitizer treatment, media and storage time on high density polyethylene (HDPE) coupon with rough surface incubated at 25°C

Effect	Num DF	Den DF	F Value	Pr>F
Nutrient	1	175	1579.52	<.0001
Sanitizer treatment	7	175	196.59	<.0001
Media	1	175	169.87	<.0001
Time	6	175	103.19	<.0001
Nutrient*Sanitizer treatment	7	175	0.99	0.4409
Nutrient*Media	1	175	401.56	<.0001
Sanitizer treatment*Media	7	175	0.96	0.4609
Nutrient*Time	3	175	70.45	<.0001
Sanitizer treatment*Time	42	175	8.53	<.0001
Media*Time	6	175	64.58	<.0001
Nutrient*Sanitizer treatment*Time	21	175	5.37	<.0001
Nutrient*Sanitizer treatment*Media	7	175	2.81	0.0085
Nutrient*Media*Time	3	175	0.32	0.8144
Sanitizer treatment*Media*Time	42	175	0.49	0.9964
Nutrient*Sanitizer treatment*Media*Time	21	175	1.00	0.4612

Appendix 4.14. Analysis of variance on the effects of nutrient, sanitizer treatment, media and storage time on high density polyethylene (HDPE) with smooth surface incubated at 25°C

Effect	Num DF	Den DF	F Value	Pr>F
Nutrient	1	175	1250.82	<.0001
Sanitizer treatment	7	175	254.13	<.0001
Media	1	175	99.25	<.0001
Time	6	175	128.18	<.0001
Nutrient*Sanitizer treatment	7	175	5.46	<.0001
Nutrient*Media	1	175	317.35	<.0001
Sanitizer treatment*Media	7	175	0.84	0.5549
Nutrient*Time	3	175	64.66	<.0001
Sanitizer treatment*Time	42	175	8.77	<.0001
Media*Time	6	175	48.92	<.0001
Nutrient*Sanitizer treatment*Media	7	175	1.31	0.2492
Nutrient*Sanitizer treatment*Time	21	175	6.69	<.0001
Nutrient*Media*Time	3	175	3.16	0.0262
Sanitizer treatment*Media*Time	42	175	0.42	0.9993
Nutrient*Sanitizer treatment*Media*Time	21	175	0.78	0.7421

Appendix 4.15. Analysis of variance on the effects of nutrient, sanitizer treatment, media and storage time on polypropylene (PP) surface incubated at 25°C

Effect	Num DF	Def DF	F Value	Pr>F
Nutrient	1	175	643.69	<.0001
Sanitizer treatment	7	175	371.75	<.0001
Media	1	175	117.24	<.0001
Time	6	175	35.32	<.0001
Nutrient*Sanitizer treatment	7	175	2.81	0.0086
Nutrient*Media	1	175	317.46	<.0001
Sanitizer treatment*Media	7	175	2.04	0.0530
Nutrient*Time	3	175	112.16	<.0001
Sanitizer treatment*Time	42	175	13.07	<.0001
Media*Time	6	175	63.58	<.0001
Nutrient*Sanitizer treatment*Media	7	175	0.68	0.6855
Nutrient*Sanitizer treatment*Time	21	175	15.47	<.0001
Nutrient*Media*Time	3	175	27.11	<.0001
Sanitizer treatment*Media*Time	42	175	0.23	1.0000
Nutrient*Sanitizer treatment*Media*Time	21	175	1.41	0.1195

Appendix 4.16. Analysis of variance on the effects of nutrient, sanitizer treatment, media and storage time on polypropylene (PP) surface incubated at 4°C

Effect	Num DF	Def DF	F Value	Pr>F
Nutrient	1	175	8.34	0.0044
Sanitizer treatment	7	175	600.91	<.0001
Media	1	175	17.22	<.0001
Time	6	175	87.81	<.0001
Nutrient*Sanitizer treatment	7	175	55.31	<.0001
Nutrient*Media	1	175	49.11	<.0001
Sanitizer treatment*Media	7	175	1.36	0.2252
Nutrient*Time	3	175	84.47	<.0001
Sanitizer treatment*Time	42	175	60.88	<.0001
Media*Time	6	175	14.10	<.0001
Nutrient*Sanitizer treatment*Media	7	175	2.59	0.0144
Nutrient*Sanitizer treatment*Time	21	175	25.33	<.0001
Nutrient*Media*Time	3	175	7.80	<.0001
Sanitizer treatment*Media*Time	42	175	1.03	0.4365
Nutrient*Sanitizer treatment*Media*Time	21	175	0.93	0.5563