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Thermal inactivation of *Salmonella* in chicken curry puff filling

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ABSTRACT

This study explores the potential risk of *Salmonella* contamination in chicken curry puff filling, focusing on chicken as a high-risk ingredient during filling preparation. It investigates cooking temperature as a critical control point to reduce *Salmonella* contamination and determines thermal inactivation kinetic parameters (*D*- and *z*-values) in chicken curry puff filling. The pH, water activity, and proximate composition of the filling were analysed. Ground chicken was inoculated with a target concentration of approximately 7 log cfu/g of *Salmonella* and its survival was assessed at pre-determined internal temperatures (60, 67, 74, and 81 ℃) using thin-layer agar plating. The *Salmonella* concentration decreased below the enumeration limit (1 log CFU/g) after 25.5 min at an internal temperature of 74°C, demonstrating a significant reduction. However, complete elimination was not confirmed in this study, suggesting potential for further investigation. Thermal inactivation parameters were determined at 55, 57.5, 60, and 62.5°C using temperature-controlled water baths. The *D*-values were 29.31 ± 0.42, 7.26 ± 0.29, 5.45 ± 0.42, and 1.34 ± 0.05 min at 55, 57.5, 60, and 62.5°C, respectively, with a *z*-value of 6.03°C. These parameters can be used to predict the time and temperature to achieve a 7-log cfu/g reduction of *Salmonella*, aligning with the current performance standard in commercial poultry processing. These findings provide essential data for food manufacturers to develop HACCP plans aimed at effectively reducing *Salmonella* in chicken curry puff filling and related products.

Introduction

Food manufacturers must provide scientific and technical evidence in HACCP plans to demonstrate effective control measures of significant hazards, reducing public health risks to consumers. Manufacturers are responsible for conducting validation studies under worst-case scenarios (CAC, 2008). This documentation is crucial for establishing a solid HACCP and food safety plan (ISO, 2018; FDA, 2016). Over the years, several guidelines have been published on control measures validation (CAC, 2008; USDA-FSIS, 2015; ISO, 2018; Ceylan et al., 2021), however many focus on specific commodities or generic guidelines, limiting applicably for diverse food manufacturers. Reviewing scientific literature can be subjective, often failing to match exact composition and processes in commercial plants (Gregory and Marcy, 2010; Panisello and Quantick, 2001). This restricts access to relevant reference materials, posing a challenge for validation studies. Scientific validation studies include microbiological challenge tests and predictive modelling, providing real-time data on food processing, distribution, and handling (CAC, 2008).

Curry puff, a popular Southeast Asian snack, especially in Malaysia, is a crescent-shaped pastry with a flaky crust. Curry puff preparation involves two stages: making pastry skin from wheat flour, butter, water, salt, and sugar, and making the filling from cooked potatoes, meat, chilli, curry powder, salt, and sugar. Curry puff can be deep-fried or baked at 180°C for 30 min. However, it is susceptible to microbial contamination such as *Salmonella*, which can originate from contaminated ingredients (Smith et al., 2004) or cross-contamination within facilities (Carrasco et al., 2012).

Thermal processing is effective in controlling foodborne pathogens but requires validation to ensure the product is exposed to a safe minimum internal temperature and time to reduce hazards and promote food safety. This study aims to investigate the cooking time and temperature of commercial chicken curry puff filling and determine the thermal inactivation parameters of *Salmonella* using inoculated ground chicken

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Table 1
Ingredients used to prepare chicken curry puff filling.

Ingredients	Cooking validation (1 %) (g)	Thermal inactivation (0.1 %) (g)
Potato	1300	169
Onion	150	20
Sugar	120	16
Salt	27	4
Curry powder	20	3
Chilli powder	10	1
Cooking oil	20	3
Water	20 (mL)	3 (mL)
Chicken	200	26

samples. The data will help manufacturers design HACCP plans and validate CCPs to effectively kill *Salmonella* spp. in chicken curry puff processing and related pastry products.

Materials and methods

Inoculum preparation

This study used a 2-serovars *Salmonella* cocktail, *S.* Typhimurium (ATCC 14028) and *S.* Enteritidis (ATCC 13076), from American Type Culture Collection (ATCC, Manassas, VA, USA). Frozen *Salmonella* cultures (-20°C) were propagated individually to prepare working cultures. A 10 μL loop was transferred into 10 mL of Tryptone Soy Broth (TSB; Oxoid Ltd; UK) and incubated at 35–37°C for 24 h. Stock cultures were streaked onto Tryptone Soy Agar plates (TSA; Oxoid Ltd; UK) to obtain isolated colonies for inoculum preparation, following protocol by Channaiah et al., (2017) with modification. A single colony from a working stock plate was cultured in TSB and 1 mL of the culture was dispensed onto TSA plates and incubated (35–37°C) to grow bacterial lawns. The lawns were harvested twice using 1 mL of 0.1 % bacteriological peptone water (Oxoid Ltd; Hampshire; UK) and dislodged with L-shaped spreaders. The harvested cultures were mixed in a 50 mL centrifuge tube and vortexed for 30 s to create a master inoculum at approximately 8–9 log cfu/mL.

Chicken inoculation

For cooking validation, 200 g of ground chicken (KLFC, Kuala Lumpur Fried Chicken (M) Sdn. Bhd., Malaysia) was inoculated, whereas for thermal inactivation, 26 g of ground chicken was inoculated with 0.2 mL of *Salmonella*, ensuring inoculation volume below 1 % of the sample weight (NACMCF, 2010). The inoculated samples were hand-massaged for 30 s for even distribution of *Salmonella* and held at 4°C.

Filling preparation

The curry puff filling was prepared using a scaled-down commercial formulation (Table 1), with ingredients supplied by a small medium enterprise (SME) company in Gombak, Selangor, to ensure consistency in sample composition.

The filling was prepared by sautéing onion, sugar, salt, curry powder, chilli powder, water, and cooking oil, before cooking with potatoes to an internal temperature of 89 ± 2°C using a non-stick wok pan (L44cm x H12cm x W32cm) on an induction cooker (HD4921; Philips; Netherland) set at 80°C. After cooling to approximately 25°C, the mixture was mixed with inoculated ground chicken for thermal studies, following procedures by Porto-Fett et al. (2019) with modifications.

Cooking validation

This study analysed *Salmonella* survival in chicken curry puff filling after cooking to internal temperatures; 60, 67, 74, and 81°C. The target temperatures were based on FSANZ's hot holding temperature (60°C) (FSANZ, 2016), USDA's minimum safe temperature for cooked poultry (74°C) (USDA-FSIS, nd.), and company standard operating procedures (SOPs) for internal temperature exceeding 80°C. The intermediate temperature of 67°C was included as a reference point for experimental purposes. A 25 g of the filling was sampled at each temperature, transferred to a sterile sample bag, and immediately placed in an ice-water slurry, where it was held for up to 30 min before *Salmonella* enumeration.

Salmonella thermal inactivation

D- and *z*-values for thermal inactivation of *Salmonella* were assessed following the method of Osaili et al. (2013). Six 25 g sample bags with a temperature reference sample, were flattened to 3 mm and vacuum-sealed to remove air pockets for even heat penetration (López-Romero et. al., 2018). Temperature monitoring was performed using a type-K thermocouple probe (PDQ400; Comark; USA) inserted at the geometric centre of the sample. Sample bags were placed on a stainless-steel wire rack and submerged in a temperature-controlled water bath (Memmert; Germany) at 55, 57.5, 60, and 62.5°C. Samples were removed at intervals, (30 min for 55°C, 7 min for 57.5°C, 4 min for 60°C, and 1 min for 62.5°C), and transferred to an ice box (0–4°C).

Salmonella enumeration

A 35 g curry puff was ground using mortar and pestle for sample homogeneity. A 25 g sample was homogenized with 225 mL of 1 % bacteriological peptone water (Oxoid Ltd; UK) using a stomacher (Bagmixer 400-P, Interscience, France) for 60 s. The suspension was serially diluted and plated using thin agar layer (TAL) (Kang and Fung, 2000), with 14 mL nonselective media (TSA) overlaid onto solidified Xylose Lysine Deoxycholate Agar (XLD; Oxoid Ltd; UK). Additional 7 mL TSA was overlaid prior to inoculation. After an overnight incubation (35–37°C), injured *Salmonella* cells appeared as visible black colonies were counted using a colony counter (Galaxy230 Colony Counter; Rocker Scientific Co. Ltd.; Taiwan). All analyses were performed in three replicates, and results reported as log cfu/g. The study involves investigating *Salmonella* survival during cooking validation with N = 18 samples and assessing thermal inactivation with N = 72 samples. The lower level of enumeration of *Salmonella* was 1 log cfu/g.

pH, water activity and proximate analysis

This study assessed sample properties for pH, water activity (a_w), and proximate compositions; ash, moisture, fat, protein, and carbohydrate content. pH was measured after diluting ground samples with 1:1 ratio of distilled water, using a digital pH metre (3505, Jenway; UK). Moisture was analysed using a moisture analyzer (XM-50; Precisa Instruments; Switzerland). a_w was determined using a water activity metre (Aqualab Series 3; Meter Group; USA). Proximate analyses were performed on 200 g composite samples by an ISO/IEC 17025-accredited laboratory in Petaling Jaya, Selangor. Carbohydrate content was calculated using the following equation: Carbohydrates (%) = 100 – (moisture + protein + ash + fat) (Ruiz-Cano et al., 2016).

D- and z- value determinations

Linear regression graphs for the *Salmonella* population were plotted to calculate *D*-values from the absolute values of the inverse slope of the linear regression line. The *z*-value was determined from absolute value of the inverse slope of a linear regression line that plotted log of *D*-values against temperatures. The thermal death time (TDT) of *Salmonella* was calculated using Douglas' (2014) equation (**Equation 2**), $F_0 = D_T$ (log *a*-log *b*), where D_T is the *D*-value at a specific temperature, *a* is the initial population and *b* is the final population. Non-reference *D*-value (D_{74}) was calculated using Horn et al. (2015) equation (**Equation 3**), log (*D*)

Table 2
Survival of *Salmonella* in chicken curry puff filling during cooking.

Filling temperature (℃)	*Salmonella* count (log cfu/g)
24.3 (*Start*)	7.51 ± 0.16[a]
60.0	2.70 ± 0.04[b]
67.0	2.00 ± 0.00[c]
74.0	<1
81.0	<1
129.5 (*Final*)	<1

Different letters indicate a significant difference at $p \leq 0.05$. Data are means of three replicate samples and error bars indicate ± SD

$= \log (D_{ref}) - [(T\text{-}T_{ref}) / z]$, where D_{ref} is the reference *D*-value of predetermined temperatures and z is the *z*-value.

Statistical analyses

One-way analysis of variance was used to determine the effect of internal temperature of the filling during cooking on the concentration of *Salmonella* (Minitab 19, Minitab Inc., USA). Means were compared among temperatures using Tukey's multiple comparison test at $p \leq 0.05$.

Results and discussion

The pH, a_w, and proximate composition of the filling sample were analysed. The sample demonstrated a pH of 6.44 ± 0.01 and a notably high water activity (a_{tx}) of 0.93 ± 0.001, indicating significant water availability that facilitates microbiological growth. Food with higher a_w requires stringent storage practices such as refrigeration or freezing to prevent deterioration. The sample exhibited a fat content of 2.07 ± 0.12 %, protein content of 4.64 ± 0.22 %, and total ash content of 2.48 ± 0.30 %. Additionally, the filling exhibited a moisture content of 70.34 ± 1.59 %. Potatoes, naturally contain high moisture levels of 75–85 % (Onu et al., 2020). The carbohydrate content was 20.48 ± 1.99 %, which may be influenced by the potato cultivars used. Firm to waxy potatoes have higher moisture and lower starch content, whereas floury potatoes have lower moisture and higher starch (Nayak et al., 2014).

Survival of *Salmonella* during cooking validation

Salmonella survival was assessed at internal temperatures of 60, 67, 74, and 81℃, with a cooking temperature of 80℃ and monitored filling temperatures. Starting at 24.3℃, the filling reached 129.5℃ by the end of 45-min cooking process. The inoculated ground chicken, initially at 4°C, was mixed with other pre-cooked ingredients, resulting in an internal filling temperature of approximately 24°C prior to cooking. The time gap between mixing and the start of cooking was carefully controlled to minimize the risk of *Salmonella* growth before the process. The final internal temperature of 129.5℃ represents the optimal quality of the cooked curry puff filling, based on the average final temperatures measured at the SME, where the formulation and ingredients were supplied. Significant reductions in *Salmonella* occurred across all internal temperatures ($p \leq 0.05$, Table 2), with an optimal reduction observed at 60℃, where the population was decreased by the mean difference of ≥ 4 log cfu/g, dropping from of 7.51 ± 0.16 log cfu/g to 2.70 ± 0.04 log cfu/g within 17 min.

The study further demonstrated that at 67℃, *Salmonella* population was significantly reduced, with a surviving population of 2.00 ± 0.00 log cfu/g, presenting a mean difference of 5.51 log reduction. Reductions below the lower limit of enumeration of *Salmonella* were achieved at 74°C, 81°C, and 129.5°C, with reductions ≥ 7-log after cooking for 25.5, 32.8, and 45 min, respectively, supporting the safe internal temperature recommendation for poultry.

Simulating the worst-case scenario is important for validating processing parameters (FDA, 2016; NACMCF, 2010). In this study, ground chicken was inoculated with a high *Salmonella* concentration (7.51 ± 0.16 log cfu/g), slightly lower than the initial inoculation level likely due to the antimicrobial effects of added onions and spices in the formulation (Karagözlü and Bozatli, 2021). Our findings indicated that cooking chicken curry puff filling for ≥ 25.5 min to 74℃, achieved the 7-log reduction of *Salmonella* required for cooked poultry products (USDA-FSIS, 2017). While this reduction brought the *Salmonella* concentration to non-detection, the potential for *Salmonella* survival should be considered, as no further analysis was conducted to verify complete inactivation. Furthermore, establishing a critical cooking time limit is essential, and *D*-value can be utilised to establish the limit.

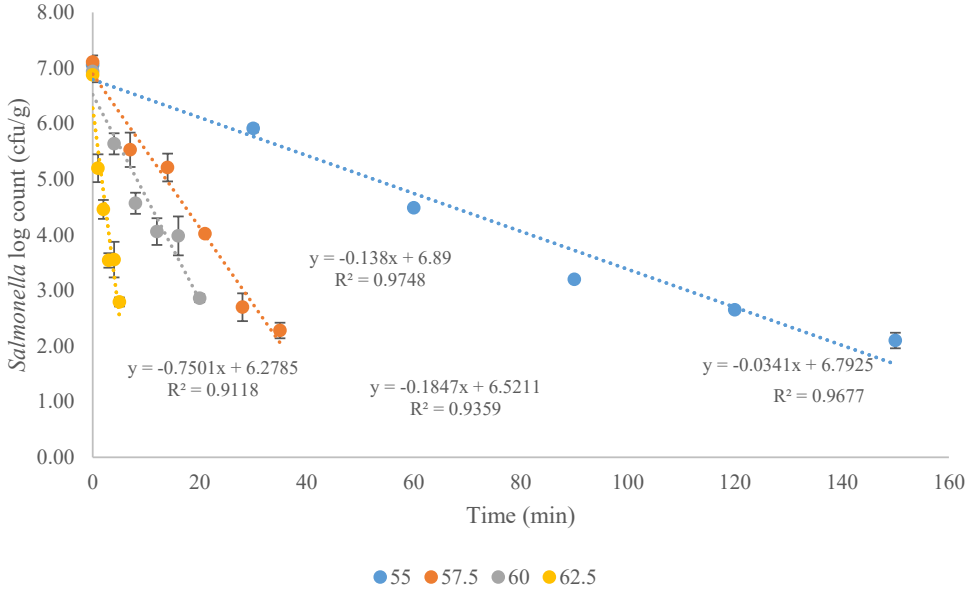

Fig. 1. Survival curves of *Salmonella* cocktail in chicken curry puff filling at 55, 57.5, 60, and 62.5°C. Data are means of three replicate samples and error bars indicate ± SD.

Table 3
D-values of a *Salmonella* cocktail in chicken curry puff filling.

Temperature (℃)	*D*-values (min)
55	29.31 ± 0.42^{a}
57.5	7.26 ± 0.29^{b}
60	5.45 ± 0.42^{c}
62.5	1.34 ± 0.05^{d}

Different letters indicate a significant difference at $p \leq 0.05$. Data are means of three replicate samples and error bars indicate ± SD

D- and z-values of Salmonella in chicken curry puff filling

This study investigated thermal inactivation of *Salmonella* by determining *D*- and *z*-values across treatment temperatures; 55, 57.5, 60, and 62.5 ℃. Survival curves (Fig. 1) revealed that inactivation followed first-order kinetics. Regression analysis showed strong linearity (R^2 > 0.9118) across the temperatures, with steeper slopes at a higher temperatures reflecting faster inactivation rate of *Salmonella* in the sample. For example, heating at 62.5℃ resulted in the most rapid *Salmonella* reduction, indicating the efficiency of higher temperatures in controlling *Salmonella* in the filling.

The study determined the *D*-values of *Salmonella* from the regression slopes of the survival curves (Fig. 1). The calculated *D*-values were presented in Table 3, reflecting the thermal resistance of *Salmonella* at the onset of cooking. A clear inverse relationship was observed, with *Salmonella*'s thermal resistance decreasing as the temperature increased: $D_{55} > D_{57.5} > D_{60} > D_{62.5}$.

Research on thermal inactivation of *Salmonella* in chicken fillings is limited, despite extensive studies in bakery and poultry products. Results showed lower *D*-values in this sample than those reported by Murphy et al. (2000, 2004), which observed greater thermal resistance of 6-serovar *Salmonella* cocktail in ground chicken breast (*D*-values of 30.10, 12.90, 5.88, and 2.51 min) and thigh/leg meat (*D*-values of 43.76, 13.66, 5.72 and 1.62 min) at 55, 57.5, 60 and 62.5℃. In contrast, López-Romero et al. (2018) reported lower thermal resistance with *D*-values of 21.85, 5.43, 2.83, and 0.58 min, respectively. These variations likely reflected differences in sample compositions, *Salmonella* strains, and other influencing factors (Doyle and Mazzotta, 2000). This study used *S*. Typhimurium and *S*. Enteritidis, following USDA-FSIS (2017) recommendation to use strains implicated in foodborne outbreaks.

Higher fat and protein contents can promote thermal resistance by forming a protective sheath around bacterial cell during heat treatment. Juneja et al. (2001) reported greater thermal resistance of *Salmonella* in ground chicken samples with higher fat content (12 %). In the current filling sample, with a fat content of 2.07 ± 0.12 %, *Salmonella* demonstrated increased thermal sensitivity. High moisture and a_w of the filling further facilitated *Salmonella* inactivation during heat treatment (Garces-Vega et al., 2019). Heat causes water molecules in *Salmonella* cells to vibrate, breaking protein bond structure, leading to their inactivation (Jin et al., 2020). These filling compositions may create sub-optimal conditions for *Salmonella* survival, resulting in lower *D*-values.

Fig. 2 illustrates the linear relationship between the log *D*-values and temperature for thermal inactivation of *Salmonella* in chicken curry puff filling. The regression slope was used to calculate the z value.

The *z*-value for *Salmonella* was determined to be 6.03℃, with R^2 of 0.9484, indicating that an increase of 6.03℃ reduces the *D*-value by one log. In comparison, *z*-values of 5.6℃ to 6.4℃ were reported by Murphy et al. (2000), López-Romero et al. (2018) and Webster (2018). Gu et al. (2022) found that *Salmonella* *z*-value decreased with higher moisture content in cornmeal, reporting 14℃ for 16 % and 11.7℃ for 28 %.

Using **Equation 2**, TDT required to achieve a 7-log reduction of *Salmonella* during the processing was 205.17 min (55℃), 50.82 min (57.5℃), 38.15 min (60℃) and 9.38 min (62.5℃). The estimated D_{74} calculated using **Equation 3**, was 1.68 s, indicating that contaminated chicken curry puff filling should be cooked to 74℃ for at least 11.78 s to achieve the target reduction.

Conclusion

This study validates cooking chicken curry puff filling at 74℃ for at least 11.78 s can achieve a 7-log reduction in *Salmonella* from contaminated ground chicken. It is imperative to control *Salmonella* through validated CCPs to support HACCP implementation in food processing. The generated *D*- and *z*- values can aid manufacturers in optimising cooking processes and designing critical limits. However, these results should not be extrapolated to different filling composition or cooking parameters.

CRediT authorship contribution statement

Rosli Nur Amira: Writing – original draft, Investigation, Formal analysis. **Mahyudin Nor Ainy:** Supervision, Resources, Methodology, Conceptualization. **Mahmud Ab Rashid Nor-Khaizura:** Methodology, Conceptualization. **Selamat Jinap:** Supervision, Methodology, Conceptualization. **Mohamad Darwi Lizawati:** Supervision, Conceptualization. **Khan Rahim:** Writing – review & editing, Data curation.

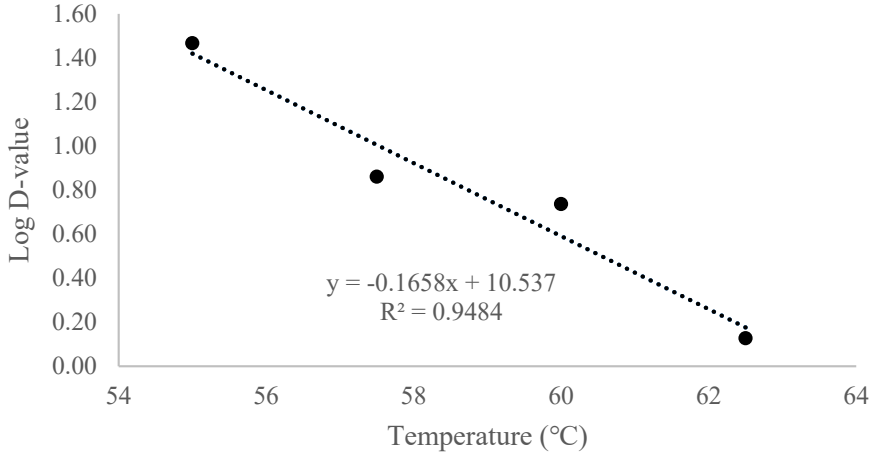

Fig. 2. Linear regression for *z*-value (°C) of *Salmonella* cocktail in chicken curry puff filling.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data will be made available on request.

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