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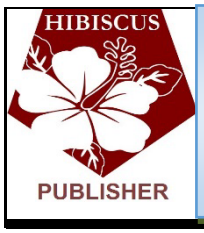

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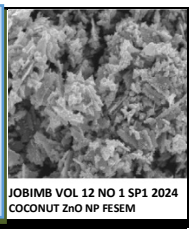

Effect of Temperature on Thermal Resistance of *Salmonella* Typhimurium in Simulated Wheat Gluten-Based Patty

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ABSTRACT

Wheat gluten-based patty, known as 'seitan', is used for alternative meat products. However, repeated *Salmonella* outbreaks linked to flour raise concerns; therefore, this study aimed to assess the effect of temperature on the thermal resistance of *Salmonella* Typhimurium in simulated wheat gluten-based patty at 60°C, 65°C and 70°C. A mixture of water (100 mL), wheat gluten flour (75 g), canola oil (10 mL) and salt (1 g) were inoculated with *Salmonella* inoculum (1 mL). Samples (5 g each) were packed, flattened, and vacuum sealed in a double bag subjected to heat treatment before two subsamples were pulled at pre-determined time intervals for *Salmonella* survivor enumeration. A linear relationship between log reduction and heat treatment time was observed at all temperatures, indicated by Root Mean Square Error (RMSE), which was < 1.3. The D values were estimated using the USDA Integrated Pathogen Modelling Program (IPMP), and yielded 5.86, 3.34, and 2.00 min for 60°C, 65°C, and 70°C, respectively. The calculated z value was 12.4°C. Both D and z values obtained in this study were higher than the meat patty reported in the literature, suggesting the need for further investigations to design more effective thermal control to inactivate *Salmonella* in wheat gluten-based patty.

INTRODUCTION

Wheat gluten-based patty has emerged as a prominent plant-based protein option, appealing to vegetarians due to its nutritional content and formation of a meat-like fibrous network [1]. They are typically made by mixing wheat gluten flour with water and other ingredients, such as herbs and spices, forming a dough and cooking. Concern arises about the safety of using wheat gluten flour in patties because there has been an alarming rise in *Salmonella* outbreaks linked to flour over the years, such as one in cake batter ice cream that occurred in the United States, with the source traced to contaminated cake mix [2], and another in New Zealand, a cluster of salmonellosis cases linked to an uncooked baking mixture containing flour contaminated with *Salmonella* Typhimurium phage type 42 [3]. Furthermore, studies have discovered that *Salmonella,* when exposed to low water activity environment, may exhibit better thermal resistance to subsequent heat treatment [4-6]. Though studies have extensively investigated the thermal resistance of *Salmonella* Typhimurium in meat patties [7-9], limited research exists on that of wheat gluten-based patties. Hence, this study addresses this gap by investigating the effect of temperature on the thermal resistance of *Salmonella* Typhimurium in simulated wheat gluten-based patty to validate thermal lethality for commercial products.

MATERIALS AND METHODS

Background microbial counts, including aerobic bacteria, yeast, mold, and *Salmonella*, were conducted using wheat gluten flour. Inoculum preparation was prepared by reviving *Salmonella* Typhimurium from frozen stock, harvesting the bacteria lawn by adding buffered peptone water, and gently scraping the agar surface to get the inoculum. The patty was prepared from a mixture of *Salmonella* inoculum (1 mL), water (100 mL), wheat gluten flour (75 g), salt (1 g), and canola oil (10 mL). The inoculated patty was in an elastic dough form.

Fig. 1. Simulated wheat gluten-based patty.

A homogeneity test was conducted on the inoculated wheat gluten-based patty. The patty sample was packed in a Whirlpak bag, flattened, and vacuum sealed in a double bag. They were immersed in the water bath at different temperatures, with corresponding time intervals for each temperature. After heat treatment, modified TSA with Yeast extract or mTSAYE was used to enumerate *Salmonella* survivors. Log CFU was calculated from the *Salmonella* colonies, and log reduction was obtained by subtracting log CFU at that particular time points from the log CFU at 0 min. IPMP 2013 generated heat inactivation kinetics and calculated D values for each temperature. A graph of log D against temperature was plotted to find z value from the inverse slope.

RESULT AND DISCUSSION

Table 1 illustrates the background microbial counts in wheat gluten flour. No black colonies were detected on mTSAYE, which indicates that the background *Salmonella* was nearly absent in WG flour; hence, it is appropriate to use it for the subsequent heat treatment. Low levels of aerobic bacteria, yeast, and mold (~ 2 log CFU/g) were also seen in WG flour, ensuring that other microorganisms would not influence the inoculated *Salmonella* population. Overall, there was no significant microbial growth in the flour sample.

Table 1. Background microbial counts in wheat gluten flour (n=2).

Type of microorganism	Microbial counts (log CFU/g)
Aerobic bacteria	2.24 ± 0.09
Yeast and mold	2.09 ± 0.12
Salmonella	nd*

Table 2 presents the homogeneity test for inoculated wheat gluten-based patty. The standard deviation for all batches of inoculated patty was below 0.3 log CFU/g [10], indicating that S*almonella* Typhimurium was homogenous in the patty samples.

Table 2. Homogeneity test for inoculated wheat gluten-based patty (n=3).

Batch	Initial *Salmonella* population in the inoculated patty (log CFU/g)
1	8.53 ± 0.14
2	8.25 ± 0.23
3	9.28 ± 0.21

Fig. 1 illustrates the *Salmonella* heat inactivation kinetics in simulated WG-based patty at 60°C, 65°C, and 70°C, which is represented by log reduction. From the graph, the greatest log reduction was observed at 60°C, achieving a 5 log reduction, which suggests that 60°C heat treatment can inactivate 99.999% of the *Salmonella* Typhimurium [11].

Overall, the observed trend of log reduction follows the linear trend. This finding aligns with Aviles et al. [9], who found that the survival of *Salmonella* cells decreased linearly as exposure heating time increased.

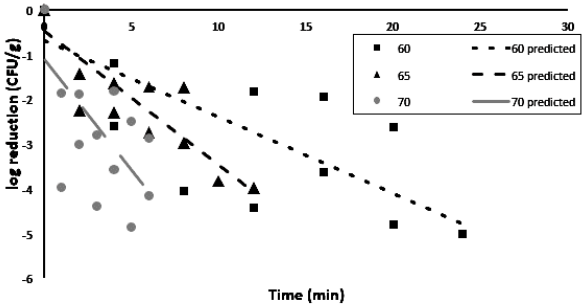

Fig. 1. Log reduction of *Salmonella* Typhimurium in simulated wheat gluten-based patty versus time at 60°C, 65°C and 70°C. Two biologically independent heat treatment experiments were performed, and duplicate samples were used for each time point (n=2).

Table 3 displays the D values with corresponding standard error, RMSE, 95% lower and upper limit obtained from IPMP 2013 for 60°C, 65°C, and 70°C. The heat inactivation kinetics of *Salmonella* Typhimurium in simulated wheat gluten-based patty was evaluated using the loglinear model. The D values obtained from this study are higher than those reported in the literature [7,9], which might be attributed to differences in the food matrix composition, pH, water activity, inoculation method, methodology used for the recovery of *Salmonella* survivors as well as cooking techniques and parameters.

Table 3. D value, standard error, RMSE, lower limit and upper limit obtained from IPMP 2013 using a log-linear model.

Temperature (°C)	D-value (min)	Standard error (min)	RMSE (log CFU/g)	95% confidence interval	
				Lower limit	Upper limit
60	5.86	1.40	1.19	2.78	8.95
65	3.34	0.52	0.65	2.19	4.49
70	2.00	0.61	1.23	0.68	3.32

Fig. **2** shows the graph of log D value against temperature. A linear relationship was observed. From the inverse slope, the z value obtained was 12.4, significantly higher than those reported in the literature [7,9].

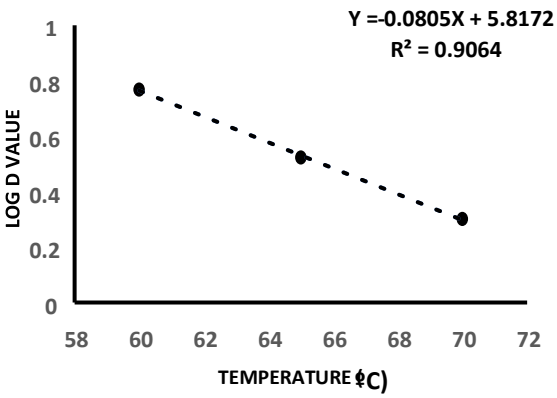

Fig. 2. Log D value of *Salmonella* Typhimurium in wheat gluten-based patty at 60°C, 65°C and 70°C**.**

CONCLUSION

In conclusion, D and z values were determined for *Salmonella* Typhimurium in the simulated wheat gluten-based patty. They are important parameters to validate thermal lethality for commercial products. Both values obtained in this study are higher than those reported for meat patty, suggesting that *Salmonella Typhimurium* shows more excellent heat resistance in wheat gluten-based patty. This knowledge is crucial for developing effective thermal control to ensure *Salmonella* inactivation in this food.

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