Influence of UV-C (254 nm) on the Inactivation of *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella Kentucky* isolated from chicken breast fillets in Egypt.

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Abstract: The study validated the efficacy of UV-C (254 nm) to inactivate *Salmonella* serotypes inoculated into chicken fillets samples obtained from different processors in Egypt with a treatment time of 2 and 4 minutes. *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella Kentucky* were successfully isolated and identified from the examined samples with a percentage of 73%, 18% and 9%, respectively from the total number of positive samples (22%) (11 out of 50). Sterile chicken fillets samples were inoculated with 8 logs of *Salmonella* serotypes (*S. typhimurium*, *S. enteritidis* and *S. Kentucky)*. The inoculated samples were treated with UV-C (254 nm) for 2 and 4 minutes and the decimal reduction times (D value, min) were determined and calculated for the *Salmonella* serotypes. The calculated D values for tested *S. typhimurium, S. enteritidis and S. kentucky* in UV-irradiated chicken fillets were 1.47, 1.67 and 2.05 min., respectively. Significant differences ($P < 0.05$) were found between control and treated samples at 2 and 4 min., also between the tested *Salmonella* serotypes. This study will better enable processors to determine the process lethality of *Salmonella* serotypes in commercial chicken fillets.
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Key words: *Salmonella typhimurium*, *Salmonella enteritidis* , *Salmonella Kentucky*, UV-C, Chicken breast fillets decontamination.
Abbreviations: UV, ultraviolet; D value, decimal reduction times; RTE, ready-to-eat; XLD, xylose lysine desoxycholate; CFU, colony forming unit.

1. Introduction:

Poultry products have become more popular as their health benefits and nutritional value have been explored. These important foods supply proteins, vitamins such as (choline, niacin, Vitamin A) and retinol, and minerals such as (potassium, phosphorus, selenium, and calcium)**.** Live poultry contain a large number of different microorganisms on their skin, feathers, and in their alimentary tract **(Kozačinski *et al.*, 2006)**. Therefore, contamination of poultry carcasses during slaughtering procedures is usually unavoidable. Foodborne illness is a major public health concern; The largest number of foodborne illness cases attributed to poultry and poultry products are caused by serotypes of *Salmonella* **(White *et al.*, 1997).** *Salmonella* are a genus of Gram-negative facultative anaerobe bacteria; they are small rod-shaped, usually motile, and non-spore-forming bacilli, Ingestion of as few as 15 to 20 cells can be enough for infection with Salmonella depending on the strain as well as the age and the health of the host (**Microorganisms and Toxins, 2005)**. After the ingestion of the contaminated food, the bacteria penetrate into intestinal epithelium where inflammation occurs. Then the bacteria may be spread from the intestine to the blood stream and then to liver and other body parts **(Isaacson and Kinsel, 1992).**

The contamination with pathogenic bacteria, such as *Salmonella*, on meat normally occurs on the surface of products during post-cook or pre-packaging processes, therefore, surface decontamination methods have been developed for inactivation of these microorganisms prior to or immediately after the packaging process **(Houben and Eckenhausen, 2006).** Thermal treatment is a critical processing step in controlling the foodborne pathogens in ready-to-eat (RTE) meat and poultry products **(O'Bryan *et al.*, 2006).** However, thermal treatment has not always been sufficient **(Henning and Cutter, 2001)**.

Hence, post-processing intervention is required for poultry products to eliminate microbial contamination which may have occurred during any stage of the processing **(Keklik, 2009).** Various non-thermal technologies have been developed for the decontamination of foods; vacuum/gas packaging, irradiation, high intensity pulsed electric field, high

pressure processing, ultrasonic energy (sonication), ultraviolet (UV) light, and pulsed light are some of the non-thermal decontamination/preservation techniques **(Aymerich *et al.*, 2008; Keklik, 2009)**

Ultraviolet light is a technology, which is defined as the electromagnetic radiation in the spectral region classified into four wavelength ranges: UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm), and Vacuum-UV (100 nm-200 nm) **(Bouslimi *et al.*, 2012)**. UV-C light possesses germicidal properties that deactivates the DNA of bacteria, viruses, and other pathogens, thus destroys their ability to multiply and cause disease, specifically, UV-C light causes damage to the nucleic acid of microorganisms by formation of lethal photoproducts and pyrimidine dimers on the microbial DNA **(Miller *et al.*, 1999; Keklik, 2009).**

2. Materials and methods

1. Samples collection:

Boneless chicken breasts (chicken fillets) were purchased from local markets and stored in refrigerator (4ºC). Samples were cut into 3 to 4 cm width, 12 to 14 cm length with a thickness of 2 to 3 cm and 25 g were weighed.

2. Salmonella species isolation and identification:

2.1. Isolation of *Salmonella*:

The following technique was recommended by **(Vassiliadis, 1983)**. Twenty five grams of each hard sample were homogenized in 225 ml of buffered peptone water (BPW), under aseptic conditions for 2 min. by using stomacher. All samples were incubated at 35˚ C for 24 + 2 hours. One ml from the pre-enriched sample was transferred to 10 ml of Rappaport Vassiliadis (RV) enrichment broth and incubated at 43˚ C for 18 hours. A Loopful from enriched RV broth was separately streaked onto xylose lysine desoxycholate (XLD) agar and was incubated at 37˚ C for 24 hours. Two or three of typical or suspected colonies? (red colonies with black center on XLD) were selected from each selective medium and were streaked onto nutrient agar slope which incubated at 37˚ C for 24 hours.

2.2. Biochemical identification and serotyping:

The obtained purified isolates were identified biochemically as reported by **(Edwards and Ewing, 1972)** and serologically according to **(Kauffmann, 1972)**.

3. Chicken meat products decontamination by UV-C (254 nm):

3.1 Preparation *of Salmonella* strain:

Salmonella strains isolated from chicken fillets samples were prepared for the current study. The bacterium was cultivated in Tryptic soy broth (Difco) at 37 ° C for 24 h. The cells were harvested by centrifugation 5000 r.p.m./10 min. and were washed twice in sterile saline (0.85% NaCl). Finally, pellets were resuspended in sterile saline (0.85% NaCl) to a final cell density of 8 logs CFU/ml.

3.2 Sample inoculation and experimental design:

Sterile chicken fillets samples were formed into 25 g (3 to 4 cm width and 12 to 14 cm length with a thickness of 2 to 3 cm) and each placed in a Polyethylene- Polyamide plastic bag. Samples were kept at room temperature for about 2 hours prior to the experiment to bring temperature to room temperature to minimize the possible effect of temperature fluctuation on treatments. Each fillet sample was inoculated with 100 µl prepared *Salmonella* strain by spreading the inoculum solution along the surface. The samples were then kept in a microbiological laminar flow hood for 1 hr. in order to allow cells to attach to the surface of the samples and the following experimental design was obtained:

First group:

Negative Control group, non-inoculated samples and not treated with UV-C light.

Second group

Positive Control group inoculated samples but not treated with UV-C light.

Third group:

The inoculated samples were placed on the shelf in the pulsed UV-light chamber and treated by 254 nm for 2 min. in a biological safety cabinet.

Fourth group:

Inoculated samples were placed on the shelf in the pulsed UV-light chamber and treated by 254 nm for 4 min in a biological safety cabinet.

3.3 Microbiological analysis:

Each of the untreated and treated samples was transferred to a filtered stomacher bag containing 225 ml of buffered peptone water (BPW) for stomaching and then tenfold serial dilution was carried out. The dilutions were plated onto xylose lysine desoxycholate (XLD) agar and were incubated at 37˚ C for 24 hours.

4. Statistical analysis:

The mean values and standard error of the means (SEM) were calculating by using the statistical package for social sciences (SPSS Inc.; Chicago, IL, USA) software. One way analysis of variance (ANOVA) at 95% level of confidence and least Significant difference (LSD) post hoc were done to determine significant differences ($P<0.05$ was considered as significant). The D-values (time to inactivate 90% of the population) were determined from the equation $D = t / \text{LogN-log}N_0$, Where N is the final spore population, t is the time at LogN and N_0 is the initial population of the straight line portion on the destruction curve produced by plotting the $\log_{10}$ numbers of survivors against time **(Harrigan, 1998).**

3. Results

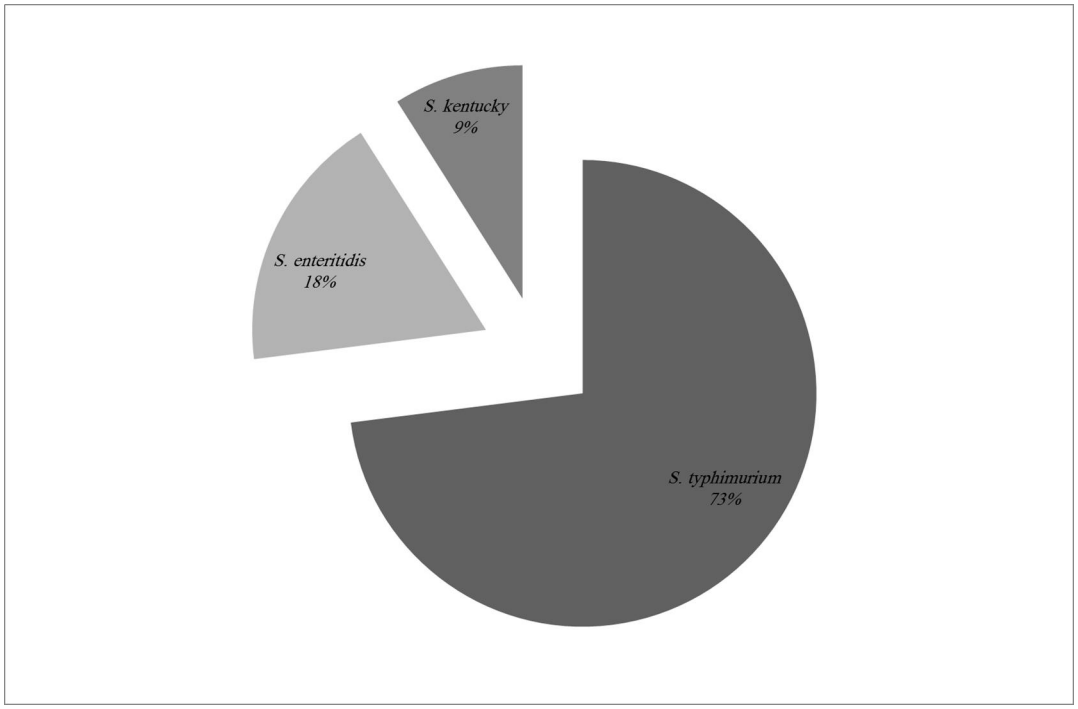


Figure 1. Prevalence of *Salmonella* serotypes, expressed as a percentage of the total number of positive samples

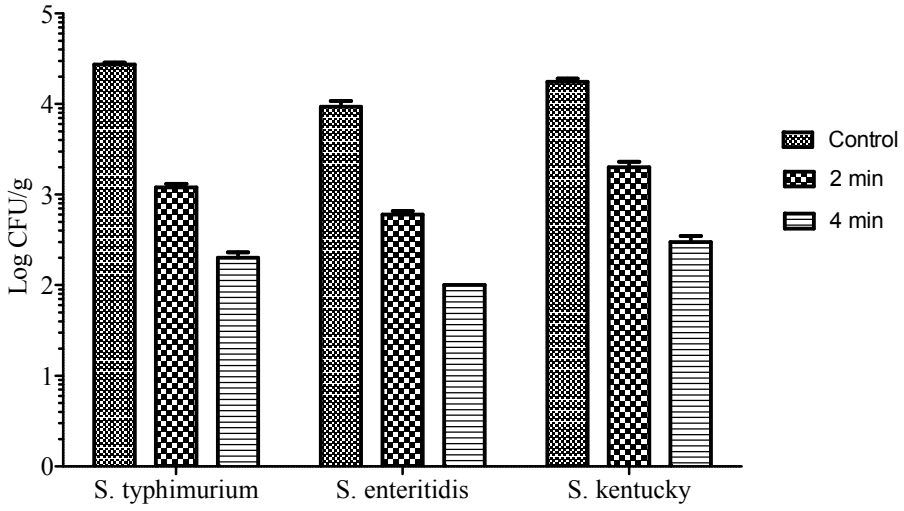
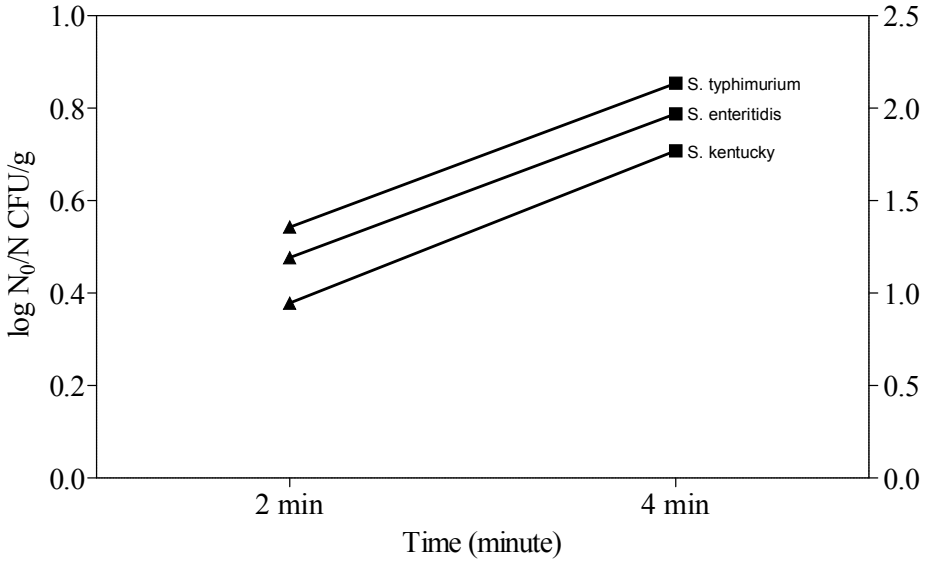


Figure 2. Effect of UV-C on *Salmonella typhimurium, Salmonella enteritidis and Salmonella Kentucky* in control and treated samples. Treatments were carried out at holding time of 2 and 4 minutes

Figure 3. Logarithmic viability reduction (log N_0/N) of *Salmonella* serotypes.



4. Discussion

Salmonella infections are worldwide and constitute an important public health problem in many parts of the world **(Erdem *et al.*, 2005).** There are several transmission routes for salmonellosis, but the majority of human infections are derived from the consumption of contaminated foods especially those of animal origin **(Hernandez *et al.*, 2005).**

Isolation results revealed that 11 out of 50 (22%) examined chicken fillets samples were contaminated with *Salmonella* species. These results were comparable with a prevalence rate of 26% in Egypt **(Ahmed *et al.*, 2014)** and 27% in Russia **(Alali *et al.*, 2012).**

Contamination of chicken meat at retail markets could be originated from the intestinal contents of the carcass during evisceration, defeathering process, handling and cross-contamination during storage **(Zhao *et al.*, 2001).**

in a study in Egypt, **(Hassanein *et al.*, 2011)** reported that 52% of frozen chicken breast fillets were contaminated with *Salmonella* spp. and in thus study, *S. enteritidis* and *S. kentucky* were identified from frozen chicken breast fillets and minced frozen meat, respectively.

Non thermal technologies have gained attention in recent years for fresh and processed meats. The UV irradiation process involves the exposure of the product to a germicidal light with a wavelength of 220–300 nm to inactivate contaminating bacteria and viruses. Exposure to UV light results in the cross-linking of neighboring pyrimidine nucleotide bases in the same DNA strand, eventually causing cell death **(Sizer and Balasubramaniam, 1999)** and also UV-C treatment of *salmonella* increases the percentage of cyclic fatty acids and induce morphological changes and alter the bacterial cell surface **(Maâlej *et al.*, 2014).**

Results of the calculated D values per test *S. typhimurium, S. enteritidis and S. kentucky* in UV-irradiated chicken fillets were 1.47, 1.67 and 2.05 minute, respectively. These results showed that *S. kentucky* strains were significantly ($P < 0.5$) more resistant than the other organisms. The test *S. typhimurium* strains were recorded to have significantly ($P < 0.5$) less resistance to UV in inoculated chicken fillets.

In the study of **(Chun *et al.*, 2010)**, UV-C irradiation of chicken breasts reduced the initial populations of *S. typhimurium* by 1.19 log CFU/g and this study suggested to use UV-C irradiation to improve the microbial safety of chicken breasts during storage. **(Haughton *et al.*, 2011)** demonstrated that UV treatment of raw chicken fillet (0.192 J/cm^2) reduced *S. Enteritidis* by 1.34 log CFU/g and they reported that the color of UV-treated chicken was not significantly affected ($P \geq 0.05$) and proposed to use UV for improving the microbiological quality of raw chicken and for decontaminating associated packaging and surface materials.

Even though all the research data indicated that UV-C (254 nm) irradiation is an effective method to eliminate pathogenic microorganisms in food products in combination with other decontamination

techniques (such as the hygienic processing of chicken breasts without causing any deleterious changes to the physicochemical and sensory qualities of the meat surface and this technology was approved by FDA **(Char *et al.*, 2010; Chun *et al.*, 2010; Sommers *et al.*, 2010; Haughton *et al.*, 2011; Park and Ha, 2015).**

Hygiene attention should be taken in poultry processing plant to reduce addressing of *salmonella* species with implementation of control strategies along with the guidelines to handle this dilemma. Therefore, the current study suggests the use of the UV-C (254 nm) in combination with other decontamination techniques to improve the microbiological quality and to control *salmonella* species in chicken fillets.

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