

Efficacy of household washing pre-treatments and cooking methods for reduction of *Listeria monocytogenes* in artificially contaminated chicken offal^{1*}Kuan, C.H., ^{2,3}Son, R., ⁴Mahyudin, N.A., ⁵Tang, J.Y.H., ¹Yeo, S.K., ⁶Kuan, C.S., ²Chang, L.S., ⁷Phuah, E.T., ²Thung, T.Y., ²Tan, C.W., ²New, C.Y. and ⁸Nishibuchi, M.¹*School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University Lakeside Campus, 47500 Subang Jaya, Selangor, Malaysia*²*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*³*Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Seri Kembangan, Malaysia*⁴*Department of Food Service and Management, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*⁵*Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (Besut Campus), 22200 Besut, Terengganu*⁶*Neogenix Laboratoire Sdn Bhd, C707, Level 7, Block C, Kelana Square, Kelana Jaya, 47301, Petaling Jaya, Selangor, Malaysia*⁷*Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR), Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia*⁸*Center for Southeast Asian Studies, Kyoto University, Kyoto, Japan***Article history:**

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Chicken offal**DOI:**[https://doi.org/10.26656/fr.2017.4\(1\).268](https://doi.org/10.26656/fr.2017.4(1).268)**Abstract**

Consumption of chicken offal is common and famous among Malaysians as it is often served as one of the side dishes with rice. Chicken offal can be a potential source of *Listeria monocytogenes* because slaughtered animals are recognized as a reservoir for foodborne pathogens. *L. monocytogenes* is a dangerous foodborne pathogen which can cause severe foodborne listeriosis with high fatality rate. This study aimed to determine the efficacy of different washing pre-treatment and cooking methods to reduce *L. monocytogenes* in artificially contaminated chicken offal. All the washing pre-treatments (dip treatment in different water sources and wash treatment with different water flow rates) showed significant reduction of the pathogen ($p < 0.05$) when the inoculated samples were treated from 2 mins onwards. Washing the inoculated samples under the water flow rate of 2 L/min was the most effective way to reduce the number of *L. monocytogenes* (approximately 1.97 log reduction after washing for 10 mins). For heat treatment study, deep-frying was the most effective cooking method followed by boiling and pan-frying to reduce *L. monocytogenes* where all *L. monocytogenes* cells (7.91 log₁₀ CFU/g) were killed within 45 s under deep-frying treatment. Overall, the study indicated that washing under running tap water (2 L/min) and deep-frying was effective in reducing and controlling the microbial populations during food preparation. The findings from this study can serve as a safe preparation step and cooking guideline. It is necessary to implement safe steps in food handling practices among food handlers to minimize the risk of foodborne infection.

1. Introduction

Chicken offal is commonly referring to the chicken gizzard, chicken heart, and chicken liver which is a cheap source of protein. In Malaysia, consumption of poultry offal is very common, and it is usually consumed as side dishes with rice. However, as compared to fruits and vegetables, the slaughtered animal has a higher

tendency as the source of foodborne pathogens (Mead, 2007; Heredia and García, 2018). This is mainly due to the release of blood and meat blood-like liquid during the slaughtering process, which served as nutrients for microorganisms to grow (Gudbjörnsdóttir *et al.*, 2004). The Ministry of Health Malaysia (MOH) enforced health certificate requirement for importation of edible poultry offal from all countries in order to protect the consumer

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against contaminated food (MOH, 2005). However, there is a lack of information regarding the safe handling procedure, pre-treatment, and cooking of the edible offal to ensure it is safe for consumption.

Listeria monocytogenes has been recognized as one of the most important foodborne pathogens as it can cause listeriosis, a severe foodborne illness, that usually affects pregnant women, newborns, elderly and immunocompromised patients (Painter and Slutsker, 2007; Scallan et al., 2011; McNeill et al., 2017). Therefore, many countries had started to impose a zero-tolerance policy towards the survival of *L. monocytogenes*. Outbreaks of *L. monocytogenes* is often associated with the consumption of meats and meat products (Arumugaswamy et al., 1994; Martín et al., 2004; Karakolev, 2009; Alonso-Hernando et al., 2012; Marian et al., 2012; Kramarenko et al., 2013; Kurpas et al., 2018). According to the Centers for Disease Control and Prevention, it is estimated that an approximately 1600 illnesses and 260 deaths (15.9% mortality rate) in the United States are caused by listeriosis annually (Nastou et al., 2012). Hence, studies concerning the reduction of *L. monocytogenes* in food should be studied extensively to ensure the safety of the consumer.

Besides, *L. monocytogenes* is ubiquitous and it is able to survive and proliferate during storage at refrigerated temperature (Lin et al., 2004; Nastou et al., 2012). Hence, it is important to apply appropriate safe procedure in food handlings and cooking prior consumption to reduce the threat posed by *L. monocytogenes* and other foodborne pathogens. Washing and cooking methods are always practiced in the domestic kitchen or restaurant because most of the food handlers believe that these steps can ensure the safety of the food. However, the effective way of pre-treatments and their outcomes are not well-known. Washing is practiced to remove dirt, blood, and blood-like liquid and most importantly to reduce the microbial load (Baur et al., 2005; Nastou et al., 2012; Pangloli and Hung, 2013). There are several ways of washing such as washing or rinsing under running water (Baur et al., 2005), stirring, dipping, or static immersion (Nastou et al., 2012). Besides, washing is always associated with sanitizer such as chlorine (Baur et al., 2005), organic acid solutions (Wu et al., 2000; Samara and Koutsoumanis, 2009), hydrogen peroxide (Ukuku and Fett, 2002) or household sanitizers (white vinegar, lemon juice, bleach and others) (Vijayakumar and Wolf-Hall, 2002) for effective washing. In general, washing treatment is always followed by cooking treatment as washing can partially remove foodborne pathogens from raw meat while cooking can destroy the pathogenic bacteria by altering their membranes and denaturing

proteins (Erkmen and Bozoglu, 2016).

Malaysian cooking methods are almost similar to those applied in any other Asian kitchens, especially the basic technique (blanching or boiling, steaming, grilling, frying and deep-frying). According to Lipoeto et al. (2013), the meal pattern in Malaysia consists of rice added with chicken or fish which was prepared by deep-frying or boiling. Home cooking methods such as boiling, pan-frying, grilling, and deep-frying are usually used to cook meat (Lahou et al., 2015; Roccato et al., 2015). Generally, cooking oil and water are served as the heating medium. Deep-frying is one of the favorite cooking methods in Malaysia as fried foods have consumer appeal in all age groups in Malaysia. Due to the high temperature (180-190°C) and the high heat conduction of cooking oil, the cooking process can be very fast. Pan-frying is another form of frying method (with minimal cooking oil usage) which is also commonly used in Malaysian cooking method. On the other hand, hotpot cooking (well known as steamboat in Malaysia) is a cooking method or eating style that is commonly found throughout China and Southeast Asia. Meats and/or vegetables are usually loaded into the hot cooking broth and the cooking time is depending on consumer preference. Hence, different cooking methods are proposed in this study for the preparation of chicken offal. The present study aimed to investigate the efficacy of different washing pre-treatments and cooking methods to reduce *L. monocytogenes* on chicken offal. This study is significant as it is the first study concerning the decontamination of pathogenic bacteria during handling of chicken offal at the household level.

2. Materials and methods

2.1 Preparation of *L. monocytogenes* inoculum

L. monocytogenes ATCC 19155 was used in this study. The pure culture was inoculated onto PALCAM agar (Merck, Germany) and incubated for 48 hrs at 30°C. Then, a single colony of *L. monocytogenes* from PALCAM agar was inoculated into 10 mL of Tryptic Soy Broth (TSB) (Merck, Germany) and incubated at 37°C for 24 hrs. The overnight cultures were then harvested by centrifugation at 13,400 × g for 5 mins and the supernatant was discarded. The cell pellets were resuspended in sterile 0.85% (w/v) saline solution (NaCl) (Merck, Germany) using a vortex and further washed twice with the saline solution. The cell suspension's absorbance at 600 nm was adjusted to 0.393 which corresponded to 9.13 log₁₀ CFU/mL (Wong et al., 2011).

2.2 Sample preparation and inoculation

Chicken gizzard was selected as the food model in the study because it posed the greatest risks as compared

to chicken heart and chicken liver (Arumugaswamy et al., 1994; Kuan et al., 2013). All the chicken gizzards were purchased from the same wet market located in Serdang, Selangor, Malaysia. Chicken gizzards with the weight between 22.5 to 25.0 g were selected to minimize the effect of variation in size on the accuracy of the result. The chicken gizzard was placed separately in plastic bags to avoid cross-contamination. Then, the samples were immediately transported to the Food Safety and Quality Laboratory, Universiti Putra Malaysia. Chicken gizzard samples were treated under UV light in a biological safety cabinet for 30 mins (15 mins for each side, with the UV light intensity of 36,000 $\mu\text{J}/\text{cm}^2$) to eliminate the native microflora (Kuan et al., 2017). It was assumed that all the chicken gizzards were free from microorganisms after being treated with UV light. Lastly, each of the chicken gizzards was then spiked with 1 mL of the pure culture of *L. monocytogenes* (0.5 mL on each side). The inoculated chicken gizzards were air-dried for 45 mins in the biosafety cabinet. The final concentration of *L. monocytogenes* cells in each chicken gizzard was approximately $7 - 8 \log_{10}$ CFU/g. Samples were prepared in three sets for each treatment including the negative control (kept aseptically, treated with UV light and without spiking).

2.3 Washing pre-treatments

The washing pre-treatments were designed to simulate the actual handling practices in a domestic kitchen. The inoculated chicken gizzards were dipped into 200 mL of different treated water: sterile distilled water (control), tap water ($22 \pm 2^\circ\text{C}$), and warm water ($50 \pm 5^\circ\text{C}$). Besides, the inoculated chicken gizzards were also washed under running tap water with the flow rate of 500 mL/ min, 1 L/ min, and 2 L/ min. The flow rate of running tap water was determined by the amount of tap water collected over a specified period of time using a bucket and a stopwatch. All these treatments were done at different exposure times which were 30 s, 1 min, 2 mins, 5 mins, and 10 mins.

2.4 Heat treatments

For the thermal study, heat treatments were designed to simulate the cooking processes that are usually carried out in a domestic kitchen. In this study, the inoculated chicken gizzards were subjected to deep-frying by submerging into 1 L of cooking oil and maintained at $180 \pm 5^\circ\text{C}$, pan-frying by using minimal oil to lubricate the frying pan and temperature control at $140 \pm 5^\circ\text{C}$, and boiling by submerging in 1 L of boiling water at 100°C for 15, 30, 45, 60 and 75 s. The temperature of cooking oil was maintained and monitored using a thermometer (TEL-TRU, USA).

2.5 Microbiological analysis

Each treated chicken gizzard sample was mixed with 0.85% saline solution in a sterile stomacher bag at the ratio of 1:10 (w/v). Then, the stomacher bag was pummeled using a BagMixer® 400P stomacher machine (Interscience, France) for 2 mins. Serial dilutions were prepared in the same diluent, plated onto PALCAM agar and incubated at 30°C for 48 hrs. A few presumptive positive colonies (grey-green with a black sunken center and a black halo on a cherry-red background) from PALCAM plates were streaked onto Tryptic Soy Agar (TSA) (Oxoid, UK) for purification and then subjected to PCR for confirmation. The quantitative data were expressed as CFU/g and transformed to logarithms before calculating the means value and standard deviations. Positive and negative control were also subjected to microbiological analysis to determine the initial contamination and validate the assumption of sterility, respectively.

2.6 Statistical analysis

All the samples in each treatment were performed in triplicate independently and the values were expressed as mean \pm SD (standard deviations). The data were subjected to a one-way analysis of variance (ANOVA) to evaluate if there was any significant difference between each treatment by Tukey's test at $p \leq 0.05$ level of significance. The computer program employed was Minitab version 14.0 statistical software (Minitab Pty Ltd, Sydney).

3. Results and discussion

Table 1 shows the microbial count of *L. monocytogenes* after subjected to different washing pre-treatments and exposure times. The results showed no significant difference ($p > 0.05$) in the efficacy on the reduction of *L. monocytogenes* between sample dipped in sterile distilled water and sample dipped in tap water. Therefore, it can be assumed that native microflora and mineral contents (e.g. iron, copper, magnesium, potassium, etc.) in tap water did not play a role to influence the accuracy of the results in the study.

Besides, samples dipped in warm water showed significant lower ($p < 0.05$) microbial count than samples dipped in tap water after 2 mins of exposure time. This indicated that water with higher temperature exhibited better efficiency in reducing *L. monocytogenes* after 2 mins of dipping. In the present study, the temperature of warm water was controlled at $50 \pm 5^\circ\text{C}$ and this was expected to impose a significant impact and stress on *L. monocytogenes* cells as it is above their normal growth temperature (Nastou et al., 2012). Baur et al. (2005)

Table 1. The microbial counts of *L. monocytogenes* (log₁₀ CFU/g) in artificially contaminated chicken gizzards after subjected to different washing pre-treatments and exposure times

Treatments	<i>L. monocytogenes</i> count (log ₁₀ CFU/g ± SD)					
	Exposure time (min)					
	0	0.5	1	2	5	10
Dipping in sterile distilled water (control)	7.24 ± 0.03	6.98 ± 0.07 ^{Aa}	6.88 ± 0.08 ^{Aab}	6.81 ± 0.08 ^{Ab}	6.77 ± 0.05 ^{Ab}	6.73 ± 0.03 ^{Ab}
Dipping in tap water (22 ± 2°C)	7.24 ± 0.03	6.99 ± 0.05 ^{Aa}	6.88 ± 0.09 ^{Aab}	6.82 ± 0.08 ^{Abc}	6.77 ± 0.02 ^{Abc}	6.71 ± 0.02 ^{Ac}
Dipping in warm water (50 ± 5°C)	7.24 ± 0.03	6.85 ± 0.09 ^{Aa}	6.76 ± 0.10 ^{Aab}	6.64 ± 0.11 ^{Bb}	6.40 ± 0.02 ^{Bc}	6.29 ± 0.04 ^{Bc}
Washing under running tap water (500 mL/min)	7.24 ± 0.03	6.77 ± 0.02 ^{Aa}	6.43 ± 0.02 ^{Bb}	6.28 ± 0.03 ^{Cc}	6.23 ± 0.02 ^{Cc}	6.10 ± 0.02 ^{Cd}
Washing under running tap water (1 L/min)	7.24 ± 0.03	6.66 ± 0.09 ^{Ba}	6.30 ± 0.03 ^{Bb}	6.01 ± 0.03 ^{Dc}	5.91 ± 0.03 ^{Dc}	5.76 ± 0.05 ^{Dd}
Washing under running tap water (2 L/min)	7.24 ± 0.03	6.41 ± 0.02 ^{Ca}	6.02 ± 0.03 ^{Cb}	5.71 ± 0.04 ^{Ec}	5.47 ± 0.04 ^{Ed}	5.27 ± 0.04 ^{Ec}

The results were expressed as mean ± SD (log₁₀ CFU/g) of triplicate measurements. Mean values in the same column with the different superscript uppercase letter (A-E) are significantly different ($p < 0.05$) and mean values in the same row with the different superscript lowercase letter (a-e) are significantly different ($p < 0.05$).

reported that applying warm water in the washing of iceberg lettuce had higher washing efficacy as compared to cold water. Therefore, the temperature can be one of the important parameters in affecting *L. monocytogenes* count and growth of other aerobic mesophilic bacteria. Although immersing in warm water was more effective than dipping in tap water, the efficacy in reduction of *L. monocytogenes* was not promising. This washing treatment can only reduce approximately 0.95 log₁₀ CFU/g of *L. monocytogenes* cells even after immersion for 10 mins.

Table 1 also shows that washing time was a significant factor ($p < 0.05$) that affects the efficacy of washing treatments. According to the present result, reduction of the microbial count was significantly increased ($p < 0.05$) as the exposure time was increased from 2 mins onwards. This might be due to the longer exposure time which improves the chance of the microbial to be washed away by the water from the surface of the chicken gizzard samples. Pangloli and Hung (2013) reported that increased washing time from 1 to 5 mins was able to reduce ($p < 0.05$) *E. coli* O157: H7 in blueberry up to 53%. To date, no study has reported on the effect of exposure time on the reduction of *L. monocytogenes* or other foodborne pathogens in chicken during washing pre-treatment.

In addition, a sharp decreased in the mean concentration of *L. monocytogenes* at the initial of washing treatments (0.5 min) regardless of the water flow rate was detected as compared to dipped treatment, signifying washing under running water had better efficacy in reduction of the microbial count. The findings also revealed that efficacy of washed under running tap water with the flow rate of 2 L/min for 1 min was greater than washed under running tap water (500 mL/min) for 10 mins. Hence, it is suggested that water flow rate and turbulence are also the main factors to influence the

efficacy of removing *L. monocytogenes*. Washing the artificially contaminated chicken gizzard samples under the flow water rate of 2 L/min was found as the most effective way to reduce the number of *L. monocytogenes*. This washing method reduced *L. monocytogenes* count by up to approximately 1.97 log₁₀ CFU/g after washing for 10 mins (Table 1). However, it was not practical to be employed by individual household because it takes a longer time and large volumes of water are used.

In this study, the efficacy of washing pre-treatment was affected by the temperature of water used, the method of washing, and the water flow rate. However, these factors might also be affected by the shape, size or surface topography of the samples (Nastou et al., 2012). As shown in this study, washing pre-treatment alone was insufficient to ensure complete removal of bacteria. Hence, the incorporation of adequate cooking is necessary, and it is the most effective way to eliminate foodborne pathogens.

There are many conventional cooking methods such as deep-frying, boiling, pan-frying, steaming, and radiant heating applied to cook food and ensure their microbiological safety. In this study, artificially contaminated chicken gizzards samples were subjected to deep-frying, pan-frying and boiling with different cooking times to evaluate the efficacy in reducing *L. monocytogenes*. Table 2 shows the microbial count of *L. monocytogenes* after subjected to different types of heat treatments and exposure times. All the cooking methods (deep-frying, boiling, and pan-frying) exhibited a significant effect ($p < 0.05$) on the reduction of *L. monocytogenes* in artificially contaminated chicken gizzard samples statistically. This indicates the effectiveness of heat treatment on the elimination of microbial in food. Wong et al. (2011) also reported cooking of chicken patty using the pan-frying method up to 6 mins was able to destroy *L. monocytogenes*. Other

researchers also reported similar findings which cooking treatment improved lethality on pathogens in ground beef (Guo *et al.*, 2006; Yoon *et al.*, 2013), egg (Fang and Huang, 2014), steak, hamburger or meat strips (Lahou *et al.*, 2015), poultry-based meat products (Roccatto *et al.*, 2015), and processed meat (McMinn *et al.*, 2018).

Table 2. The microbial counts of *L. monocytogenes* (\log_{10} CFU/g) in artificially contaminated chicken gizzards after subjected to different types of heat treatments and exposure times

Exposure time (s)	Heat treatments (\log_{10} CFU/g \pm SD)		
	Deep-frying	Boiling	Pan-frying
0	7.91 \pm 0.04	7.91 \pm 0.04	7.91 \pm 0.04
15	4.03 \pm 0.06 ^{Aa}	5.41 \pm 0.02 ^{Ab}	6.05 \pm 0.05 ^{Ac}
30	2.63 \pm 0.05 ^{Ba}	3.83 \pm 0.02 ^{Bb}	4.58 \pm 0.05 ^{Bc}
45	ND	2.57 \pm 0.01 ^C	3.29 \pm 0.07 ^C
60	ND	ND	1.86 \pm 0.08 ^D
75	ND	ND	ND

The results were expressed as mean \pm SD (\log_{10} CFU/g) of triplicate measurements. Mean values in the same column with the different superscript uppercase letter (A-D) are significantly different ($p < 0.05$) and mean values in the same row with the different superscript lowercase letter (a-c) are significantly different ($p < 0.05$).

ND – Not detected by direct plating.

The regression analysis between survivor concentration of *L. monocytogenes* (\log_{10} CFU/g) on artificially contaminated chicken gizzard samples after deep-frying, boiling and pan-frying time are shown in Figures 1, 2 and 3, respectively. All the figures show a negative correlation of microbial count with the exposure time with R^2 value higher than 0.99, indicating high correlation. The straight line (Figures 1, 2 and 3) is commonly known as the survival curve and it indicates that microbial inactivation by thermal processes in this study followed the first-order kinetics. This also signifies that heat destruction on *L. monocytogenes* is an irreversible process (Hassani *et al.*, 2005). A similar finding was also reported by Wong *et al.* (2011) in pan-fried chicken patties.

In the present study, dramatic reductions of *L. monocytogenes* counts in all thermal treatments in the first 15 s were observed with approximately 3.88 log reduction, 2.50 log reduction, and 1.86 log reduction on artificially contaminated chicken gizzard samples that treated with deep-frying, boiling and pan-frying, respectively (Table 2). The initial mean concentration of inoculated samples was approximately 7.91 \log_{10} CFU/g and it was reduced to an undetectable level at the end of treatment. This observation was in agreement with the finding of Murphy *et al.* (2002) in which the destruction of *L. innocua* and *Salmonella enterica* serovar Senftenberg in beef patty was more intense as the time of

deep-frying increases. This also signifies that cooking time is an important parameter to ensure the core of food reaches a desirable temperature to destroy the bacteria. Sufficient cooking is important to prevent outbreak caused by consumption of undercooked food which the bacteria have higher chances to survive in it. However, prolonged cooking time is not a favorable alternative as it might affect the quality of food such as water loss (Schlisselberg *et al.*, 2013), cooking loss (Pathare and Roskilly, 2016) or overcook of the food surface (Shirsat *et al.*, 2004; Tian *et al.*, 2019).

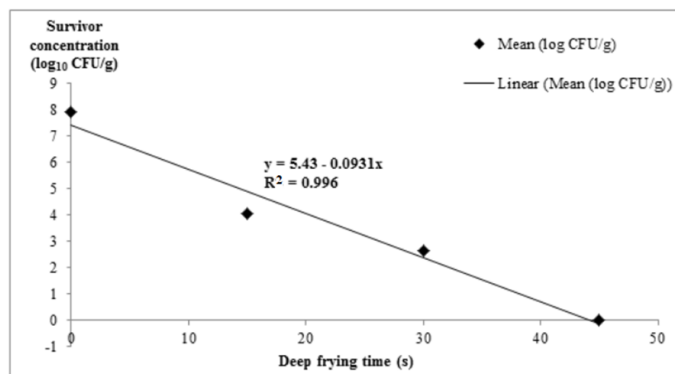


Figure 1. Survival curve showing the negative linear regression correlation ($R^2 = 0.996$) between survivor concentration of *L. monocytogenes* (\log_{10} CFU/g) and the deep-frying time for the artificially contaminated chicken gizzards

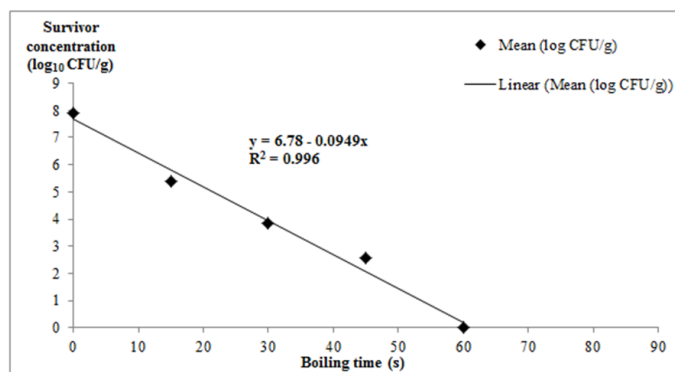


Figure 2. Survival curve showing the negative linear regression correlation ($R^2 = 0.996$) between survivor concentration of *L. monocytogenes* (\log_{10} CFU/g) and the boiling time for the artificially contaminated chicken gizzards

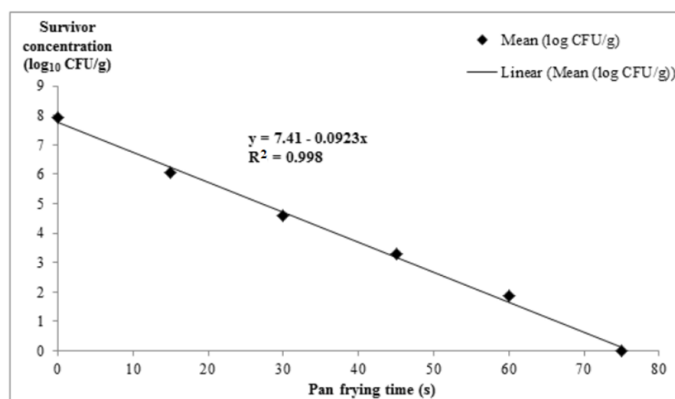


Figure 3. Survival curve showing the negative linear regression correlation ($R^2 = 0.998$) between survivor concentration of *L. monocytogenes* (\log_{10} CFU/g) and the pan-frying time for the artificially contaminated chicken gizzards

Overall, deep-frying can be considered as the most effective cooking method in reducing *L. monocytogenes* as it killed about 7.91 log₁₀ CFU/g of *L. monocytogenes* within 45 s as compared to boiling (60 s) and pan-frying (75 s). This might be due to deep-frying was using the hot oil (temperature up to 180±5°C) as the heating medium and temperature of 180°C was the highest temperature among the three cooking methods. During deep frying, the artificially contaminated chicken gizzards were immersed completely in hot oil. Therefore, the heat was transferred and penetrated uniformly to all the surfaces of chicken gizzard via conduction process. The *L. monocytogenes* cell was damaged due to exposure to the high heat medium. The investigation conducted by Murphy *et al.* (2002) also reported that deep-frying was more effective as the oil was able to speed the reduction of *S. enterica* serovar Senftenberg and *L. innocua* as compared to air convection cooking.

In addition, the boiling method was found more effective as compared to pan-frying in microbial inactivation. From Table 2, boiling achieved greater reduction as compared to pan-frying at the same cooking time although the heating temperature in pan-frying was much higher. Pan-frying was the least effective cooking method as it needs about 75 s to destroy all the *L. monocytogenes* in one artificially contaminated chicken gizzard. The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS, 2002) also reported that pan-frying was less effective in the reduction of microbial for every 5.6°C increase of cooking temperatures as compared to other cooking methods such as boiling and grilling. It should be taken into consideration that when more food is required to pan-fry at once, the quality of the food might be affected as the longer cooking time is required. The food might also be unsafe because when a large quantity of food is cooked at once, it will lower the cooking temperature.

Various factors that influence heat transfer should be considered to determine the efficacy of cooking methods on the elimination of foodborne pathogens in foods. These factors are the size and shape of the food, surface area exposed to heating medium and specific heat capacity of the heating medium (Vijay *et al.*, 2008). During heating, the physical size and shape of foods will affect the temperature distribution and heat transfer from the heating medium to foods. On the other hand, the thickness of food will influence the heat penetration rate as thicker food led to difficulty in heat penetration and might cause the inner/center part of the foods undercooked but the surface was overcooked (Laycock *et al.*, 2003). Samples with the biggest surface area exposed to the heating medium can be heated up faster. In this study, artificially contaminated chicken gizzard

that was immersed completely into the heating medium (hot oil or hot water) during deep-frying and boiling showed convincing results as all the microbes were killed completely in a shorter time. Besides, the specific heat capacity of the heating medium also plays an important role in determining the cooking time. Oil can be heated up as high as 180-190°C while boiling water can only reach a maximum of 100°C. The heat capacity and amount of the heating medium play a critical role in the heating process (Vijay *et al.*, 2008).

In this study, all the cooking methods imposed significant ($p<0.05$) effect in reduction of *L. monocytogenes* counts but differently depending on the exposure time and cooking process. Deep-frying is the most effective method, but it is always considered as an unhealthy and costly method due to a lot of oil is required. Pan-fried food is healthier as less oil is used. However, this form of cooking method always contributed to inadequate cooking because pan-frying mainly relies on cooking oil as the heat transfer medium; therefore, correct cooking temperature and sufficient cooking time to retain the moisture in the food at the same time to destroy the pathogen had to be known. Furthermore, due to partial coverage by cooking oil, the food must be flipped frequently in order to ensure both sides are cooked. Based on the findings in this study and the characteristics of each cooking method used, deep-fried foods are considered as safer food in term of microbiological quality than foods cooked by boiling or pan-frying. This is because it required a much shorter time to fully eliminate the foodborne pathogens while foods cooked by boiling or pan-frying have higher potential risks for *L. monocytogenes* to survive.

4. Conclusion

The results of this study suggested that washing the chicken gizzards prior to cooking under running tap water at the flow rate of (2 L/min) for 1-2 mins was the most effective and practical household handling practice. Although washing pre-treatment was ineffective to remove the contaminated microbes with the limited 1-2 log reduction, it was able to reduce the initial microbial load and help to lower the risk of bacterial cross-contamination. Besides, deep-frying is the most effective and the fastest way to fully cook the food and reduce the foodborne pathogens as compared to boiling and pan-frying. However, the choice of cooking methods depends on the types of cuisines and consumer preferences in term of sensory qualities, the degree of doneness, and retention of nutrient content. This study showed that *L. monocytogenes* is still able to survive if there is insufficient cooking time. All in all, the findings from this study can serve as a safe preparation step and

cooking guideline for food handlers to ensure the safe consumption of chicken offal and other meats.

Conflict of Interest

Authors declare no conflict of interest.

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References

- Alonso-Hernando, A., Prieto, M., García-Fernández, C., Alonso-Calleja, C. and Capita, R. (2012). Increase over time in the prevalence of multiple antibiotic resistance among isolates of *Listeria monocytogenes* from poultry in Spain. *Food Control*, 23(1), 37-41. <https://doi.org/10.1016/j.foodcont.2011.06.006>
- Arumugaswamy, R.K., Rahamat Ali, G.R. and Abd Hamid, S.N. (1994). Prevalence of *Listeria monocytogenes* in foods in Malaysia. *International Journal of Food Microbiology*, 23(1), 117-121. [https://doi.org/10.1016/0168-1605\(94\)90227-5](https://doi.org/10.1016/0168-1605(94)90227-5)
- Baur, S., Klaiber, R., Wei, H., Hammes, W.P. and Carle, R. (2005). Effect of temperature and chlorination of pre-washing water on shelf-life and physiological properties of ready-to-use iceberg lettuce. *Innovative Food Science and Emerging Technologies*, 6(2), 171-182. <https://doi.org/10.1016/j.ifset.2005.02.002>
- Erkmen, O. and Bozoglu, T.F. (2016). Food Preservation by High Temperature. In Erkmen, O. and Bozoglu, T.F. (Eds). *Food Microbiology: Principles into Practice*. Vol. 2, p. 12-16. Oxford, UK: John Wiley and Sons, Ltd. <https://doi.org/10.1002/9781119237860.ch28>
- Fang, T. and Huang, L. (2014). Growth and survival kinetics of *Listeria monocytogenes* in cooked egg whites. *Food Control*, 36(1), 191-198. <https://doi.org/10.1016/j.foodcont.2013.08.034>
- Gudbjörnsdóttir, B., Suihko, M.L., Gustavsson, P., Thorkelsson, G., Salo, S., Sjöberg, A.M., Niclasen, O. and Bredholt, S. (2004). The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the Nordic countries. *Food Microbiology*, 21(2), 217-225. [https://doi.org/10.1016/S0740-0020\(03\)00012-1](https://doi.org/10.1016/S0740-0020(03)00012-1)
- Guo, Q., Piyasena, P., Mittal, G.S., Si, W. and Gong, J. (2006). Efficacy of radio frequency cooking in the reduction of *Escherichia coli* and shelf stability of ground beef. *Food Microbiology*, 23(2), 112-118. <https://doi.org/10.1016/j.fm.2005.02.004>
- Hassani, M., Álvarez, I., Raso, J., Condón, S. and Pagán, R. (2005). Comparing predicting models for heat inactivation of *Listeria monocytogenes* and *Pseudomonas aeruginosa* at different pH. *International Journal of Food Microbiology*, 100(1-3), 213-222. <https://doi.org/10.1016/j.ijfoodmicro.2004.10.017>
- Heredia, N. and García, S. (2018). Animals as sources of food-borne pathogens: A review. *Animal Nutrition*, 4(3), 250-255. <https://doi.org/10.1016/j.aninu.2018.04.006>
- Karakolev, R. (2009). Incidence of *Listeria monocytogenes* in beef, pork, raw-dried and raw-smoked sausages in Bulgaria. *Food Control*, 20(10), 953-955. <https://doi.org/10.1016/j.foodcont.2009.02.013>
- Kramarenko, T., Roasto, M., Meremäe, K., Kuningas, M., Pöltšama, P. and Elias, T. (2013). *Listeria monocytogenes* prevalence and serotype diversity in various foods. *Food Control*, 30(1), 24-29. <https://doi.org/10.1016/j.foodcont.2012.06.047>
- Kuan, C.H., Goh, S.G., Loo, Y.Y., Chang, W.S., Lye, Y.L., Puspanadan, S., Tang, J.Y.H., Nakaguchi, Y., Nishibuchi, M., Mahyudin, N.A. and Son, R. (2013). Prevalence and quantification of *Listeria monocytogenes* in chicken offal at the retail level in Malaysia. *Poultry Science*, 92, 1664-1669. <https://doi.org/10.3382/ps.2012-02974>
- Kuan, C.H., Lim, L.W.K., Ting, T.W., Rukayadi, Y., Ahmad, S.H., Wan Mohamed Radzi, C.W.J., Thung, T.Y., Ramzi, O.B., Chang, W.S., Loo, Y.Y., Kuan, C.S., Yeo, S.K., and Son, R. (2017). Simulation of decontamination and transmission of *Escherichia coli* O157: H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* during handling of raw vegetables in domestic kitchens. *Food Control*, 80, 395-400. <https://doi.org/10.1016/j.foodcont.2017.05.029>
- Kurpas, M., Wiczorek, K. and Osek, J. (2018). Ready-to-eat meat products as a source of *Listeria monocytogenes*. *Journal of Veterinary Research*, 62(1), 49-55. <https://doi.org/10.2478/jvetres-2018-0007>
- Lahou, E., Wang, X., De Boeck, E., Verguldt, E., Geeraerd, A., Devlieghere, F. and Uyttendaele, M. (2015). Effectiveness of inactivation of foodborne pathogens during simulated home pan-frying of steak, hamburger or meat strips. *International Journal of Food Microbiology*, 206, 118-129. <https://doi.org/10.1016/j.ijfoodmicro.2015.04.014>

- Laycock, L., Piyasena, P. and Mittal, G.S. (2003). Radio frequency cooking of ground, comminuted and muscle meat products. *Meat Science*, 65(3), 959-965. [https://doi.org/10.1016/S0309-1740\(02\)00311-X](https://doi.org/10.1016/S0309-1740(02)00311-X)
- Lin, Y.T., Labbe, R.G. and Shetty, K. (2004). Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. *Applied and Environmental Microbiology*, 70(9), 5672-5678. <https://doi.org/10.1128/AEM.70.9.5672-5678.2004>
- Lipoeto, N.I., Khor, G.L. and Angeles-Agdeppa, I. (2013). Food consumption patterns and nutrition transition in South-East Asia. *Public Health Nutrition*, 16(9), 1637-1643. <https://doi.org/10.1017/S1368980012004569>
- Marian, M.N., Sharifah Aminah, S.M., Zuraini, M.I., Son, R., Maimunah, M., Lee, H.Y., Wong, W.C. and Elexson, N. (2012). MPN-PCR detection and antimicrobial resistance of *Listeria monocytogenes* isolated from raw and ready-to-eat foods in Malaysia. *Food Control*, 28(2), 309-314. <https://doi.org/10.1016/j.foodcont.2012.05.030>
- Martín, B., Jofré, A., Garriga, M., Hugas, M. and Aymerich, T. (2004). Quantification of *Listeria monocytogenes* in fermented sausages by MPN-PCR method. *Letters in Applied Microbiology*, 39(3), 290-295. <https://doi.org/10.1111/j.1472-765X.2004.01580.x>
- McMinn, R.P., King, A.M., Milkowski, A.L., Hanson, R., Glass, K.A. and Sindelar, J.L. (2018). Processed meat thermal processing food safety - generating D-Values for *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli*. *Meat and Muscle Biology*, 2(1), 168-179. <https://doi.org/10.22175/mmb2017.11.0057>
- McNeill, C., Sisson, W. and Jarrett, A. (2017). Listeriosis: A Resurfacing Menace. *The Journal for Nurse Practitioners*, 13(10), 647-654. <https://doi.org/10.1016/j.nurpra.2017.09.014>
- Mead, G.C. (2007). Sampling Methods for Poultry-Meat Products. In Mead, G.C. (Ed). *Microbiological Analysis of Red Meat, Poultry and Eggs*, p. 148-164. Washington: CRC Press. <https://doi.org/10.1533/9781845692513.148>
- Ministry of Health Malaysia (MOH). (2005). Guideline on Health Certificate Requirement for Importation of Meat, Edible Meat Offal, Poultry, Edible Poultry Offal, Shrimps and Prawns into Malaysia. Retrieved on July 26, 2019 from Ministry of Health Malaysia Website: http://fsis2.moh.gov.my/UploadFosim/BULETTIN/200815094629B3558Lampiran_Garispanduan%20HC.pdf
- Murphy, R.Y., Duncan, L.K., Johnson, E.R., Davis, M.D. and Marcy, J.A. (2002). Thermal inactivation of *Salmonella* Senftenberg and *Listeria innocua* in beef/turkey blended patties cooked via fryer and/or air convection oven. *Journal of Food Science*, 67(5), 1879-1885. <https://doi.org/10.1111/j.1365-2621.2002.tb08740.x>
- Nastou, A., Rhoades, J., Smirniotis, P., Makri, I., Kontominas, M. and Likotrafiti, E. (2012). Efficacy of household washing treatments for the control of *Listeria monocytogenes* on salad vegetables. *International Journal of Food Microbiology*, 159(3), 247-253. <https://doi.org/10.1016/j.ijfoodmicro.2012.09.003>
- Painter, J. and Slutsker, L. (2007). Listeriosis in Human. In Ryser, E.T. and Marth, E.H. (Eds). *Listeria, Listeriosis, and Food Safety*, p. 85-110. Boca Raton, FL: CRC Press. <https://doi.org/10.1201/9781420015188.ch4>
- Pangloli, P. and Hung, Y.C. (2013). Reducing microbiological safety risk on blueberries through innovative washing technologies. *Food Control*, 32(2), 621-625. <https://doi.org/10.1016/j.foodcont.2013.01.052>
- Pathare, P.B. and Roskilly, A.P. (2016). Quality and energy evaluation in meat cooking. *Food Engineering Reviews*, 8, 435-447. <https://doi.org/10.1007/s12393-016-9143-5>
- Roccatto, A., Uyttendaele, M., Cibin, V., Barrucci, F., Cappa, V., Zavagnin, P., Longo, A. and Ricci, A. (2015). Survival of *Salmonella* Typhimurium in poultry-based meat preparations during grilling, frying and baking. *International Journal of Food Microbiology*, 197, 1-8. <https://doi.org/10.1016/j.ijfoodmicro.2014.12.007>
- Samara, A. and Koutsoumanis, K.P. (2009). Effect of treating lettuce surfaces with acidulants on the behaviour of *Listeria monocytogenes* during storage at 5 and 20 °C and subsequent exposure to simulated gastric fluid. *International Journal of Food Microbiology*, 129(1), 1-7. <https://doi.org/10.1016/j.ijfoodmicro.2008.10.023>
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M. (2011). Foodborne illness acquired in the United States-Major pathogens. *Emerging Infectious Diseases*, 17(1), 7-15. <https://doi.org/10.3201/eid1701.P11101>
- Schlisselberg, D.B., Kler, E., Kalily, E., Kisluk, G., Karniel, O. and Yaron, S. (2013). Inactivation of foodborne pathogens in ground beef by cooking with highly controlled radio frequency energy. *International Journal of Food Microbiology*, 160(3), 219-226. <https://doi.org/10.1016/>

- j.ijfoodmicro.2012.10.017
- Shirsat, N., Lyng, J.G., Brunton, N.P. and Mckenna, B.M. (2004). Conductivities and ohmic heating of meat emulsion batters. *Journal of Muscle Foods*, 15, 121-137. <https://doi.org/10.1111/j.1745-4573.2004.tb00716.x>
- Tian, X., Shao, L., Yu, Q., Yang, H., Li, X. and Dai, R. (2019). Comparative study of survival of *Escherichia coli* O157: H7 inoculated in pork batter after ohmic cooking and water bath cooking. *International Journal of Food Microbiology*, 304, 11-18. <https://doi.org/10.1016/j.ijfoodmicro.2019.05.019>
- Ukuku, D.O. and Fett, W. (2002). Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *Journal of Food Protection*, 65(6), 924-930. <https://doi.org/10.4315/0362-028X-65.6.924>
- United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS). (2002). Interpretation of the Risk Estimates for Intact and Non-Intact Beef Cooked to Temperatures 140°F. Retrieved on July 26, 2019 from FSIS Website: http://www.fsis.usda.gov/wps/wcm/connect/9edab015-a06b-4627-b295-af13f1dcb20e/Non-intact_Beef_Risk_Estimates_RAD.pdf?MOD=AJPERES
- Vijay, K., Shiowshuh, S. and Gaurav, T. (2008). Intervention Technologies for Food Safety and Preservation. In Wilson, C, L. (Ed). *Microbial Food Contamination*, 2nd ed., p. 373-375. Boca Raton, FL: CRC Press.
- Vijayakumar, C. and Wolf-Hall, C.E. (2002). Evaluation of household sanitizers for reducing levels of *Escherichia coli* on iceberg lettuce. *Journal of Food Protection*, 65(1), 1646-1650. <https://doi.org/10.4315/0362-028X-65.10.1646>
- Wong, W.C., Pui, C.F., Tuan Chilek, T.Z., Noorlis, A., Tang, J.Y.H., Nakaguchi, Y., Nishibuchi, M. and Radu, S. (2011). Survival of *Listeria monocytogenes* during frying of chicken burger patties. *Food and Nutrition Sciences*, 2(5), 471-475. <https://doi.org/10.4236/fns.2011.25067>
- Wu, F.M., Doyle, M.P., Beuchat, L.R., Wells, J.G., Mintz, E.D. and Swaminathan, B. (2000). Fate of *Shigella sonnei* on parsley and methods of disinfection. *Journal of Food Protection*, 63(5), 568-572. <https://doi.org/10.4315/0362-028X-63.5.568>
- Yoon, Y., Geornaras, I., Mukherjee, A., Belk, K.E., Scanga, J.A., Smith, G.C. and Sofos, J.N. (2013). Effects of cooking methods and chemical tenderizers on survival of *Escherichia coli* O157: H7 in ground beef patties. *Meat Science*, 95(2), 317-322. <https://doi.org/10.1016/j.meatsci.2013.04.056>