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The effect of emulsifier type and oil fraction on *Salmonella* Typhimurium growth and thermal inactivation in oil-in-water emulsion

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Abstract

High water activity oil-in-water emulsions can promote survival and growth of *Salmonella* Typhimurium. Nevertheless, the precise effect of emulsifier type and oil content on bacterial growth and inactivation is not fully understood. Here, emulsions were prepared using different emulsifiers (Tween 20, Tween 80, and Triton X-100) and different oil fractions (20%, 40%, and 60% (v/v)). TSB (control), emulsifier solutions, and emulsions were inoculated with *S*. Typhimurium. Bacterial growth rate was measured at 7, 22, and 37°C, whereas thermal inactivation was performed at 55°C. Growth and inactivation data was fitted into Logistic and Weibull models, respectively. At an incubation temperature of 37°C, the presence of high amount of oil (60%) in Tween 20 and Triton X stabilized emulsions extended the lag phase (5.83 ± 2.20 and 9.43 ± 1.07 h, respectively, compared to 2.28 ± 1.54 h for TSB, $p < 0.05$), whereas individual emulsifiers had no effect on growth behavior compared to TSB. This effect was also prevalent but attenuated at 22°C, whereas no growth was observed at 7°C. In thermal inactivation, we observed protective effect in Tween 80 and Triton X-100 solutions, where time required for five-log reduction was 1914.70 ± 706.35 min and 795.34 ± 420.09 min, respectively, compared to 203.89 ± 10.18 min for TSB ($p < 0.05$). Interestingly, the presence of high amount of oil did not offer protective effect during thermal inactivation. We hypothesize that oleic acid in Tween 80 and lower hydrophobicity value of Triton X-100 help maintain membrane integrity and improve the resistance of bacteria to heat inactivation.

KEYWORDS
emulsion, *Salmonella*

1 | INTRODUCTION

Foodborne illness is a significant global concern (Bennett et al., 2018; Callejón et al., 2015; Scallan et al., 2011). *Salmonella* is a pathogen that causes a high number of foodborne illnesses in the United States (Finstad et al., 2012). To prevent *Salmonella* outbreaks, it is critical to understand their growth and inactivation in various food systems. Aside from fresh meat and produce, emulsion is another prominent category affected with *Salmonella*.

Salmonella can survive and grow in oil-in-water emulsions with 2–3 log CFU/mL growth after 2 days incubation at 2% oil content emulsion at temperatures below 30°C (Fabian & Pivnick, 1953; Pivnick et al., 1954). In water-in-oil emulsion with low water activity, such as peanut butter and ground beef, *Salmonella* can not only grow but also show increased thermal resistance (Shachar & Yaron, 2006; Smith et al., 2001). On the other hand, milk is one of the few o/w emulsions studied for *Salmonella* growth and inactivation. *Salmonella* growth showed no difference from 0% to 15% of fat content in emulsion system (Warren, 1998). In contrast, tailing effect was observed under 55°C thermal treatments, especially when the fat content was increased in the milk formula (Warren, 1998). Because oil-in-water emulsion is a large food category, including dairy products, such as ice cream, dipping sauces, and flavor systems, there is an opportunity to understand the factors that affect *Salmonella* growth and inactivation in emulsion system.

The influence of emulsion parameters on bacteria growth and inactivation is not fully known. Emulsifier type is one of the essential factors that need further investigation. Food emulsifiers are classified as anionic, cationic, nonionic (ionic), high molecular weight, low molecular weight (size), and high hydrophobicity, low hydrophobicity (HLB value) (McClements & Jafari, 2018; Ozturk & McClements, 2016; Zhang et al., 2015). Although the application of different emulsifiers has been studied extensively, the correlation between emulsifier type and bacteria growth and inactivation is not well articulated. On the other hand, as the oil fraction in emulsion system changes, it is plausible that the growth and inactivation rate of *Salmonella* changes too. As a result, it is critical to assess the impact of emulsifiers and oil concentration in oil-in-water emulsion food systems on *Salmonella* growth and inactivation.

The goal of this study was to see how different emulsifiers (Tween 20, Tween 80, and Triton X-100) and oil concentration affected *Salmonella* growth and inactivation in an oil-in-water emulsion system. The Gompertz and Logistic models, as well as the Weibull model, were used to observe the growth and inactivation effects, respectively.

2 | MATERIALS AND METHODS

2.1 | Materials

Salmonella enterica serotype Typhimurium (CVM98) was cultured from the stock collection of the department of Nutrition and Food Science at University of Maryland, College Park. Tryptic soy agar (TSA) and tryptic soy broth (TSB) were purchased from BD Biosciences (236,920, 211,825; Franklin Lakes, NJ, USA). Phosphate buffer saline tablets were ordered from Fisher Scientific (BP2944-100; Waltham, MA, USA). Buffered peptone water was obtained from Thermo Scientific Remel Agar (R452672). Emulsifiers Tween 20, Tween 80, and Triton X-100 were obtained from Fisher Scientific (BP337-500, BP338-500), and Acros Organics (21,568-2500; Geel, Belgium), respectively. Vegetable oil was purchased from local grocery store and stored at room temperature in dark.

2.2 | Sample preparation

Overall, 2.0% (v/v) of Tween 20, Tween 80, or Triton X-100 was added into different volume of TSB (10, 8, 6, and 4 mL) and uniformly dispersed. To prepare 0%, 20%, 40%, and 60% (v/v) oil-in-water emulsion, specific amount of vegetable oil was added into TSB containing emulsifiers (0, 2, 4, and 6 mL, respectively) to obtain total 10 mL of solutions. Solutions were then homogenized via ultrasonic processor (FB505; Fisher Scientific; 200 W, 3 min) to prepare stable emulsions.

2.3 | Physical and chemical properties

Emulsifier solutions of 10 mL and emulsion without *S.* Typhimurium were prepared for pH value and water activity measurement. pH value and water activity were tested by pH meter (AB15; Fisher Scientific) and HygroPalm (HP23-A; Rotronic), respectively. Particle size was determined by dynamic light scattering (DLS) (BI-200SM, Brookhaven Instruments Corp., Holtsville, NY, USA). The method was modified from Zhang et al., 2020. Before detecting the size of emulsion samples, samples were diluted 1000-fold by distilled water. A 35 mW HeNe laser beam with 637 nm wavelength was applied to detect the diameters of emulsion samples. All measurements by DLS were measured at 25°C and in triplicate.

2.4 | Bacterial growth and enumeration

S. Typhimurium was recovered from frozen culture, inoculated on TSA plates overnight (∼20 h) at 37°C, and stored at 4°C for up to a month. Before experiments, *S.* Typhimurium isolated from the plate was inoculated into 15 mL sterile tube with TSB and incubated overnight (∼20 h) at 37°C to reach early stationary phase (∼9 log CFU/mL). With appropriate dilution, ∼5 log CFU/mL of bacteria were inoculated into TSB (control), emulsifier solutions, and emulsions. These inoculated solutions were then incubated at different temperatures of 7, 22, and 37°C, and samples were collected at specific time points (0, 1, 2, 3, 4, 5, 6, and 7 days for 7°C; 0, 3, 6, 9, 12, 18, and 24 h for 22°C; and 0, 2, 4, 6, 8, 12, 18, and 24 h for 37°C). To quantify bacterial growth and inactivation, samples were inoculated on TSA plates using a spiral plater (Eddy Jet; Neutec Group Inc., Farmingdale, NY, USA) and incubated overnight (~20 h) at 37°C. Colony counts were determined using an IUL Flash & Go automated colony counter (6010 colony counter; Neutec Group Inc.).

2.5 | Thermal treatment

S. Typhimurium was inoculated in TSB solution, emulsifier solutions, and emulsions overnight (~20 h) at 37°C. Aliquots of 200 µL were then placed into 55°C water bath (Model: 28L-M; Fisher Scientific) and collected at specific time point (0, 15, 30, 45, 60, 75, and 90 min). Treated aliquots were plated on TSA plates, incubated overnight (~20 h) at 37°C, counted by Plate Counter.

2.6 | Statistical analysis

All experiments were performed in triplicate. Gompertz (1) and Logistic (2) models were used to describe the growth curve:

$$\log N_t = \log N_0 + Ce^{\left(-e^{(-\mu(t-d)}\right)} \tag{1}$$

$$\log N_t = \log N_0 + \frac{C}{1 + e^{-\mu(t-d)}} \tag{2}$$

At time t, the number of surviving bacteria, N_t, can be predicted using these models, where N_0 is the initial bacterial number, C is log bacterial number increase from t_0 to t_{MAX}, μ is the maximum growth rate, and d is the inflection point (Dalgaard & Koutsoumanis, 2001; Tjørve & Tjørve, 2017). According to Buchanan and Cygnarowicz (1990), the duration of lag phase can be determined by the following formula:

$$\lambda = d - \left(\frac{1}{\mu}\right) \tag{3}$$

where λ is the duration of lag phase.

In addition, Weibull model (3) was applied to describe the inactivation at 55°C heat treatment:

$$\log N_t = \left(\frac{-1}{2.303}\right)\left(\frac{t}{\alpha}\right)^{\beta} \tag{4}$$

Surviving bacterial number N_t can be predicted using this formula, where α is the shape parameter, and β is the scale parameter (van Boekel, 2002). According to van Boekel (2002), 5D time, or the time required to achieve a 5-log reduction in bacterial population, can be calculated by the following equation:

$$5D = \alpha\left(-\ln\left(10^{-5}\right)\right)^{\frac{1}{\beta}} \tag{5}$$

Models were built by JMP Pro 15.2.0. The significant difference between each group was evaluated using a two-way ANOVA with Tukey's honestly significant difference post hoc test at a significance level of $\alpha = 0.05$.

3 | RESULTS AND DISCUSSION

3.1 | Physical and chemical properties

The pH value and water activity of 0%, 20%, 40%m, and 60% (v/v) o/w emulsion with 2.0% (v/v) Tween 20, Tween 80, and Triton X-100 were tested separately (Table 1). The pH value for all samples was approximately 7.0 with no significant difference among the samples ($p > 0.05$). Likewise, water activity values were approximately 0.95 with no significant difference between them ($p > 0.05$). Moreover, the size of the emulsion particles detected by the particle size analyzer varied from 200 to 700 nm (Table 1). The mean droplet size generally increased with an increase in the volumetric fraction of the oil phase inside the emulsion, irrespective of the emulsifier selected. Thus, regardless of their composition, all emulsion samples had similar pH, water activity, and droplet size.

3.2 | Effect of oil fraction and emulsifier type on the growth rate of *S*. Typhimurium

The effect of the emulsifier type and oil fraction in an emulsion on the growth behavior of *S*. Typhimurium was assessed at 37°C. Gompertz (Supporting information) and logistic models were applied to this primary data to describe and predict bacterial growth with a specific growth rate (log CFU/h), inflection point (h), and the duration of lag phase (h). We found that Logistic model fit the data slightly better than Gompertz model ($R^2 > 0.97$, compared to $R^2 > 0.96$). In addition, previous studies have shown that Gompertz model is more precise for predicting and describing tumor growth, whereas Logistic model is better for modeling bacterial growth (Akın et al., 2020; Annadurai et al., 2000). As a result, we performed the statistical comparison only on the parameters generated

TABLE 1 Summary of emulsion characteristics.

Emulsifier	Oil fraction (v/v) (%)	pH	Aw	Particle size (nm)
TSB	0	6.96 ± 0.01	0.997 ± 0.000	–
Tween 20	0	7.04 ± 0.02	0.964 ± 0.001	–
	20	7.07 ± 0.03	0.975 ± 0.011	323 ± 69
	40	7.03 ± 0.03	0.969 ± 0.001	320 ± 29
	60	6.99 ± 0.05	0.966 ± 0.006	645 ± 25
Tween 80	0	7.03 ± 0.00	0.953 ± 0.003	–
	20	7.02 ± 0.01	0.977 ± 0.004	214 ± 79
	40	7.00 ± 0.01	0.972 ± 0.010	261 ± 83
	60	6.99 ± 0.02	0.978 ± 0.012	746 ± 110
Triton X-100	0	7.06 ± 0.01	0.951 ± 0.001	–
	20	7.01 ± 0.02	0.993 ± 0.001	268 ± 30
	40	0.96 ± 0.00	0.982 ± 0.013	410 ± 87
	60	6.91 ± 0.02	0.984 ± 0.012	487 ± 55

Note: Each data point is an average of at least triplicate measurements ± standard deviation (SD).
Abbreviation: TSB, tryptic soy broth.

by Logistic model. The growth curve for *S*. Typhimurium in Tween 20 emulsifier had similar trend compared with that in TSB (Figure 1a). Presence of oil phase in Tween 20 stabilized emulsion had little impact on bacterial growth, except for 60% emulsion where visibly extended lag phase was observed. Figure 1b,c shows qualitatively similar trends in emulsions stabilized by Tween 80 and Triton X-100, respectively.

Lag phase was considered the reference to determine the effects of the factors in this study (Table 2). Among all cultured environments, there was a significant extension in the lag phase of bacteria cultured in every 60% emulsions, compared with *S*. Typhimurium cultured in TSB ($p < 0.05$). Specifically, the lag phase was 2.5-fold higher with Tween 20 stabilized 60% emulsion, 3.5-fold higher with Tween 80 stabilized 60% emulsion, and fourfold higher with Triton X-100 stabilized 60% emulsion compared to that in TSB. However, there was no significant difference in the lag-phase between bacteria inoculated in the TSB and the three emulsifier solutions ($p > 0.05$) indicating that the addition of Tween 20, Tween 80, and Triton X-100 to TSB did not have any significant effect on bacteria growth ($p > 0.05$). Interestingly, the specific growth rate was not significantly different for all samples ($p > 0.05$) than that in TSB.

When comparing three types of emulsifiers, there were no significant difference among emulsions at 0% and 20% emulsion system. At 40% emulsion, *Salmonella* cultured in Triton X-100 had significantly longer lag phase than in Tween 20 or Tween 80 ($p < 0.05$); at 60% emulsion, *Salmonella* inoculated in Tween 80 and Triton X-100 had significantly longer lag phase than in Tween 20 ($p < 0.05$). Thus, in addition to the oil fraction, the type of emulsifier also affects bacterial lag phase in emulsions containing 60% oil fraction.

Previous studies have shown that Tween 20, Tween 80, or Triton X-100 have no antimicrobial effects on *S*. Typhimurium growth within the range of 0.5%–10.0% (w/v) concentration (D'Aoust et al., 1982; Dikici et al., 2013; Zivanovic et al., 2004). Consistent with these findings, 2.0% v/v emulsifiers used in this study did not significantly affect bacterial growth. However, Prachaiyo and McLandsborough (2003) found an extended lag phase (higher inflection point) in *Escherichia coli* O157:H7 growth when Tween 20 was used as the emulsifier and the volume fraction of oil phase of the emulsion was over 40%, which is also consistent with our findings. The result indicated that the type of emulsifier and the oil fraction in the emulsion influence bacterial growth, although the emulsifiers themselves may not affect bacterial growth. Several hypotheses may be proposed to explain this apparent inconsistency: according to previous research, bacterial growth is known to primarily occur in the aqueous phase of an emulsion (Cápiro et al., 2014). As the oil fraction increases in the emulsion, the total aqueous volume available for bacterial growth decreases. Consequently, the lag phase becomes longer than that under normal conditions (inoculated at TSB). Similarly, the bacterial metabolites are also excreted within the aqueous phase and due to lower available aqueous volume, their concentration would be higher in emulsions while the high oil fraction. It is known that metabolite accumulation in the extracellular environment may affect bacterial growth (Babynin, 2006; Shayanfar et al., 2018). This may explain the observed outcomes. The nutrient concentration in the cultured media is a

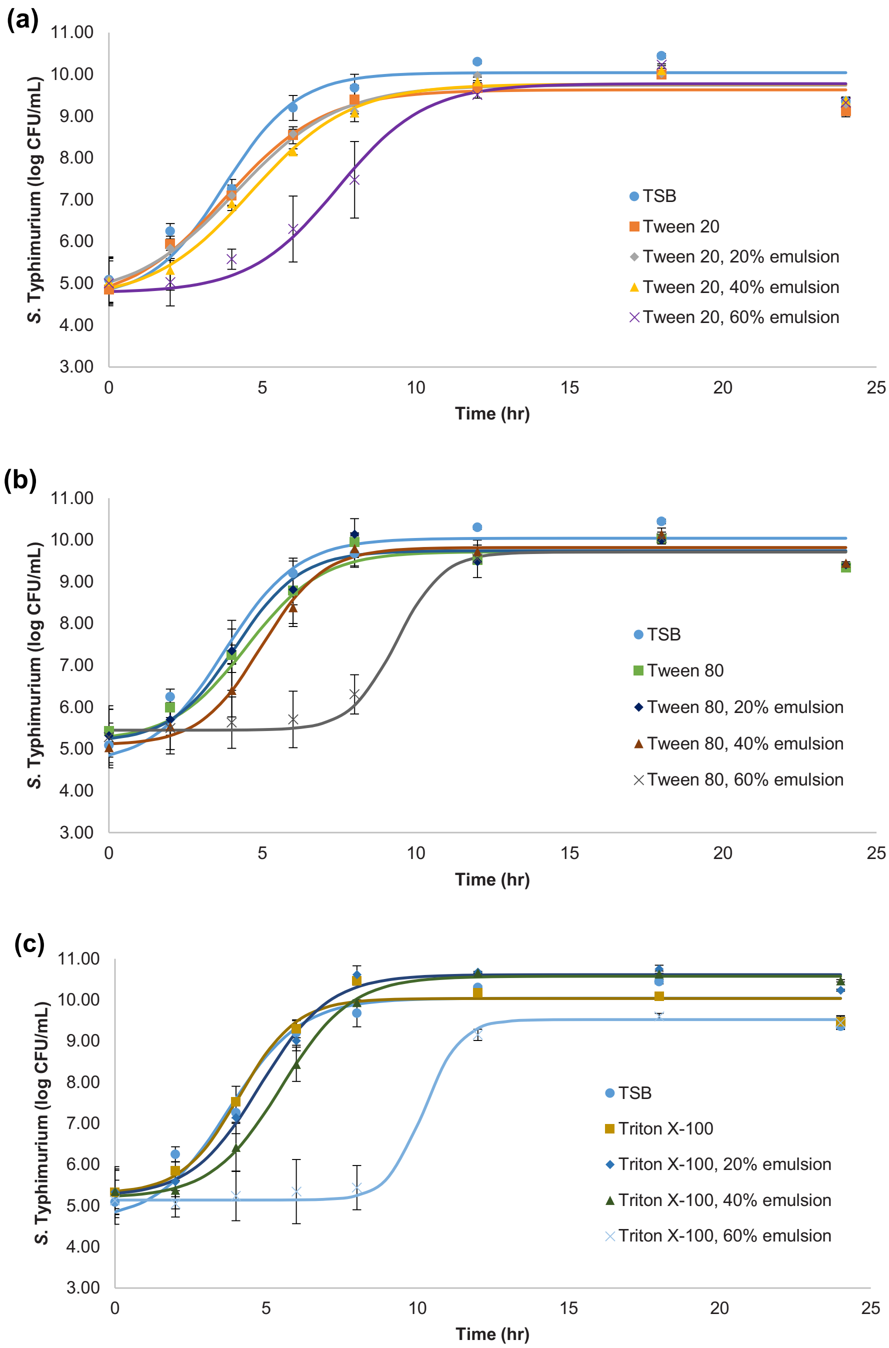

FIGURE 1 The growth curve of *Salmonella* Typhimurium at optimal growth temperature (37°C) in (a) tryptic soy broth (TSB), TSB supplemented with Tween 20 and 20%, 40%, and 60% (v/v) emulsions prepared using Tween 20; (b) TSB, TSB supplemented with Tween 80, and 20%, 40%, and 60% (v/v) emulsions prepared using Tween 80; (c) TSB, TSB supplemented with Triton X-100, and 20%, 40%, and 60% (v/v) emulsions prepared using Triton X-100. The experimental data was modeled using the Logistic model, and the average model fit for each growth curve is shown with a solid line. Each data point presented is the average of at least three measurements with standard deviation (SD).

potential factor that influences the growth behavior of *S.* Typhimurium. However, it is sufficient for bacteria to ultimately grow to at least 9–10 log CFU/mL. Additionally, even within the emulsions with highest concentration of oil phase (60%) had 50% aqueous phase comprised of TSB. Thus, TSB was generally present in excess and was not a limiting factor.

Subsequently, to evaluate whether the temperature of growth affects this observed behavior, additional experiments were performed at 22 and 7°C (Figures 2 and 3, respectively). Table 3 summarizes the growth rate and lag phase of *S.* Typhimurium in TSB, emulsifier solutions, and emulsions at 22°C, which is a common model of room temperature in microbiological studies (Alshammari et al.,

TABLE 2 Summary of Salmonella Typhimurium growth model in tryptic soy broth (TSB), emulsifier solutions, and emulsions at 37°C.

	TSB	Tween 20	Tween 20, 20% emulsion	Tween 20, 40% emulsion	Tween 20, 60% emulsion
R-square	0.97 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.99 ± 0.01	0.98 ± 0.01
Growth rate	0.84 ± 0.52^{a}	0.62 ± 0.06^{a}	0.60 ± 0.05^{a}	0.62 ± 0.12^{a}	0.70 ± 0.31^{a}
Inflection point	3.76 ± 0.85	3.80 ± 0.51	4.13 ± 0.83	4.62 ± 0.64	7.44 ± 1.64
Lag phase (h)	2.28 ± 1.54^{C}	2.18 ± 0.64^{C}	2.47 ± 0.97^{C}	2.98 ± 0.93^{C}	5.83 ± 2.20^{B}
		Tween 80	**Tween 80, 20% emulsion**	**Tween 80, 40% emulsion**	**Tween 80, 60% emulsion**
R-square		0.98 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.97 ± 0.03
Growth rate		0.82 ± 0.07^{a}	0.95 ± 0.20^{a}	1.00 ± 0.28^{a}	1.33 ± 0.87^{a}
Inflection point		4.49 ± 0.68	4.13 ± 0.52	4.97 ± 0.46	9.37 ± 0.65
Lag phase (h)		3.26 ± 0.59^{C}	3.06 ± 0.66^{C}	3.91 ± 0.74^{BC}	8.42 ± 1.04^{A}
		Triton X-100	**Triton X-100, 20% emulsion**	**Triton X-100, 40% emulsion**	**Triton X-100, 60% emulsion**
R-square		0.98 ± 0.01	0.99 ± 0.01	0.99 ± 0.01	0.98 ± 0.02
Growth rate		1.06 ± 0.22^{a}	0.88 ± 0.05^{a}	0.87 ± 0.12^{a}	1.65 ± 1.09^{a}
Inflection point		4.11 ± 0.19	4.80 ± 0.42	5.50 ± 0.60	10.21 ± 0.67
Lag phase (h)		3.14 ± 0.28^{C}	3.66 ± 0.49^{C}	4.34 ± 0.76^{BC}	9.43 ± 1.07^{A}

Note: Each data point is an average of at least triplicate measurements ± standard deviation (SD). Lower case letter labels (a, b, c, etc.) were used to indicate significant differences of growth rate in different growth environment, based on two way ANOVA. Upper case letter labels (A, B, C, etc.) were used to indicate significant differences of lag phase in different growth environment, based on two way ANOVA. Groups with the same letter were not significantly different ($p > 0.05$), whereas those with different letters were significantly different ($p < 0.05$).
Abbreviation: TSB, tryptic soy broth.

TABLE 3 Summary of *Salmonella* Typhimurium growth model in tryptic soy broth (TSB), emulsifier solutions, and emulsions at 22°C.

	TSB	Tween 20	Tween 20, 20% emulsion	Tween 20, 40% emulsion	Tween 20, 60% emulsion
***R*-square**	0.98 ± 0.01	0.99 ± 0.01	0.99 ± 0.01	0.98 ± 0.01	0.97 ± 0.01
Growth rate	0.42 ± 0.04^{a}	0.41 ± 0.12^{a}	0.37 ± 0.06^{a}	0.47 ± 0.07^{a}	0.49 ± 0.19^{a}
Inflection point	7.21 ± 0.22	6.49 ± 0.45	7.61 ± 0.66	7.59 ± 0.95	8.68 ± 0.87
Lag phase (h)	4.79 ± 0.33^{CD}	3.88 ± 1.18^{D}	4.85 ± 1.03^{CD}	5.44 ± 1.26^{CD}	6.44 ± 1.56^{BC}
		Tween 80	**Tween 80, 20% emulsion**	**Tween 80, 40% emulsion**	**Tween 80, 60% emulsion**
***R*-square**		0.99 ± 0.01	0.99 ± 0.01	0.97 ± 0.02	0.98 ± 0.01
Growth rate		0.45 ± 0.05^{a}	0.35 ± 0.07^{a}	0.45 ± 0.10^{a}	0.57 ± 0.20^{a}
Inflection point		6.77 ± 0.30	7.26 ± 0.97	7.80 ± 1.16	8.38 ± 1.45
Lag phase (h)		4.54 ± 0.52^{CD}	4.30 ± 1.52^{CD}	5.52 ± 1.63^{CD}	6.47 ± 2.03^{BC}
		Triton X-100	**Triton X-100, 20% emulsion**	**Triton X-100, 40% emulsion**	**Triton X-100, 60% emulsion**
***R*-square**		0.97 ± 0.02	0.98 ± 0.01	0.98 ± 0.01	0.99 ± 0.01
Growth rate		0.51 ± 0.07^{a}	0.44 ± 0.03^{a}	0.58 ± 0.33^{a}	0.76 ± 0.23^{a}
Inflection point		7.75 ± 0.33	7.75 ± 1.17	8.59 ± 0.77	9.75 ± 0.45
Lag phase (h)		4.97 ± 0.53^{CD}	5.49 ± 1.30^{CD}	6.52 ± 1.63^{BC}	8.36 ± 0.79^{AB}

Note: Each data point is an average of at least triplicate measurements ± standard deviation (SD). Lower case letter labels (a, b, c, etc.) were used to indicate significant differences of growth rate in different growth environment, based on two way ANOVA. Upper case letter labels (A, B, C, etc.) were used to indicate significant differences of lag phase in different growth environment, based on two way ANOVA. Groups with the same letter were not significantly different ($p > 0.05$), whereas those with different letters were significantly different ($p < 0.05$).
Abbreviation: TSB, tryptic soy broth.

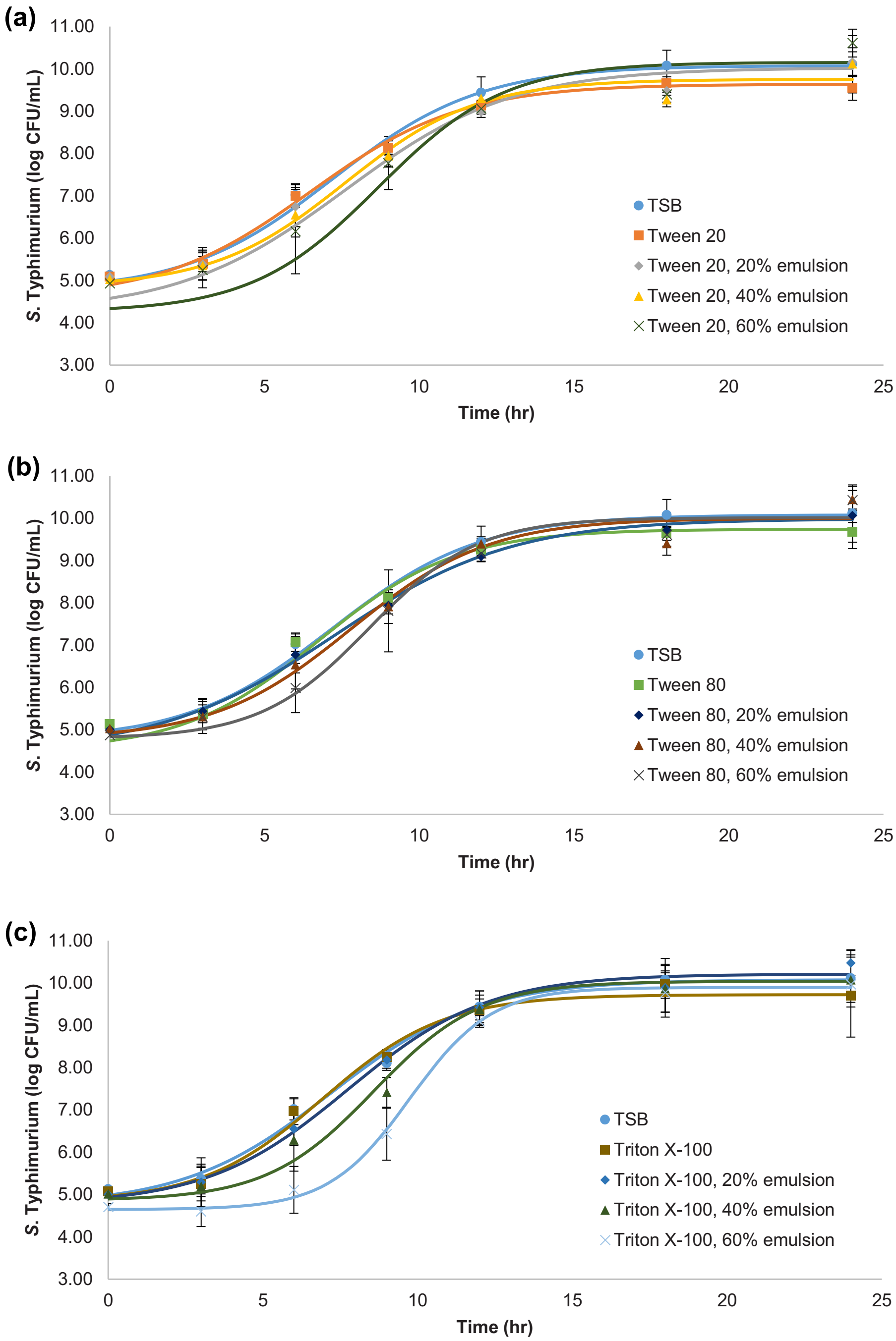

FIGURE 2 The growth curve of *Salmonella* Typhimurium at room temperature (22°C) in (a) tryptic soy broth (TSB), TSB supplemented with Tween 20 and 20%, 40%, and 60% (v/v) emulsions prepared using Tween 20; (b) TSB, TSB supplemented with Tween 80, and 20%, 40%, and 60% (v/v) emulsions prepared using Tween 80; (c) TSB, TSB supplemented with Triton X-100, and 20%, 40%, and 60% (v/v) emulsions prepared using Triton X-100. The experimental data was modeled using the Logistics model, and the average model fit for each growth curve is shown with a solid line. Each data point presented is the average of at least three measurements with standard deviation (SD).

2021; Cho et al., 2016). There was a lack of significant difference in the growth rates between emulsifier solutions, emulsions, and TSB ($p > 0.05$). However, a significantly higher lag phase was observed in Triton X-100 stabilized 60% emulsion compared to TSB ($p < 0.05$), similar to that observed at 37°C.

On the other hand, according to Table 3, some of the lag phase duration was shorter at 22°C than 37°C. However, after conducting the statistical analysis (two-way ANOVA with fisher's LSD, $p = 0.05$) between lag phase duration between 22 and 37°C, majority of samples did not have significant difference in their lag phase duration (emulsifier

TABLE 4 Comparison of *Salmonella* Typhimurium lag phase duration (h) at 22 and 37°C.

	TSB	Tween 20	Tween 20, 20% emulsion	Tween 20, 40% emulsion	Tween 20, 60% emulsion
22°C	4.79 ± 0.33	3.88 ± 1.18	4.85 ± 1.03	5.44 ± 1.26	6.44 ± 1.56
37°C	2.28 ± 1.54*	2.18 ± 0.64	2.47 ± 0.97*	2.98 ± 0.93*	5.83 ± 2.20
		Tween 80	**Tween 80, 20% emulsion**	**Tween 80, 40% emulsion**	**Tween 80, 60% emulsion**
22°C		4.54 ± 0.52	4.30 ± 1.52	5.52 ± 1.63	6.47 ± 2.03
37°C		3.26 ± 0.59	3.06 ± 0.66	3.91 ± 0.74	8.42 ± 1.04*
		Triton X-100	**Triton X-100, 20% emulsion**	**Triton X-100, 40% emulsion**	**Triton X-100, 60% emulsion**
22°C		4.97 ± 0.53	5.49 ± 1.30	6.52 ± 1.63	8.36 ± 0.79
37°C		3.14 ± 0.28	3.66 ± 0.49	4.34 ± 0.76*	9.43 ± 1.07

Note: *: Significant difference compared with bacteria inoculated in 22°C ($p < 0.05$).
Abbreviation: TSB, tryptic soy broth.

solutions, 60% emulsion stabilized with Tween 20, 20% and 40% emulsion stabilized with Tween 80, and 20% and 60% emulsion stabilized with Triton X-100) (Table 4). There were five samples (TSB, 20% and 40% emulsion stabilized with Tween 20, 60% emulsion stabilized with Tween 80, and 40% emulsion stabilized with Triton X-100) that did have significant difference ($p < 0.05$) in their lag phase durations. However, four of those samples (TSB, 20% and 40% emulsion stabilized with Tween 20, and 40% emulsion stabilized with Triton X-100) had significant lower lag phase duration at 37 than 22°C, which is consistent with our understanding. Only one sample, 60% emulsion stabilized with Tween 80, showed longer lag phase at 37 than at 22°C. The reason and mechanism are still unclear. We can only hypothesize that as the formulation of membrane was affect by the oleic acid released from Tween 80, optimal growing temperature might be also changed in higher oil fraction environment. However, the specific effect and mechanism need further investigation.

The growth behavior of *S*. Typhimurium at 7°C was analyzed over a 7-day incubation period and the results were illustrated in Figure 3. No significant changes were observed in bacterial cultures except for those in Triton X-100 stabilized emulsions ($p > 0.05$). A decline in bacterial numbers was detected in Triton X-100 stabilized 20% and 40% emulsions on the third and fourth day of incubation at 7°C ($p < 0.05$). Bacterial numbers subsequently recovered to the same level as on the first day of incubation ($p > 0.05$). Furthermore, a notable decrease in bacterial numbers was observed in Triton X-100 stabilized 60% emulsion after 3 days of incubation at 7°C as compared to the first day ($p < 0.05$). The growing temperature for *S*. Typhimurium has been recoded as 5–46°C, and the optimistic range has been reported as 35–43°C (Yates, 2011). Thus, at 7°C, which was the temperature associated with the temperature in fridge, *S*. Typhimurium was merely able to survive instead of growing in TSB and emulsifier solutions.

3.3 | Effect of emulsifier type on thermal inactivation rate of *S*. Typhimurium in high oil fraction emulsion

It is known that the environment in which bacteria grow influences their survival and inactivation in subsequent treatments (Birk et al., 2016). As a result, we hypothesized that just as emulsions affect bacterial growth rate, they also affect bacterial inactivation rate. Because only oil fraction emulsions at 60% (v/v) had a significant impact on bacterial growth, inactivation experiments were conducted using only those emulsions, in addition to emulsifier and TSB solutions. Bacterial numbers reached 9–10 log CFU/mL after an overnight (20-h) incubation in various media (TSB, emulsifier solutions, and 60% emulsions). Figure 4 shows the bacterial inactivation data in TSB, individual emulsifiers, and 60% oil-in-water emulsions prepared using those emulsifiers. It was discovered that bacteria inactivation in a 55°C water bath did not follow the log-linear inactivation kinetics, which was consistent with a previous study conducted by Warren (1998). The tailing effect of inactivation curve was found during thermal treatment, and the effect became more significant as the increase of oil fraction in emulsion (Warren, 1998). Therefore, inactivation data was fitted into the Weibull model (Table 5). Based on the Weibull model parameters, $\beta < 1$ indicated that the remaining bacteria had a proclivity to adapt to heat. Furthermore, α was an analogue to the traditional D value (van Boekel, 2002). As a result, the 5D concept was chosen as the standard for evaluating effects on bacterial inactivation. Bacteria grown in

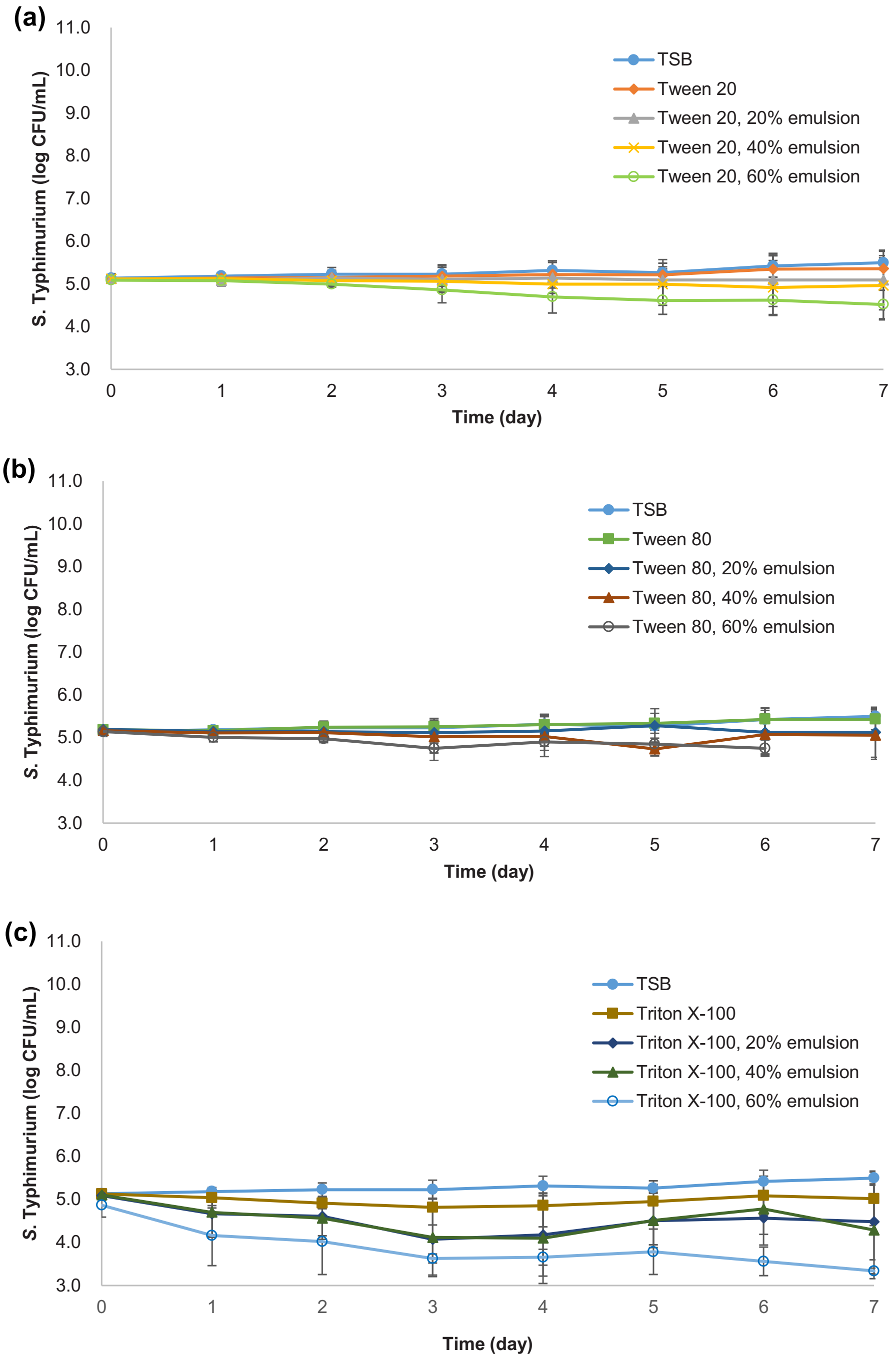

FIGURE 3 The growth curve of *Salmonella* Typhimurium at 7°C (corresponding to refrigerating temperature) in (a) tryptic soy broth (TSB), 2% (v/v) Tween 20 solution and 20%, 40%, and 60% (v/v) emulsions prepared using Tween 20; (b) TSB, 2% (v/v) Tween 80 solution and 20%, 40%, and 60% (v/v) emulsions prepared using Tween 80; (c) TSB, 2% (v/v) Triton X-100 solution and 20%, 40%, and 60% (v/v) emulsions prepared using Triton X-100. Each data point is an average of at least triplicate measurements ± standard deviation (SD).

emulsifier solutions had a significantly lower ($p < 0.05$) reduction in bacterial number after 90 min of thermal treatment than bacteria grown in TSB (Figure 4). *S.* Typhimurium inoculated in Tween 20 had a comparable 5D value (1.3-fold lower) to bacteria inoculated in TSB, a 9-fold higher 5D in Tween 80, and a 4-fold higher 5D in Triton X-100, indicating that bacterial resistance significantly increased particularly when incubated with Tween 80 and Triton X-100 emulsifiers ($p < 0.05$). However, 5D value was only 1.4-fold higher in bacteria cultured in Tween 20 emulsion than in bacteria cultured in TSB ($p > 0.05$). Furthermore, when *Salmonella* was inoculated in Tween 80 and Triton X-100 emulsions, 5D decreased 1.5-fold and increased 1.01-fold, respectively ($p > 0.05$). Thus, a protec-

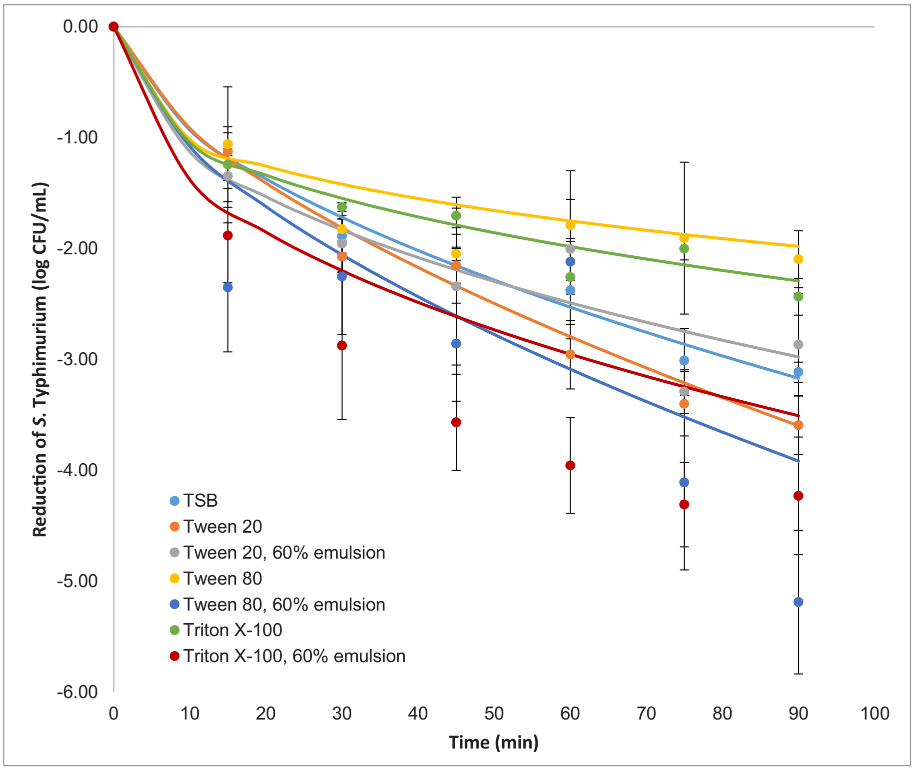

FIGURE 4 Thermal inactivation of *Salmonella* Typhimurium at 55°C inoculated in tryptic soy broth (TSB), Tween 20, Tween 80, and Triton X-100 emulsifiers and 60% (v/v) oil-in-water emulsions prepared using those emulsifiers. Experimental data was fitted into Weibull model and average model fit for each growth curve is depicted using the solid line. Each data point is an average of at least triplicate measurements ± standard deviation (SD).

tive effect of emulsifiers against bacterial inactivation was found in 2% (v/v) emulsifier solution. According to Yang (2020), thermal treatment had a protective effect on bacteria when oil was present. On the contrary, our findings revealed that the protective effect was found in emulsifier solutions rather than in emulsion systems.

As β was less than 1 in all the cases, we can conclude that *Salmonella* was able to adapt to thermal stress at 55°C. Furthermore, tailing effect was also observed across all groups as the 90-min thermal treatment progressed. Thus, it is apparent that a subset of *S*. Typhimurium was transferred to heat-resistant bacteria. Furthermore, the obvious protective effects were demonstrated when bacteria were cultured in Tween 80 and Triton X-100 emulsifier solutions using the 5D concept, which has not been noticed in previous studies. However, previous research has shown that oleic acid released from Tween 80 can enhance tolerance to stress-related scenarios. For example, Reitermayer et al. (2018) found that with high hydrostatic pressure treatment, Tween 80 supplementation increased the content of oleic acid and showed protective effect under stress. In addition, in 2023, Xia et al. (2023) reported that bacterial membrane integrity and fluidity were increased with the addition of oleic acid, which was consistent with the inclusion of Tween 80 in the environment during freeze drying. Thus, it has been proved that the oleic acid released from Tween 80 had the tendency to increase the tolerance toward stress. Another study concerned with HLB value has also discovered that cells cultured in Triton X-100 (lower HLB value) had lower membrane permeability compared to cells grown in Tween 20 (higher HLB value) at the same concentration (BadrulHaswan et al., 2022). This finding indicates that Triton X-100 can help to protect cells from external stress due to its lower HLB value. It is also possible that presence of different emulsifiers and mild heat (55°C) trigger different bacterial stress response genes that are able to protect the bacteria against inactivation. Additionally, previous study suggested that a slower heating rate, or longer come-up time, may contribute to increased heat resistance within the food matrix (Juneja & Marks, 2003). The use of water bath in our experiment possibly provided longer come up time (5 min) as well as less absolute value of treating temperature (55°C) for bacteria to adapt to certain treatments, which affected their thermal sensitivity. Although the effects of our approach may vary across different strains of bacteria, this research still provides valuable insights into the parameters that affect bacterial growth and inactivation

TABLE 5 Thermal Inactivation Model Parameters (Weibull Model) For Of Salmonella Typhimurium At 55°C In Tryptic Soy Broth (Tsb), Tween 20, Tween 80, And Triton X-100 Emulsifiers And 60% (V/V) Emulsions Prepared Thereof.

	TSB	Tween 20	Tween 20, 60% emulsion	Tween 80	Tween 80, 60% emulsion	Triton X-100	Triton X-100, 60% emulsion
α (min)	2.543 ± 0.228	3.027 ± 1.417	1.155 ± 0.565	0.602 ± 0.747	2.119 ± 1.709	0.864 ± 0.488	0.660 ± 1.012
β	0.557 ± 0.007	0.623 ± 0.070	0.442 ± 0.061	0.303 ± 0.065	0.587 ± 0.180	0.358 ± 0.057	0.425 ± 0.180
Avg. 5D concept predicted by Weibull model (min)	203.89 ± 10.18	152.56 ± 14.42	290.79 ± 73.66	1914.70 ± 706.35****	136.45 ± 30.56	795.34 ± 420.09***	207.23 ± 60.03
RMSE	0.206	0.194	0.476	0.309	0.862	0.176	0.301

Note: Each data point is an average of at least triplicate measurements ± standard deviation (SD).*: significant difference compared with inoculation in TSB ($p < 0.05$).
Abbreviation: TSB, tryptic soy broth.

in emulsions. More research is needed to evaluate the impact at faster heating rates, such those observed during pasteurization.

4 | CONCLUSION

Current research shows that *S*. Typhimurium can thrive in high water activity oil-in-water emulsions. However, the impact of emulsifiers and oil content on bacterial growth and inactivation is unclear. The aim of this research was to gain a better understanding of these variables. Bacterial growth and thermal inactivation rates were measured in bacteria inoculated in TSB, Tween 20, Tween 80, and Triton X-100 emulsifiers and 20%, 40%, and 60% (v/v) oil-in-water emulsions prepared using those emulsions. The results showed that the impact of emulsifier type and oil fraction became more pronounced at the optimal growing temperature (37°C). At 37°C, although the presence of individual emulsifiers did not affect the duration of lag phase or the growth rate of the bacteria, the presence of high amount of oil phase (60%) significantly extended the lag phase but not the growth rate and ultimate bacterial population. Among high oil fraction (60% (v/v)) emulsions, Tween 80, and Triton X-100 stabilized emulsions had significantly longer lag phase than Tween 20 emulsion. Thermal inactivation of bacteria was found that Tween 80 and Triton X-100 solutions provided significant protection against inactivation at 55°C, whereas the presence of oil (emulsion) had no protective effect. We hypothesize that the oleic acid in Tween 80 and the reduced HLB value of Triton X-100 can help maintain membrane integrity and thus improve the resistance of bacteria to thermal inactivation. It was also found that after 30 min of thermal treatment at 55°C, a subpopulation of *S*. Typhimurium had become heat resistant. The mechanism behind this will be further investigated. This information could be used by food manufacturers to reconsider the inactivation process toward emulsion products with specific emulsifiers, so that the food safety could be improved.

AUTHOR CONTRIBUTIONS

Shawn Tsai: Methodology; investigation; formal analysis; visualization; writing—original draft; writing—review and editing; conceptualization; data curation; software. **Rohan V. Tikekar**: Conceptualization; methodology; supervision; funding acquisition; project administration; resources; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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