**Inactivation of *Staphylococcus saprophyticus* in Chicken Meat and
Purge Using Thermal Processing, High Pressure Processing,
Gamma Radiation, and Ultraviolet Light (254 nm)**

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Abstract

Staphylococcus saprophyticus is a common contaminant in meat and poultry, and causes urinary tract infections after colonization of the gastrointestinal tract, followed by accidental transfer of contaminated feces to the urethra. There is limited information regarding the inactivation kinetics of *S. saprophyticus* in meat and poultry. When *S. saprophyticus* was suspended in ground chicken meat (GCM) the thermal processing D_{10} was 6.26, 0.60 and 0.09 min at 55, 60 and 65 °C, respectively. When *S. saprophyticus* was inoculated into GCM and subjected to high pressure processing (5 °C, 0 - 25 min) at 200, 300 or 400 MPa the HPP D_{10} was 15.5, 9.43, and 3.54 min, respectively. When the *S. saprophyticus* cocktail was inoculated into GCM and irradiated (5 and -20 °C) the gamma radiation D_{10} were 0.64 and 0.77 kGy, respectively. When *S. saprophyticus* was inoculated into chicken purge which was then placed on food contact surfaces including stainless steel, and high density polyethylene and polypropylene and treated with UV-C (0-60 mJ/cm²) the UV-C D_{10} ranged from 14.9 to 18.5 mJ/cm². These results indicate the inactivation kinetics for *S. saprophyticus* are consistent with those for other foodborne pathogens and could be controlled in poultry meat and purge without difficulty.

Keywords: *S. saprophyticus*; urinary tract infection; thermal processing; high pressure; gamma radiation; ultraviolet light; chicken meat; purge.

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1. Introduction

Urinary tract infections (UTI) account for ca. 10 million illnesses in the United States annually as well as 100, 000 hospitalizations and ca. 23,000 deaths (Flores-Mireles et al., 2015; Nordstrom et al., 2013). Recent food microbiology research has focused on the incidence and prevalence of emerging foodborne pathogens in meat and poultry such as Uropathogenic *Escherichia coli* (UPEC), *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus* which do not cause diarrheal illnesses, but instead cause urinary tract infections (Davis et al., 2015; Mitchell et al., 2015; de Sousa et al., 2013; Poulsen et al., 2012;). Public health policy and consumer groups have called for increased study of these often antibiotic resistant foodborne uropathogenic bacteria, and their relationship to illness in humans (Bennington-Castro, 2016; PCAST, 2014; PEW Commission, 2013).

S. saprophyticus is genetically distinct from *S. aureus*, and possesses different virulence factors (Flores-Mireles, 2015). *S. saprophyticus* causes ca. 5 % (500, 000 cases) of UTI annually in the U.S., is harmless in the gastrointestinal tract, but cause illness when they are accidentally transferred from the anus, via feces, to the urethra, bladder and kidneys (Raz et al., 2005; Rupp et al., 1992; Hedman et al., 1991; Latham et al., 1983). Isolates which cause UTI typically match those from the individual's own feces. *S. saprophyticus* is also a common contaminant in meat and poultry products, although the fraction of uropathogenic types among the total population is uncertain (Goja et al., 2013; Gundogan et al., 2013; Marino et al., 2010; Coton et al., 2010; Hedman et al., 1990).

The Centers for Disease Control and Prevention (CDC, 2013) has recommended foods treated with processing technologies such as Thermal processing (TP), High Pressure Processing (HPP) and Gamma Radiation (GR) for people who are “at risk” for illness because of underlying

99 medical conditions. These technologies, including ultraviolet light (UV-C), are of particular
100 interest to the meat processing industry (Salvage, 2014) as they are sustainable and do not
101 require the use of exogenous water during food processing. Thermal processing kills
102 microorganism through denaturation of proteins and other cellular components (Smelt and Brul,
103 2014). HPP subject food to an elevated pressure of 100–1000 MPa typically at temperatures
104 below 60°C. The mechanism by which HPP inactivates foodborne pathogens include; ribosome
105 dissociation, enzyme inactivation, cell membrane and structure damage, and dissociation of
106 DNA (Ganzle et al., 2015). GR kills bacteria by damaging their DNA indirectly through
107 radiolysis of water and induction of oxidative damage or direct damage through breakage of the
108 phosphodiester backbone in addition to oxidative damage to proteins and cell membranes (Diehl,
109 1995). TP, HPP, and GR are the three primary commercialized technologies which can kill
110 bacteria internalized within food matrices such as ground meat and poultry, thus determination of
111 inactivation kinetics for emerging foodborne pathogens is essential.

112 UV-C kills microorganisms through formation of 6-4 photoproducts and cyclobutane
113 pyrimidine dimers in their DNA in addition to causing protein damage (Rastogi et al., 2010;
114 Krisko and Radman, 2010). Unlike the previously mentioned technologies UV-C is primarily
115 used for decontamination of food surfaces, food contact surfaces, and UV-C transmissible
116 liquids, as these photons have limited penetration ability and are affected by both particulate
117 matter and shadowing affects.

118 Uropathogenic microorganisms in foods are now receiving scrutiny from scientific policy
119 and consumer groups. The purpose of this study was to determine the TP, HPP and GR
120 inactivation kinetics of *S. saprophyticus* in ground chicken meat; and 2) determine the

inactivation kinetics of *S. saprophyticus* on poultry meat surfaces and in poultry meat purge on food contact surfaces using ultraviolet light (UV-C).

2. Materials and Methods

2.1. Chicken

Ground chicken (95% lean) was freshly prepared and purchased from a local wholesaler (Lansdale, PA) and evenly portioned into 90 g aliquots in polynylon pouches (Uline, Inc., Philadelphia, PA), vacuum sealed to 50 millibars using a Multi-Vac A300 packager (Multi-Vac Inc., Kansas City, MO) and then frozen (-70 °C). Chicken purge was obtained from a local poultry processor and frozen (-70 °C) until ready for use. The samples was tested for presence of *Staphylococci* as described below and was found to be ca. 1 log CFU/g after freezing.

2.2. Staphylococcus saprophyticus isolates

Isolates 49907, 3552, 49453, BAA-750 and 15305 were obtained from the American Type Culture Collection (Manassas, VA). A multi-isolate cocktail was used as recommended for appropriate validation of nonthermal processing technologies (NACMCF, 2006).

2.3. S. saprophyticus growth and inoculation

The *S. saprophyticus* isolates were cultured independently in 10 ml Tryptic Soy Broth (TSB) without dextrose to avoid development of acid resistance (BD-Difco, Sparks, MD) using 50 ml sterile tubes at 37°C (150 rpm) for 18 - 24 h using a New Brunswick Model G34 Environmental Shaker (New Brunswick, Edison, NJ). The bacteria were then sedimented by centrifugation (1,200 x g, Hermle Model Z206A, Hermle Labortechnik, Germany) and resuspended as a cocktail in 20 ml sterile 0.1 % peptone water (SPW, BD-Difco).

Thawed ground chicken was aliquoted (10 g) into 2 oz. Nasco (Ft. Atkinson, WI) Whirl-Pak bags and inoculated with 0.1 ml of *S. saprophyticus* cocktail, mixed manually for 1 min, and then sealed using the Multi-Vac A300 Packager. The final concentration of *S. saprophyticus* in the

ground chicken was ca. 8 log CFU/g. The sample bags were then sealed in a second bag and stored at 5°C for 1 hr prior to HPP treatment and irradiation (refrigerated) or 2 days (-20 °C) for gamma irradiation. One g samples were used for thermal inactivation experiments.

2.4. High pressure processing treatment

The HPP process is based on our well established and previously published methodology (16). HPP was performed in a laboratory scale pressure unit (Mini Food lab FPG5620, Stansted Fluid Power Ltd., Essex, UK), which comprised of a double-jacketed thick-wall stainless steel cylinder (approximate volume of 0.3L) having an internal stainless steel sample holder of 25.4 mm × 254 mm (diameter × length). The thick-wall cylinder was maintained at a set-point temperature in which heat transfer fluid continuously circulated from a refrigerated liquid chiller (Proline RP 855, Lauda, Germany). The HPP unit with packed samples in the sample holder, locked onto the breech nut, then, lowered in product chamber was shown by Hsu et al. (2015, 2014). The refrigerated chiller was set at 4°C which indirectly cooled the pressure transmitting medium (a mixture of ethylene glycol and water, 50/50 v/v, 2.3 l/min flow rate). The pressure come-up rate was 6.67 MPa/s and the release rate was 11.11 MPa/s. Samples were pressure-treated at 200, 300, and 400 MPa at 5 minute intervals for up to 25 min. The initial temperature in the processing chamber with samples was ca. 5 °C.. In our experiments the temperature did not exceed ca 22, 28 or 35 °C at 200, 300, or 400 MPa, respectively. The chamber temperature was monitored by the built-in sensor (a T-type thermal couple device) which was calibrated against a standard mercury thermometer (to ±0.5°C accuracy). The thermal sensor was immersed in the working chamber near food samples filled with the recirculation fluid. Temperature profile was recorded by the data acquisition/recording device at one second interval during HPP.

2.5. Gamma Radiation

A Lockheed Georgia Company (Marietta, GA, USA) self-contained ^{137}Cs irradiator, with a dose rate of 0.065 kGy/min, was used for all exposures. The radiation source consisted of 23 individually sealed source pencils in an annular array. The 22.9 cm $\times$ 63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. Inoculated samples were placed vertically and centrally in the sample chamber, using a 4 mm thick polypropylene bucket, to ensure a good dose uniformity (DUR<1.1:1.0). The temperature during irradiation (4°C), monitored by thermocouple, was maintained (4 or -20 °C) by introduction of the gas phase from a liquid nitrogen source directly into the top of the sample chamber. The radiation doses were at 0.3 and 0.6 kGy increments at 4 or -20 °C, respectively. The absorbed dose was verified using temperature tempered 5 mm alanine pellets that were then measured using a Bruker eScan EPR Analyzer (Bruker, Billerica, MA).

2.6 Exposure to Ultraviolet Light (UV-C)

A custom built UV-C apparatus (intensity of 1 mW/cm²) (Sommers et al., 2010) was used to treat chicken purge inoculated with ExPEC on stainless steel (SS, 304L), High Density Polypropylene (HDPP) and High Density Polyethylene (HDPE) coupons (5 x 10 cm). Chicken purge was thawed in a refrigerator overnight and 0.5 ml of *S. saprophyticus* cocktail was inoculated into 4.5 ml purge, which was then mixed by vortexing for 30 s, 0.1 ml of inoculated purge was placed on the coupons, and spread to a 4 cm² area using an inoculating loop. The coupons were placed in a refrigerator for 30 min and then placed on a cold pack (4 °C) for UV-C exposure. UV-C doses of 10, 20, 40, and 60mJ/cm². UV-C intensity was monitored using a calibrated UVX Radiometer (UVP Inc., Upland, CA).

2.7. Recovery of the *S. saprophyticus*

The individual chicken samples were added to 9x weight of 0.1% SPW and then stomached for 2 min (Model Bag Mixer 100W, Inter science Co., France). The coupons with 0.1 ml exudate were placed in stomacher bags with 9.9 ml SPW and hand massaged for 1 min. Then 0.1 mL, after proper decimal dilutions, was distributed on duplicate Baird-Parker Medium plates (BD-Difco, Sparks, MD). The plates were maintained at room temperature for 4 hours to allow the injured cells to recover (Hsu et al., 2015) and then incubated at 37 °C for 2 days. Colonies (CFU) were enumerated for determination of log reduction and D_{10} .

2.8. Statistical Analysis

The mean plate counts of the treated samples (N) were divided by the average control plate counts (N_0) to give a survivor ratio (N/N_0). The $\log_{10} (N/N_0)$ of the ratios was then used for determination of D_{10} -values and other statistical analyses. D_{10} are the processing conditions needed to reduce the population by 1 log (90%) D_{10} -values were determined by the reciprocal of the slope calculated from the log reductions (Diehl, 1995) and can be determined for a variety of food processing technologies including TP (FDA, 2015), HPP (Sheen et al., 2015), GR (Sommers et al., 2015) and UV-C (Koutchma, 2009) providing inactivation kinetics are first order in nature. Single samples were used for each experimental condition (e.g. time/pressure or radiation dose/temperature, etc.), and each experiment (D_{10} determination) was conducted independently three times. Statistical analysis functions of MS Excel (Microsoft Corp., Redmond, WA) were used for routine calculations (D_{10} determination), descriptive statistics, analysis of variance (ANOVA, 95% confidence).

3. Results and Discussion

3.1 Thermal processing

The thermal D_{10} for *S. saprophyticus* in ground chicken was 6.26, 0.60 and 0.09 min at 55, 60 and 65 °C, respectively (Figure 1, Table 1). These D_{10} are consistent with those obtained for other vegetative foodborne pathogens including *Salmonella* spp, *Listeria monocytogenes*, *E. coli*, and *S. aureus*. (Smelt and Brul, 2014; FDA, 2015). Thermal inactivation parameters used for common foodborne pathogens should be able to inactivate *S. saprophyticus* in meat and poultry.

3.2 High pressure processing

The HPP D_{10} (5 °C, 0-25 min) for *S. saprophyticus* suspended in chicken meat was 15.5, 9.43, and 3.54 min at 200, 300 or 400 MPa, respectively (Table 1, Figure 1) and were significantly different from each other (ANOVA, $p < 0.05$). *S. aureus* has been the traditional foodborne pathogen of study for food safety reasons. Jofré et al. (2008) obtained a 3.5 log reductions of *S. aureus* in cooked ham and marinated beef with HPP (600 MPa, 31 °C, 6 min). Garriga et al. (2002) did not obtain significant log reductions for *S. aureus* in model systems with HPP (400 MPa, 17 °C, 10 min). Moerman et al. (2005) found no inactivation of *S. aureus* in pork (400 MPa, 20 °C, 20 min). Our study indicates that *S. saprophyticus* can be killed in meat and poultry more easily than *S. aureus* per the published literature. However, as noted in our previous research the quality of poultry meat can be adversely affected by pressure treatment above 400 MPa (Sheen et al., 2015). HPP at 300 MPa was able to inactivate 2.6 log of *S. saprophyticus* (Figure 1).

3.2 Gamma Radiation

Meat and poultry are typically irradiated at refrigerated (4 °C) or frozen temperatures (-20 °C). The radiation D_{10} for microorganisms are typically higher in frozen meat as opposed to refrigerated meat due to the inability of hydroxyl radicals to cause indirect cellular damage because of their inability to diffuse in a frozen matrix (Bruns and Maxcy, 1979; Taub et al.,

1979). When the *S. saprophyticus* cocktail was inoculated into ground chicken and gamma irradiated (5 and -20 °C) the GR D₁₀ were 0.64 and 0.77 kGy, respectively (ANOVA, p < 0.05) (Table 1, Figure 1.) Thayer et al. (1997, 1995) found the D₁₀ for a multi-isolate cocktail of *S. aureus* to range from 0.34 to 0.46 kGy in eleven different refrigerated meat and poultry products. Thayer and Boyd (1991) found the D₁₀ of *S. saprophyticus* to be 0.51 in refrigerated vs. 0.88 kGy in frozen ground beef. In our study the D₁₀ of *S. saprophyticus* in frozen chicken meat was 0.77 kGy. It should be possible to inactivate 3-4 log of *S. saprophyticus* in refrigerated and frozen chicken meat without affecting product sensory quality (Mantilla et al., 2011).

3.4 UV-C

When *S. saprophyticus* was inoculated on the surface of skinless chicken breast pieces only ca. 0.4 log was inactivated by UV-C (data not shown). The relatively low log reduction obtained on meat and poultry surfaces is expected due to shadowing and shielding effects from UV-C due to the surface topology of those foods (Sommers et al., 2010; Gardner and Shama, 2000). In this study we focused on determination D₁₀ using a lower intensity 1 mW/cm²/s system for *S. saprophyticus* suspended on chicken exudates placed on SS, HDPE, and HDPP surfaces. D₁₀ ranged from 14.9 – 18.5 mJ/cm² (Figure 1, Table 1), and there was no significant difference between the D₁₀ (ANOVA, p > 0.05). As noted in previous research UV-C reduces vegetative microorganisms in purge by > 5 log, but will not completely eliminate them, most likely do to shielding of the bacteria by particulate matter (Sommers et al., 2016; Sommers et al., 2010).

4. Conclusions

The CDC has noted that foods treated using intervention technologies can reduce the risk of illness for at-risk individuals. Emerging science has now determined that uropathogenic bacteria

in foods may be a form of extraintestinal foodborne illness. Inactivation of foodborne pathogens, whether they are gastrointestinal or uropathogenic, may provide health benefits to at-risk individuals. The inactivation kinetics of *S. saprophyticus* in suspended in chicken meat and purge to TP, HPP, GR and UV-C are consistent with those for other vegetative foodborne pathogens (e.g. *Listeria monocytogenes*, *S. aureus*, *Salmonella* spp., UPEC and Shiga toxin-producing *E. coli*).

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Table 1. D_{10} for *Staphylococcus saprophyticus* in chicken meat and purge using high pressure processing, gamma radiation, thermal processing, and ultraviolet light.

Technology	Parameter	D_{10} (SEM)
High Pressure	200 MPa, 5 °C	15.5(±0.65) min ^a
	300 MPa, 5 °C	9.43 (±0.22) min ^b
	400 MPa, 5 °C	3.54 (±0.21) min ^c
Gamma Radiation	5 °C	0.64 (±0.01) kGy ^a
	-20 °C	0.77 (±0.01) kGy ^b
Thermal Processing	50 °C	6.26 (±0.21) min ^a
	55 °C	0.60 (±0.03) min ^b
	60 °C	0.09 (±0.01) min ^c
254 nm Ultraviolet Light	SS	18.5 (±1.27) mJ/cm ^{2a}
	HDPE	16.6 (± 1.54) mJ/cm ^{2a}
	HDPE	14.9 (±1.88) mJ/cm ^{2a}

Single samples were used for each experimental condition, and each D_{10} experiment was conducted independently 3 times. Standard error of the mean is shown in parenthesis. Linear regressions (R^2) were all > 0.95. For High Pressure Processing, Gamma Radiation, and Thermal Processing of *S. saprophyticus* was suspended in ground chicken. For ultraviolet light *S. saprophyticus* was inoculated into chicken purge and placed on stainless steel (SS), High Density Polyethylene (HDPE), or High Density Polypropylene (HDPP) coupons. D_{10} with different letters (within each technology) are statistically significant (ANOVA, $p < 0.05$).

Figure 1. Inactivation of *Staphylococcus saprophyticus* on chicken meat and chicken purge by nonthermal processing technologies. HPP 400 (●), 300 (○) and 200 (▼) MPa; gamma radiation at 5 (●) and -20 (○) °C; Thermal processing 55 °C (●), 60 (○) and 65 °C (▼). For UV-C inactivation of *S. saprophyticus* on chicken purge on SS (●), HDPE (○) and HDPP (▼) are shown. A single sample was used for each experimental condition, and each D₁₀ determination was conducted independently three times. The standard error of the mean for each condition is shown as error bars. The linear regressions are shown as solid lines.