THE EFFICACY OF ELECTROLYZED WATER FOR CLEANING AND SANITIZING RUBBER PICKER FINGERS SOILED WITH CHICKEN FAT AND *SALMONELLA* TYPHIMURIUM.

by

VEDAS K.BURKEEN

(Under the Direction of Yen-Con Hung)

ABSTRACT

Rubber picker fingers used to remove feathers from broiler carcasses have been considered potential cross-contamination sites during poultry processing. The effects of alkaline electrolyzed (EO) water and other cleaners on chicken fat removal from rubber picker fingers over elapsed time (0,1,4, 8, and 24 hr) and repeated daily soiling and cleaning cycles (1-4 days) were examined. In addition, the effects of fat absorption on attachment and inactivation of *Salmonella* Typhimurium by various sanitizers, including acidic EO water, on soiled rubber fingers were investigated. In the first study, results suggested alkaline EO water can effectively remove chicken fat from fingers. However, when using a different type of rendered fat, results suggested that cleaning every 4 hours with a commonly used chlorinated-alkaline cleaner (50% solution) can effectively remove fat from fingers. This may be due to the chlorinated-alkaline cleaner higher pH (13.5) and the usage of a higher concentration which resulted in better fat removal when compared to alkaline EO waters lower pH (pH 11.6) and dilute sodium hydroxide concentration. Results also suggested that fat does not play a significant role on the attachment of *S.* Typhimurium to rubber fingers. However, the presence of cracks and crevices along with rubber degradation of used fingers collected from a local poultry plant can affect the cleanability

and sanitation of soiled fingers when compared to new ones. When comparing bacterial inactivation by various sanitizers, heated acidic EO was the most effective sanitizing solution by reducing *S.* Typhimurium to non-detectable levels on unsoiled used fingers. Overall, the presence of fat on the rubber fingers decreased the efficiency of all sanitizing agents examined. The chlorinated-alkaline cleaner and 10% sodium hypochlorite combination (cleaning followed by sanitizing) was the most effective treatment for soiled fingers and achieved 4.88 log CFU per rubber piece reductions of *S.* Typhimurium.

INDEX WORDS: cleanability and sanitation, electrolyzed oxidizing (EO) water, *Salmonella* Typhimurium, rubber picker fingers, fat, soil, defeathering, poultry processing

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DEDICATION

My dissertation is dedicated to my Lord and Savior, Jesus Christ, my grandmother, the late Rev. Lucy M. Burkeen, and to all my family and loved ones.

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CHAPTER 1

INTRODUCTION

Defeathering practices have been identified by the International Commission on Food Microbiological Specifications as the first major cross contamination site during poultry processing. Microbial increase on carcasses after defeathering has been illustrated with food borne pathogens such as *Campylobacter jejuni*, *Staphylococcus aureus* and *Salmonella.* Although there are many sources of contamination via picker machines, the picker fingers have been identified as ideal vectors for contamination since they are located in warm, humid environments, and they are very difficult to clean and disinfect. They also come into direct contact with the surface of the carcass throughout plucking procedures.

It has been demonstrated that cleaning efficacy is surface dependent and soil dependent. Inadequate cleaning of surfaces can lead to minimal inactivation of unwanted bacteria because sanitizing efficiency is hindered in the presence of organic matter. The type and amount of soil accumulated in the food processing environment and on food contact surfaces can determine the species of bacteria present and how readily they are inactivated by cleaning and sanitizing agents. Thus, the evaluation of the type and an adequate estimation of the amount of soils present are crucial.

During defeathering practices, feathers are removed along with a large portion of the outer layer of the skin, called the stratnum corneum. The stratnum corneum is made up or cornea cells and fat vacuoles. Although chicken skin is thought to be made of mostly protein, fat is a major portion of the skin. The skin of chickens is a secretory organ producing a lipoid or sebaceous material that is similar to oil that is produced by an oil gland. Lipids are present throughout epidermis of chickens in the form of droplets in the cells and vacuoles spaces in the stratum corneum. While feathers and protein from skin contribute highly to the cross contamination of rubber finger surfaces, the fat component of the skin can be a very important

factor. One of the main reasons for this is that feathers and protein components of the skin can be more readily rinsed away during rinsing procedures. Fat on the other hand, is not water soluble and is very hard to remove without using a detergent or surfactant. Fat may also serve as protection for bacteria by shielding them from the sanitizers and cleaners. Thus, it is very important that effective methods of removing the fatty film from food contact surfaces be utilized. Moreover, an area largely unexplored, is fat absorption into the porous surface of picker fingers. Absorbed fat may better protect bacteria than the fat film on the surface which can decrease inactivation by cleaning and sanitizing procedures to a greater degree.

The major functions of cleaning compounds are to lower the surface tension of water so that soils may be dislodged and loosened and to suspend soil particles for subsequent flushing away. Deciding what cleaner to use is not as simple as one might think. Alkaline cleaners are most effective on organic soils and are most often used to remove fats from surfaces. The cleaning mechanism of alkali cleaners are behind their high pH (>11), which allows for neutralization of acidic soils, saponification of fats and lipids, and dispersion of oils and lipids on various surfaces.

Sanitation of surfaces is greatly influenced by soil and bacterial attachment to a particular surface. With this in mind, it is imperative that adequate cleaning is done before the sanitation process to help eliminate pathogens such as *Salmonella* Typhimurium from food surfaces. There are many sanitizers that can be used to inactivate unwanted microorganisms, however, there is still room for improvement and exploration of innovative chemical sanitation methods along with the identification of more efficient disinfectants.

Using Acidic electrolyzed oxidizing water in agriculture, livestock management, medical sterilization and food sanitation is a relatively new concept. This water has high antimicrobial

qualities associated with its high chlorine content, low pH and high oxidation-reduction potential (ORP). Acidic EO water consists primarily of hypochlorous acid (HOCl), which is the most bactericidal of all chlorine species (80 times more effective than OCL^-). Acidic EO water has exhibited bactericidal affects against various pathogens such as *L. monocytogenes, E. coli, Campylobacter* and *Salmonella* on various food substrates and food contact surfaces. This unique water is also safer to use than many other chlorine based sanitizers because it uses NaCl as its sole source of chlorine and is generated by passing a dilute NaCl solution through an electrode cell. Because using EO water is a relatively new concept and has many safety attributes**,** it is imperative that we explore viable uses in the food industry more thoroughly. It is equally important to define concrete uses for its alkaline counterpart, which is generated at the same time as acidic EO water. Alkaline EO water is made up of dilute sodium hydroxide and has surfactant and degreaser properties because of it high pH (11.6). These properties may prove instrumental in cleaning processes, especially with the removal of fat. In the following studies, the use of alkaline EO and acidic EO waters will be evaluated on the cleaning and disinfection of rubber picker fingers soiled with fat and contaminated with *Salmonella* Typhimurium, respectively.

CHAPTER 2

LITERATURE REVIEW

Poultry processing

Poultry processing has significantly changed over the last two decades due to large demand for poultry products. Currently, the scale of production has increased with an intensification of farming and slaughtering/processing facilities to ensure that the demand is met (Bell, 2001). The speed and intensity of current poultry processing methods are highly efficient and are cost effective with modern processing lines processing up to 200 birds per minute (Mastroeni, 2006). These intensified conditions of poultry processing offer greater opportunities for pathogens to spread under intensive growing conditions (Mastroeni, 2006). Each poultry processing step should be analyzed intensely for potential hazards and considerations that need to be made to control cross-contamination. Primary processing steps include: stunning, killing, scalding, defeathering (plucking), evisceration, and chilling. These processes will be briefly discussed in this section.

Broilers (5-8 weeks) are transported to processing plants in cages. Once at the plant, the birds are stored in a collection chamber until they are placed upside down onto shackles by hand. The birds are stunned, usually by an electrical stunner. Next, the birds are killed by cutting the neck, rupturing the blood vessel, and are bled out before they enter the scalding tank. The scald tank contains a large amount of water that is usually between 50-60^{o}C (Cason et al., 2004; Mastroeni, 2006). The main purpose of the scalding practice is to help loosen feathers so that they are more readily removed by plucking. After scalding, birds are defeathered via mechanical picker machines with rubber picker fingers. Once the feathers have been removed, birds are eviscerated (removal of viscera) via cutting a hole in the caecal vent by the evisceration unit. Other

organs may be removed also, depending on the system. Finally, after washing inside and outside with automatic sprayers, carcasses are moved to the chiller where they are chilled (to less than 5^{o}C) (Mastroeni, 2006).

Cross-contamination during poultry processing

Broilers entering the processing plant are contaminated by large, diverse, populations of microorganisms. Bacteria are attached to the feathers and skin, and they are found in the fecal contents of birds. Feathers may harbor 10^{8} total bacteria/g, the skin may carry 10^{6} bacteria/cm^{2}, and fecal matter may also harbor extremely high numbers of bacteria (Cason et al., 1999). Overall, poultry processing may decrease the number of undesirable microorganisms that are present on poultry carcasses (Hinton et al., 2002, 2004). Yet, throughout the process, cross-contamination may spread bacteria between processing steps. This variability is exhibited early on in processing during scalding and defeathering steps. There is a decrease in the total number of microorganisms present on chicken carcasses after the hot water scalding step (Cason et al., 2004). However, the step after scalding (defeathering) leads to a significant increase in the number of bacteria recovered from carcasses. For these reasons, the defeathering process has been a point of cross-contamination in the poultry industry for many years.

The International Commission on Food Microbiological Specifications has identified defeathering as the first major cross-contamination site during poultry processing (Clouser et al., 1995). Hinton (2004) showed that many spoilage bacteria such as, *Acinetobacter* and *Aeromonas*, increased in numbers on chicken carcasses after scalding/defeathering operations. Over a series of four trials, bacteria species present on carcasses were tracked throughout processing steps. Specific bacteria were identified by

using the MIDI Sherlock Microbial Identification System that was able to identify bacteria by using their fatty acid profiles. Results showed that *Aeromonas spp.* and *Acinetobacter spp.* that were only present on previously processed flocks could also be isolated from flocks processed in the same facility at a later date. These findings implicated that bacteria could colonize feather picker machines and other processing equipment to later cross-contaminate carcasses during different processing dates in the same plant facility.

Microbial increase on carcasses after defeathering has also been illustrated with foodborne pathogens such as *Campylobacter jejuni*, *Staphylococcus aureus* and *Salmonella* Typhimurium (Berrang et al., 2001; Kim and Doores, 1993a; Purdy et al., 1988). *Campylobacter* numbers can increase from 1 CFU/cm^2 on skin before scalding and defeathering practices to 10^4 or 10^5 CFU/cm^2 after defeathering (Berrang and Dickens, 2004; Berrang et al., 2001). The increase in *Campylobacter* recovery after defeathering was attributed to the escape of fecal contents via the cloaca (Berrang et al., 2001). Further investigation proved that *Campylobacter* cross-contamination was also possible via soiled surfaces of feather picker fingers machines (Berrang and Dickens, 2004). Dodd (1988) observed a 1000-fold increase in *Staphylococcus aureus* counts on carcasses during plucking practices. He further proved this point by demonstrating that this organism can contaminate picking machines at different levels and different points (Dodd et al., 1988). This was exemplified when *S. aureus* was recovered at the entrance of machines at lower counts of approximately 10^3 CFU/ml, while at the exit of the machine this organism was found at a level of 10^7 CFU/ml.

Rubber picker fingers

Defeathering machines contain many potential sites and sources for bacterial contamination, including rotating disk, picker fingers, plastic curtains, aqueous aerosols, and water sprays (Allen et al., 2003a; Dodd et al., 1988; Lindsay et al., 1996). When examining picker machines, non-metallic surfaces such as rubber fingers and plastic curtains (at the entrance and exit of machines) have been found to be contaminated by large populations of bacteria. The picker fingers have been identified as ideal vectors for contamination "since they are located in warm, humid environments, and they are very difficult to clean and disinfect" (Purdy et al., 1988). They also come into direct contact with the surface of the carcass throughout plucking procedures.

Studies have proven that new rubber feather picker fingers can prevent bacterial attachment and biofilm formation due to attributes of the rubber surface (may be contributed to the presence of sulfur and zinc) (Arnold, 2007; Arnold and Bailey, 2000). Bacterial attachment to picker finger rubber can be significantly less than attachment to stainless steel and other surfaces. This is due to the ability of the picker finger rubber to naturally inhibit growth of bacteria (Arnold and Bailey, 2000; Helke and Wong, 1994).

Rubber

Natural rubber is derived from a milky colloidial suspension called latex. Latex is present in the sap of many plants but is specifically obtained from the tree of *Hevea brasiliensi*, commonly known as the rubber tree. Natural rubber is essentially a polymer of isoprene units, a hydrocarbon diene (double bonded) monomer (Dickens, 1989; Kauffman, 1990a). Rubber can also be composed synthetically. Synthetic rubbers are made by the polymerization of a single monomer or mixture of monomers to produce

polymers. Some examples of synthetic rubber include styrene-butadiene rubber (buna rubber), butadiene rubber, chloroprene rubber (CR) and ethylene-propylene-diene rubber (EPDM) (Absolom, 1983). Rubber in its natural state is stringy and elastic which is not easily molded. As a result, most rubber in everyday use is vulcanized. Vulcanization refers to a specific curing process of rubber involving high heat and the addition of sulfur. The addition of sulfur atoms and the high temperatures cause polymer molecules to link to other polymer molecules due to sulfur cross linking (Kauffman, 1990a). This cross linkage determines the hardness, durability, and chemical resistance that a rubber sample may have (Kauffman, 1990b).

Sulfur addition to rubber may be one of the main reasons for rubber's innate ability to inhibit bacterial attachment. Although sulfur is essential for living cells and is present in two important amino acids (methionine and cystiene), it is also present in many bacterial defense molecules. For example, although sulfur is not a part of the lactam ring, it is a part of most beta lactam antibiotics, including the penicillins, cephalosporins, and monobactams. In addition, many sulfur containing compounds, such as sulfur dioxide and its salts (potassium and sodium sulfites, potassium and sodium bi-sulfite, and potassium and sodium metabisulfites) are used as preservatives and as antimicrobials on fruits and vegetables. Sulfites also act as antioxidants and inhibit enzymatic and non-enzymatic browning in a variety of foods (Doyle, 2001; Mafu et al., 1990).

The sulfur cross linkage between polymers may be degraded during prolong usage and chemical attack. This may be a major factor that contributes to the loss of firmness and bacterial resistance that is observed in used fingers. In addition, zinc is another primary element in rubber and is considered an antimicrobial component that

may be influential. However, the presence of these elements cannot totally explain the inhibitory attributes of rubber (Arnold and Silvers, 2000; Chmielewski and Frank, 2006).

Bacterial attachment to rubber

When evaluating the hazards of poultry processing and the potential of surface cross-contamination to carcasses, it is important to understand mechanisms of bacterial attachment to the surfaces of concern. In general, bacterial attachment to surfaces involves two basic stages. In the first step, the loosely attached cells that are present in the environment adhere to surface via Van der Waals interaction. This stage usually involves the presence of a water film on the surface that assists in bacterial adherence. The second stage involves irreversible attachment with the production of an extracellular polysaccharides layer known as the glococalyx which allows bacteria to more firmly attach to surface (Doyle, 2001; Krysinski et al., 1992).

When it comes to interaction with the surface, bacterial adhesion is affected by physico-chemical properties, such as, surface charge and hydrophobicity. Bacteria have a net negative charge on the cell wall although the magnitude of this charge varies from strain to strain (Dickens, 1989; Gibbs et al., 1978). Most bacteria have a hydrophobic surface and the contact surface's hydrophobicity has an affect on the ability for a particular bacteria strain to attach. Absolom (1983) noted that bacterial adhesion is more extensive to hydrophobic surfaces with relatively low surface energy when compared to more hydrophilic surfaces. For example, studies have shown that bacterial strains, *Listeria* and *Salmonella,* are both more adherent to hydrophobic surfaces than hydrophilic surfaces (Chmielewski and Frank, 2006; Sinde and Carballo, 2000). Rubber, in particular, is a hydrophobic surface with lower energy than other surfaces such as

stainless steel (Mafu et al., 1990). Bacterial attachment to this surface may be contributed highly to the hydrophobic-hydrophobic interaction between the cells and rubber surface. However, rubber hydrophobicity can decrease during aging and continual use. This may lower the interaction between the bacterial cells and rubber (Chmielewski and Frank, 2006).

Attached bacteria have an increase resistance to sanitization than planktonic cells (Krysinski et al., 1992). This resistance is further increased when bacteria are attached to rubber when compared to other commonly used food contact surfaces such as stainless steel. Ronner and Wong (1993) tested biofilm development of *Listeria monocytogenes* and *Salmonella* Typhimurium on stainless steel and buna-N-rubber, a gasket material commonly used in food processing. In the study, buna-N-rubber had a strong bacteriostatic affect on *L. monocytogenes* but exhibited less inhibitory affect on *S.* Typhimurium. Biofilms of both pathogens were treated with four types of detergents and non-detergent sanitizers. Results showed that stainless steel had very little effect on biofilm formation and bacterial populations were reduced by all sanitizers by approximately 3-5 logs. However, the buna-N-rubber surface influenced bacterial resistance to sanitizers. Results on the buna-N- rubber surface showed that there were only 1-2 log reductions in bacterial populations by sanitizers for both pathogens studied (Ronner and Wong, 1993).

Increased bacterial resistance to sanitizers and cleansers on rubber surfaces may be a major contribution to cross-contamination during defeathering. Although biofilms do not readily form on the rubber picker fingers, the increased resistance can prevent the rubber surface from being adequately cleaned and disinfected (Gibbs et al., 1978),

leaving bacteria to survive and contaminate poultry carcasses later. The decrease in cleaning and disinfection of rubber surfaces due to increased microbial resistance can be further enhanced by aging and continual use.

Used rubber fingers

Choosing the surface material for food processing equipment is of great importance in food processing facilities. The ability of bacteria and food to attach to the surface and the surface cleanabilty are significant factors in respect to hygiene. If the surface materials used for the processing equipment are smooth and the integrity of the material is preserved, then surfaces will be easy to clean. Irregularities in the surface, such as roughness, cracks, pits, and crevices decrease the feasibility of attached cells to be removed by cleaning (Storgards et al., 1999). Storgards (1999) demonstrated that a variety of rubber materials used in food processing gaskets (ethylene propylene diene monomer rubber (EPDM), nitrile butyl rubber (NBR also known as buna-N), polytetrafluoroethylene (PTFE) and Viton (fluoroelastomer)) can be attacked by cleaners and food particles. In most of the gasket materials tested, the attack led to brittleness followed by crack formation and eventually to dissolution and softening of gaskets (Storgards et al., 1999). This is a form of surface corrosion caused by chemical reactions between sanitizers/cleansers and the contact surfaces (Ayebah and Hung, 2005). Alkaline cleaning agents frequently contain complex formers and antifoaming agents and do not corrode most rubber materials. However, many rubber surfaces, such as gasket materials, are more or less prone to attack by oxidizing agents such as chlorine, hydrogen peroxide, peracetic acid and nitric acid (Storgards et al., 1999). This deterioration of the

surface can caused increase biofilm formation and decrease cleanablity by offering better attachment surfaces and protection from cleaning operations (Storgards et al., 1999).

The continual usage of rubber fingers causes the rubber to wear, cracks and crevices to form, and shape deformation (curved fingers and smoothness of rings). Rubber fingers that have been worn and soiled during extended uses have increased biofilm formation and offer better bacterial attachment surfaces than new rubber fingers (Arnold, 2007). Scanning Electron Microscope (SEM) images (Figure 2.1) demonstrated how readily bacteria cells adhere to the surface of used picker fingers from poultry plants (Arnold, 2007). In that study, fingers were gathered from three different processing plants and SEM pictures showing attached bacteria cells on the finger were taken. Data showed that the number of bacterial cells that were attached to fingers differed from plant to plant. Two of the process plants showed an average of approximately 3 log CFU per finger. Numbers were greatly increased in the third plant with numbers ranging from approximately 3 to 7 log CFU. The difference in bacterial numbers may have been a result of insufficient cleaning and sanitizing practices (Arnold, 2007).

Soil

Bacteria need a continual source of nutrients and water to thrive. Food processing facilities are good environments for bacteria to live for many reasons which include: their introduction into the facility via the food substrate, the continual source of nutrients supplied by food particles and the large amount of water used to clean and rinse food products and equipment. The establishment of biofilms will usually occur on soiled surfaces in contact with liquid (Arnold, 2007). During processing, organic and inorganic materials that are present in liquid are able to sediment onto food contact surfaces. As a

result, bacteria will attach to the interface of surfaces. The type and amount of soil accumulated in the food processing environment and on food contact surfaces can determine the species of bacteria present and how readily they are inactivated by cleaning and sanitizing agents. Thus, the evaluation of the type and an adequate estimation of the amount of soils present are crucial.

Conventional defeathering, which is used by the majority of commercial processors, includes immersion of birds into a hot scalding tank (58^{o}C) followed by automatic picking by mechanical pickers (Kim et al., 1993). The time required and the number of birds processed varies depending on the size and capacity of the processing plant. As many as 3000 to 6000 birds can be processed per hour per processing line (Allen et al., 2003b; Dodd et al., 1988; Purdy et al., 1988), however, numbers can be less or higher depending on plant size. Allen (2003) and Mead (1994) noted that the estimated times for each bird to become totally defeathered and move through the machines are 45 seconds and 1 minute, respectively. The large volume of birds processed per hour can lead to high amounts of soil retention on picker fingers before adequate cleaning can be done. This large amount of soil is another vector contributing to cross-contamination (Berrang and Dickens, 2004).

One explanation for large accumulation of soil present is the removal of the skin during conventional defeathering processing. Feathers are removed relatively quickly from carcasses during defeathering processes with the majority of feathers being removed in approximately the first ten seconds of processing (Allen et al., 2003a). This leaves the skin of carcasses exposed to the picker fingers for the remainder of the defeathering

process. As a result, the epidermis can be damaged and removed exposing dermal tissue (Kim and Doores, 1993a; Thomas and McMeekin, 1980).

Poultry skin is divided into the epidermis and dermis which are separated by a basal membrane. The epidermis of chicken skin is composed mainly of two parts: the superficial corneous layer (stratum corneum) and the deeper germinative layer (stratum germinativum) (Thomas and McMeekin, 1980). The germinative layer is the deeper layer of skin that provides new cells. The corneous layer is the superficial layer of cornified germinative cells which are mainly made up of keratin and lipid material. The dermis, on the other hand, is mainly composed of connective tissue (Thomas and McMeekin, 1980).

Liquid film

Studies have demonstrated that skin microtopography and the presence of a liquid film are major contributing factors to contamination of poultry (Lillard, 1986). During the defeathering process, most of the epidermis is removed. However, small epidermal cell fragments attached to intact basal lamina can still remain on the exposed dermal skin (Thomas and McMeekin, 1980). Thomas and McMeekin (1980) described this new surface as “smoother and less hydrophobic than that of the stratum corneum, but covered with capillary-sized channels and crevices associated with dermal intercellular spaces and epidermal fragments”. This exposed dermal layer can be subsequently contaminated during later processing operations (Kim and Doores, 1993a).

One of the major ways that the skin is contaminated is by the liquid film that forms on the poultry skin after water treatments, such as immersions treatments and spraying during defeathering (Lillard, 1986). This water film forms on the outside of the

poultry carcasses as a thin layer directly above the skin. The film consists of water, protein, and fat components that are in folds and channels in the skin, with thinner layers present on the smooth dermal surface. This water film is of great importance because it serves as the major medium for bacterial penetration into the skin and also aids in cross-contamination onto processing equipment. Lillard (1986) showed that the liquid film forms on skin due to immersion in water during processing. After short immersion periods (15 and 25 seconds) in inoculated water, results showed that of the total percentage of *Salmonella* Typhimurium cells that were present on the skin (after immersion), 95% were of the cells retained as surface water film and the remaining 5% were attached firmly to the surface of skin. A trend was evident with increasing immersion times. Once the bacteria were entrapped by the water film, they could be absorbed into the skin, and then firmly attached to skin (Lillard, 1986). Since the scalding practices are not very long (1.3 to 1.83 minutes) (Allen et al., 2003b; Clouser et al., 1995), bacteria are still present in large numbers in the liquid film. This allows for easy cross-contamination to other equipment, such as picker fingers via the surface water film on the skin of the carcasses.

Although immersion during scalding is the main source of this water film formation, the water in the sprayers used during defeathering also aids in its formation. Water spraying allows easy removal of the feathers and helps in product decontamination (Mulder et al., 1978) during plucking. However, this spraying can impact cross-contamination because the spray water used in the machines can become contaminated with microorganisms derived from the carcasses (Mead et al., 1995). It has also been demonstrated that water draining from the picker to the machines contained high numbers

of bacteria with a total viable count of 6.5 log CFU/ml of water. Spray water was in constant contact with the rubber fingers and was dispersed by the rotating fingers as they removed the feathers.

Fatty liquid film

Since a large portion of the epidermis, mainly the stratum corneum, is removed during defeathering, we could conclude that it makes up most of the soil build-up on picker fingers. With this in mind, it is important to look into the main components of the skin in more detail. Although chicken skin is thought to be made of mostly protein, fat is a major component of the skin. The skin of chickens is a secretory organ producing a lipoid or sebaceous material that is similar to oil that is produced by an oil gland (Thomas and McMeekin, 1980). Lipids are present throughout the epidermis of chickens in the form of droplets in the cells and vacuoles spaces in the stratum corneum. When the water film is formed on the skin, the fat mixes with the film forming a “fatty liquid film”.

The fatty liquid film also serves as protection for microorganism engulfed in it or attached to the surface of the skin. Kim and Doors (1993a) viewed turkey skin specimens using an electron microscope during different defeathering practices (conventional, kosher, and steam spray). Attached *S.* Typhimurium cells were observed to be two to three times larger than the original size after the conventional defeathering process. These cells were also two to three times larger on the surface of turkey carcasses that were treated with other defeathering systems, such as kosher (lower temperatures) and steam spray, that did not produce the liquid film on the skin. The enlarged bacteria cells may have resulted from swelling due to water uptake as was evident in the swollen poultry tissue. However, the fact that the salmonellae cells did not shrink during

preparations of the electron skin micrographs was of concern. This protection from shrinkage was attributed to the fat film being present. The mechanism of this protection by the liquid film is not fully understood, however.

Cleaning and sanitizing picker fingers

Cleaners

The major functions of cleaning compounds are to lower the surface tension of water so that soils may be dislodged and loosened and to suspend soil particles for subsequent flushing away. Deciding what cleaner to use is not as simple as one might think. Some of the major considerations are the nature of the soil to be cleaned, water characteristics and the application method. Soil is material in the wrong place at the wrong time. Many soil types are water soluble soils which will dissolve in tap water and do not need a cleaning compound. Examples of this soil types include inorganic salts, sugars, starches and minerals. Soils such as zinc carbonates, calcium oxalates, metal oxides, and films on stainless steel are soluble in acidic solutions with pH below 7.0. An acid cleaning compound is most appropriate for inorganic deposits. Fatty acids, blood, proteins, and other organic deposits are made soluble by alkaline solutions. Unlike acidic compounds, alkaline cleaners are most effective on organic soils and are most often used in the food industry (Tables 2.1-2.2) (Marriot, 1999). Some commonly used cleaners administered in the poultry processing plants are alkaline cleaners, chlorine cleaners, and soaps.

1. Alkaline cleaners: In meat processing plants, alkaline cleaners are routinely used to remove fats and proteins from processing equipment. These cleaners are divided into subclasses from strong to mild cleaners. Strong alkaline cleaners, such as caustic sodas

(sodium and potassium hydroxides), are used to remove difficult organic loads that accumulate in smokehouse and commercial ovens (Katsuyama, 1980; Marriot, 1999). They are very dangerous to humans and are extremely corrosive to surface materials. Less concentrated alkaline cleaners are referred to as heavy-duty alkaline cleaner. These cleaners are frequently used with high pressure or mechanized systems and are very good for soil emulsion activity. Examples of heavy-duty cleaners are sodium hexametaphosphate and sodium carbonate. Mild cleaners, such as sodium bicarbonate and tetradodium pyrophosphate, frequently exist in solutions and are used for hand cleaning lightly soiled areas (Katsuyama, 1980; Marriot, 1999).

The chemistry behind the usage of alkaline cleaners is their high alkalinity (pH values ranging form 12-14) which allows for neutralization of acidic soils, saponification of fat and lipids, and dispersion of oils and lipids on various surfaces. Under alkaline conditions fat reacts with the alkali to form a soap compound. Saponification is the formation of a soap compound from a fatty acid molecule and an alkaline salt. The soap formed from this reaction is soluble and will act as a solubilizer and dispersant for the remaining soil. Dispersion is a process that allows the alkaline cleaner to penetrate oil film and wet the surface, thereby penetrating the oil film (Marriot, 1999). Alkaline agents can also act as emulsifiers or surfactants by breaking down the surface tension between molecules that repel each other such as oil and water. Currently, alkaline cleaners are most commonly used on metal surfaces and applied in temperature ranges of 38-93^{o}C. Higher temperatures increase the efficiency of these cleaners (Marriot, 1999; Teo, 1996).

2. *Chlorine cleaners*: Active chlorine containing cleaners have the ability to break chemical bonds, leading to the formation of smaller, more soluble molecules and increase cleaning efficiency. Active chlorine compounds, such as hypochlorite, attacks the large, complex carbohydrates and degrades them to smaller more soluble and readily removed derivatives. When protein molecules are allowed to react with hypochlorite, hydrogen atoms attached to nitrogen in amides are replaced by chlorine (Banwart, 1979). This replacement of nitrogen-bonded hydrogen improves solubility. This further explains why active chlorine degrades proteins to render them soluble and enhance their removal from soiled surfaces (Marriot,1999).

Chlorine cleaners are more reactive at lower pH (<8). For example, sodium hypochlorite (bleach) is enhanced at lower pH due to the hypochlorite ion being in the form of hypochlorous acid (Banwart, 1979). Hypochlorous acid is much faster at diffusing into the bacteria and organic residues than the hypochlorite ion, which increases the cleaning reaction rate. Commercial bleach usually has a pH of 8.5-10 and has a slower reaction rate, thus it is usually heated and used at high concentrations to help compensate for its deficiencies (Marriot, 1999).

3. *Soap*: A basic soap contributes to the removal of fats, oils, and greases via emulsion. Soap serves as the medium that breaks down and suspends these insoluble materials in water allowing flushing of these materials to be done easily (Katsuyama, 1980). The suspension process of insoluble matter via the interaction with soap is known as the emulsion process. Alkaline soaps, in particular, are manufactured by the reaction of an alkaline compound with a fatty acid and serve as a better remover of fats and oils than

other soaps. Some disadvantages to using soaps in the food industry are that they are generally inactivated by hard water (Marriot, 1999).

Factors that affect cleaning

Other factors that affect cleaning by cleaners are temperature, concentration, time, and mechanical force/applications. As the temperature and concentrations of cleaners increase, the efficiency and activity of the cleaners increase also. However, protein denaturation of soil can occur with extreme temperature increases (>55 ^{o}C) and cleaner usage that is above manufactures recommendations, resulting in the decrease of effectiveness of the cleaner. In addition, if the time that the cleaner is on the surface is increased, than the cleaning ability will also increase causing the surface to become cleaner. The method in which the cleaner is applied also affects the efficiency of the cleaner along with the exposure time. The application of cleaner is most useful when mechanical force is applied because it supplies energy that can assist in the dispersement of soil. The amount of mechanical energy in the form of agitation and high pressure spray will affect the penetration of the cleaning compound and physical separation of soil from the surface (Katsuyama, 1980; Marriot, 1999). All these factors, along with soil disposition, should be considered carefully to ensure high cleaning efficiency.

Disinfectants/Sanitizer

A sanitary environment is obtained by thoroughly removing soil deposits and subsequently applying a sanitizer to destroy residual microorganisms. If soil residues are present, they protect microorganisms from contact with chemical sanitizing agents. Soil deposits can reduce the effectiveness of a sanitizer through diluting the solutions and

reacting with the sanitizing compound (Doyle, 2001). Thus, adequate cleaning before sanitizing is of great significance in the inactivation of bacteria.

Sanitizing does not mean that all living bacteria cells are destroyed or removed. A sanitized surface does mean, however, that pathogenic or disease producing microorganisms, as well as a large percentage of non-pathogenic ones, are killed (Banwart, 1979). The major methods of sanitization of food substrates and surfaces are thermal sanitizing (steam and hot water), radiation sanitizing, and chemical sanitizing. Thermal and radiation practices are less practical for food production facilities than are chemical sanitizers (Banwart, 1979). Thus, chemical sanitation practices and commonly used sanitizers will be highlighted. The most popular types of sanitizers are the chlorine compounds, iodine or idophors, and quaternary ammonium compounds. However, quaternary ammonium compounds are mostly used for environment control such as walls, drains, and tiles (Marriot, 1999).

1. *Chlorine sanitizers:* Of the chemical sanitizers, chlorine cleaners are ideal because they not only aid in removal of soils and residue, but they can kill bacteria. Examples of chlorine compounds used in the food industry are gaseous chlorine and sodium hypochlorite (Banwart, 1979). The use of these compounds as sources of chlorine can be very hazardous to humans. Chlorine has an unpleasant odor, causes skin irritation, and it can also be corrosive to equipment used in processing. Yet, chlorine and chlorine compounds are still widely used as disinfectants during poultry processing because they are economical, quick, colorless, and the concentration of chlorine is easily determined (Table 2.3). Most importantly, they break down into many different chemicals such as hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) when combined with water.

HOCl and OCl^- are known as free chlorine compounds and are available to oxidize bacterial cells more quickly and effectively than other molecules. The hypochlorites are considered to be more effective against gram negative than against gram positive bacteria. Viruses are more resistant than bacteria to the action of chlorine (Banwart, 1979).

Despite the high bactericidal effect of hypochlorites, there are some disadvantages associated with the use of these and other chlorine compounds as antimicrobials in poultry plants (Table 2.3). Even at elevated levels of chlorine (from 40-100 ppm), significant numbers of pathogens remain on skin and processing surfaces after processing and cleaning. This may be attributed to the high pH levels that are commonly found in washing waters (8.0 or higher), the ability of microorganisms to hide in the intricate sites of the skin, and the inactivation of HOCl which will react with organic matter. Most hypochlorites (such as HOCl and OCl^-) are inactivated by organic matter (Lillard, 1980) and their effects are decreased with increasing pH (Banwart, 1979). Hypochlorous acid is almost completely hydrolyzed at pH 6 and below. With increasing pH the hypochlorous acid molecule dissociates into the less bactericidal hypochlorous ion (OCl^-) (Banwart, 1979; Kim, 2000).

2. Iodophors: The major iodine compounds used for sanitizing are iodophores, alcohol-iodine solutions, and aqueous iodine solutions. Iodophors are used as water treatments while the two other solutions are usually used to disinfect skin. Iodophors are the most popular form of iodine compounds used today and have better bactericidal effects under acidic ($pH \geq 3$) conditions (Katsuyama, 1980). The iodophore complex releases an intermediate triiodide ion (I_3), which, in the presence of acid, is rapidly converted to

hypoiodous acid (HIO) and diatonic iodine (I_2). Both the hypoiodous acid and diatonic iodine are active antimicrobial forms of iodophor sanitizers.

Some advantages of using iodophors are that these sanitizers are more stable in the presence of organic matter than chlorine compounds. Because iodine complexes are stable at very low pH, they can be used at very low concentrations (12.5-25 ppm). They are very effective against viruses, vegetative cells, and many spores. Against vegetative cells, the activity of 25 ppm of iodine at low pH is roughly equivalent to that of 200 ppm of chlorine at neutral pH (Katsuyama, 1980). Also, since chlorine is corrosive and can remove the carbon out of the surface of rubber, iodophores are commonly recommended sanitizers to be used on rubber surfaces.

Some disadvantages of using iodine are that it still can be inactivated in the presence of organic matter. These compounds also loose activity during storage and need to be generated at the time of use to ensure higher efficiency. Other disadvantages are that these compounds are vaporized at approximately 50^oC, they have poor efficiency at lower temperatures, they are usually more expensive than chlorine compounds, and are pH sensitive (Marriot, 1999).

3. Quaternary ammonium compounds: The quaternary ammonium compounds (commonly called quats) are prepared by condensation of a tertiary amine and an alkali salt (Collins, 1981; Finch, 1958). The quaternary compounds are frequently used on floors, walls and furnishings. They are good penetrating agents and thus have value for porous surfaces (Collins, 1981; Finch, 1958). The most common quaternary agents are the cationic detergents, which are poor detergents but excellent germicides.

Quaternary compounds act against microorganisms differently than do chlorine and iodine compounds. They form a bacteriostatic film after being applied to surfaces. Although the film is bacteriostatic, these compounds are selective in the destruction of various microorganisms and do not kill microorganisms but inhibit growth. Quaternary compounds, like chlorine and iodine sanitizers, are impaired by organic soils, however, they are more stable in the presence of organic matter than the other two types of sanitizers (Marriot, 1999).

Some advantages to the use of quaternary compounds are they are odorless, non-irritating, non-corrosive and their concentrations are easily measured. In addition, they are stable to temperature increase and are effective at higher pH levels. Some of their disadvantages are their limited effectiveness on gram negative bacteria, their inability to be combined with some synthetic type detergents, and the formation of a film on food contact surfaces (Collins, 1981; Finch, 1958; Marriot, 1999).

Factors that affect sanitizing

In addition to the factors that affect cleaning (concentration, time, temperature, soil deposition and cleanliness), bacterial attachment to surface is also important in the sanitizing ability of disinfecting compounds. When selecting a specific sanitizer, these factors need to be considered carefully. The role of pH of the sanitizing agent is of great significance because the concentration of bacteriacidal agents may be altered with changes in pH. For example, HOCl is present at higher levels at pH 4 to 5 in chlorine solutions however, pH values above or below 4-5 may result in the formation of less bacterialcidal chlorine species of OCl^- and Cl_2, respectively. In addition, increasing a sanitizer's concentration and temperature can greatly enhance a sanitizer's antimicrobial

activity (Katsuyama, 1980). One of the other factors that affect sanitizer efficiency is the amount of soil or bacterial attached to surfaces. Failure to clean surfaces properly results in inactivation of sanitizers due to the presence of organic matter. Bacterial attachment to specific surfaces can also increase resistance to sanitizers decreasing the efficiency (Frank and Koffi, 1990; Krysinski et al., 1992; Sinde and Carballo, 2000).

Currently used cleaning and sanitizing process

There are specific methods in which food processing equipment, depending on the type, are cleaned and sanitized. Each machine and food contact surface are usually cleaned and sanitized according to plant Good Manufacturing Practices (GMP's) and Sanitation Standard Operating Procedures (SSOP). Marriot (1999) described a method of cleaning and sanitizing rubber picker fingers as follows:

1. Picking up all large debris and transfer the matter to receptacles.
2. Covering electrical equipment with plastic sheeting.
3. Briefly pre-rinsing equipment with 50^{o}C to 55^{o}C water.
4. Appling a heavy-duty alkaline cleaner through a centralized or portable foam system on the shower cabinets. Shackles should going into the tank with the same cleaner.
5. After cleaning compound exposure for approximately 20 minutes, rinse with approximately 50^{o}C water.
6. Remove residual feathers and other debris by hand.
7. Apply 25 ppm iodophor as a sanitizer through a centralized sanitizing unit (Marriot, 1999).

This method gives a general idea of how to clean and sanitize feather picker machines, however, practices may differ from plant to plant. When observing the cleaning and sanitizing practices of a nearby poultry plant in Athens, GA, there were some distinct differences in methodologies. One of the main differences was the temperature of all cleaning and sanitizing agents, including water, was 65^{o}C. At this particular processing plant, a 50% concentration of an chlorinated-alkaline cleaner, Chill Kleen (Duchem, Atlanta, GA), was used to spray the machines during cleaning practices**.** The chlorinated-alkaline cleaner used was primarily made up of potassium hydroxide and sodium hypochlorite which is the active agent in bleach. For sanitation, a 10%- 12.5% sodium hypochlorite solution was sprayed onto the machines and then rinsed. One of the major problems with current practices used for cleaning and sanitizing feather picker machines at poultry plants is the use of high concentration of chlorine sanitizers. As discuss earlier, the use of chlorine based chemicals results in rubber deterioration which increase the ability for bacteria to harbor in cracks and crevices and proliferate on the surface. After cleaning and sanitizing, fingers are visually inspected by sanitation personal and are replaced if needed. Fingers are replaced when broken or when it appears that the ribbed or picking portion has worn to the point that feather removal deteriorates (Arnold, 2007). This poses a major problem because finger replacement is based solely on the judgment of the person making the inspection, many fingers that are worn and in need of replacement can be easily overlooked. Worn fingers can serve as frequent sources of cross-contamination on poultry carcasses.

Electrolyzed water

Principles of generation of EO water

Using acidic electrolyzed (EO) water in agriculture, livestock management, medical sterilization and food sanitation is a relatively new concept. This water has high antimicrobial qualities associated to its hypochlorous acid (HOCl) content and high oxidation-reduction potential (ORP), and it is safer to use than most other chlorine based sanitation practices, such as chlorine gas or hypochlorous acid. The safety of acidic EO water is a result of the use of sodium chloride as its sole source of chlorine.

During the generation of acidic EO water, a dilute concentration of sodium chloride is passed through positive and negatively charged electrodes separated by a membrane (Park et al., 2002). By subjecting the electrodes to direct voltage of about 8 - 10 V, sodium chloride is ionized into four types of ions (sodium ion, Na^+, chloride ion, Cl^-, Hydrogen ion, H^+, hydroxide ion, OH^-) (Fabrizio et al., 2002; Kim, 2000; Kim et al., 2000). These ions are present in the sodium chloride solution. When the pair of electrodes are introduced into the salt solution and a voltage is applied, negative ions, Cl^- and OH^-, are attracted to the anode and positive ions, Na^+ and H^+, are attracted to cathode (Kim, 2000). Two different types of electrolyzed water are produce at each node. At the anode, the negative ions are attracted, an electron (e^-) is released and radicals are produced. These radicals are combined to form hypochlorous acid (HOCl). In addition, dilute hydrochlorous acid is also generated by the reaction of gas (Cl_2) with water. The dilute HCl formed during the process lowers the pH of the solution forming an acidic solution with high levels of HOCl, referred to acidic EO water. From the cathode side of the electrode, alkaline EO water is produced by attracting the cations that react with

water. The result is the production of hydrogen (H_2) gas, and sodium hydroxide (NaOH) (Fabrizio et al., 2002; Kim, 2000), which is the compound that increases the alkalinity of this water (Figure 2.2.).

Properties of acidic EO water

Acidic water produced by the EO generator has high antimicrobial activity and is commonly used for sanitation practices. The primary active chlorine molecule in the acidic EO water is hypochlorous acid (HOCl), which is a weak acid that has a high oxidizing capacity. Hypochlorous acid kills microbial cells by inhibiting glucose oxidation by oxidizing sulfhydryl groups of certain enzymes important in carbohydrate metabolism (Banwart, 1979; Kim, 2000). This high oxidizing capacity of hypochlorous acid is equal to two equivalents of chlorine or one mole of Cl_2 and also 80 times more effective as a sanitizing agent then an equivalent concentration of hypochlorite ion (Anonymous, 1997), which makes it the most effective form of chlorine compounds.

Using acidic EO water as a sanitizer

Some of the other advantageous attributes of acidic EO water are its low pH and high oxidation-reduction potential (ORP). As stated earlier, HOCl is more active at pH levels below 6. In addition, the acidic environment assists in the inactivation of bacteria since most bacteria can not survive below a pH of 4. The ORP is defined as the ability to oxidize or reduce substances and is expressed in mV. At high ORP, the substance oxidizes or removes electrons from the counterpart while the substance itself is reduced. Hence, the oxidation of bacterial molecules at elevated ORP levels greatly enhances the bactericidal attributes of acidic EO water.

When electrolyzed oxidizing water is generated by the ROX-20TA model (Hoshizaki Electric Inc., Toyoake, Aichi, Japan) machine at an amperage of 14, the acidic EO water has a pH of approximately 2.50, an oxidation-reduction potential of approximately 1150 mV, and a total chlorine content of approximately 45-55 ppm. This novel sanitizer is highly effective in the reduction and inactivation of bacteria such as *E. coli* O157:H7 (Len, 2001; Sharma and Demirci, 2003; Venkitanarayanan et al., 1999); *L. Monocytogenes, Salmonella* Enteritidis (Park et al., 2005, Venkitanarayanan et al., 1999a), *Bacillus cereus* (Len, 2001), and *Campylobacter jejuni* (Park et al., 2002; Park et al., 2005) on various food substrates, such as poultry, and a variety of surface materials, such as plastic cutting boards (Venkitanarayanan et al., 1999b) and stainless steel (Park, 2002).

Alkaline EO water

Although many uses for acidic EO water have emerged, efficient uses of alkaline EO water have not been thoroughly identified. As discussed earlier, acidic EO water has proven to be an effective sanitizer. Alkaline EO water has some antimicrobial activity, but it is not as effective at sanitizing and inactivating bacteria as its acidic counterpart. However its high pH and chemical makeup makes it a promising degreaser and soil remover. Recently, it has been shown to significantly reduce the attachment of fecal matter to chicken skin (Kim et al., 2005).

Alkaline EO water is a caustic agent. Sodium hydroxide (caustic soda) and potassium hydroxide (caustic potash) can be produced by using dilute sodium chloride or potassium chloride solutions, respectively. Both caustic agents have many uses and are most commonly used as the alkaline builders for alkaline cleaners. At 14 A and 10 V,

alkaline EO water has an elevated pH (11.6) and ORP of -835 mV (Fabrizio et al., 2002). Because of the high pH, alkaline EO water may also act as an emulsifier or a surfactant by breaking down the surface tension between molecules that repel each other such as oil and water.

Utilizing alkaline EO water as a degreaser

In poultry processing plants, chlorine water is used as the primary sanitizing agent, inactivating microorganisms on carcasses and equipment. However, the accumulation of organic matter reduces the effectiveness of the chlorine because insoluble hydrophobic compounds, such as fat, do not rinse away and are left to bind with chlorine molecules and shield microbes from inactivation and supply nutrients for bacterial survival. To help eliminate this problem, alkaline EO water can be utilized in food processing plants as a degreaser and/or organic soil remover on metal, rubber or plastic surfaces. This application will be useful in processing plants were acidic EO water could be used as the primary sanitizing agent on carcasses during spraying (i.e. during defeathering) and immersion applications. With large amounts of acidic EO water generated on site, there will be large amounts of alkaline EO water that will be generated also. Excess alkaline EO water can be stored and used between processing shifts as a soil remover and/or degreasing agent that will allow insoluble organic matter to be removed and easily rinsed away.

Acidic and alkaline EO water synergy

When acidic EO water was used alone on a variety of surfaces (glass, stainless steal, ceramic tile), it was able to inactivate pure cultures of *Enterobacter aerogenes* and *Staphylococcus aureus* more so with agitation than without. In this study, there was little

to no organic matter present on surfaces, leaving bacteria to be completely exposed to the sanitizer. In a recent study done at the University of Pennsylvania, acidic EO water and alkaline EO water were used in combination to remove soil and inactivate bacteria that accumulate in milking pipelines. Surfaces commonly used in milking pipelines, (rubber rings, stainless steel, plastic pipeline e.q.) were soiled with milk and bacteria inoculum. Results showed that the combination of both EO waters effectively removed soil and inactivated bacteria (Walker et al., 2005). The same synergistic effect was observed when both EO waters were applied to *Listeria* biofilms attached to stainless steel coupons (Ayebah et al., 2005). Ayebah et al. found that when *L. monocytogene*s biofilms were treated with acidic EO water, alone, bacterial counts were reduced by more than 5 logs on the stainless steel surface. This effect was further enhanced by an additional 1.2 logs reduction when biofilms were treated with alkaline EO water first, followed by acidic EO water (Ayebah et al., 2006).

Acidic EO water and its alkaline counterpart can be efficient when used in combination. Yet, when a mixture of acidic EO and alkaline EO water applications was used on poultry carcasses, there was little synergistic effect observed. The reasoning behind this is not fully understood. It may be due to neutralization (pH changes) of the solution and salt formation that can occur when these two compounds are mixed together with each other in combination applications. As a result, determining independent uses for both the acidic and alkaline waters are advantageous in food processing applications.

Organism used in this study

Salmonella are gram negative, facultatively anaerobic, nonsporeforming bacterial rods that are present in nature, with the intestines of humans and animals being their

primary reservoirs. There are approximately 2300 serovars in this genus and many of them are pathogenic to humans. These bacteria cause diseases such as typhoid fever and gastroenteritis (salmonellosis). Salmonellosis can be caused by a variety of strains of salmonellae and usually is contracted by fecal-oral route, animal contact, or food transmission. Common symptoms associated with this disorder are abdominal pain, non-bloody diarrhea, vomiting, nausea, and fever.

Salmonellosis constitutes a major public health burden and represents a significant cost to society in many countries. Very few countries report data on economic cost of the disease. According to the World Health Organization (WHO), in the United States of America, an estimated 1.4 million non-typhoidal *Salmonella* infections occur, resulting in 168,000 visits to physicians, 15,000 hospitalizations and 580 deaths annually (World Health Organization, 2005). Estimated cost per case of human salmonellosis range from approximately $40 to $4.6 million respectively for uncomplicated cases to cases ending with hospitalization and death. The total cost associated with *Salmonella* is estimated at $3 billion annually in the United States (United States Department of Agriculture, 2007). *Salmonella* Enteriditis and *Salmonella* Typhimurium are two of the most important serotypes for salmonellosis transmitted from animals to humans (World Health Organization, 2005). Jay (1998) reported that *S*. Typhimurium is invariably the most commonly found foodbrone serovar in the world. However, *S*. Enteriditis numbers have increase significantly over the pass 5 to 10 years due to its high association to outbreaks via poultry eggs.

Salmonellosis can be transmitted through a large variety of food vectors such as milk, dairy products, sauces, cake mixes, raw meats, etc. However, poultry and eggs

remain a predominant reservoir for *Salmonella* spp. and in many countries poultry and eggs over shadow the importance of other meats, such as pork, beef and, mutton, as potential vehicles of infection. A very large number of broiler carcasses have been found to be contaminated with *Salmonella* (Bell, 2001).

Due to this organism's ability to cause foodborne illness and its high correlation with poultry, it is a pathogen of concern among poultry producers. The elimination and reduction of this organism is a difficult task due to its persistence throughout processing steps. Mead (1987) has documented that there is a positive relationship between the initial amount of *Salmonella* positive birds before processing and contamination of carcasses at the end of the processing line. More importantly, *Salmonella* Typhimurium numbers increase after defeathering steps and the defeathering process has been proven to be a major contributing factor to cross-contamination of poultry carcasses by this organism.

Overall objective

The overall objective of this research was to evaluate efficiency of electrolyzed oxidizing water for cleaning and sanitizing of rubber picker fingers soiled with chicken fat and *S.* Typhimurium attached cells.

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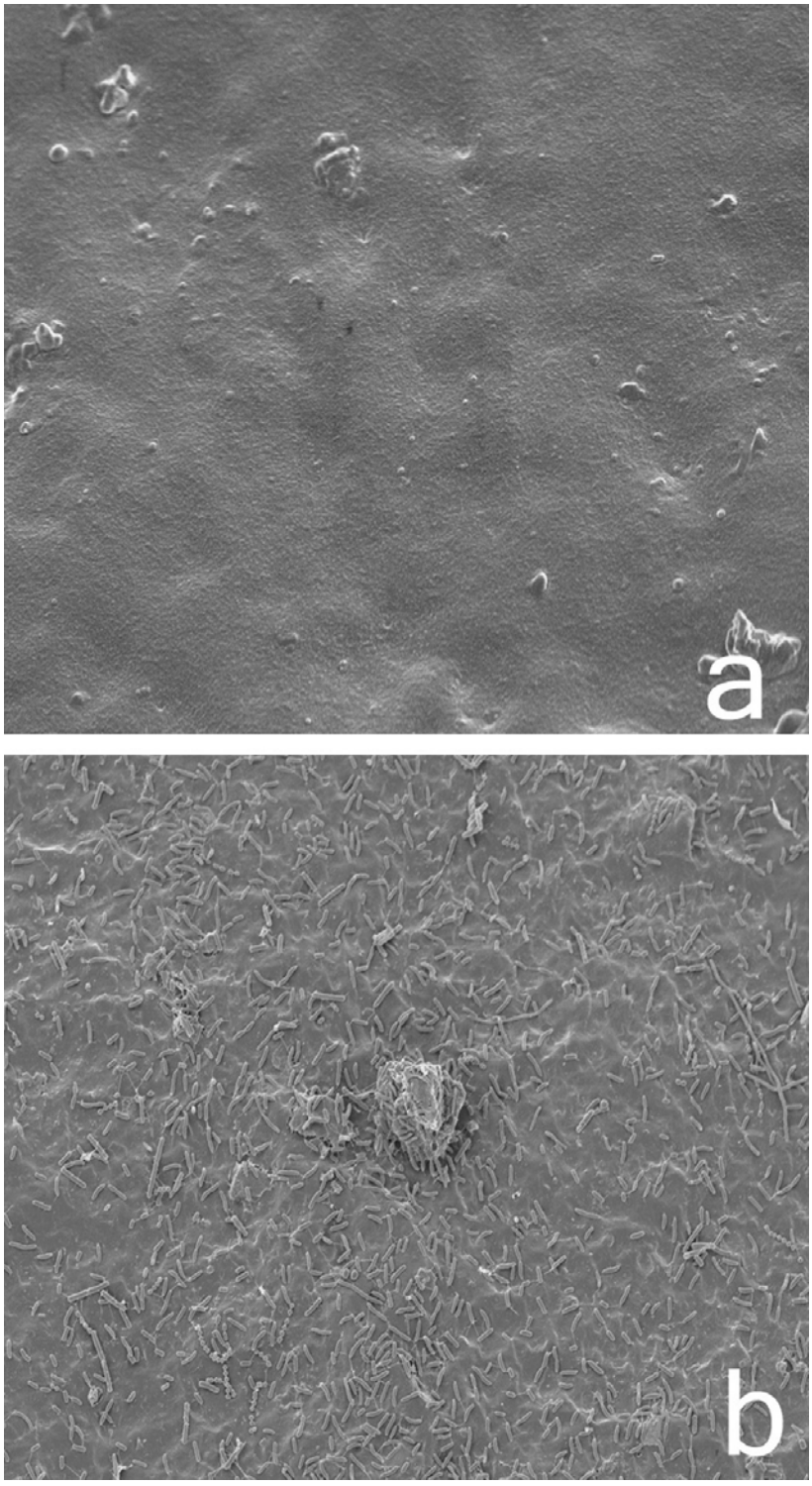

FIGURE 2.1 Scanning electron micrograph of the surface of a rubber picker finger after use in a commercial defeathering machine from plant 1 (a) and from plant 3 (b), magnification = 1,000· (Arnold, 2007)

TABLE 2.1 Solubility characteristics of various soils (Marriott 1999)

Type of salt	*Solubility Characteristics*	*Removal Ease*	*Changes Induced by Heating Surface*
Monovalent Salts	Water-soluble, acid-soluble	Easy to difficult	Interaction with other constituents with removal difficulty
Sugar	Water-soluble	Easy	Carmelization and removal difficulty
Fat	Water-insoluble, alkali-soluble	Difficult	Polymerization and removal difficulty
Protein	Water-insoluble, slightly acid-soluble, alkali-soluble	Very difficult	Denaturation and extreme difficulty in removal

TABLE 2.2 Classification of soil deposits (Marriott 1999)

Type of Soil	Soil Subclass	Deposit Examples	Required Cleaning Compound
Inorganic soil	Hard-water deposits	Calcium and magnesium carbonates	Acid-type cleaner
	Metallic deposits	Common rust, other oxides	
	Alkaline deposits	Films left by improper rinsing after use of alkaline cleaner	
Organic soil	Food deposits	Food residues	Solvent-type cleaner
	Petroleum deposits	Lubrication oils, grease, other lubrication products	Alkaline-type cleaner
	Non-petroleum deposits	Animal fats and vegetable oils	

TABLE 2.3: Advantages and disadvantages of hypochlorites as sanitizers (Banwart, 1979)

Advantages	Disadvantages
Relatively inexpensive	Unstable during storage
Quick acting	Inactivated by organic matter
Not effected by hard water salts	Corrosive if misuses
Harmless residue does not form a film	Irritates skin
Effective at high dilution	Odor may be undesirable
Active against a wide variety of microorganisms including spores and phages	Precipitates in iron waters
Relatively non-toxic at use dilutions	Effectiveness decreases with increasing pH of solution
Non-staining	May remove carbon from rubber parts of equipment
Colorless	
Easy to prepare and apply	
Concentration easily determined	
Can be used for water treatment	

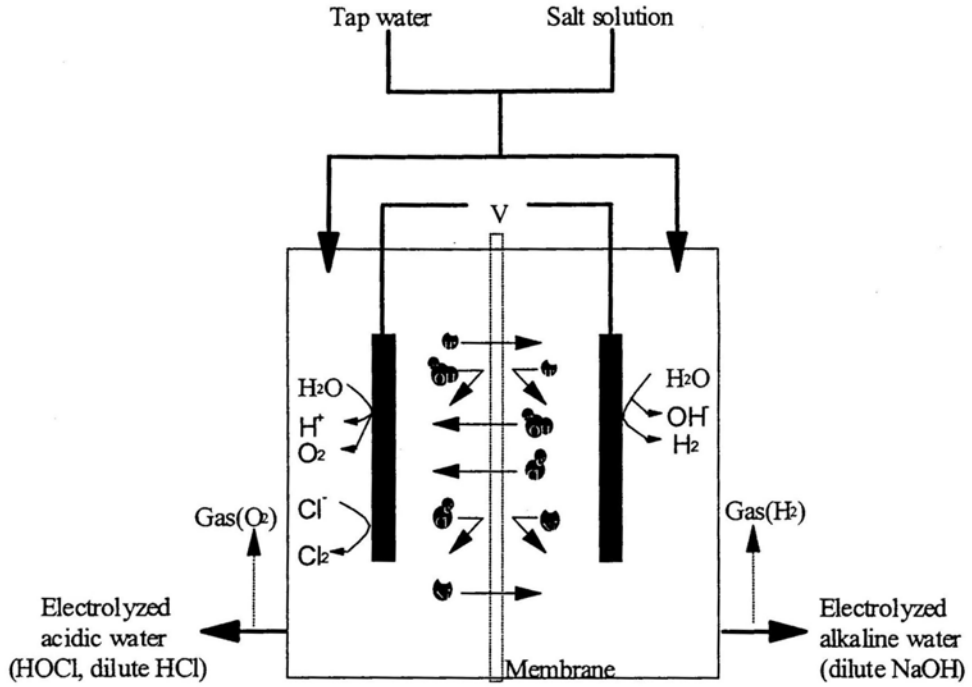

FIGURE 2.2: Schematic of Electrolyzed oxidizing water generator (Kim, 2000a)

CHAPTER 3

EVALUATION OF ALKALINE ELECTROLYZED WATER AND OTHER CLEANERS ON THE REMOVAL OF CHICKEN FAT FROM RUBBER PICKER FINGERS

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ABSTRACT

This study investigates the use of alkaline electrolyzed (EO) water on the removal of chicken fat soiled onto rubber picker fingers. Soiled rubber picker fingers were treated with either alkaline EO water (pH of 11.6, oxidation-reduction potential (ORP) of -813 mV) or deionized water by vortexing (3 sec and 10 sec) or immersion (10 sec and 60 sec) applications. Vortexing for 10 sec with 10 ml of alkaline EO water was the most effective treatment and removed 62.58% of fat from the fingers. In addition, alkaline EO water was compared to: 10% sodium hypochlorite solution, 50% chlorinated-alkaline cleaner solution, acidic EO water (45 ppm chlorine, pH of 2.6, ORP of 1157 mV), dieonized water (single and double applications) and a combination of alkaline EO water followed by an application of acidic EO water, for fat removal. Alkaline EO water was the most effective treatment for fat removal from rubber picker fingers.

INTRODUCTION

Defeathering machines contain many sites and sources of bacterial contamination, including rotating disk, picker fingers, plastic curtains, aqueous aerosols, and water sprays (Allen et al., 2003; Dodd et al., 1988; Lindsay et al., 1996). When examining machines, non-metallic surfaces such as rubber fingers and plastic curtains (at the entrance and exit of machines) are contaminated by large populations of bacteria (Allen et al., 2003; Lindsay et al., 1996). During feather picking, populations of pathogens such as *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Staphylococcus aureus* increased 1-2 logs CFU/cm^2 after scalding (Berrang and Dickens, 2000; Kim and Doores, 1993; Purdy et al., 1988). This increase in pathogens during defeathering has made this processing step a major concern for the poultry industry. Although there are many modes of cross contamination during poultry processing, picker fingers have been identified as an ideal vector for contamination since they are located in warm, humid environments, and they are difficult to clean and disinfect (Purdy et al., 1988).

During the defeathering process, feathers are removed along with a large portion of the outer layer of the skin, called the stratum corneum. The stratum corneum is made up of cornea cells and fat vacuoles (Thomas and McMeekin, 1980). While feathers and protein from skin significantly contribute to cross-contamination of rubber finger surfaces, the fat component of the skin is also a very important factor. One of the main reasons for this is that feathers and protein components of the skin may be easily removed during rinsing. Fat, on the other hand, is not water soluble and may leave a fatty film (Kim and Doores, 1993) that can be difficult to remove without using a detergent or surfactant. Fat may also provide protection for bacteria that are attached to chicken skin

and processing equipment by shielding them from the sanitizers and cleaners (Kim and Doores, 1993). Thus, it is very important that effective methods of removing the fatty film from food contact surfaces, such as feather picker fingers, be utilized.

The major functions of cleaning compounds are to lower the surface tension of water so that soils may be dislodged and loosened and to suspend soil particles for subsequent flushing away during rinsing. Deciding which cleaner to use is not as simple as one might think. Some of the major considerations are the nature of the soil to be cleaned, water characteristics, and the application method. Many cleaning agents can be used during cleaning operations such as water, soap, and alkali detergents. An alkali is the principle detergent ingredient of most cleaner formulae. When it comes to organic soil removal, alkaline cleaners are effective because alkalis can combine with fats to form soaps and with protein to form soluble compounds that are more easily rinsed away (Katsuyama, 1980). The cleaning mechanism of alkali cleaners are behind their high pH (>11), which allows for neutralization of acidic soils, saponification of fats and lipids, and dispersion of oils and lipids on various surfaces (Marriot, 1999). The combination of high pH and elevated temperatures further increases the efficiency of these cleaners to remove soil and results in better sanitation of contaminated surfaces, such as animal skin (Teo, 1996). In addition, solutions such as caustic sodas (sodium hydroxide and potassium hydroxide), trisodium phosphate, and other cleaners with pH above 11, have been successful in the destruction of gram negative microorganisms when heated (Dickens, 1994; Humphrey, 1981; Teo, 1996). Coppen (1993) demonstrated that these solutions were effective because of the removal of the fatty layer from the animal skin due to their intrinsic high pH. Trisodium phosphate (TSP), in particular, has been noted

to prevent cross contamination by removing a thin layer of fat that forms on carcasses during defeathering (Kim et al., 2005; Vareltzis, 1997). Although TSP and other alkaline cleaners are effective cleaners and disinfectants, there are some disadvantages accompanied with their use. For example, TSP has been known to increase waste treatment cost by producing large amounts of phosphates that are difficult and expensive to dispose of (Fabrizio et al., 2002). In light of these findings, the investigation of current cleaning efficiency on processing surfaces, such as rubber picker fingers, is imperative. Furthermore, the exploration of other alkaline cleaners, such as alkaline electrolyzed (EO) water, could be beneficial to the improvement of cleaning and sanitizing practices.

Acidic and alkaline EO water

Acidic electrolyzed (EO) water is generated by pumping a dilute salt (sodium chloride) solution through an electrolytic cell with a membrane separating an anode and cathode. Hypochlorous acid is produced on the anode side of the generator. Hypochlorous acid contributes to the low pH and high oxidation-reduction potential (ORP) of acidic EO water which allows acidic EO water to function as a highly effective sanitizer. Acidic EO water has exhibited bactericidal affects against many pathogens such as *Listeria monocytogenes*, *E. coli, Campylobacter* and *Salmonella* on various food substrates and food contact surfaces (Park et al., 2001; Venkitanarayanan et al., 1999). An alkaline solution of sodium hydroxide is produced on the cathode side of the EO water generator (Len, 2001). Alkaline EO water has a very high pH (11-12) and low ORP. Although many usages of acidic EO water have emerged, efficient usage of the alkaline EO water has not been thoroughly investigated.

Alkaline EO water's low ORP level limits its role as a sanitizer; however, its high pH makes it a promising degreaser and soil remover. Alkaline EO water contains a caustic agent, sodium hydroxide that is commonly used as the alkaline builders for alkaline cleaners. The use of alkaline EO water as a degreaser or soil remover will be useful in poultry processing plants where acidic EO water could be used as the primary sanitizing agent on carcasses and during defeathering spraying and immersion chilling applications. With large amounts of acidic EO water generated on site, there will be a large amount of alkaline EO water available. Excess alkaline EO water can be stored and used as a soil remover and/or degreasing agent that will allow insoluble organic matter to be removed and easily rinsed away during cleaning shifts. Kim and Hung (2005) demonstrated that alkaline EO water was equally effective as 10% TSP in preventing the attachment of feces and in the removal of feces from chicken carcasses. However, its ability to remove fat attached to carcasses and coated on food contact surfaces, such as rubber picker fingers, has not been fully explored.

The objective of this experiment was to determine the efficiency of alkaline EO water and other commonly used cleaners on the removal of chicken fat from the rubber surface of picker fingers. The effect of finger hardness levels and cleaning applications were also determined.

MATERIALS AND METHODS

The effect of rubber hardness

Finger preparation

The level of hardness that is exhibited by a rubber sample results from the process of vulcanization. Vulcanization is the processes of heating and adding sulfur bonds to the

rubber matrix to ensure rubber durability and hardness (Kauffman, 1990). This study, compares two different finger hardness levels that are common in use. New fingers (type 2075) of different hardness levels (D and E) were ordered from William Goodyear Company (Durham, NC). Fingers were cut into approximately 4 cm (12 rings) pieces by use of a Model 14 band saw (Rockwell International Inc., Pittsburg, PA) from the top portion of the finger (Figure 3.1). Pieces were washed twice in an automatic dishwasher with a detergent (Alcojet, Alconex, Inc., White Plains, NY) dried and stored at 22 $\pm$ 2^{o}C before use. On the day of the experiment, fingers were immersed in ethanol for 5 min to remove fat or oil residue on finger pieces. Pieces were allowed to dry under air flow hood until use.

Soil preparation

Chilled rendered chicken fat used in a previous study (Chmielewski and Frank, 2006) was placed in a glass beaker and allowed to liquefy on a hot plate (Model PC-520, Fisher Scientific Co., Pittsburgh, PA) for 10 min at 65^{o}C with agitation. The liquid fat was then allowed to cool to room temperature (22$\pm$ 2^{o}C). Using an analytical weighing scale (Model A-160, Denver Instrument Company, Denver, CO), the initial weight of each rubber piece was taken (Huff et al., 1984; Shupe et al., 1982). Approximately 1$\pm$0.2 g of fat was coated onto each finger by placing the fingers in the fat and rolling it from one side to the other. Coated fingers were held in an incubator at 42^{o}C for 30 min and then air dried under flow hood for 1 h to allow for fat attachment.

Treatment solutions

Alkaline EO water was generated using a ROX-20TA machine (Hoshizaki Electric Inc, Toyoake, Aichi, Japan) at settings of 18 A (at this setting, the acidic EO

water's chlorine concentration is approximately 45-50 ppm, pH 2.7 and ORP 1157 mV). Alkaline EO water had a pH of 11.6 and ORP of -813 mV at the time of generation. A 5% soap (Liquid-nox, White Plains, NY) solution (pH 8.2 and ORP 47.1 mV) was prepared by mixing the appropriate amounts of soap with deionized (DI) water. DI water (pH 6.8 and ORP 202.2 mV) was also used as a control. The ORP and pH levels of all solutions were measured using a dual scale pH meter (Accumet model 15, Fisher Scientific Co.). Treatment solutions were heated in closed glass containers in a water bath (Model WB1120-A, Lindberg/Blue M, Ashville, NC) to 65^{o}C and held until use. One liter of alkaline EO water was heated in glass container until visible white clusters of NaOH were formed in solution (for approximately 1.5 hours). Alkaline EO water was then mixed by vigorously shaking the bottle by hand to ensure all clusters of NaOH were dissolved. Ten ml of each heated treatment solution was poured into 50 ml centrifuge tubes at time of application.

Treatment of soiled fingers

Fat coated fingers were weighed before treatment to determine how much of the fat was actually attached onto finger. All treatment tubes were held at 65^{o}C in a water bath and temperatures were determined by a standard (mercury) thermometer before use. Fingers were then immersed into 10 ml of the respective treatment solution and vortexed (Model 6-560, Scientific Industries, Bohemic, NY) at low settings for 3 sec to simulate finger washing action at the poultry plant. During vortexing, treatment tubes were tilted slightly to allow for swirling of the liquid cleaner around finger pieces (Figure 3.2: B and C). After treatment, the treatment solutions were immediately decanted. Each finger piece was then removed and set horizontally in the cap of the centrifuge tube for 30

seconds to allow excess treatment water to drain. Fingers were weighed after 24 hours of drying at room temperature (22 $\pm$ 2^{o}C). Preliminary work was conducted by following the weight change of washed fingers four days at room temperature. Results indicated that there were no further weight changes after 24 hours (data not shown), hence, 24 hour room temperature drying was used for all treatment studies. Two fingers were treated with each cleaning solution and the experiment was repeated 3 times. The percent fat removed after each treatment was calculated as:

$$\textbf{Percent Fat Removed} = \left[\frac{W_s - W_t}{W_s - W_i} \right] \times 100$$

W_s= weight of soiled finger before treatment

W_t = weight of soiled finger after treatment

W_i = weight of unsoiled finger

The effect of rubber finger soiling time

Soiling of fingers

Rubber fingers were prepared as previously described. To simulate two (8 hours) shifts of chicken processing (approximately 350,000 birds) before cleaning exercises, the soiled pieces were allowed to set overnight (approximately 16-18 hours) in a desiccator at room temperature (22 $\pm$ 2 ^{o}C) to allow for fat attachment.

Treatment of fingers

Two different amounts (10 ml and 25 ml) of heated alkaline EO water (pH of 11.43 and ORP of -852 mV) and DI water (pH 6.74 and ORP of 198 mV), as described above, were poured into 50 ml centrifuge tubes. In this study, vortexing at low setting

(simulating washing treatment action at poultry processing plant) for 3 and 10 sec were compared with immersion treatments. For immersion, soiled fingers were immediately placed in treatment solution for either 10 sec or 60 sec. Treatment tubes were held in a water bath at 65^{o}C during the immersion treatment. Two fingers were used for by each cleaning solution and the experiment was replicated four times.

The effect of finger usage and cleaning solutions

Rubber fingers were cut, washed and soiled as described before. New fingers that were used for preliminary studies (denoted as re-used) were compared to new fingers to determine the effect of previous soiling, washing applications, and rubber deterioration on cleanability. Eighteen hour fat attachment time was also used for this study.

Treatment solutions

Seven treatment solutions were compared in this study: acidic EO (45-50 ppm chlorine) water, alkaline EO water, sequential combination of alkaline and acidic EO waters, single and double applications of DI water, a chlorinated-alkaline cleaner (50% solution) (Chill Kleen, Duchem, Atlanta, GA) and a 12.5% sodium hypochlorite (Clorox, Oakland, CA) solution. Poultry plants commonly use concentrations of cleaners and sanitizers in excess of concentrations recommended by manufactures. High concentrations of the chlorinated-alkaline and sodium hypochlorite cleaners were used in this study to evaluate the actual solution concentrations that are used during cleaning in a local poultry plants. Acidic and alkaline EO water were generated at 18 A. Solutions of 12.5% sodium hypochlorite and 50% chlorinated-alkaline cleaner were prepared by diluting with DI water. DI water was used as a control for this study. All treatments, with exception of acidic EO, were heated as previously described. In preliminary

experiments, acidic EO water showed diminished fat removal capacity due to chlorine loss when heated (data not shown) and was therefore used at room temperature (22±2°C) in this study. Properties (ORP and pH) of each solution were also measured. The total chlorine of acidic EO water was determined with a chlorine test kit (Hach, Co., Ames, Iowa). All treatment solutions were thoroughly mixed by vigorously hand shaking the bottles before pouring into tubes.

Treatment of soiled finger

Fat coated fingers were treated with 10 ml of each solution with 10 sec of vortexing to simulate washing treatments at poultry plant. The percent fat removed after treatment was calculated. Two fingers were used for each cleaning solution and this experiment was repeated three times.

Data analysis

Data for all experiments were analyzed using the general liner model (GLM) procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.). Comparisons of means were performed using Duncan multiple range test. All statistical analysis was done at $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Chicken fat

The chicken fat (Chmielewski and Frank, 2006) that was used in this study was different than common rendered chicken fat in temperature stability, color and thickness. This fat remained a white solid substance at room temperature (22± 2°C), while rendered chicken fat is usually a yellow free flowing liquid. This fat also exhibited an unique

thickness that allowed it to more readily attach to the rubber surface than other forms of rendered chicken fat.

Comparison of finger hardness

The mean values of percent fat removed from both finger types D (soft) and E (hard) after 90 min soiling time is presented in Table 3.1. There was no significant difference in the overall removal of fat from finger surfaces due to hardness level indicated in this study (data not shown). When comparing the effect of different treatment solutions on fingers (average of finger type), DI water was significantly less effective on fat removal than the other treatments (Table 3.1). Alkaline EO water and soap water exhibited similar effectiveness.

When comparing fat removal on hardness levels, water was significantly less effective on the soft finger (type D) than the hard finger (type E). Although there was a slight increase in the removal of fat for all cleaners when used on harder fingers (type E) than soft fingers (type D), this difference was not significant except for DI water. This slight increase in cleanablity may be due to the increase in the amount of sulfur bonds that are associated with harder rubber by making it less penetratable by fat and hence easier to clean (Table 3.1). Overall, both alkaline EO water and soap water were equally effective at fat removal (Table 3.1) for both finger hardness levels.

Washing method comparison

In this study, there was a significant difference between the overall means of vortex and immersion for fat removal. However, there was no significant difference between immersion and vortexing applications for DI water treatment (Table 3.2) and vortexing was more effective application using alkaline EO water than DI water. When a

centrifugal force is applied to a solution, it will move the solution along a solid boundary and incur a shear stress on that boundary. Vortexing applies shear force on the finger which in turn causes the treatment to swirl around the finger, such as would happen with a spray washing treatment. This shear force can be beneficial and aid in the cleaning process. Bridget (1998) illustrated that immersion treatments alone were ineffective at beef soil removal and inactivation of *Escherichia coli* O157:H7 attached to meat grinders. He concluded that a combination of both mechanical and chemical cleaning is important for successful removal of soil and bacteria.

Both volume and time, when evaluated independently, did not significantly affect overall fat removal, yet, the volume-time combinations were significant. There was a slight decrease in cleanability (approximately 11%) of alkaline EO water when the volume increased to 25 ml for vortexing applications at both 3 and 10 sec time intervals. This may be a result of the larger volume (25 ml) of solution not having adequate mechanical force during vortexing to aid in cleaning. Figure 3.2 illustrates how the 25 ml vortex treatment using a 50 ml centrifuge tube may have had similar results as immersion. This affect of higher volumes may have resulted in lower fat removed when compared to the lower volume of 10 ml due to less shear force during vortexing.

Results indicated that water shows the same effectiveness of fat removal regardless of volume-time combinations (Table 3.2). The only water application that was significantly different was the 10 ml solution for 10 sec vortexing. The 10 ml alkaline EO water at 10 sec vortexing was also significantly better (62.58% fat removal) than all other alkaline EO water treatments making it the most effective application. This may be due to the long vortexing time and the appropriate amount of solution which allowed for

more movement of solution around finger due to shear force. Overall, alkaline EO water was the most effective treatment with an average fat removal of 41.92% compared to 22.09% for water (data not shown). The percents fat removed by DI water, for this study, (16.77% to 33.63%, Table 3.2) were significantly lower than in Table 3.1 (62.32% to 73.31%). This may be due to the longer attachment time (18 hr) before cleaning (Table 3.2) than used for studies reported in Table 3.1 (1.5 hours).

Cleaner comparison

Ten ml treatment solution with the 10 sec vortex was used in the cleaner comparison study. Alkaline EO water had a pH of 11.54 and a low ORP of -872 mV while, the chlorinated-alkaline cleaner had a higher pH of 13.70 and OPR of 412 mV when produce. The higher pH of the chlorinated-alkaline cleaner may have enhanced its ability to effectively remove fat from fingers when compared to all other solutions. However, alkaline EO water was more effective on fat removal than the chlorinated-alkaline cleaner. The reasoning for alkaline EO water's greater ability to remove fat is not fully understood. DI water had a neutral pH of 6.98 and an ORP 225.4 mV. The properties of all treatment solution are presented in Table 3.3.

Alkaline EO water was significantly more effective than all other treatments except for the alkaline and acidic EO water combination treatment for both new fingers and re-used fingers and sodium hypochlorite and chlorinated-alkaline cleaner treatments for re-used fingers. This implies that alkaline EO water may be equally or even more effective on fat removal than current cleaners used in poultry processing facilities. Double DI water treatments only increased fat removal approximately 8.0 and 16.0 percent than DI water (single treatment) for new and re-reused fingers, respectively

(Table 3.3). There was not a significant difference in the fat removal between alkaline EO water alone and combination of alkaline EO and acidic waters supporting preliminary data (not shown) that acidic EO water treatment has little affect on fat removal. The low activity of acidic EO water on fat removal may be a result of its low pH (2.5).

The new and re-used fingers did not have a significant difference on cleaning ability by different treatment solutions (Table 3.3). Results indicated that previous soiling, washing applications, and rubber deterioration have little affect on the removal of fat by cleaners.

CONCLUSIONS

Finger hardness levels had no significant affect on the removal of fat by alkaline EO water and should not be an area of concern when cleaning. Although washing time and solution volume had no significant affect on the fat removal when analyzed individually, the combinations of the 10 sec time interval, 10 ml of solution, and the shear force applied by vortexing (spray) was highly effective for removal of chicken fat. Thus, 10 sec/10 ml spray application may be the most effective application for administering alkaline EO water as a degreaser of rubber picker fingers.

Alkaline EO water proved to be an effective cleaner for the removal of chicken fat from the rubber surface of picker fingers. It had the highest percent fat removal of all cleaners and treatment solutions studied. Alkaline EO water may be more effective than sodium hypochlorite and chlorinated-alkaline cleaners even at high concentrations of 12.5% and 50%, respectively. Due to the unique nature of the chicken fat utilized in this study, further investigation is needed to evaluate the role of alkaline EO water as a cleaner for other soil types.

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TABLE 3.1 Average percent removal of chicken fat from rubber fingers of different hardness levels

	Average Percent Fat Removal (%)		
Treatment	**Finger type D**	**Finger type E**	**Total average for finger types (D & E)**
Deionized Water	$_{B}$ 62.32 ^{b}	$_{A}$ 73.91 ^{a}	68.11 ^{b}
Alkaline EO	$_{A}$ 74.44 ^{a}	$_{A}$ 78.58 ^{a}	76.51 ^{a}
Soap Water	$_{A}$ 78.09 ^{a}	$_{A}$ 81.58 ^{a}	79.78 ^{a}

Values in columns sharing a common letters $^{(a-b)}$ after the mean value are not significantly different ($p \geq 0.05$)
Values in a row sharing common letters $_{(A-B)}$ before the mean value are not significantly different ($p \geq 0.05$)

TABLE 3.2: Average percent removal of chicken fat after vortexing and immersion

Treatment	Average percent fat removal (%)							
	Vortex				Immersion			
	10 ml		25 ml		10 ml		25 ml	
	(3 sec)	(10 sec)	(3 sec)	(10 sec)	(10 sec)	(60 sec)	(10 sec)	(60 sec)
Deionized Water	19.28 ^{b}	33.63 ^{a}	20.94 ^{b}	19.21 ^{b}	16.77 ^{b}	19.82 ^{b}	22.47 ^{b}	22.56 ^{b}
Alkaline EO	52.07 ^{b}	62.58 ^{a}	41.52 ^{c}	50.66 ^{b}	32.30 ^{de}	33.40 ^{de}	26.54 ^{e}	36.27 ^{cd}
Average	*35.68* ^{b}	*48.11* ^{a}	*31.23* ^{bcd}	*34.94* ^{bc}	*24.54* ^{d}	*26.61* ^{d}	*25.51* ^{cd}	*29.41* ^{bcd}

Values in rows sharing a common letter are not significantly different ($p \geq 0.05$).
The row noted "*Average*" is the combine effect of both water and alkaline EO water on percent removal of fat

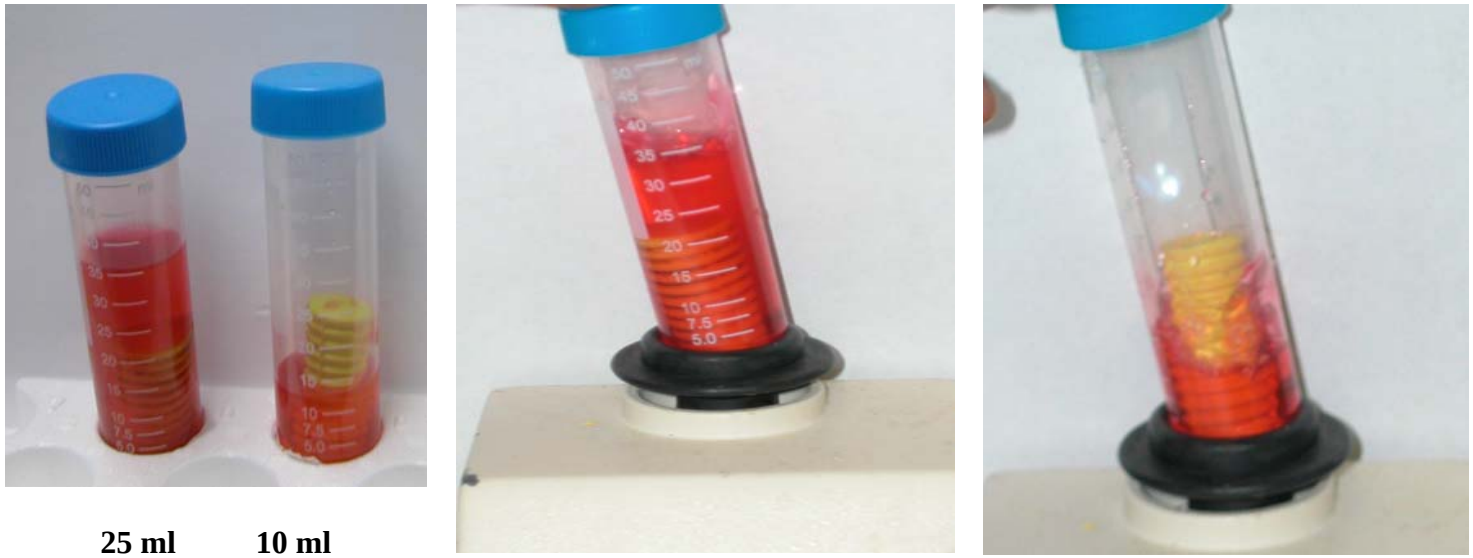
TABLE 3.3 Percent chicken fat removed by different cleaners for new and re-used fingers.

Treatment	Solution property			Fat Removal (%)	
	pH	ORP (mV)	Chlorine (ppm)	New finger	Re-used finger
Deionized Water	6.98	225.4	0	$_{A}$ 29.37^{d}	$_{A}$ 20.44^{c}
Double deionized water	6.98	225.4	0	$_{A}$ 37.38^{bcd}	$_{A}$ 36.00^{bc}
Acidic EO	2.62	1149.7	48.23	$_{A}$ 21.42^{d}	$_{A}$ 17.72^{c}
Alkaline EO	11.54	-872	0	$_{A}$ 64.34^{a}	$_{A}$ 53.22^{ab}
50% chlorinated-alkaline cleaner	13.70	412	NA	$_{A}$ 45.10^{bc}	$_{A}$ 47.99^{ab}
12.5% Sodium Hypochlorite	11.65	650	5963	$_{A}$ 46.39^{bc}	$_{A}$ 48.28^{ab}
Combo	-	-	-	$_{A}$ 58.05^{ab}	$_{A}$ 56.97^{a}

Values in column sharing a common letters $^{(a-b)}$ after the mean value are not significantly different ($p \geq 0.05$)
Values in a row sharing common letters $_{(A-B)}$ before the mean value are not significantly different ($p \geq 0.05$)
Item marked "Combo" is of the sequential application of alkaline EO water followed by acidic EO water

FIGURE 3.1: Rubber picker finger piece (12 rings)

A) **B)** **C)**



25 ml **10 ml**

FIGURE 3.2: Washing rubber finger in A) 25 ml and 10 ml cleaning solutions B) vortexing in 25 ml solution and C) vortexing in 10 ml solution

Treatments were dyed red to highlight effect

CHAPTER 4

CHICKEN FAT ABSORPTION INTO RUBBER PICKER FINGERS AND ITS EFFECT ON *SALMONELLA* TYPHIMURIUM ATTACHMENT

[1] **Burkeen, V.K., Y-C. Hung, A. Hinton Jr., and J. Arnold To be submitted to the Journal of Food Science**

ABSTRACT

This study investigates the effects of attached/absorbed chicken fat over time on the cleanability of rubber picker fingers and the effect of fat on the attachment of *S*. Typhimurium to the rubber finger surface. Finger pieces (12 rings) were examined after they were coated with rendered chicken fat and allowed to hold for various lengths of time (0, 1, 4, 8, and 24 hour). In addition, repeated daily soiling and cleaning cycles (1-4 days) on fat absorption were also studied. Cleaning treatment solutions included: deionized (DI) water (control), alkaline electrolyzed (EO) water, sodium hypochlorite (10% solution), a soap (5% solution) and a chlorinated-alkaline cleaner (50% solution). Results demonstrated that as elapse time before cleaning increased (longer fat attachment time), the amount of fat remaining after cleaning also increased for all treatments solutions, with washing after 24 hours having the highest percent fat remaining on the surface. Results suggested that cleaning every 4 hours with a chlorinated - alkali cleaner may be adequate control of fat accumulation on finger. The culmative percent fat remain on finger's gradually increased each day after washing practices from average of 51.9% (day 1) to 106.45% (day 4) for used finger and 47.2% (day 1) and 88.78% (day 4) for new fingers. New and used fingers gained an average of 0.64 g and 0.77 g, respectively, by day 4 of soiling and cleaning cycles, indicating that fat absorption does occur during poultry processing practices and that used fingers absorb a significantly larger amount of fat than new fingers. Fat attachment and absorption did not play a significant role on attachment of *S.* Typhimurium. Bacteria readily attached to the surface at approximately 6 log CFU per finger with or without the presence of fat.

INTRODUCTION

Defeathering practices have been identified by the International Commission on Food Microbiological Specifications as the first major cross contamination site during poultry processing (Clouser et al., 1995). Many pathogens such as *Salmonella* Typhimurium, have been noted to be transmitted through this processing step via picking machines (Purdy et al., 1988). There are many modes of bacterial transmission by picker machines including, plastic curtains, metal surfaces, water sprays and rubber picker fingers to name a few (Allen et al., 2003; Dodd et al., 1988; Lindsay et al., 1996). The rubber picker fingers, in particular, can be contaminated during defeathering by feathers, the fatty liquid film on the skin and the epidermal tissue of birds that harbor microorganisms. Used rubber fingers are also difficult to clean and disinfect. This may be a result of rubber degradation and cracks and crevices that developed in the rubber during prolong usage. The deterioration of the rubber surface after prolonged usage can cause increased biofilm formation and decrease cleanablity by offering better attachment surfaces and protection from cleaning operations (Storgards et al., 1999).

Somers and Wong (2004) demonstrated that cleaning efficacy is surface dependent and, to some degree, residue dependent. Inadequate cleaning of surfaces can lead to minimal inactivation of unwanted bacteria because sanitizing efficiency is greatly hindered in the presence of organic matter (Best, 1990). Therefore, adequate evaluation of the surface/soil interaction on cleanibility is of great importance. During defeathering practices, feathers are removed along with a large portion of the outer layer of the skin, called the stratnum corneum. The stratnum corneum is made up of cornea cells and fat vacuoles (Thomas and McMeekin, 1980). Fat is an organic soil of concern because it is not water soluble and may leave a fatty film (Kim and Doores, 1993). This fatty film can be difficult to remove and may serve as protection

for bacteria that are attached to chicken skin or processing equipment by shielding them from cleaning and sanitizing procedures (Kim and Doores, 1993). Moreover, an area largely unexplored, is fat absorption into the porous surface of picker fingers. Absorbed fat may better protect bacteria than the fat film on the surface and reduce inactivation during cleaning and sanitizing procedures to a greater degree.

Pathogens such as *S.* Typhimurium and *L. monocytogenes* have exhibited increased resistance to sanitizers when attached to rubber (Storgards et al., 1999). The presence of food residue on rubber can affect microbial attachment (Chmielewski and Frank, 2006). Helke (1994) demonstrated that milk and its constituents inhibit the attachment of pathogens like *L. monocytogenes* and *S*. Typhimurium on buna-N-rubber. The presence of fat on rubber fingers may reduce or promote bacterial attachment. Fat may increase attachment and boifilm formation as demonstrated with *Pseudomonas* species in the presence of cream and fat (Storgards et al., 1999). Bridget (1998) demonstrated that beef fat did not have a significant role on the attachment of *Escherichia coli* O157:H7 to meat grinder surface. The role of poultry fat on bacterial attachment to rubber finger surfaces is not understood and needs further investigation.

When it comes to organic soil, alkaline cleaners are effective because of their high pH (>11), which allows for neutralization of acidic soils, saponification of fats and lipids, and dispersion of oils and lipids on various surfaces (Marriot, 1999). The combination of high pH and elevated temperatures increases the efficiency of these cleaners ability to remove soil which results in better sanitation of contaminated surfaces, such as animal skin (Teo, 1996). Cleaning solutions such as caustic sodas (sodium hydroxide and potassium hydroxide), trisodium phosphate, and other cleaners with pH above 11, have also been successful in the destruction of gram negative microorganisms when heated (Dickens, 1994; Humphrey, 1981; Teo, 1996).

Coppen (1993) demonstrated that these solutions were effective because of the removal of the fatty layer from the animal skin and their intrinsic high pH. In light of these findings, the investigation of current cleaning processes and their efficiency on processing surfaces, such as rubber picker fingers could be valuable. Trisodium phosphate (TSP), in particular, has been noted to prevent cross contamination by removing a thin layer of fat off from carcasses during defeathering (Kim et al., 2005; Vareltzis, 1997). Furthermore, the exploration of other alkaline cleaners, such as alkaline electrolyzed (EO) water, could be beneficial to the improvement of cleaning and sanitizing practices. Alkaline EO water properties include low oxidation-reduction potential (ORP), high pH (>11) and contains dilute concentrations of NaOH. These properties make this solution a promising degreaser and soil remover.

The objectives of the studies were to examine the effects of time between cleanings on fat absorption and daily cleaning procedures on cleanibility of picker fingers and the attachment of *S.* Typhimurium to the rubber surface. Efficacy of alkaline EO water for cleaning rubber picker fingers was also compared to other cleaners commonly used in poultry plants.

MATERIALS AND METHODS:

Preparation of feather picker fingers

Used feather picker fingers (type E 2075, yellow) were collected from a local poultry processing plant in Athens, GA. The used fingers were transported to the laboratory in plastic bags and then washed with a detergent (Alcojet, Alconex, Inc., White Plains, NY) in an automatic dishwasher twice on regular cycle. New rubber fingers (the same type) were obtained from William Goodyear Company (Durham, NC) and were also washed as described above. Fingers were then cut into approximately 4 cm (12 rings) pieces by use of a Model 14 band saw (Rockwell International Inc., Pittsburg, PA) from the top portion of the finger (Figure 3.1). On

the day of experiment, fingers were immersed in ethanol for 5 min to remove fat or oil residues. Pieces were allowed to dry under air flow hood until use.

Soiling of fingers

Frozen rendered chicken fat (Tip Top Poultry, Atlanta, GA) was obtained and stored at -70^{o}C until use. At least 48 hours before experiment, the fat was allowed to liquefy at room temperature (22 $\pm$ 2^{o}C). The liquid fat was then poured into an empty medium size weighing dish. The initial weight of each rubber piece was obtained using an analytical balance (Model A-160, Denver Instrument Company, Denver, CO). Approximately 1$\pm$0.2 g of fat was applied onto each finger by placing the fingers in the fat and rolling it from one side to the other. Coated fingers were allowed to sit at room temperature for 0, 1, 4, 8 and 24 hour time periods in a desiccator for attachment. Weights of coated fingers were taken before treatment. It should be noted that when calculating percent fat remaining on fingers the difference between the weight before treatment and the initial weight of the finger were used as the total amount of fat on finger instead of the 1 g that was applied (Huff et al., 1984; Shupe et al., 1982).

Treatment solutions

Fingers were washed using 5 different treatment solutions: deionized (DI) water, 5% soap (Liquid-nox, White Plains, NY) solution, 10% sodium hypochlorite (Clorox, Oakland, CA) solution, 50% chlorinated-alkaline cleaner (Chill Kleen, Duchem, Atlanta, GA) solution, and alkaline EO water. Chlorinated-alkaline cleaners are commonly used in commercial poultry plants to clean fingers. Poultry plants commonly use concentrations of cleaners and sanitizers in excess of concentrations recommended by manufactures. Recommended levels for alkaline cleaners range from 0.5% to 5% at temperatures of 60^{o}C or higher (Farrell et al., 1998; Somers and Wong, 2004). Recommend levels for chlorine based sanitizers range from 200 ppm on a

non-porous surface to 800 ppm on a porous surface (Mafu et al., 1990; Marriot, 1999). High concentrations of the chlorinated-alkaline and sodium hypochlorite cleaners were used in this study to evaluate the actual solution concentrations that are used during cleaning in a local poultry plant. Alkaline EO water was generated by a ROX-20TA machine (Hoshizaki Electric Inc, Toyoake, Aichi, Japan) at settings of 18 A. At this setting, alkaline EO water has a pH of 11.6 and ORP of -813 mV (Table 4.1). Solutions of 5% soap, 10% sodium hypochlorite and 50% chlorinated-alkaline were prepared by diluting with DI water. DI water was used as a control for this study.

The oxidation-reduction potentials (ORP) and pH levels of all solutions were measured using a dual scale pH meter (Accumet model 15, Fisher Scientific Co., Pittsburgh, PA)(Table 4.1). Treatment solutions were heated in closed glass containers in a water bath (Model WB1120-A, Lindberg/Blue M, Ashville, NC) to 65^{o}C and held until use. One liter of alkaline EO water was heated in glass container until visible white clusters of NaOH were formed in solution (for approximately 1.5 hours). Alkaline EO water was then mixed by vigorously shaking the bottle by hand to ensure all clusters of NaOH were dissolved before pouring. Ten ml of each heated treatment solution was poured into 50 ml centrifuge tubes at time of application.

Effect of elapse time between cleaning on fat attachment after soiling

Preliminary results indicated that there was no significant difference between used and new fingers on fat attachment at the time intervals studied. Therefore, only new fingers were used in this study. Cleaning solutions were applied by either brushing or vortexing (simulating spray washing step at poultry processing plants) protocols at 45^{o}C and 65^{o}C, respectively. Coated fingers were brushed with a standard cleaning brush in 400 ml of cleaning solutions in a small metal container to simulate the most aggressive cleaning action. The solution temperature

was kept constant by placing the container in a water bath which was held down with a weight. The temperature of cleaning solutions was monitored by a standard (mercury) thermometer. Fingers were submerged in cleaning solution and then brushed with 3 (up and down) strokes on each side. After brushing, rubber fingers were allowed to drip for 30 sec to remove excess cleaning solution.

Coated fingers were also vortexed (Model 6-560, Scientific Industries, Bohemic, NY) at low setting in 10 ml of cleaning solution for 45 seconds. All treatment tubes were held at 65^{o}C in a water bath and the temperature was determined by a thermometer before use. During vortexing, treatment tubes were tilted slightly to allow swirling of the liquid around finger pieces. After vortexing, the treatment solutions were immediately decanted. Each finger piece was removed and set horizontally in the cap of a centrifuge tube for 30 seconds to drain excess treatment solution. All treated fingers were weighed after 24 hours of drying at room temperature (22$\pm$ 2^{o}C). The percent fat remaining on fingers after treatment was calculated (Shupe et al., 1982) as follows:

$$\textbf{Percent Fat Remain} = 100 - \left\{ \left[\frac{W_s - W_t}{W_s - W_i} \right] \text{X } 100 \right\}$$

W_s= weight of soiled finger before treatment

W_t = weight of soiled finger after treatment

W_i = weight of unsoiled finger

Effect of daily cleaning on fat removal

New and used picker fingers were coated with chicken fat as described previously and held for 24 hour before being washed. Soiling and washing cycles were repeated up to four

times throughout the experiment. On day 0, fingers were coated and dried for 24 hours. The coated fingers were washed and re-coated on days 1, 2, and 3. On day 4, fingers soiled from day 3 were washed. Each day, coated fingers were weighed and then washed with one of the 5 solutions described above. Each solution was also evaluated by the following cleaning protocols: brushing in 400 ml of treatment solution at 45^{o}C, 45 sec vortexing with 10 ml of treatment solution at 45^{o}C, and 45 sec vortexing with 10 ml of treatment solution at 65^{o}C. After treatment, fingers were allowed to dry under a sterile flow hood for 1 hour. Fingers were then re-coated with approximately 1$\pm$0.2 g of fat. The percent fat remain after cleaning was calculated as described previously. Two fingers were used for each washing treatment and the experiment was repeated 3 times.

Preparation of inocula

S. Typhimurium was obtained from the culture collection of the United States Department of Agriculture (USDA), Russell Research Center, Athens GA. Two hundred µl of frozen aliquots (-70^{o}C) of *S.* Typhimurium was cultured overnight in trypticase soy broth (TSB) (Becton Dickerson, Sparks, MD) at 37^{o}C. Two hundred µl of the overnight culture was transferred into 20 ml of TSB in 50 ml centrifuge tubes. Cultures were incubated at 37^{o}C on a shaker at 90 rpm (Model 6576D, New Brunswick Scientific, Edison, NJ) (Sinde and Carballo, 2000) for 18 hours.

Bacterial attachment

Fat was coated onto new and used fingers and washed after every 24 hours of fat attachment. To evaluate the effect of residual fat on bacterial attachment of rubber finger, fingers that were treated with one soiling and cleaning cycle (1 day) and fingers that were treated with 3 soiling and cleaning cycles (day 3) were selected based on the calculated percent fat

remaining after washing with DI water in the effect of daily finger cleaning on fat removal study. The fat was not sterile but contributed less than 10 CFU/cm^2 of microbial load.

Fingers (washed and unwashed) were inoculated in an 18 hour culture of *S*. Typhimurium (approximately 9.3 log CFU/ml of TSB) for 3 hours at 37^{o}C on a shaker at 90 rpm to allow for bacterial attachment. After attachment, pieces were washed in 10 ml of sterile peptone water (1 g of peptone per liter of DI water (0.1%)) twice to remove loosely attached microorganisms. Inoculated samples were then place into empty petri dishes and allowed to dry for 30 min under a boisafety hood.

To enumerate attached *S*. Typhimurium cells, the rubber fingers were placed in sterile medium size stomacher bag containing 80 ml of sterile peptone water (0.1%). The fingers were then stomached on high setting in a stomacher (Model 80 Laboratory Blender, Sew and Limited, London, UK) for 2 min. During preliminary studies, inoculated rubber samples were viewed before and after stomaching procedures using a fluorescent microscope (Nikon Eclipse E600, Southern Micro Instruments, Marrietta, GA). Results demonstrated adequate removal of attached bacteria from surface with approximately one cell per image remaining after stomaching procedures (data not shown). Serial dilutions of the peptone water were made after stomaching. The surviving bacteria from the control and treated rubber pieces were enumerated by plating 0.1 ml diluents on tryptic soy agar (TSA) (Becton Dickerson, Sparks, MD). Plates were incubated at 37^{o}C for 48 hours and colonies were counted and recorded as log CFU per rubber piece. Three samples were used per treatment solution and the experiment was replicated 3 times.

Data analysis

Data for all experiments were analyzed using the general liner model (GLM) procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.). Comparisons of means were

performed using Duncan multiple range test. All statistical analysis was done at $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Elapse time before cleaning

The average amounts of fat that remained on new fingers after holding at room temperature (22±2°C) for various lengths of time before washing were: 0.867 g at 0 hours, 0.721 g at 1 hour, 0.672 g at 4 hours, 0.691 g at 8 hours, and 0.661 g at 24 hours. Findings indicate that 12 ring fingers pieces can hold approximately 0.7 g of fat in and on its surface before cleaning. This is important finding because as many as 3000 to 6000 birds can be processed per hour per line in a poultry processing plant (Allen et al., 2003; Dodd et al., 1988; Purdy et al., 1988), which allows for large amounts of accumulated soil (fat) on rubber finger surface in the first hour of processing. This soil can be left to further contaminate subsequent carcasses throughout the processing day with cleaning procedures occurring only once a day.

In this study, there was no significant difference in the amount of fat removed by brushing or vortexing applications at either temperature. Figure 4.1 shows cleaning fingers with the chlorinated-alkaline cleaner using brushing or vortexing had no significant affect on the amount of fat remain. Shupe (1982) noted that fat removal was not significantly different at various water pressures or temperatures when a detergent was used during spray applications. Hence, data was further analyzed by posting all three washing protocals for each cleaning solution together.

After cleaning, there was a significant removal of the coated fat from the fingers. At 0 hours (washed immediately after soiling), there was an average fat removal for all cleaners of approximately 84.4% (Table 4.2). The sodium hypochlorite and chlorinated-alkali solutions

were the most effective cleaners with only 8.92% and 9.44% fat remaining at 0 hour, respectively (Table 4.2). Water, alkaline EO water, and soap water all had significant higher amounts of fat remain after washing at 0 hours than the other two cleaners with water being the least effective. The amount of fat that was still on fingers at 0 hours indicates that there is an immediate presence of a fatty film that is not easily removed with cleaning applications, even when high concentrations of cleaners were utilized. It also suggests a possibility of fat absorbing into rubber immediately after soiling.

With increase of elapse time (longer fat attachment time), the amount of fat removed by cleaning treatment decreased (Table 4.2). At 1 hour, the average amount of fat that remained on the finger after cleaning increased to 31.82% from 15.6% further indicates the absorption of fat into the pores of rubber occurs early on. At this period, fat removal by water, alkaline EO water and soap water were not significantly different from one another. The sodium hypochlorite and chlorinated-alkali solutions were still more effective than the other three cleaners on removing fat from rubber fingers with only 24.19% and 22.17% fat remain, respectively. At 4 and 8 hour time periods, the mean values of percent fat remain on fingers was not significantly different. However, the chlorinated-alkaline cleaner remained the most effective cleaner throughout the 8 and 24 hour elapse time. The 4 hour period is also the time that soap water and sodium hypochlorite had similar efficiency on fat removal. At 24 hours, there was a combined average of 47% of fat remain after cleaning which is approximately 0.33 g when calculated from an initial soil amount of 0.70 g. This significant amount of soil remains after standard cleaning operation poses a serious threat on cross contamination. Fingers cleaned by the chlorinated-alkaline cleaner at 4 hour elapsed time had only 28.4% fat remaining and was not significantly

higher than DI water at 0 hour elapsed time (26.8%). Results suggest that rubber picker fingers may need to be cleaned at least every 4 hours.

Fat absorption into fingers

Although 1 g of fat was coated onto the finger on each day, after the 24 hour fat attachment period, a combined average amount of 0.7± 0.08 g remained on each finger before treatment regardless of finger type and the day applied (data not shown). On day 1, there was a significantly larger amount of fat remaining after cleaning for all cleaning solutions ranging from approximately 40 to 55 % depending on the cleaning agent and finger type (new or used) and is in agreement with Table 4.2 at 24 hr elapse time (Figure 4.2). Day 2 had a significantly lower amount of fat than day 1 remain on fingers after washing and different cleaning solutions had no significant affect on percent fat remaining on finger. By day 2, the data indicate a trend for all cleaners. After each day of cleaning the amount of fat that was left after washing decreased with increase of the number of soiling and washing cycles, while the total finger weight continued to increase (Figure 4.2 and 4.3). This strongly suggests that there was a build up of fat from previous days. By the 3rd and 4th days, the fat built up to a point of saturation (Figure 4.3). This was further supported by the significant weight gain in rubber fingers over the study (data not shown).

Both new and used fingers increased in weight with average weight gains of 0.642 g and 0.745 g, respectively, by day 4. Each day, fingers gained more weight from the initial mass after the soiling and washing cycles. For example, the cumulative weight gain over the four day period for new fingers was 0.313 g (day 1), 0.458 g (day 2), 0.548 g (day 3) and 0.642 g (day 4) (Figure 4.3). Used fingers had cumulative weight gains of 0.344 g (day 1), 0.529 g and (day 2), 0.633 g (day 3), and 0.775 g (day 4). These results indicated that used fingers can absorb

significantly higher amounts of fat than new fingers as also indicated in Table 4.3. These finding were further supported by totaling the amounts of percent fat remaining after each day. When adding the average percent fat remain for new fingers over the four days of soiling and washing cycles, the combine percentage is 88.78 % (47.19 (day 1) +19.06 (day 2)+10.87 (day 3)+11.66 (day 4))(Table 4.3). Used fingers, on the other hand, reached a point of 91.4% fat remaining by day 3. By day 4, used fingers reached over 100% (106.45%). Further evidence that strongly supports soil absorption into the surface of rubber picker fingers during usage at the processing plant was that used fingers were on average 1± 0.3 g heavier in weight than new fingers.

The ability to absorb more fat was also observed in another study (data not shown). New and old fingers were soiled and washed as in fat absorption study over a 4 day soiling and cleaning period. Washed fingers after the experiment (from day 5) were left at room temperature ($22\pm2^{o}C$) for 30 days before the weight was taken again. There was no significant difference in weight change over the month. When these fingers were resoiled they were still able to absorb a similar amount of fat (about 45% after 24 hr) that was observed in previous studies (Table 4.3). While this mechanism is not totally understood, the absorption could be due largely to the porous surface of the rubber. In addition, cleaning agents may cause rubber deterioration and may be the reason that used fingers are able to harbor more soil. Storgard (1999) demonstrated that a variety of rubber materials used in food processing gaskets (ethylene propylene diene monomer rubber (EPDM), nitrile butyl rubber (NBR also known as buna-N), polytetrafluoroethylene (PTFE) and Viton (fluoroelastomer)) can be attacked by cleaners and food particles. In most of the gasket materials tested, the degradation of rubber by chemical agents led to brittleness followed by crack formation and eventually to dissolution and softening (Storgards et al., 1999).

This is a form of surface corrosion caused by chemical reactions between sanitizers/cleansers and the contact surfaces (Ayebah and Hung, 2005).

The softening and deterioration of the rubber matrix may allow for more absorption of fat into the pores of used finger more readily than new fingers due to larger pores in the upper portion of the used finger surface. When fingers are used in the poultry processing facility, the exterior surfaces of fingers are in direct contact with the poultry carcasses during defeathering operations. Theoretically, if the surfaces of the used fingers are degraded by continual use and cleaning agents, then the outer surface of the finger will have larger holes than the new fingers. This would explain the more rapid absorption of fat by used fingers when compared to new fingers (smaller pores on surface) and provides a reasonable explanation for why fat can continually be absorbed into finger surfaces even after 30 days. As indicated by this study, fat is absorbed quickly over 4 days into the rubber surface of fingers and reaches a point of saturation. Over time, however, the absorbed fat may sink deeper inside of rubber finger pores, leaving more room for more fat to be absorbed at the surface during subsequent soiling (30 days later). To test this theory, scanning electron micrographs were taking of the interior of both new (Figure 4.4) and used (Figure 4.5) rubber fingers. Using a sterile razor, fingers were cut horizontally down the middle from the tip of the finger to the end which is a 4 inch cut leaving two equal halves of a finger (each with a flat side and a side with 12 partial rings on surface). Figures 4.4 and 4.5 are horizontal cross sections from the interior side (flat side) of new and old fingers, respectively. Results illustrate that new fingers have fewer holes (approximately 1 hole per image) in the interior of the rubber (Figure 4.4), while used fingers have approximately 6 holes per image (Figure 4.5) that were also bigger (approximately 3 μm larger) than the ones found in new fingers (Figure 4.4). Vertical cuts were also made between every 2 rings of the fingers

totaling 6 cross sections per finger. Further supporting evidence that holes may run from the ring surface (exterior) to the interior of the finger was that the vertical cross sections showed higher presence of crevices rather than holes on the inside of the used rubber finger.

Bacterial attachment

Fat in and on the surface of the rubber did not have a significant effect on the attachment of *S*. Typhimurium. All samples regardless of soil or cleaning treatment had approximately 6 log CFU per finger bacterial attachment (Table 4.4). There was a significant increase in the attachment of bacterial cells to non-soiled/unwashed rubber samples (controls) when compared to soiled rubber (both washed and unwashed sample). However, these differences were less then 0.5 log CFU per finger. Furthermore, when washed samples were compared to unwashed, there was no significant difference.

There was also no significant difference between the effects of the 1 soiling and washing cycle and the 3 day soiling and washing cycles on the bacterial attachment of *S.* Typhimurium. After 3 cycles of daily washes with heated DI water (with fat absorption percents reaching 77-91%), *S.* Typhimurium cells still readily attached to rubber. Although there was a clear distinction between the amounts of fat accumulated on new and used fingers in previous fat absorption studies (Figure 4.3 and Table 4.3), this difference was not a factor on bacterial attachment. These results confer with the data presented by Bridget (1998) stating that fat (beef) had no significant affect on bacterial attachment to meat grinders.

CONCLUSIONS

Attached/absorbed chicken fat is a significant factor on the cleanibility of rubber picker fingers but does not influence attachment of *S.* Typhimurium. Daily soiling by defeathering processes contributes significantly to the accumulation of soil on the surface of fingers. Results

indicated that fat attached/absorbed reached a point of saturation in fingers after 4 days and that washing once a day does not adequately remove fat from fingers. In addition, used fingers (obtained from poultry processing plants) absorb significantly higher amounts of fat when compared to new fingers. This may be a result of rubber degradation by cleaning and continual use which may produce larger holes on the surface of the used fingers when compared to the newer ones. This is important because fingers are manually replaced when sanitation personal identifies that finger as cracked or deformed (Arnold, 2007). This can lead to damaged fingers being easily overlooked and become a source of cross-contaminate on other poultry carcasses down the line. Results from the fat attachment time studies suggest that rubber picker fingers may need to be cleaned at least every 4 hrs.

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TABLE 4.1: Average pH and ORP levels of cleaners

Cleaner	pH value	ORP (mV)
Deionized water	7.01	252.2
Soap solution (5%)	8.62	42.2
Chlorinated-alkaline (50%)	13.54	375.9
Sodium hypochlorite (10%)	11.65	680
Alkaline EO water	11.56	-813.3

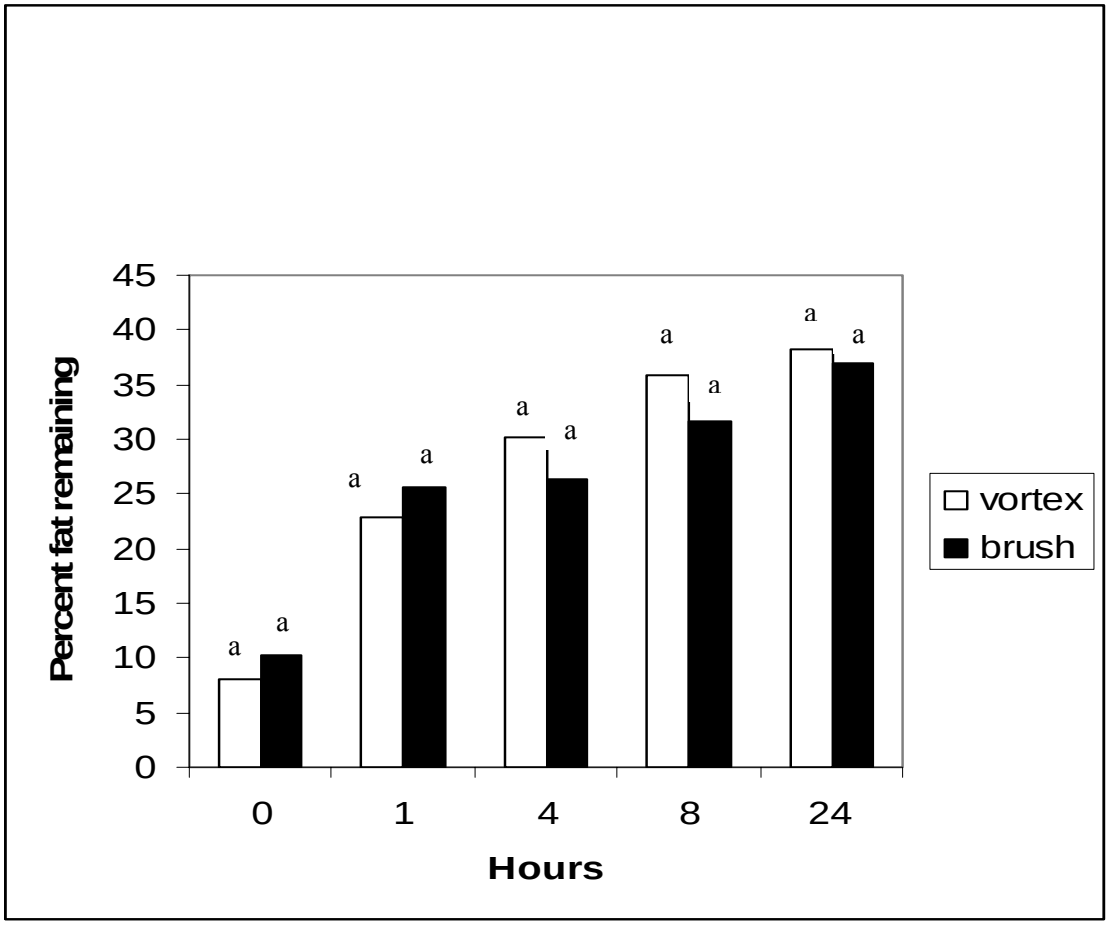

FIGURE 4.1: Effect of washing protocol on fat removal using a chlorinated-alkaline cleaner for various fat attachment times.

Bars with the same letter are not significantly different ($P \geq 0.05$)
Brushing treatment temperature was 45°C
Vortexing treatment temperature was 65°C

TABLE 4.2: Percent fat remaining after washing after various fat attachment times

Percent Fat Remaining After Washing (%)						
Elapse Time (hours)	**Deionized Water**	**Soap Water**	**Sodium hypochlorite**	**Chlorinated-alkali**	**Alkaline EO**	***Average***
0	26.77 ^{a}	13.77 ^{c}	8.92 ^{d}	9.44 ^{d}	19.10 ^{b}	*15.60* ^{z}
1	40.60 ^{a}	33.24 ^{a}	24.19 ^{b}	22.17 ^{b}	36.80 ^{a}	*31.82* ^{y}
4	50.06 ^{a}	37.61 ^{c}	35.75 ^{c}	28.40 ^{d}	44.74 ^{b}	*39.03* ^{x}
8	52.71 ^{a}	40.42 ^{b}	42.08 ^{b}	42.08 ^{b}	44.78 ^{b}	*42.45* ^{x}
24	53.65 ^{a}	41.62 ^{bc}	49.41 ^{ab}	49.41 ^{ab}	52.05 ^{a}	*46.90* ^{w}
Average total fat remaining	*44.57* ^{a}	*33.27* ^{c}	*31.9* ^{c}	*26.68* ^{d}	*39.40* ^{b}	

For each row, mean values with the same letter $^{(a\text{-}e)}$ are not significantly different ($P \geq 0.05$)
The row denoted "Average total fat remaining " is the total percent fat remaining for all five elapse times
The column denoted " *Average***"** is the total average of fat remaining of all treatments for a specific time (hours) and mean values with the same letter are not significantly different $^{(w\text{-}z)}$ ($P \geq 0.05$)

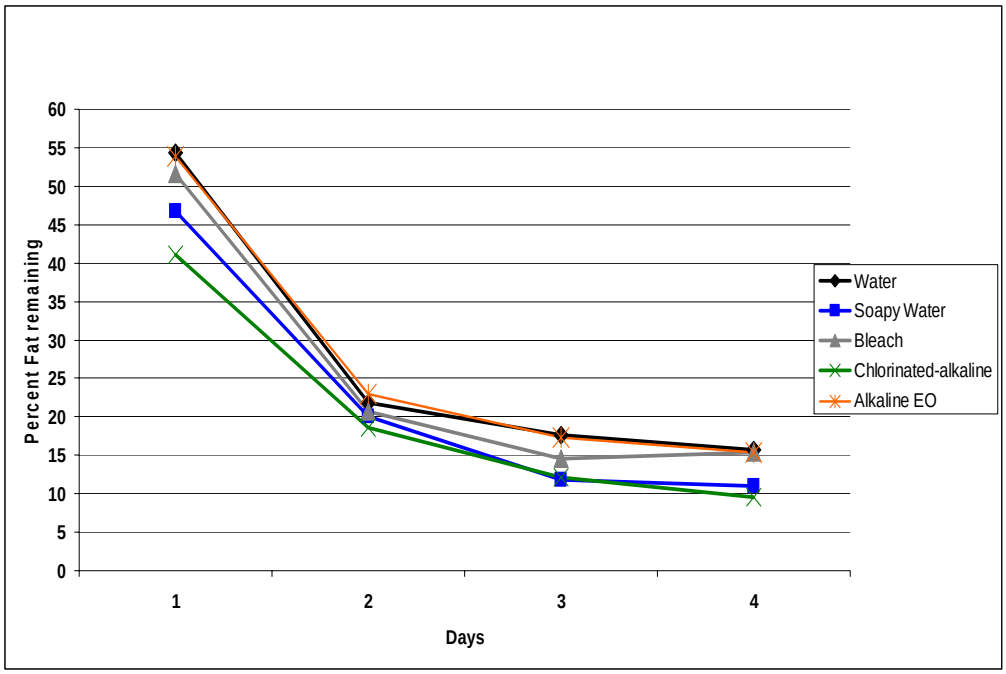

FIGURE 4.2: Percent fat remaining after daily soiling and washing with various cleaners

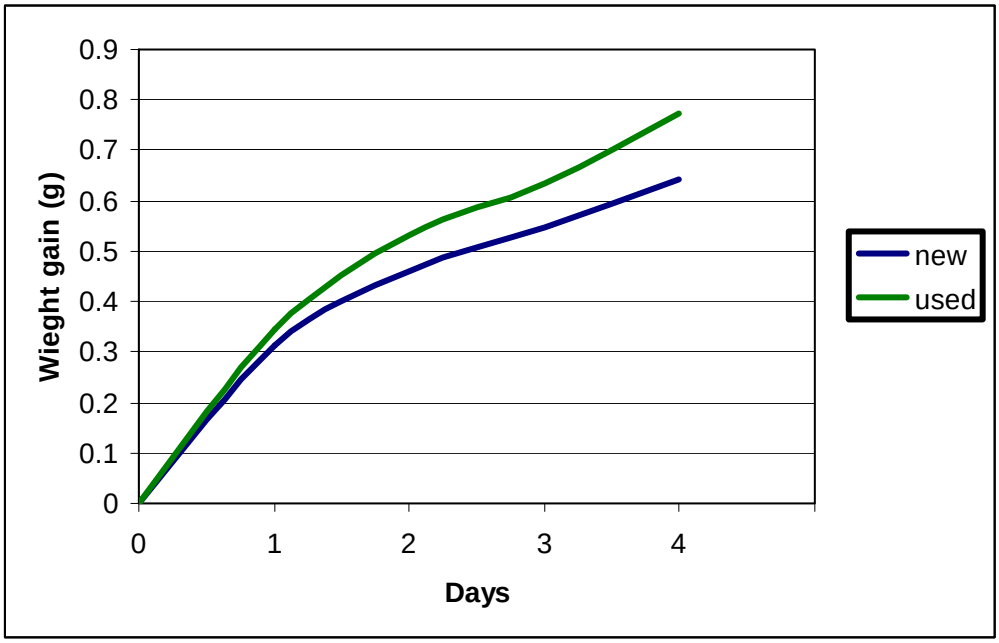

FIGURE 4.3: The average weight gain per day of soiled new and used picker fingers after washing

TABLE 4.3: Percent fat remaining on used and new fingers after 4 repeated soiling and cleaning cycles with various cleaners

Days	Finger	Water		Soap Water		Sodium hypochlorite		Chlorinated-alkali		Alkaline EO		*Average*	
1	**New**	52.1 ^{a}		43.6 ^{a}		50.6 ^{a}		38.1 ^{b}		51.5 ^{a}		*47.2 ^{a}*	
	Used	56.6 ^{a}		50.0 ^{a}		52.6 ^{a}		44.0 ^{a}		56.5 ^{a}		*51.9 ^{a}*	
2	**New**	20.4 ^{b}	(72.5)	18.4 ^{bc}	(62.1)	18.8 ^{b}	(69.5)	18.3 ^{c}	(56.2)	19.5 ^{c}	(71.0)	*19.1 ^{b}*	*(66.3)*
	Used	23.2 ^{b}	(79.8)	21.7 ^{b}	(71.6)	22.5 ^{b}	(75.1)	18.4 ^{c}	(62.3)	26.5 ^{b}	(82.7)	*22.5 ^{b}*	*(74.3)*
3	**New**	15.5 ^{cd}	(88.0)	10.5 ^{d}	(82.1)	11.8 ^{d}	(81.4)	9.4 ^{e}	(65.6)	15.8 ^{cd}	(86.7)	*10.9 ^{d}*	*(77.2)*
	Used	19.7 ^{bc}	(99.5)	13.8 ^{cd}	(85.4)	17.1 ^{c}	(92.2)	14.7 ^{cd}	(77.1)	18.8 ^{c}	(101.5)	*16.8 ^{bc}*	*(91.2)*
4	**New**	12.8 ^{d}	(100.7)	9.3 ^{d}	(91.5)	14.7^{d}	(96.1)	8.6 ^{e}	(74.3)	12.81 ^{d}	(99.7)	*11.7 ^{d}*	*(88.8)*
	Used	18.6 ^{bc}	(118.1)	12.2 ^{cd}	(97.7)	16.1 ^{cd}	(108.3)	10.8 ^{e}	(87.9)	18.80 ^{c}	(120.3)	*15.3 ^{c}*	*(106.5)*
Total Fat remaining		*25.2 ^{x}*		*20.5 ^{x}*		*24.0 ^{x}*		*18.5 ^{x}*		*24.8 ^{x}*			
		29.5 ^{x}		*24.5 ^{x}*		*27.1 ^{x}*		*22.0 ^{x}*		*29.9 ^{x}*			

Columns with the same letter $^{(a\text{-}c)}$ are not significantly different ($P \geq 0.05$)
Rows with the same letter are not significantly different $^{(y\text{-}z)}$ ($P \geq 0.05$)
The row denoted "Total" is the total fat percent remaining for all five treatments
Values in parentheses are culmative amount of fat remain on the fingers after each soling and cleaning cycle

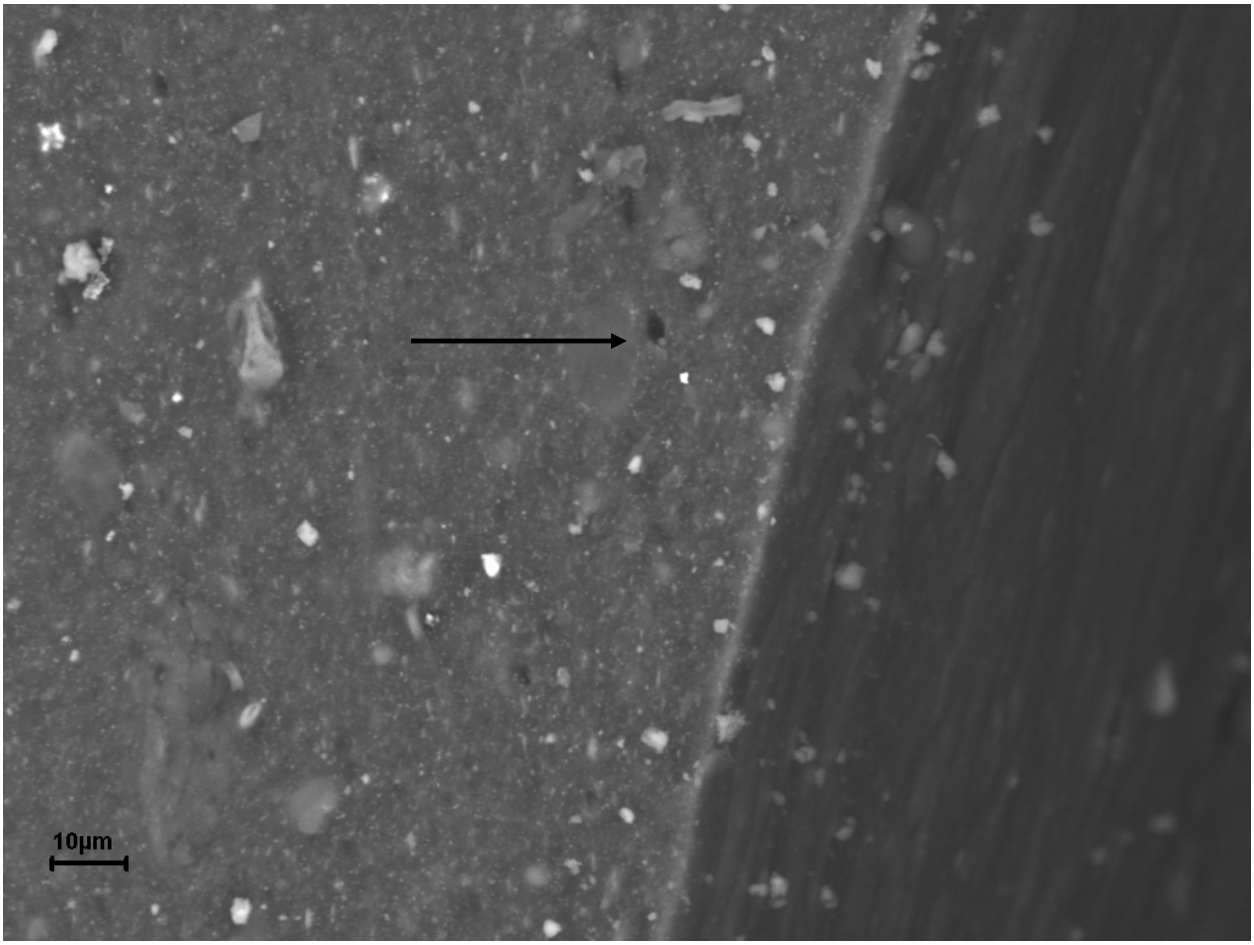

FIGURE 4.4: Scanning Electron Microscope image showing small holes in the edge of new rubber fingers.

Magnification = 1,000
Arrow highlights hole in surface of rubber

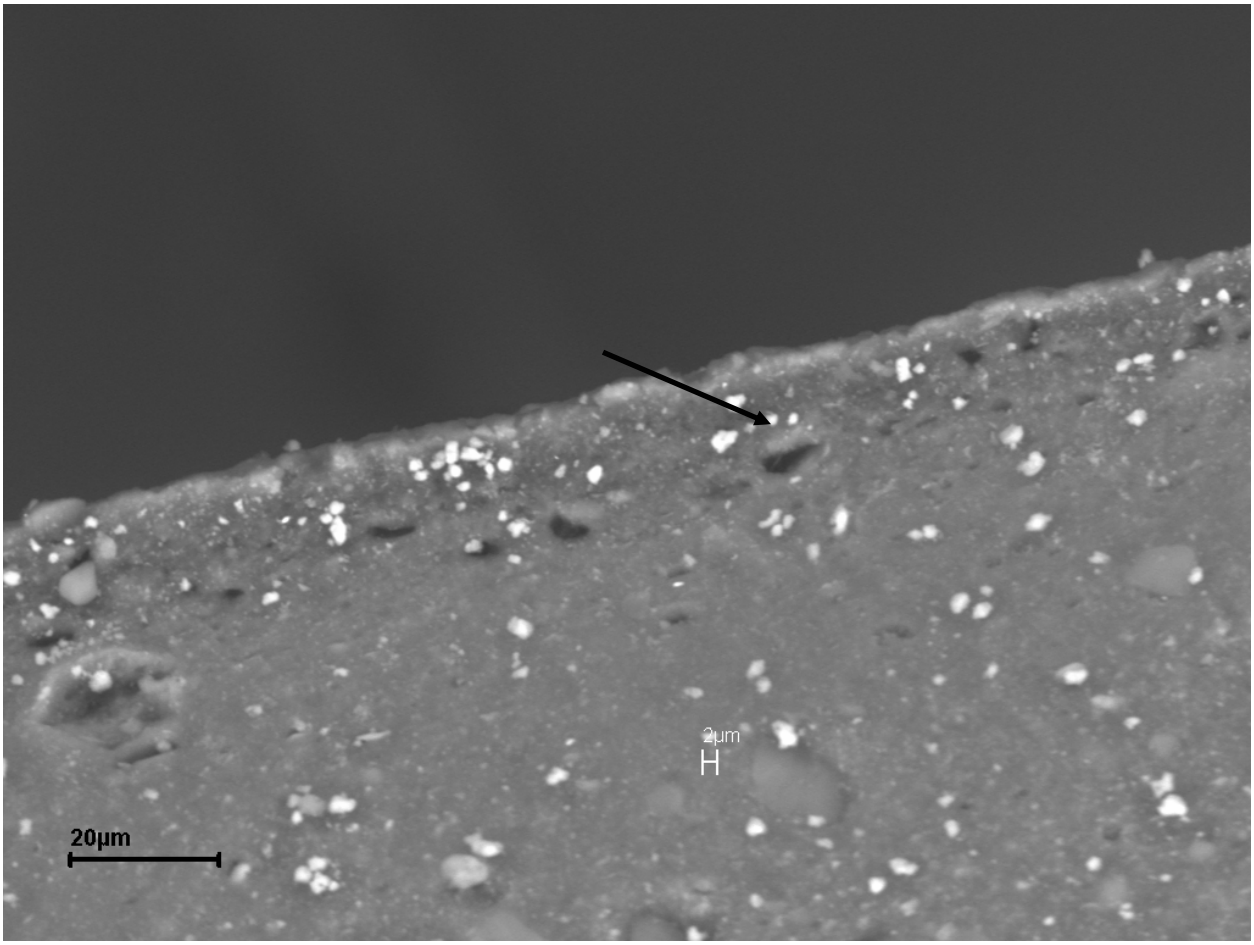

FIGURE 4.5: Scanning Electron Microscope image showing numerous large holes in the surface of used picker fingers.

Magnification =1,000
Arrows highlight holes in surface of rubber

TABLE 4.4: Log CFU/ finger piece recovered of *S.* Typhimurium attached to new and used rubber finger pieces soiled with chicken fat after washing

Soiling with fat	Washing with DI water	Bacteria attachment (Log CFU per finger)	
		New fingers	Used fingers
No	No	6.42[a]	6.40[a]
Yes (1 soiling cycle)	No	6.29[ab]	6.30[a]
Yes (1 soiling cycle)	Yes (1 washing cycle)	6.07[b]	6.22[a]
Yes (3 soiling cycles)	Yes (3 washing cycles)	6.37[a]	6.27[a]

Each value within the same column that has the same latter [(a-c)] are not significantly different ($P \geq 0.05$)

CHAPTER 5

EFFICACY OF CLEANING AND SANITIZING ON THE INACTIVATION OF *SALMONELLA* TYPHIMURIUM ATTACHED TO RUBBER PICKER FINGERS

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ABSTRACT

The effect of different sanitizers on the inactivation of *Salmonella* Typhimurium on rubber picker fingers in the presence of rendered fat was investigated. *S.* Typhimurium was inoculated onto new and used rubber pieces and allowed to attach for 3 hours. Inoculated rubber pieces were treated for 1 min with both heated (65±1^{o}C) and unheated (22± 2^{o}C) solutions of peptone water (0.1%), acidic electrolyzed (EO) water, modified EO (MEO) water (pH 5.8), sodium hypochlorite (50 ppm) solution, and unheated iodophor (25 ppm) and unheated 10% sodium hypochlorite solutions. Inoculated rubber fingers were also soiled with render chicken fat for 24 hours and then treated with cleaners followed by sanitizing agents. Finger pieces were first cleaned with either alkaline EO water or a commonly used chlorinated-alkaline cleaner (50% solution) by vortexing on low setting in 10 ml of treatment solution for 45 sec. After cleaning with alkaline EO water, either heated or unheated acidic EO water or heated MEO water were administered as sanitizers for 1 min. The combination of a 50% chlorinated-alkaline cleaner (cleaner) followed by heated 10% sodium hypochlorite solution (sanitizer) also was evaluated. Results indicated that heated acidic EO was the most effective sanitizing solution with less then 1 log CFU per rubber piece and non-detectable recovery of *Salmonella* on non-soiled new and used finger pieces, respectively. The presence of fat on the rubber fingers decreased the efficiency of all sanitizing agents. The chlorinated-alkaline cleaner and 10% sodium hypochlorite (cleaning followed with sanitizing) combination was the most effective treatment for inoculated fat coated fingers and achieved 4.88 log CFU per rubber piece reductions on *S.* Typhimurium.

INTRODUCTION

The defeathering step during poultry processing increases microbial loads on poultry carcasses. This increase in the microbial load has been illustrated with food borne pathogens such as *Campylobacter jejuni*, *Staphylococcus aureus* and *Salmonella* Typhimurium (Berrang et al., 2001; Kim and Doores, 1993a; Purdy et al., 1988). Rubber feather picker fingers used to remove feathers from broiler carcasses are potential cross-contamination source during poultry processing. Arnold (2007) noted microbial loads on used poultry fingers collected from processing facilities ranged from approximately 3 to 7 log CFU/cm ^{2} depending on plant sanitation practices (Arnold, 2007; Purdy et al., 1988). Studies have demonstrated that the rubber on new feather picker fingers can prevent bacterial attachment and biofilm formation due to attributes (antimicrobial activity of sulfur and zinc in rubber) of the rubber surface (Arnold, 2007; Arnold and Bailey, 2000; Kauffman, 1989). Although newer picker fingers exhibit resistant to bacteria attachment (Purdy et al., 1988), after continual use, cracks and crevices are formed in the rubber. In addition, cleaning and sanitizing agents have corrosive affects on the surface of rubber picker fingers causing softening and degradation over time (Storgards et al., 1999). These cracked and degraded surfaces can harbor bacteria making pickers harder to clean and disinfect (Purdy et al., 1988).

During feather removal, the epidermal tissue of the skin is ruptured and torn, and fat is released onto surfaces where it mixes with water from the sprayers to form a "fatty liquid film" on the surface of the carcasses (Lillard, 1986; Thomas and McMeekin, 1980). This can pose a huge problem since 3000-6000 birds can be defeathered per hour per line at poultry processing plants (Allen et al., 2003; Dodd et al., 1988; Purdy et al.,

1988). Soil accumulated on the surfaces of the rubber during processing may protect bacteria and decrease the effect of sanitizers (Berrang and Dickens, 2004). According to European Standards (EN 13697) (Anonymous, 2001), the bactericidal activity of a disinfectant should achieve 4 log reductions on both clean and soiled surfaces. The definition of a soiled surface is however, not reflecting the true situation of food processing since lipids are not included in the soil described and are not taken in account (Gram, 2007). Fat is an organic soil of concern because it is insoluble in water, and it can remain as a film on carcasses and contact surfaces, while other water soluble soils (e.g. blood and muscle) may be readily rinsed away. Gram (2007) reported that greased surfaces protect bacteria such as *L. monocytogenes* better than broth based soiling. Somers and Wong (2004) found bacteria biofilm on a variety of food contact surfaces were more readily inactivated than those developed in the presence of frankfurter and fat residues. This study further concluded that the contact surface itself may have more effect on bacteria reduction and resistance than the actual soil (Somers and Wong, 2004).

Bacterial attachment to different surfaces has been duly noted in many studies (Frank and Koffi, 1990; Krysinski et al., 1992; Sinde and Carballo, 2000). *S.* Typhimurium and *L. monocytogenes* have shown increased resistance to sanitizing agents when attached to rubber (Storgards et al., 1999). *S.* Typhimurium, a pathogen associated with poultry, is an organism that is difficult to eliminate due to its persistence throughout poultry processing environment. *S.* Typhimurium numbers have also been shown to significantly increase during defeathering (Berrang et al., 2001). This may be a result of the protective properties of the "fatty liquid film" that has been observed when this pathogen is attached to poultry skin (Kim and Doores, 1993b) and other surfaces.

Typical sanitation programs involve the use of detergents to remove soil and the application of sanitizers to inactivate bacteria (Ayebah et al., 2006). Dunsmore (1981) considered the detergent as the most important factor for controlling bacterial count. This is because detergents assist greatly in lowering the surface tension of water so that soils may be dislodged and loosen and suspend soil particles for subsequent flushing away during rinsing (Marriot, 1999). Alkaline cleaners are commonly used to clean organic soils due to their high pH which allows for neutralization of acidic soils, saponification of fat and lipids, and dispersion of oils and lipids on various surfaces. Iodophors (25 ppm) (Marriot, 1999) and sodium hypochlorite solutions (50 ppm to 12.5%) are common disinfectants used to sanitize rubber fingers. Higher concentrations than manufacture recommendations of commonly used cleaners and sanitizers are needed for porous surfaces such as rubber (Mafu et al., 1990a).

Acidic electrolyzed (EO) water is an innovative and highly effective chlorine-based sanitizer that is safer to produce and utilize than sodium hypochlorite solutions. The safety of EO water is a result of the use of sodium chloride as its source of chlorine, as compared to sodium hypochlorite, chlorine dioxide, and other chlorine based sanitizers. By passing a dilute concentration of sodium chloride through positive and negatively charged electrodes separated by a membrane, sodium chloride can be ionized into acidic and alkaline EO waters. Acidic EO water has high antimicrobial activity and can be used for sanitation. The primary active chlorine molecule in the acidic EO water is hypochlorous acid (HOCl), which is a weak acid that has a high oxidizing capacity. This sanitizer is effective for the reduction and the inactivation of bacteria such as *E. coli* O157:H7 (Len, 2001; Sharma and Demirci, 2003; Venkitanarayanan et al., 1999a), *L.*

Monocytogenes , Salmonella Enteritidis (Park et al., 2005; Venkitanarayanan et al., 1999a), *Bacillus cereus* (Len, 2001), and *Campylobacter jejuni* (Park et al., 2002) on various food substrates, such as poultry, and a variety of surface materials, such as plastic cutting boards (Venkitanarayanan et al., 1999b) and stainless steel (Park et al., 2002).

Alkaline EO water has a high pH which makes it a promising degreaser and soil remover. Alkaline EO water contains a caustic agent, sodium hydroxide that is commonly used as the alkaline builders for alkaline cleaners. The chemistry behind its usage is the high alkalinity (pH values ranging form 11-12) which allows for neutralization of acidic soils, saponification of fat and lipids, and dispersion oils and lipids on various surfaces (Marriot, 1999). The use of alkaline EO water as a degreaser or soil remover could be useful for poultry processing plants where acidic EO water is used as a sanitizing agent on carcasses and during spraying defeathering and immersion chilling applications. With large amounts of acidic EO water generated on site, there will be also be large amounts of alkaline EO water t available. Excess alkaline EO water can be stored and used as a soil remover and/or degreasing agent to allow insoluble organic matter to be removed and easily rinsed away during cleaning.

In a recent study done at the University of Pennsylvania, acidic EO water and alkaline EO water were used in combination to remove soil and inactivate bacteria that accumulate in milking pipelines. Surfaces commonly used in milking pipelines, (rubber rings, stainless steel, plastic pipeline) were soiled with milk and bacteria inoculum. Results showed that the combination of both acidic and alkaline EO waters effectively removed soil and inactivated bacteria (Walker et al., 2005). The same synergistic effect was observed when both EO waters were applied to *Listeria* biofilms attached to stainless

steel coupons and when washing shell eggs (Ayebah et al., 2005; Park et at., 2005). Ayebah et al. (2005) found that when *L. monocytogene*s biofilms were treated with acidic EO water alone, bacterial counts were reduced by more than 5 log on stainless steel surface. This effect was further enhanced by an additional 1.2 log reduction when biofilms were treated with alkaline EO water first, followed by acidic EO water (Ayebah et al., 2006).

The overall objective of this study was to evaluate the effect of different sanitizers to inactivate *Salmonella* Typhimurium on rubber picker fingers in the presence of fat.

MATERIALS AND METHODS

Sanitation experiment

Preparation of feather picker fingers

Used feather picker fingers (type E 2075, yellow) were collected from a local poultry processing plant in Athens, GA and transported to laboratory in plastic bags. Fingers were then washed with a detergent (Alcojet, Alconex, Inc., White Plains, NY) in an automatic dishwasher twice on regular cycle. After cleaning, used fingers were inspected for visible cracks and crevices. In general, used fingers have smooth surfaces due to ring deterioration with few visible cracks. To ensure uniformity, used fingers with large (over 2 rings) observable cracks and/or irregularities in the surface were not used. New rubber fingers (of the same type) were obtained from William Goodyear Company (Durham, NC) and were also washed as stated above. Both new and used fingers were cut with a surgical scissor into approximately 1 cm (3 rings) pieces (excluding the ring at the tip). The pieces were submerged in deionized (DI) water, sterilized by autoclaving at 121^{o}C, and allowed to cool over night.

Treatment solutions

Acidic EO waters was generated using a ROX-20TA machine (Hoshizaki Electric Inc, Toyoak, Aichi, Japan) at 18 A to obtain a chlorine concentration of approximately 45-50 ppm. Modified EO water was made by adjusting the pH of acidic EO water to a pH of approximately 5.8 with a 10 molar NaOH solution. A solution of 10% sodium hypochlorite (Clorox, Oakland, CA) was made by diluting with sterile DI water. A 50 ppm sodium hypochlorite solution was also made by adding 1 ml of commercial bleach (Clorox, Oakland CA) to 1 L of sterile DI water. A common iodine cleaner/sanitizer, Zepidine (Zep Cleaning Company, Atlanta, GA), was diluted to 25 ppm according to manufactures recommendations. Peptone water (1 g of peptone per liter of DI water) (pH of approximately 7) was used as a control. All treatment solutions were prepared at room temperature (22 ± 2^{o}C). Acidic EO water, modified EO water, 50 ppm sodium hypochlorite solution and peptone water were also heated in a water bath to 65^{o}C (Model WB1120-A, Lindberg/Blue M, Ashville, NC) after preparation and held until use. To preserve the chlorine content, the solutions were collected in glass bottles with no air space and capped tightly before heating (Bialka et al., 2004). The oxidation-reduction potentials (ORP) and pH of all treatment solutions were measured using a dual scale pH meter (Accumet model 15, Fisher Scientific Co., Pittsburgh, PA). The total chlorine concentration was determined by the iodometric method using a total chlorine test kit (Hach Co., Ames, IA). The iodine concentration of the Zepidine solution was determined by the iodine test kit method (LaMotte, Chestertown, MD). Properties of solutions were also measured after heating (Table 5.1.)

Preparation of inocula

S. Typhimurium was obtained from the culture collection of the United States Department of Agriculture (USDA), Russell Research Center, Athens, GA. Two hundred µl of frozen aliquots (-70^{o}C) of *S.* Typhimurium was cultured overnight in 10 ml of trypticase soy broth (TSB) (Becton Dickerson, Sparks, MD) at 37^{o}C. After 18 hours, 100 µl of the culture was transferred into 10 ml of fresh TSB in 50 ml centrifuge tubes and incubated at 37^{o}C on a shaker at 90 rpm (Model 6576D, New Brunswick Scientific, Edison, NJ)**(**Sinde and Carballo, 2000) for another 18 hours. Ten ml *S*. Typhimurium cultures with a population of approximately 9.3 log CFU/ml of TSB were used as inoculum of rubber finger pieces.

Inoculation of rubber fingers

Sterile rubber finger pieces were placed into each inoculation tube and allowed to incubate for 3 hours at 37^{o}C on a shaker at 90 rpm to allow for bacterial attachment. After attachment, the inoculum were poured out of tubes. Each finger piece was rinsed twice with approximately 5 ml of peptone water (0.1%). The fingers were then transferred to clean petri dishes and allowed to dry under a biosafety hood for 30 min.

Treatment of inoculated rubber fingers

Thirty ml of each treatment solution were poured into 50 ml centrifuge tubes. Each inoculated finger piece was submerged into the solution for 1 min after vortexing for approximately 2 sec. Heated treatment solutions were held in a water bath at 65^{o}C to keep the treatment temperatures constant for the duration of the treatment. After treatments, solutions were decanted. Three inoculated fingers were used per treatment and the experiment was replicated 3 times.

To enumerate attached *S.* Typhimurium cells, the rubber fingers were placed in a medium size sterile stomacher bag containing 45 ml of sterile neutralizing buffer (Becton Dickerson, Sparks, MD). The fingers were then stomached on high setting in a stomacher (Model 80 Laboratory Blender, Sew and Limited, London, UK) for 2 min. During preliminary studies, inoculated rubber samples were viewed before and after stomaching procedures by use of a fluorescent microscope (Nikon Eclipse E600, Southern Micro Instruments, Marrietta,GA). Results demonstrated adequate removal of attached bacteria from surface with approximately one cell per image remaining after stomaching procedures (data not shown). Serial dilutions of neutralizing buffer were made after stomaching. The surviving bacteria from the control and treated rubber pieces were enumerated by plating 0.1 ml of diluents on tryptic soy agar (TSA) (Becton Dickerson, Sparks, MD). Plates were incubated at 37^{o}C for 48 hours, and colonies were counted and recorded as log CFU per rubber piece. The neutralizing buffer was also enriched for the presence of surviving *S.* Typhimurium cells by adding one ml of neutralizing buffer to 9 ml of TSB and incubating at 37^{o}C for 24 hours. Tubes that exhibited growth were streaked unto *Salmonella spp.* selective agar XLT4 (Becton Dickerson, Sparks, MD) and incubated for 24 hours at 37^{o}C and the presence of *Salmonella* typical colonies were noted.

Cleaning and sanitizing procedures

Preparation of feather picker fingers

New and used fingers were obtained and washed as previously described. However, fingers were cut into approximately 4 cm (12 rings) pieces by use of a Model 14 band saw (Rockwell International Inc., Pittsburg, PA) from the top portion of the

finger (Figure 3.1). On the day of experiment, fingers were immersed in ethanol for 5 min to remove fat or oil residue. Pieces were allowed to dry under air flow hood until use.

Inoculation of rubber pieces

S. Typhimurium inoculum were prepared as described above except 20 ml of *S.* Typhimurium cultures with a population of approximately 9.0 log CFU/ml of TSB were used to inoculate rubber finger pieces.

New and used rubber finger pieces were placed into each inoculation tube containing 20 ml of inoculum and allowed to incubate for 3 hours at 37^{o}C on a shaker at 90 rpm to allow for bacterial attachment. After attachment, the inoculum were decanted and each finger piece was rinsed twice with approximately 10 ml of peptone water (0.1%). The fingers were then transferred to sterile petri dishes and allowed to dry under a biosafety hood for 30 min.

Soiling of inoculated fingers

Frozen rendered chicken fat (Tip Top Poultry, Atlanta, GA) was obtained and stored at -70^{o}C until use. The fat was allowed to warm to room temperature (22 $\pm$ 2^{o}C**)** at least 48 hours before experiment**.** The inoculated finger pieces were coated with approximately 3 ml of liquid chicken fat by using a sterile pipette. This procedure was modified from earlier studies due to potential cross contamination with continual reuse of the same soiling fat. During soiling, each finger was gently slanted over a sterile petri dish and 1.5 ml of fat was applied to each side of finger. Excess fat was allowed to drip off of finger for 10 sec, leaving approximately 1$\pm$ 0.2 g of fat on finger. The fat coated

finger was then placed into a sterile petri dish, covered and allowed to sit in biosafety hood at room temperature (22 ^{o}C $\pm$ 2) for 18 hours before treatment.

Treatment preparation

A chlorinated-alkaline cleaner (Chill Kleen, Duchem, Atlanta, GA) commonly used for cleaning rubber fingers and alkaline EO water were used as cleaning solutions in this study. Cleaning solutions were used as single treatments and as combination treatments with select sanitizing agents. Alkaline EO water and acidic EO water (45-50 ppm chlorine, pH 2.6, and ORP 1157 mV) were generated at a setting of approximately 17 A simultaneously. Alkaline EO water had a pH of approximately 11.5 and ORP level of -820 mV at the time of generation. The chlorinated-alkaline solution was prepared by mixing with sterile DI water. To mimic common industrial cleaning practices, the chlorinated-alkali solution (50%) was used as a cleaning agent followed by a sodium hypochlorite solution (10 %) to sanitize the fingers. Poultry plants commonly use concentrations of cleaners and sanitizers in excess of concentrations recommended by manufactures. Recommended levels for alkaline cleaners range from 0.5% to 5% at temperatures of 60^{o}C or higher (Farrell et al., 1998; Somers and Wong, 2004). Recommend levels for chlorine based sanitizers range from 200 pm on a non-porous surface to 800 ppm on a porous surface (Mafu et al., 1990; Marriot, 1999). Higher concentrations of the chlorinated-alkali and sodium hypochlorite cleaners were used in this study to evaluate the efficacy of actual solution concentrations that were used during cleaning in a local poultry plant. Alkaline EO water in combination with acidic EO water (both heated and unheated) and modified EO water (heated) were also evaluated. Modified EO water was produced by adding NaOH (10 molar) solution to acidic EO

water (pH approximately 2.5) to adjust the pH to 5.6-5.8. A double combination of water-water (DI) treatment was used in this study as a control. The oxidation-reduction potentials (ORP) and pH levels of all solutions were measured using a dual scale pH meter (Accumet model 15, Fisher Scientific Co.). Treatment solutions were heated in closed glass containers in a water bath at 65^{o}C and held until use unless stated other wise. The total chlorine concentration of chlorine solutions was determined by the iodometric method using a total chlorine test kit (Hach Co., Ames, IA) before and after heating.

Treatment application

The inoculated and soiled fingers were washed in cleaning solutions by placing the finger in a 50 ml centrifuge tube containing 10 ml of cleaning solution and vortexing on low setting for 45 sec. The cleaning solutions were then decanted and the finger was then immersed in 40 ml of sanitizer for another 1 min. The sanitizer was also discarded after treatment before microbial analysis of rubber sample.

To enumerate attached S. Typhimurium cells, the rubber fingers were placed in a medium size sterile stomacher bag containing 80 ml of neutralizing buffer. The fingers were then stomached on high setting in a stomacher (Model 80 Laboratory Blender, Sew and Limited, London, UK) for 2 min. Serial dilutions of the neutralizing buffer were made after stomaching. The surviving bacteria were enumerated by plating 0.1 ml diluents on TSA. Plates were incubated at 37^{o}C for 48 hours and colonies were counted and recorded as log CFU per rubber piece. Two samples were treated by each solution/combination treatment and the experiment was replicated 3 times.

Data analysis

Data were analyzed using the general liner model (GLM) procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.). Comparisons of means were performed using Duncan multiple range test. All statistical analysis was done at $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Sanitizing

The sanitizing agents used in this study exhibited a broad range of properties. Acidic EO water had a pH of 2.57 and ORP of 1157.33 mV. Acidic EO water, modified EO water (pH of 5.83 and ORP of 941mV) and the sodium hypochlorite solution (pH of 8.89 and ORP of 764.9 mV) had similar chlorine concentrations of 47.07, 47.13 and 47.67, respectively, at room temperature (22±2^{o}C). However, after heating, acidic EO water's chlorine concentration decreased while modified EO water and sodium hypochlorite solution (50 ppm) concentrations remained the same (± 2 ppm)(Table 5.1).

Unheated sanitizers

When analyzing the effect of sanitizers on the inactivation of *S.* Typhimurium attached to rubber fingers without soil, unheated treatment solutions were less effective than heated (Table 5.2). The total number of bacteria attached to rubber fingers were about 6.8 log CFU per finger (peptone water). The number of CFU per finger recovered from peptone water was significantly higher (6.80 to 6.91 log CFU) than all other treatments. All room temperature chlorine treatments (acidic EO water, modified EO water and 50 ppm sodium hypochlorite solution) worked at the same efficiency level with a 1.5 to 1.7 log reductions when compared to peptone water treatments. The iodophor

had significantly lower reduction than EO water and was the least effective sanitizer with the highest log recovery. The 10% sodium hypochlorite solution was the most effective unheated treatment with approximately average reductions of 4.81 and 4.28 log CFU per finger on new and used fingers, respectively (Table 5.2). This may be due the higher chlorine concentration (5364 ppm) (Table 5.1).

Results demonstrates that the sanitizing of rubber picker fingers with room temperature iodophors at their suggested concentrations (25 ppm) (Marriot, 1999) may be inadequate. This same ineffectiveness by iodophor and chlorine sanitizers at manufactures recommended levels was also demonstrated by Krysinski (1992) on stainless steal surfaces. In addition, Krysinski (1992) found that none of the sanitizers (including acid quaternary ammonia, peracetic acid, and chlorine dioxide) were effective at sanitizing the surface of polyester/polyurethane. This may be an indication of the inability of these sanitizers to inactivate attached *Salmonella spp*. Ronner and Wong (1993) showed that *S.* Typimurium biofilms were not completely inactivated by iodophors, quaternary ammonium, or other sanitizers although sanitation with chlorine resulted in lower recovery.

Both acidic EO water and 10% sodium hypochlorite solution achieved higher reduction on new fingers than used fingers (Table 5.2). This may be a result of bacteria being present in the cracks and crevices of the rubber surfaces which may shield them from the sanitizers on used rubber fingers.

Heated sanitizers

It is evident that the temperature of the sanitizer and the nature of the food surface affects the performance of sanitizing agents (Mafu et al., 1990b). Gelinas (1984) also

noted that an increase in temperature greatly enhances sanitizing activity profile. Heated peptone water reduced the population of bacteria by approximately 2 logs (Table 5.2). The number of bacteria recovered from samples treated with heated acidic EO water, modified EO water and 50 ppm sodium hypochlorite solution were not significantly different from one another but were significantly lower than the heated peptone solution. There was a significant difference in sanitizing effect of heated modified EO water on new and used fingers. Modified EO water exhibited better inactivation on used fingers than new, with log reductions of 6.28 per finger verses 5.41 logs per finger, respectively. Heated acidic EO water lost some of it chlorine content (8 ppm) (Table 5.1) during heating, however, it was one of the most effective treatment with less then 1 log (0.83) recovery on new fingers and achieved undetectable levels on used (Table 5.2). This may be due to the low pH value (2.3) of acidic EO water, which allows for more active species of hypochlorous acid to be present in solution and its high oxidation-reduction potential (1157mV) (Table 5.1) that actively oxidizes (remove electrons) bacterial cells when compared to the heated 50 ppm sodium hypochlorite solutions properties (pH of 9.23 and ORP of 712.2 mV).

Although there were clear differences in the reduction of *S.* Typhimurium on new and used picker fingers due to different sanitizers, there was not a significant difference between new and used fingers on overall inactivation (data not shown). Results suggested that sanitation of rubber fingers can work at the same efficacy on new and used fingers that are adequately cleaned (without soil) before sanitation (Table 5.2).

Cleaning and sanitizing

In this study, cleaning and sanitizing efficiency on fat soiled fingers inoculated with *S.* Typhimurium were examined. Results from the previous study illustrated the increased ability of heated sanitizers to reduce bacteria attached to finger surface, therefore, the most effective sanitizers were employed at 65^{o}C for this study. Data from a previous study showed that after 24 hour soiling, 30-50% of the fat remains in and/or on the finger surface after washing (Table 4.3). These values were used to estimate the amount of fat that remains on finger after cleaning before sanitizing practices.

S. Typhimurium cells attached to the surface of soiled fingers at an average 5.54 and 6.29 log CFU per rubber piece for new and used fingers, respectively (Table 5.3). With the exception of cleaning with alkaline EO water, used fingers had lower reduction than new fingers after treatment (Table 5.3). This may be due to cracks and crevices that are present in the rubber or the present of larger pores on surface of rubber of used fingers, which can provide places for bacteria to hide. Cracks and crevices form in the rubber during continual usage, however, large pores in used rubber fingers may also be a result of softening and surface corrosion of rubber by cleaning agents (Storgards et al., 1999). Although there were significantly larger amounts of bacteria recovered on the used fingers, the log reduction of bacteria by treatment solutions was approximately the same for both new and used fingers (Table 5.3). This is primarily due to the initial population of bacteria on used fingers being significantly higher (6.29 logs) than new fingers (5.54 logs) (Table 5.3). This demonstrated that used fingers surfaces may be easier to attach for *S.* Typhimurium cells than new. The similarities in log reduction by cleaning and sanitizing practices on both new and used fingers demonstrated that there is

no significant difference in cleaning efficacy due to rubber finger usage. Although used fingers had similar log reduction after cleaning as new fingers, used fingers still had higher total number of bacteria on fingers than the new finger after cleaning. This is important because fingers are individually replaced sanitation personal identifies that finger as cracked or deformed (Arnold, 2007). This can lead to used fingers being easily overlooked harboring large amount of soil and embedded microorganisms that can be a potential source of contamination of other poultry carcasses down the line.

Cleaner applications with alkaline cleaners demonstrated a significant log reduction when administered alone. The chlorinated-alkaline solution achieved a significantly higher reduction than alkaline EO water with an approximately 4 log reductions on both new and used fingers compared to Alkaline EO water's 1.6 log reductions. This bacterial reduction by both alkaline cleaners may be attributed to the synergistic effect of high pH (>11) and elevated temperature. The combination of high pH and elevated temperatures increases the efficiency of these cleaners ability to remove soil which results in better sanitation of contaminated surfaces, was also reported by Teo (1999) for animal skin. Literature has noted solutions such as caustic sodas (sodium hydroxide and potassium hydroxide), trisodium phosphate, and other cleaners with pH above 11, have been successful in the destruction of gram negative microorganisms when heated (Dickens, 1994; Humphrey, 1981; Teo, 1996). Ten percent NaOH and KOH solutions can reduce *S.* Typhimurium by 4 logs on lean and beef fat tissues (Humphrey, 1981). Coppen (1993) demonstrated that these solutions were effective because of the removal of the fatty layer from the animal skin and their intrinsic high pH. The chlorinated-alkaline solution's significant reduction effect can be attributed to its higher

pH (13.5) when compared to alkaline EO waters (11.5). In addition, the chlorinated-alkaline cleaner is primarily made up of potassium hydroxide with the addition of sodium hypochlorite, which is the active agent in commercial bleach, thus increasing its sanitizing effect. Alkaline EO water, on the other hand, is a dilute sodium hydroxide solution.

The sequential treatments of DI water served as the control for the cleaning-sanitation combination. This control exhibited about 1 log reduction from the initial attachment amounts. The alkaline EO water and acidic EO water (heated and unheated acidic EO water and heated modified EO water) cleaning-sanitizing combinations all work at the same efficiency when compared to one another. Regardless of the type of acidic EO water used, all EO water treatment combinations exhibited an additional 1-1.5 log reductions after the application of alkaline EO water. However, when modified EO water was utilized on used fingers, there was not significant difference than the alkaline alone application. Data suggested that modified EO water may have little to no effect on the reduction of *S.* Typhimurium on fat coated used fingers although results from earlier study indicated (Sanitizing study, Table 5.2) that it was effective in the absence of fat. Acidic EO waters (both heated and unheated) exhibited diminished inactivation capacity in the presence of fat. Ayebah et al. (2006) noted the decrease in efficiency of acidic EO water in the presence of organic matter (chicken serum) on the inactivation of *L. monocytogenes.* In the absence of serum, acidic EO water (chlorine concentrations of 44 ppm and 85 ppm) produced a >6 log reductions in *L. moncytogenes* cells after 1 min treatment. However, in the presence of 5 ml of serum per liter of acidic EO water, bacterial reductions were decreased to 0.33 log (Ayebah et al., 2006).

The chlorinated-alkali solution's sanitizing ability was not significantly increased with the addition of the 10% sodium hypochlorite treatment (Table 5.3). This may be due to the hindering capabilities of absorbed fat that remains in finger even after cleaning. The finger also may provide better sanitizing protection with fat present because bacteria may be logged inside of the rubber, covered by fat, and shielded from sanitizer. The fat may hinder sanitizing by decreasing chlorine efficiency due to chlorine compounds binding with the organic matter. The lower efficiency of alkaline and acidic EO water combinations may also be a result of the neutralizing effect of the cleaner on the sanitizer.

CONCLUSIONS

Results suggested that iodophor sanitizers may not be effective at the manufacturer recommended temperature (22 ±2^{o}C) and concentration (25 ppm) to inactivate *S.* Typhmurium attached to rubber picker fingers. In addition, unheated chlorine solutions at 50 ppm were also less effective than heated solutions to inactivate S. Typhimurium. Acidic EO water, when heated, resulted in the lost of some of its chlorine content, however, its low pH and high ORP contributed highly to is disinfection capabilities. Heated acidic EO water was the most effective sanitizer overall, with below the detectable level of bacteria recovered on used fingers after treatments. Results suggested that sanitizing agents should be heated or used at higher concentrations, such as 10% sodium hypochlorite, to give adequate reductions ($\geq$4 log) of bacteria.

Chlorinated-alkaline cleaners are effective at high concentrations. However, fat may play a significant role in the inactivation of bacteria by chlorine sanitizers even when heated. New and used fingers exhibited the same levels of log reductions of bacteria after the combined cleaning and sanitizing treatments. However, significantly higher numbers

of bacteria remained on used fingers after sanitation treatment. This is a result of the higher number of bacteria that were attached to used finger surfaces than new which also indicates that *S.* Typhimurium cells may more readily attach to used rubber fingers than newer ones. This is important factor because although cleaners and sanitizers may work at the same efficiency on both new and used fingers, used fingers may pose a greater threat to cross-contamination with higher microbial loads being present.

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TABLE 5.1 Average properties of treatment solutions

Treatment	pH	ORP (mV)	Concentration of active agent (ppm)
Peptone	6.77	361	Na
Iodine	2.49	569.5	25 (Iodine)
Sodium hypochlorite (50 ppm)	8.89	764.9	47.67
EO water	2.57	1157.3	47.07
Modified EO water	5.83	941.1	47.13
10% Sodium hypochlorite	11.14	678.8	5364.38
Heated peptone	6.94	574.0	Na
Heated Modified EO	5.69	941.4	45
Heated Sodium hypochlorite (50 ppm)	9.23	712.2	49.95
Heated acidic EO water	2.30	1148.0	38.9

All values represent total chlorine concentration unless indicated otherwise

TABLE 5.2: Average log CFU of *S.* Typhimurium per rubber piece recovered after sanitizing treatment

Treatment	New finger		Used finger	
	Log CFU recovered	Log CFU reduction	Log CFU recovered	Log CFU reduction
Peptone	$_{A}6.80^{a}$	0	$_{A}$ 6.91^{a}	0
Iodine	$_{A}$ 5.72^{b}	1.08	$_{A}$ 5.97^{b}	0.94
Sodium hypochlorite (50 ppm Cl)	$_{A}$ 5.32^{bc}	1.48	$_{A}$ 5.42^{bc}	1.49
EO water	$_{B}$ 4.88^{c}	1.92	$_{A}$ 5.50^{bc}	1.41
Modified EO water	$_{A}$ 5.01^{bc}	1.79	$_{A}$ 5.28^{c}	1.63
10% Sodium hypochlorite	$_{B}$ 1.99^{d}	4.81	$_{A}$ 2.63^{e}	4.28
Heated Peptone	$_{A}$ 4.73^{c}	2.07	$_{A}$ 4.61^{d}	2.30
Heated Modified EO	$_{A}$ 1.39^{de}	5.41	$_{B}$0.65^{f}	6.26
Heated Sodium hypochlorite (50 ppm Cl)	$_{A}$ 0.78^{e}	6.02	$_{A}$ 1.19^{f}	5.72
Heated EO	$_{A}$ 0.83^{e}	5.97	$_{B}$ ND^{g} (0/6)	NA

Columns with the same letter $^{(a\text{-}e)}$ after the mean values are not significantly different ($P \geq 0.05$)
Rows with the same letter $_{(A\text{-}B)}$ before mean values are not significantly different ($P \geq 0.05$)
Numbers in parenthesis represent the number of positive enrichments per total number of enrichments analyzed.

TABLE 5:3*:* Average log CFU of *S*. Typhimurium per rubber piece recovered after treatments

Cleaning treatment (65° C)	Sanitizing treatment	New		Used	
		Log CFU recovered	Log CFU reduction	Log CFU recovered	Log CFU reduction
No	No	$_{B}$ 5.54 ^{a}	0	$_{A}$ 6.29 ^{a}	0
Alkaline EO water	No	$_{B}$ 3.94 ^{bc}	1.60	$_{A}$ 4.61 ^{bc}	1.68
Chlorinated-alkali (50%)	No	$_{B}$ 1.52 ^{e}	4.02	$_{A}$ 2.37 ^{f}	3.92
Water	Water (65 °C)	$_{B}$ 4.47 ^{b}	1.07	$_{A}$ 5.40 ^{b}	0.89
Alkaline EO	Acidic EO (23± 1 °C)	$_{A}$ 3.07 ^{cd}	2.47	$_{A}$ 3.39 ^{d}	2.15
Alkaline EO	Acidic EO (65 °C)	$_{B}$ 2.58 ^{d}	2.96	$_{A}$ 3.89 ^{cd}	2.4
Alkaline EO	Modified EO (65 °C)	$_{B}$ 3.23^{cd}	2.31	$_{A}$ 4.35 ^{c}	1.94
Chlorinated-alkali (50%)	Sodium hypochlorite (10%) (65 °C)	$_{B}$ 0.86 ^{e}	4.68	$_{A}$ 2.00 ^{f}	4.21

Columns with the same letter $^{(a\text{-}e)}$ are not significantly different ($P \geq 0.05$)
Rows with the same letter $_{(A\text{-}B)}$ are not significantly different ($P \geq 0.05$)

CHAPTER 6

SUMMARY AND CONCLUSIONS

Results from these studies indicate that rubber picker fingers can serve as potential cross contaminated sources due to the “fatty film” that forms on the rubber fingers. The large amount of fat that is absorbed into fingers between daily cleaning can serve as a major contributor to the harboring and protection of pathogens such as *Salmonella* Typhimurium from adequate inactivation by chemical sanitizers. Current practices (spraying and brushing) and chemicals used (chlorinated-alkaline cleaners and sodium hypochlorite) can remove the surface fat and bacteria; however, evidence in this study has demonstrated that absorbed fat can shield *S.* Typhimurium from inactivation and stresses the importance of sufficient cleaning practices. Cleaning efficiency may be increased with more frequent cleaning during processing shifts and adequate removal of used finger from picker machines. Due to the increase percentage of fat accumulated on finger with increasing elapsed times before cleaning, cleaning every 4 hours with an chlorinated-alkaline detergent may be needed to achieve adequate fat removal form rubber picker fingers. Acidic EO water may be a promising sanitizer when heated, but showed diminished effectiveness in the presence of fat. This was most likely caused by inadequate removal of chicken fat by alkaline EO water and the inactivation of chlorine in the presence of organic matter. Further research needs to be done to strengthen the effectiveness of alkaline EO water’s cleaning capacity.