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Inactivation kinetics of *Salmonella enterica* serovar Typhimurium NCTC 12023 in Chokanan mango (*Mangifera indica* L.) fruit juice by aqueous ozone treatment

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Abstract

Food processing industries worldwide focus on maintaining and enhancing food quality and security, particularly microbial food safety, to fulfil customer requirements. This study examined the efficacy of aqueous ozone on the inactivation of *Salmonella enterica* serovar Typhimurium NCTC 12023 in Chokanan mango juice (MJ) diluted with distilled water (DW) ratios (100MJ:0DW, 75MJ:25DW, and 50MJ:50DW). The inoculated MJ samples were treated at 10, 20, 30, and 40 mins with ozone doses 0.33, 0.67, 1.00, and 1.33 mg/mL. The results showed that the Bx values and mango pulp presence in the various MJ samples interfered with the *S. enterica* ser. Typhimurium disinfection activity by ozone. The *S. enterica* ser. Typhimurium inactivation and the survival curves' concavity are more reliably measured in the kinetic Weibull model. Validation of the Weibull model produced coefficient determination (R^2) of 0.94–0.99, RSME of 0.1 to 0.4, bias and accuracy factor of 0.7 to 1.4, and shape parameter $p<1$. A fast inactivation rate for 5-log reduction of *S. enterica* ser. Typhimurium was observed in 50MJ:50DW sample (24.82 mins, 0.82 mg/mL) and in 75MJ:25DW sample (28.39 mins, 0.95 mg/mL), respectively. However, in the 100MJ:0DW sample, the 5-log reduction was achieved at 36.39 min with an ozone dose of 1.20 mg/mL. Nevertheless, this study's ozone dose range from 0.33 mg/mL to 1.33 mg/mL with a treatment time of 10 to 40 mins has proven to lower and achieved a 5-log reduction of *S. enterica* ser. Typhimurium in all MJ samples.

1. Introduction

The fruit juice industry is the world's fastest-growing beverage industry, with fruit juice taking a salient part in the human diet and the consumer's most desired food product for consumption (Ağçam *et al*., 2018). Mango (*Mangifera indica* L.) fruit's ability to fit into a balanced human diet has increased the mango production and market size globally and domestically. The market for value-added mango products has shown progressive growth due to consumers' acceptability to seek a new indulgence and awareness of a healthy lifestyle. Grand View Research (2019) announced that the size of the global demand for mango processed products was estimated at 16.55 billion USD in 2018 and expects a 6.4% Compound Annual Growth Rate (CAGR) over the 2019-2025 forecast period.

The value of mango exports from Malaysia to various countries in the Asia Pacific, Middle East, and the European Union is about USD 5.2 million (International Trade Centre, 2017). In line with the government's effort to boost gross national income (GNI), twelve National Key Economic Areas (NKEAs), which include the agricultural sector, was launched by the Performance Management and Delivery Unit (PEMANDU) agency in September 2010 (PEMANDU, 2010). In addition, the Economic Planning Unit agency under the Prime Minister Department of Malaysia has prepared the National Agrofood Policy (DAN) 2011-2020, specifically for the agro-food industry (FAMA, 2011). One of this policy's main ideas is to increase agricultural modernization by research and development (R and D) technology and strengthening fruit industry production. Department of Statistic Malaysia also recorded mango consumption in 2019 as 1.5 kg/year of per capita consumption (DOSM, 2020). In further realizing this policy and report, diversifying mango fruit production, exportation, and processing into juice

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products are highly encouraged.

However, the increase in the frequency of fresh fruit outbreaks could occur as there were increments in cross-country shipping and/or importing products from other countries. Fresh produce, including fruits, has been recognized as a common source for *Salmonella* since the bacteria can attach and internalize in fruit (Kilonzo-Nthenge and Mukuna, 2018). The *S. enterica* ser. Typhimurium outbreaks linked to fresh fruits have occurred in 2012 and 2010 at multistate locations in the United States (CDC, 2010; CDC, 2012a). The outbreaks were caused by Cantaloupe melon and frozen Mamey fruit pulp consumption, respectively, with 228 and 9 infected individuals. The infection of *S. enterica* ser. Typhimurium has led to typhoid fever or, in the worst cases, caused death if no prompt action is taken. The outbreak of foodborne diseases associated with mango occurred in 2012 where 127 people were infected with *S. Braenderup* in the United States (CDC, 2012b). In Malaysia, the exact number of *Salmonellosis* incidences associated with fresh fruits remains unknown as a chain of events needs to be addressed first before it is brought to the authority.

Furthermore, the lack of continuous efforts by the affected consumers, the healthcare workers and local authorities have made such incidents hard to investigate. Nevertheless, in 2018, the Health Ministry of Malaysia reported that the incidence rate of food and water-borne disease for typhoid/paratyphi was recorded as 0.53 per 1000 live birth (MOH, 2019). Studies done locally on such matters were also found to be modest. The only *Salmonella* cases in fruit juice were recorded locally in a research study by Diana *et al*. (2012). The study had detected that the prevalence and concentration of *S. enterica* ser. Typhimurium in apple juice was 30% and 9.20 MPN/g, meanwhile for orange juice was 10% and 6.10 MPN/g and in starfruit juice at 10% and 3.00 MPN/g, sold by fruit juice vendors and hawker stall in Serdang and Klang Valley. It was proven that fruits could be a reservoir for *Salmonella*. Hence, more prevalence studies on *Salmonella* in fresh produce and its by-products are needed to create awareness on the consumed food's safety and quality.

Consequently, the juice industry is encouraged to implement the proper pasteurization and preservation technique in its processing line to avoid food safety issues. This includes the promising non-thermal technology, ozone. Ozone is known for its potent antimicrobial properties with a powerful oxidizing agent (+2.07 eV) (Asokapandian *et al*., 2018), efficient as bactericidal, viricidal, and sporicidal in fruit juice microbiological activity. Despite that, it is challenging to predict ozone behaviour in juices as it depends not only on the ozone processing parameter (time and concentration) but also on the juice condition. Several studies have proven the microbial reductions in ozone-treated fruit juices, including cantaloupe melon, peach, apple, and orange juice (Patil *et al*. 2010b; Torlak, 2014; Garcia Loredo *et al*., 2015; Sroy *et al*., 2019). However, previous research mainly focused on the microbial reduction perusing ozone processing parameters and studies are limited on the intrinsic parameters of the juice. Prabha *et al*. (2015) reported that ozone molecules' reaction in fruit juice could be disturbed by the presence of organic or inorganic matter in juice. The solid content of the juice contributes to the limitation of ozone's synergistic towards its bactericidal effect. Furthermore, certain microorganisms are able to survive the acidic condition of the juice, including Gram-negative bacteria *S. enterica* ser. Typhimurium (Pandey and Negi 2018). An acid-loving microorganism such as *Salmonella* can adapt and survive for a few days or even weeks in an acidic environment formed by organic acids of the fruit juices.

Therefore, it is vital to study the influence of juice conditions on aqueous ozone efficacy for antimicrobial activity. Specifically, the objective of this research was to evaluate the effect of aqueous ozone on the *S. enterica* ser. Typhimurium in Chokanan mango juice diluted with distilled water (DW) ratios. In addition to that, the inactivation kinetic model approach incorporating the aqueous ozone treatment effect on microbial growth of *S. enterica* ser. Typhimurium was also studied to predict the influence of ozone control parameters on the safety and quality of mango juices.

2. Materials and methods

2.1 Preparation of mango (Mangifera indica L.) juice

Ripe mango fruits (stage 5-fully yellow colour) (DOA, 2009) Chokanan variety were purchased from the local market in Serdang, Selangor, and were extracted using Slow Cold-Pressed juicer (SJ-33, Russell Taylors, Malaysia). Then, the juices were kept in a high-density polyethylene bottle and stored at -20°C. Prior to experimental work, the juices were thawed at room temperature for less than 24 hr and prepared by mixing with distilled water according to the ratio % (v/v) of mango juice (MJ): distilled water (DW). The samples prepared were 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW, respectively.

2.2 Determination of pH and total soluble solids

The juice pH and total soluble solids (TSS) were measured using a digital refractometer (D Series, Graigar Technology, China) and pH meter (PB – 10, Sartorius, Germany), respectively, without further dilution. All measurements were done in triplicate.

2.3 Preparation of inoculum and inoculation process

Freeze-dried culture of *S. enterica* ser. Typhimurium NCTC 12023 was obtained from the Evergreen Engineering and Resources, Selangor, Malaysia. The culture was recovered with Difco^{TM} Universal Pre-enrichment Broth (UPB) (Difco Laboratories, Becton, Dickinson and Company Sparks, USA) and incubated for 24 hrs at 37°C (Nam *et al*., 2004). The stock cultures were then inoculated onto Difco^{TM} Bismuth Sulphite Agar (BS) (Difco Laboratories, Becton, Dickinson and Company Sparks, USA) and incubated 37°C for 24-48 hrs to confirm the colonies. After confirming, the working cultures were sub-cultured into UPB, incubated at 37°C for 24 hrs. Then, the cell was harvested by centrifugation at 4000 rpm for 20 mins at 4°C and washed three times with Millipore 107228 Buffered Peptone Water; acc. ISO 6579 (BPW) (Merck KGaA, Darmstadt, Germany and EMD Millipore Corporation, USA). Finally, after three washes with 0.1% BPW, the cell pellet was re-suspended in BPW. Bacterial concentration was determined by using UV Spectrophotometer (Ultra Spec 3100 Pro. Amersham Pharmacia Biotech, UK) at a wavelength of 600 nm (Alwi and Ali, 2014) and also compared with 0.5 Mc Farland standard (Patil *et al*., 2010a), resulted in cell density of approximately 1.5×10^{8} CFU/mL. Approximately 3 mL of *S. enterica* ser. Typhimurium NCTC 12023 suspensions were added into 200 mL of fruit juice and shaken for 2 hrs before ozone treatment.

2.4 Ozone treatment

Ozone gas was generated using the ozone generator (Model GL-3189, China), with an ozone output of 400 mg/hr. Ozone dosage rates at an exposure time of 10, 20, 30, and 40 min was calculated based on the juice flow rate (GPM) and ozone generator output (mg/hr) (Oxidation Technologies, 2017). The measured ozone doses were given as 0.67 mg/mL, 0.33 mg/mL, 1.00 mg/mL and 1.33 mg/mL. Experiments were carried out with gaseous ozone pumped directly into mango juice (MJ) samples through the delivery tube into a beaker. Juice samples were stirred at 3000 rpm using a magnetic stirrer to ensure homogenous dispersal of ozone. All experiments were carried out in replicates.

2.5 Detection and enumeration of Salmonella enterica serovar Typhimurium

Salmonella enterica ser. Typhimurium NCTC 12023 was confirmed by plating onto DifcoTM Bismuth Sulphite Agar (BS) (Difco Laboratories, Becton, Dickinson and Company Sparks, USA). Approximately 1 mL portions of the ozone-treated juice sample were serially diluted (10-fold) in 9 mL 0.1% of buffered peptone water (BPW) tubes. Then, one-tenth (0.1 mL) from each dilution was streaked onto BS agar plates and incubated for 24-48 hr at 37°C. After incubation, colonies grown on BS agar plates were counted, and *S. enterica* ser. Typhimurium NCTC 12023 was calculated as log CFU/mL. The experiment was done in triplicates.

2.6 Inactivation kinetics

The inactivation curve behavior of *S. enterica* ser. Typhimurium was fitted to the Log-Linear models and Weibull models. The regression analysis of the *S. enterica* ser. Typhimurium inactivation data was performed by Minitab and MS Excel software.

2.5.1 Log-linear model

Log-linear models describe the survival growth curve for a single strain population and assume the inactivation followed the first-order kinetics (Van Impe *et al*., 2018);

$$\frac{dN(t)}{dt}=-k\cdot N(t) \quad (1)$$

Then, the parameterization of the Log-Linear equation employs (Bevilacqua *et al*., 2015) was:

$$\log N=\log N_o-\frac{t}{D} \quad (2)$$

N is the number of microorganisms, N_o is the initial number of microorganisms, k is the inactivation rate (min^{-1}) and t, describes the ozone treatment time (min), respectively. The D value (min) represents the time needed for one log or 90% reduction of *S. enterica* ser. Typhimurium population in mango juice (MJ) samples. The experimental data were fitted to Equation 2 (where ozone treatment time on the x-axis and $log\ (S)$ or $log\ (N/N_o)$ is on the y-axis). The inactivation rate was determined from the D-value, where $D = k/ln10$ and the time required for a 5-log reduction, t_{5d} was calculated using $t_{5d} = n*D$, where n = 5-$\log_{10}$ (Mazzola *et al*., 2003).

2.5.2 Weibull model

Weibull model describes the inactivation kinetic on curvilinear semi-logarithmic survival curve, where there was the formation of the shoulder (concave upward) or tail (concave downward) (Cullen *et al*., 2009; Sorour *et al*., 2014).

$$\ln S(t)=-\left(\frac{t}{\alpha}\right)^{\beta} \quad (3)$$

The Weibull model can be cast in the decimal logarithmic form to analyze it (Mafart *et al*., 2002; Van Boekel, 2002). The experimental data were fitted to this

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equation (where ozone treatment time on the x-axis and *log (S)* or *log (N/N_o)* is on the y-axis):

$$\log N = \log N_o - \left(\frac{t}{\delta}\right)^p \quad (4)$$

where N is the number of microorganisms, N_o (CFU/mL) is the initial number of microorganisms, t (min) is the time of treatment, δ (min) is the time required to attain a 1-log or 90% reduction of cell count, and p is the parameter related to the inactivation curve's scale and shape, respectively. The Weibull denotes a concave upward survival curve if $p<1$, concave downward if $p>1$, and straight-line if $p = 1$. The estimated parameters δ and p were used to calculate the desired 5-log reduction. The time required to obtain 5 log reductions (t_{5d}) was calculated, where x = 5-$\log_{10}$ using:

$$t_{5d} = \delta \times (x)^{\frac{1}{p}} \quad (5)$$

2.6 Statistical analysis

A root mean squared error (RMSE) (Choi *et al*., 2012), R^2 (coefficient determination), accuracy factor (*AF*), and bias factor (*BF*) (Halmi *et al*., 2014) were calculated for the fit ability of the tested models. The *RSME* was used to measure the differences between values predicted by the model and the values observed; the smaller *RSME* value was expected from the models. R^2 was used to determine the data's closeness to the fitted model; thus, it must be close to 1. An ideal predictive model would have *AF* = *BF* = 1. A *BF* with values <1 indicates a fail-dangerous model, while a *BF* with values >1 indicates a fail-safe model, and the higher *AF* values indicate less precise prediction. All the equations for *RSME*, *AF*, and *BF* were shown below:

$$\text{RSME} = \sqrt{\sum_{i=1}^{nt}\left(\frac{y_{expi} - y_{pred}}{n_t - n_p}\right)^2} \quad (6)$$

Where y_{expi} are experimental observations, y_{pre} are model predictions, n_t are the number of data points, and n_p is the number of estimated model parameters.

$$\text{AF} = 10^{\left(\frac{\sum\left|\log_{10}\frac{predicted}{observed}\right|}{n}\right)} \quad (7)$$

$$\text{BF} = 10\left(\frac{\sum \log_{10}\frac{predicted}{observed}}{n}\right) \quad (8)$$

Where n is the number of validation data used to make the calculations.

3. Results and discussion

3.1 Comparison of total soluble solids and pH various mango juice samples

Table 1 shows the physicochemical properties and initial population of *S. enterica* ser. Typhimurium of fresh mango juice (MJ) with various distilled water ratios without ozone treatment. The TSS and pH values at different MJ samples were substantially different (p<0.05). The distinction was due to the highest amount of mango pulp content in 100% MJ compared to other MJ samples. In addition, 100% MJ would have a high hydrogen ion (H^+) concentration. The addition of water would create an event where the donation of hydroxide (^-OH) from distilled water and combined with H^+, thereby raising the pH of MJ. Fruit juices with a pH below 4.0 are the focus for juice production as this will curb microbial growth and enzyme inactivation (Aneja *et al*., 2014). The initial population of *S. enterica* ser. Typhimurium presents a significant difference (p<0.05) in all untreated MJ samples, indicating that this microorganism can survive in the low pH in the range of 4.0 to 4.5. A positive correlation was found between bacterial growth to total soluble solid (TSS) and pH of the MJ samples; R^2 = 0.954, -0.902, and significant (p<0.05), respectively. This finding shows that the colonization factor of *S. enterica* ser. Typhimurium in MJ is not only due to 'MJ's pH but includes the presence of juice's components.

3.2 Effect of aqueous ozone treatment on the population of Salmonella enterica serovar Typhimurium *in mango juice*

The ozone application was found to have highly corresponded to the reduction of *S. enterica* ser. Typhimurium in MJ samples. Treatment for 40 mins with ozone dose (1.33 mg/mL) decreased *S. enterica* ser. Typhimurium counts by 5.39, 6.78, and 7.05 log cycles in 14.53°Bx of 100MJ:0DW, 11.10°Bx of 75MJ:25DW, and 7.53°Bx of 50MJ:50DW samples, respectively (Figure 1). This showed that ozone has the potential to be used as an antimicrobial agent for *S. enterica* ser. Typhimurium within the given exposure time with ozone dose in all MJ samples. The Food and Drug Administration issued a final rule in the Federal Register on January 19, 2001, requiring juice processors to incorporate Hazard Analysis and Control Point (HACCP) systems for their fruit juice processing operations (USFDA, 2003). Under this rule, the juice processor also must obey the 5-log reduction performance standard requirement to treat the fruit juice. The 5-log reduction must be directed at "pertinent pathogen" usually the most resistant microorganisms of public health concern that occurred in the juice. This includes Gram-negative bacteria, *Salmonella,* which may cause Typhoid fever, or in the worst-case scenario, death (CDC, 2018). In this study, the *S. enterica* ser. Typhimurium inactivation by aqueous ozone has successfully achieved a 5-log or 99.999% reduction

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Table 1. Physicochemical and population of *S. enterica* ser. Typhimurium of non-ozonated (control) mango juice (MJ)

Juice ratio (%)	100MJ:0DW	75MJ:25DW	50MJ:50DW
pH	4.11±0.01[c]	4.23±0.01[b]	4.31±0.01[a]
Total soluble solid (°Bx)	14.35±0.06[a]	11.10±0.00[b]	7.53±0.06[c]
Population of *S. enterica* ser. Typhimurium (CFU/mL)	1.90×10^{11a}	1.80×10^{11b}	1.50×10^{11c}
Population of *S. enterica* ser. Typhimurium ($\log_{10}$ CFU/mL)	11.2780[a]	11.2553[b]	11.1761[c]

Values are expressed as the mean±standard deviation, n = 3. Values with different superscripts within the same row are significantly different (p<0.05).

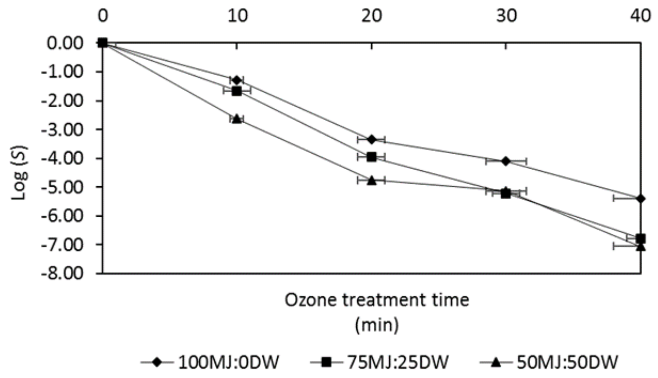

Figure 1. Survival curve of *S. enterica* ser. Typhimurium at different treatment time with ozone dose (10 mins, 0.33 mg/mL; 20 mins, 0.67 mg/mL; 30 mins, 1.00 mg/mL and 40 mins, 1.33 mg/mL)

within 40 and 30 mins, which is the minimum requirement of USFDA for all fruit juice manufacturing (USFDA, 2003).

The findings also observed that the inactivation of *S. enterica* ser. Typhimurium in MJ samples is highly dependent (R^2 = 0.951 and R^2 = 0.954 with p<0.05) on juice ratio and solid content presence in juice. Choi *et al*. (2012) reported that the population of *S. enterica* ser. Typhimurium in 36°Bx of apple juice solid content was able to achieve a 5-log reduction within 10 mins after exposure to ozone concentration at 900 mg/hr. The previous study indicates that at a higher ozone dose, inactivation can be done at minimal treatment time, even when the Bx content of treated juice is much higher (36 °Bx). This contrasts with the time taken in the current study for *S. enterica* ser. Typhimurium inactivation by ozone, which can be explained by the difference in ozone concentration (400 mg/hr) and the use of fruit juice samples – where the solid content also played a role in achieving microbial inactivation. The solid content of apple juice may emulate that of distilled water. It can assist the effectiveness of ozone pasteurization, as opposed to mango fruit juice, which may reach up to 36°Bx of solid content values. Nevertheless, this study has shown that the *S. enterica* ser. Typhimurium in all MJ samples and apple juice perfectly achieved the 5-log reduction within the given ozone parameters.

Thus, it can be concluded from the results of this study, ozone dose negatively correlates to the growing population of *S. enterica* ser. Typhimurium in MJ samples (R^2 = 0.960, p<0.05). The reduction of *S. enterica* ser. Typhimurium in MJ was due to ozone acting as an oxidizing agent, attacking the microbial cell, which leads to a decreasing number of bacteria in juice. The inactivation of microorganisms by ozone is complex as ozone acts on various cellular components of cell bacteria. Nath *et al*. (2014) reported that ozone's microbial inactivation involved two major mechanisms where ozone will oxidize the sulfhydryl groups, enzyme amino acids, peptides, and proteins, while during the second mechanism, the polyunsaturated fatty acids were oxidized to acid peroxides. The potent oxidation capacity of ozone also caused damage to a nucleic acid (cytosine and especially thymine, which was more susceptible to ozone) in cell bacteria's cytoplasm (Miller *et al*., 2013). This further leads to a microbial inability to propagate and subsequently grow in treated juice.

The inactivation of *S. enterica* ser. Typhimurium in MJ samples were due to the ozone molecules' ability to invade the outer membrane of *S. enterica* ser. Typhimurium. Alwi and Ali (2014) reported that in Gram-negative bacteria (*S. enterica* ser. Typhimurium), lipopolysaccharide and lipoproteins are the prime sites of ozone's attack. Then, the disruption by ozone resulting in the increases in cell permeability, leading to disruption of the cell wall (a single layer of peptidoglycan), penetration to vital components (proteins, enzymes, and nucleic acids), and eventually caused cell lysis (Patil *et al*., 2009). The effectiveness of ozone in microbial inactivation relies on targeted microorganisms' morphology (Fundo *et al*., 2018). Gram-positive bacteria (*Staphylococcus, Streptococcus, and Listeria*) appeared to be more resistant to ozone (thick layer of peptidoglycan) compared to Gram-negative bacteria (*E. coli*, *S. enterica* ser. Typhimurium, and *Pseudomonas aeruginosa*), and both bacteria were more susceptible than yeast (Miller *et al*., 2013).

3.3 Inactivation kinetics of Salmonella enterica serovar Typhimurium *in mango juice*

The resulting microbiological data is then fitted to the kinetic model to predict the influence of ozone control parameters for the safety and quality of a fruit juice product. The inactivation kinetics of *S. enterica* ser. Typhimurium in mango juice (MJ) samples were fitted

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using the Log-Linear and Weibull model, estimating the microbial inactivation parameters (Figure 2). The models represent different types of assumptions; (1) the inactivation of *S. enterica* ser. Typhimurium in MJ samples obeyed the first-order kinetic, which present a negative and linear correlation between cell count and lethal treatment/inactivation rate (Log-linear), and (2) describe the non-log-linear modelling structures of *S. enterica* ser. Typhimurium in MJ samples where the shoulder or tail phenomenon occurred.

As shown in Table 2, both models depicted good fits for the experimental data analyzed, given that a high value of R^2 and *RSME* value was not larger than 1 in all MJ samples. For 100MJ:0DW, the R^2 value were 0.9628 and 0.9667, and the RSME value were 0.2745 and 0.2371 for Log-linear and Weibull models, respectively. Based on the data, there were no significant differences between both models. Despite that, the *p*-value in the Weibull's model parameter showed the inactivation curve's shape was 0.8816 ($p<1$). Cullen *et al*. (2009) indicated that if the survival curve shape parameter is $p = 1$, it followed the first-order inactivation kinetic. The same case was observed in the 75MJ:25DW sample, where the R^2, *RSME* of log-linear and Weibull models given out 0.9827, 0.2315, and 0.9861, 0.1939, respectively. The shape parameter, the *p*-value of the survival curve shape, was 0.9120 ($p<1$). Thus, it was concluded that the Weibull model could accurately estimate the values of Log_{10} (*S*) for both 100MJ:0DW and 75MJ:25DW samples compared to the Log-linear model.

Furthermore, the *AF* and *BF* values of the Weibull models were found to be more accurate compared to the log-linear models, 100MJ:0DW (AF = 1.1044, BF = 1.0393) and 75MJ:25DW (AF = 1.0684, BF = 1.0260). Halmi *et al*. (2014) stated that the accuracy factor (AF) and bias factor (BF) present the precision of the predicted value and observed value. The Weibull model *BF* value in 100MJ:0DW and 75MJ:25DW shows that $BF = 1$ indicates a perfect match model.

In 50MJ:50DW, the Weibull model exhibits the *AF* and *BF* of 1.4969 and 0.7366. Thus, it is suggested that the Weibull model has a less precise prediction compared to Log-linear. Nevertheless, in the 50MJ:50DW sample, it was found that the Log-linear model and Weibull model gave out R^2 and *RSME* values distinct from each other (Table 2). The smallest *RSME* (0.3286) and high R^2 (0.9457) value (close to 1) were observed in Weibull model compared to Log-linear model ($RSME$ = 0.6376) and (R^2 = 0.8963). It was also observed that the *p*-value was 0.6613 ($p<1$), indicating the upward concave (Van Impe *et al*., 2018) shape the survival curve of *S. enterica* ser. Typhimurium. With that justification, the Weibull model is considered, as it shows better performance in describing the inactivation kinetic of *S. enterica* ser. Typhimurium in 50MJ:50DW.

The t_{5d} values were lowest as the amount of pulp present or TSS in the 50MJ:50DW sample decreased (t_{5d} = 24.82 min) (see Table 3). However, the inactivation of

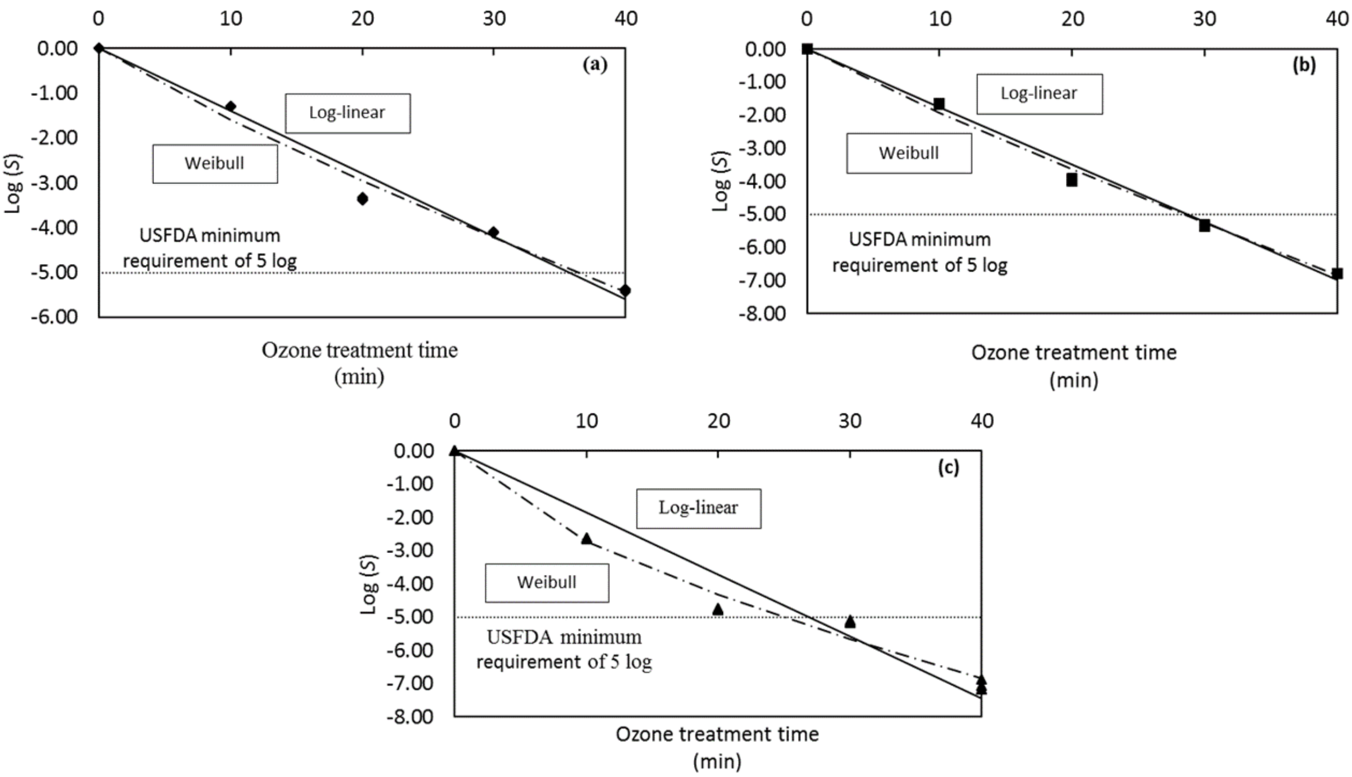

Figure 2. Ozone inactivation of *S. enterica* ser. Typhimurium in different MJ (a) 100MJ:0DW, (b) 75MJ:25DW and (c) 50MJ:50DW at 10 mins (0.33 mg/mL), 20 mins (0.67 mg/mL), 30 mins (1.00 mg/mL) and 40 mins (1.33 mg/mL)

Table 2. The goodness of fit for Log-linear model and Weibull model of *S. enterica* ser. Typhimurium of ozone-treated mango juice (MJ)

Juice ratio (%)	Kinetic model	R^2	*RSME*	*AF*	*BF*
100MJ:0DW	Log-linear	0.9628	0.2745	1.0859	0.9931
	Weibull	0.9667	0.2371	1.1044	1.0393
75MJ:25DW	Log-linear	0.9827	0.2315	1.0955	0.9128
	Weibull	0.9861	0.1939	1.0684	1.0260
50MJ:50DW	Log-linear	0.8963	0.6376	1.1436	0.8744
	Weibull	0.9457	0.3286	1.4969	0.7366

R^2 is the coefficient determination, *RSME* (root mean square error) is the difference between values predicted by a model or an estimator and the values observed, and *AF* is the accuracy factor, and *BF* is the bias factor.

Table 3. Weibull model parameter for the inactivation of *S. enterica* ser. Typhimurium in ozone-treated mango juice (MJ)

Juice ratio (%)	ρ	δ (min)	t_{5d} (min)
100MJ:0DW	0.8816	5.8631	36.3883
75MJ:25DW	0.9120	4.8597	28.3835
50MJ:50DW	0.6613	2.1765	24.8176

δ is the time needed for one log reduction or 90% reduction of *S. enterica* ser. Typhimurium population, *p* is the parameter related to the inactivation curve's scale and shape, and t_{5d} is the time required to obtain a 5-log reduction or 99.999% reduction of *S. enterica* ser. Typhimurium population

S. enterica ser. Typhimurium in the 100MJ:0DW sample showed the highest t_{5d} values (t_{5d} = 36.39 min). Further corroborates that the organic and inorganic compounds in the 100MJ:0DW sample could impact the ozone's dissolution rate, reducing ozone efficiency to inactivate *S. enterica* ser. Typhimurium. Patil and Bourke (2012) implied that the ozone disinfection rate could be affected due to the dissolved organic and inorganic matter that competed with microorganisms for disinfectant. Ozone is highly attracted to the double bond of organic compounds, and the oxidation of this compound by ozone causes no or weak bactericidal activity in juice.

Patil *et al*. (2009) also observed that ozone treatment 0.075-0.078 mg/mL successfully reduces the *E. coli* population in orange juice by 5-logs where t_{5d} for unfiltered juice is between the range of 15 to 18 min, whereas no pulp juice at 5 mins. These observations corroborated that the solid content of the fruit juice will affect the ozone's rate of efficacy in order to reduce the microbial count. Thus, it further supports the theories regarding the interference of ozone efficacy in achieving 5-log reduction caused by the presence of solids content in all MJ samples.

4. Conclusion

This work has shown that the maximum value at 1.33 mg/mL of ozone dose and 40 mins of ozone exposure time can inactivate the *S. enterica* ser. Typhimurium, where increment in ozone dose reduced the population *S. enterica* ser. Typhimurium in various MJ samples. The results presented in this research also stated that the inactivation of *S. enterica* ser. Typhimurium in MJ samples obeyed the Weibull model trend. The distinction in t_{5d} value was observed between the MJ samples due to the organic and inorganic compounds present in the juice competing with microorganisms for total disinfection. The 100MJ:0DW samples had a longer time (36.38 mins, 1.20 mg/mL) to achieve 5-log reduction compared to the other two samples. Generally, the juice condition (pulp content/ Brix content), ozone concentration level, and type of microorganism (morphology of bacteria) highly affected the efficacy of aqueous ozone treatment toward inactivation *S. enterica* ser. Typhimurium in MJ, as proven in this study.

Nevertheless, this study's ozone dose has proven to lower and achieved a 5-log reduction of *S. enterica* ser. Typhimurium in all MJ samples. Thus, it is recommended that the implementation of ozone technology in the food industry should further consider juice's parameter value that might affect the aqueous ozone efficacy on pasteurizing the fruit juice to inactivate any pertinent microorganism to ensure food safety is not threatened.

Conflict of interest

The authors declare no conflict of interest.

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