



Thermal resistance of *Salmonella* in low-moisture high-sugar products

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ABSTRACT

Recent foodborne outbreaks in low-moisture foods were caused mostly by *Salmonella*. The main common carbohydrates in low-moisture high-sugar products are D-glucose and D-fructose. There is a lack of information on the thermal resistance of *Salmonella* in pure sugar and high-sugar products with low water activity (a_w) levels. The objective of this study was to study the thermal resistance of *Salmonella* Enteritidis PT30 in sugar products. Two pure sugars (glucose and fructose) and honey powder were selected for this research. The initial a_w and moisture content for glucose, fructose, and honey powder are 0.30, 0.30, 0.40 and 0.02, 0.15, and 0.50% db., respectively. The *D*-values of *Salmonella* in different sugar products are determined at low a_w levels (0.18, 0.30, 0.40, and 0.50 measured at 80 °C). The results showed that the $D_{80\text{ °C}}$ -values of *S. Enteritidis* PT30 decreased with increasing a_w of all samples. At $a_{w80\text{ °C}}$ 0.18, *S. Enteritidis* PT30 in glucose powder has higher *D*-value than those in fructose and honey powder whereas, at $a_{w80\text{ °C}}$ 0.30, 0.40, and 0.50, *S. Enteritidis* PT30 in fructose powder has higher *D*-values than glucose and honey powder at 80 °C. Z_{a_w} values of *S. Enteritidis* PT30 in fructose and honey powder are comparable but was lower in glucose because of the phase change. The results of this study provide insights on understanding the thermal resistance of *Salmonella* in sugars and guidance on thermal process design to improve the safety of low-moisture high-sugar products.

1. Introduction

Low-moisture foods refer to foods with water activity (a_w) less than 0.6 (Labuza, 1980). Low-moisture foods are produced by removing moisture in dehydration to prevent the growth of microorganisms and hence reduce foodborne illnesses (Labuza, 1970). Yet, outbreaks were reported to be associated with low-moisture foods that were contaminated with foodborne pathogens, in particular *Salmonella* (CDC, 2020). *Salmonella* caused around 11% of foodborne illnesses annually (Scallan et al., 2011). *Salmonella* was widely isolated from eggs, vegetables, and poultry (Tauxe, 1991). But, in the last few years, several high-sugar content products with low- a_w levels have been linked to *Salmonella*; these low-moisture and high-sugar products include chocolate (Werber et al., 2005), dried fruits (Witthuhn, Engelbrecht, Joubert, & Britz, 2005), honey smacks cereal (CDC, 2018), and marshmallow confectionery (Lewis et al., 1996). Brockmann, Piechotowski, and Kimmig (2004), and Unicom et al. (2005) reported *Salmonella* contamination in a high-sugar, sesame seed-based product. De Jong et al. (2001) reported an outbreak of *Salmonella* Typhimurium from contaminated jars of halva; and Raimi Olayinka (2013) reported contamination of pathogenic bacteria such as *Salmonella* species and

Escherichia coli in date fruits.

Salmonella does not grow in dry environments, but it can survive for years (Juven et al., 1984; and; Podolak, Enache, Stone, Black, & Elliott, 2010). Kotzekidou (1998) reported that *S. Enteritidis* can survive in halva (a_w of 0.17 with sugar content 49.5%) at 18–20 °C for at least 8 months. *Salmonella* were detected in high-sugar, peanut butter-flavored candy fondant (with a_w 0.65 to 0.69 at room temperature) stored at room temperature for one year (Nummer, Shrestha, & Smith, 2012). Beuchat and Mann (2014) reported that *Salmonella* can survive in dried cranberries, date paste, and raisins stored at 4 °C for at least 8 months. However, very limited studies were reported on the thermal resistance of *Salmonella* in low-moisture and high-sugar products. Limcharoenchat et al. (2018) reported a study on the thermal resistance of *S. Enteritidis* PT30 in date fruits at a_w 0.45 measured at 25 °C and found the $D_{80\text{ °C}} = 1.2$ min. K. Mattick et al. (2001) reported studies on thermal resistance of *S. Enteritidis* PT4 and *S. Typhimurium* DT104 in high sugar content broths and found the $D_{80\text{ °C}} = 3.6$ min. Kwast and Verrips (1982) studied the thermal resistance of *S. Senftenberg* 775 W at 60 °C in sucrose concentration between (0–2.20 mol l⁻¹) in different agar.

The a_w has been noticed as one of the main factors influencing the thermal resistance of *Salmonella* in low-moisture foods (Syamaladevi

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Nomenclature

a_w	Water activity
CFU	Colony forming unit
CUT	Come up time
MC	Moisture content
RH	Relative humidity

RMSE	Root mean square error
R^2	Regression coefficient
TAC	Thermal water-activity cells
YE	Yeast extract
TSA	Tryptic soy agar
TSB	Tryptic soy broth

et al., 2016). Heat resistance of *Salmonella* was influenced by the changing a_w levels at treatment temperatures (Syamaladevi et al., 2016; Liu, Tang, Tadapaneni, Yang, & Zhu, 2018; and; Xu et al., 2019). Gibson (1973) reported that the heat resistance of *Salmonella* in sugar solutions increased with decreasing a_w . However, the range of a_w at room temperature ($\sim 23^\circ\text{C}$) tested in this study were 0.706–0.995, which are not in the category of low-moisture foods. There is a lack of information on how a_w at treatment temperature influences the heat resistance of *Salmonella* in low-moisture and high-sugar content matrices.

S. Enteritidis PT30 was chosen in this study because this strain caused a raw almond outbreak in 2001. Almond Board of California developed a protocol for process validation using a surrogate for control of *S. Enteritidis* PT30 in almonds (Danyluk et al., 2007). Moreover, many published studies targeted *S. Enteritidis* PT30 in low-moisture foods, such as almond, wheat flour, and shell walnuts (Blessington, Theofel, Mitcham, & Harris, 2013; Tadapaneni, Syamaladevi, Villa-Rojas, & Tang, 2017; and; Xu et al., 2019). Previous report also identified high survivability of *S. Enteritidis* spp. in high-sugar products (Kotzekidou, 1998). Therefore, the objective of this study was to determine the *D*-values at 80°C of *S. Enteritidis* PT30 in glucose powder, fructose powder, and honey powder at four a_w levels 0.18, 0.30, 0.40 and 0.50 measured at the treatment temperature.

2. Material and methods

2.1. Food materials

D-glucose powder (Fisher Scientific, PA), D-fructose powder (Fisher Scientific, PA), and spray-dried honey powder (Augason Farm, UT) were purchased from Fisher scientific or local Walmart. The chemical and physical properties of the sample are presented in Table 1.

2.2. Particle size analyses

The particle size of sugar samples was analyzed using a sonic sifter (Model L3P, ATM Corporation, Milwaukee, WI, USA). A 50 g of each sample was sieved and separated by six different sieve sizes. After sieving each separate sieve was weighted. The measurements were made in duplicates.

2.3. Test cells for heat treatment

The thermal water-activity cells (TACs) manufactured at Washington State University were used in the heat treatment to determine thermal resistance of *S. Enteritidis* PT 30 inoculated into the sample powders. The TAC cell has two parts (base and lid), the base has two cavities: the inner cavity for the inoculated samples while the

surrounding cavity for LiCl solutions. An O-ring between the base and lid was used to prevent leakage of moisture during heat treatments. Detailed design of TACs is described in Tadapaneni et al. (2017) and Tadapaneni, Xu, Yang, and Tang (2018). The LiCl solutions with different molalities were selected to control the relative humidity inside the TAC cells during the heat treatments of the samples using an oil bath. Before each test, 1.0 ± 0.05 g of inoculated sample was spread with a thin layer on the center of the cell, the surrounded cavity was filled with 3 mL of LiCl solution at different a_w levels. The molalities of LiCl solution for the target a_w between 0.18 and 0.50 ranged between 9.4 and 18.9 mol/kg, as shown in Fig. 1. In preparing the lithium chloride (LiCl) solution, the proper LiCl powder was weighed and dissolved in distilled water to reach a target a_w that was measured by a water activity meter (Aqualab, Meter Group, Inc., Pullman, WA, USA). Fig. 1. shows information on how the water activity of LiCl changes when temperature increases from 20°C to 80°C . The relative humidity created by the LiCl solution in the TAC cell as indicated in Fig. 1 controlled the water activity of the inoculated samples during the thermal treatments.

2.4. Cultural and inoculated sample preparation

Stock culture of *S. Enteritidis* PT 30 was obtained from Linda Harris' Lab of the University of California, Davis. The culture was kept at -80°C in tryptic soy broth with 20% glycerol before use. The lawn-grown inoculum was prepared by following the procedure described previously (Hildebrandt et al., 2016; Xu et al., 2018). A loop of thawed culture stock was subjected to two consecutive transfers in 9 mL of tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) and incubated for 24 h at 37°C . Then, 1 mL of incubated culture was evenly spread over a plate (150×15 mm) made of tryptic soy agar supplemented with 0.6% of yeast extract (TSAYE) to obtain a uniform lawn. After incubation at 37°C for 24 h, the bacteria lawn was harvested by flooding with 20 mL of sterile 0.1% peptone water. The culture was centrifuged at $8,000 \times g$ for 15 min at 4°C , then the pellet was resuspended in 3 mL of 0.1% peptone water with a final concentration of $11.52 \log \text{CFU/mL}$. Silicon dioxide (SiO_2 ; 0.2- to 0.7-mm in diameter granules) was purchased from Umicore (Brussels, Belgium) and used as a carrier to prepare inoculated sugars and honey powder, and the detailed method was described in Liu et al. (2018) and Liu, Tang, et al. (2018). In brief, 1 mL of resuspended culture was added to a sterile plastic bag containing 30 g of sterile silicon dioxide (SiO_2) and mixed by hand massage for 3 min. The inoculated SiO_2 was dried for 12 h in a biosafety hood at room temperature ($\sim 23^\circ\text{C}$) with relative humidity (RH) 15% (Liu et al., 2018). Twenty grams of respective samples were completely mixed with 3 g of inoculated SiO_2 . Inoculated samples were transferred to TAC cells with different relative humidity LiCl solutions,

Table 1
Chemical and physical properties of samples.

Sample	Chemical formula	Molecular weight ($\text{g}\cdot\text{mol}^{-1}$)	Melting point ($^\circ\text{C}$)	T_g^* ($^\circ\text{C}$)	Initial a_w	Moisture content (%db)
D-glucose	$\text{C}_6\text{H}_{12}\text{O}_6$	180.16	147	5	0.30	0.02
D-fructose	$\text{C}_6\text{H}_{12}\text{O}_6$	180.16	103	31	0.30	0.15
Honey powder	–	–	200	63	0.40	0.50

*Glass transition temperature.

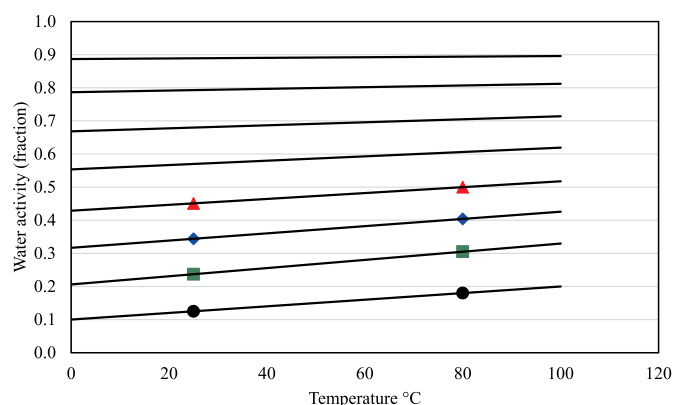


Fig. 1. Prediction of a_w of LiCl of different molarities in the range of temperatures between 0 and 100 °C. This figure was adopted from (Tadapaneni et al., 2017).

the cells were sealed and were stabilized at room temperature (~ 23 °C) for 2 h to reach the equilibration inside the TACs.

2.5. Thermal inactivation

Thermal inactivation study was performed at four water activity levels (a_w 0.18, 0.30, 0.40, and 0.50 at 80 °C) which was estimated from Fig. 1. The water activity was limited because of the state change (solid to liquid) in the samples of fructose and honey powder when conditioned at relative humidity above 50% at ambient temperature, as shown in Fig. 2. The sealed TAC cells were submerged in a preheated oil bath at 80 °C (Isotemp 5150 H11, Fisher 180 Scientific, Inc., Pittsburgh, PA, USA). The come-up time (CUT) for all three samples to reach the target temperature (80 °C) in TAC cells was 95 s, which was measured by a T-type thermometer (Omega Engineering, Inc., Stamford, CT). Two TAC cells were removed at fixed time intervals and immediately cooled down in an ice-water bath for 30 s to stop thermal treatment.

2.6. Recovery and enumeration

The treated samples (1.0 ± 0.05 g) were transferred into 9 mL of 0.1% (w/v) peptone water to establish a 10-fold dilution and then

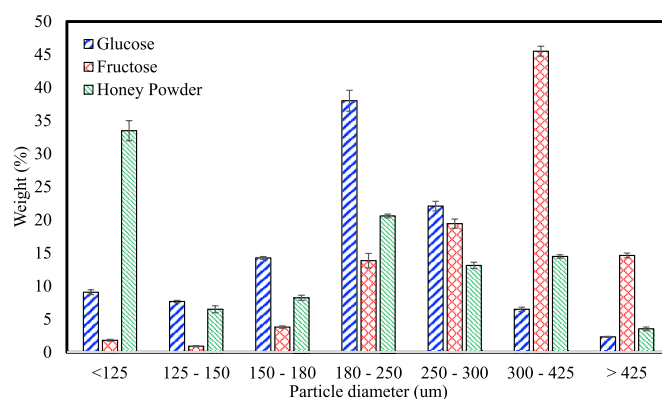


Fig. 3. Particle size distribution of glucose, fructose and honey powder ($n = 3$).

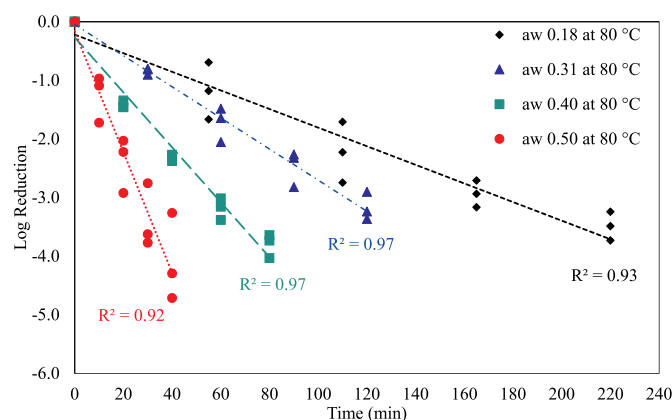


Fig. 4. Representative survival curves of *Salmonella* Enteritidis PT30 in glucose powder at four a_w levels at 80 °C.

stomached for 3 min at 260 rpm in a Seaward stomacher (Seaward, London, UK) (Harris, Uesugi, Abd, & McCarthy, 2012). Suitable 10-fold serial dilutions were enumerated in duplicate on tryptic soy agar supplemented with 0.05% ammonium ferric citrate and 0.03% sodium thiosulfate pentahydrate (TSAYE⁺) plates to determine the population



Fig. 2. Appearance of samples after 14 days of conditioning in different relative humidity at ~ 23 °C.

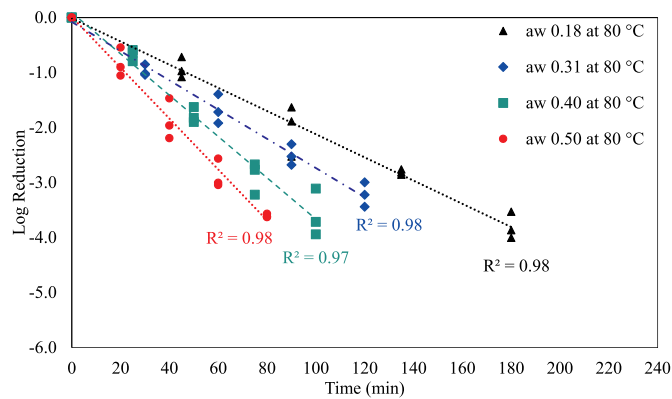


Fig. 5. Representative survival curves of *Salmonella* Enteritidis PT 30 in fructose powder at four a_w levels at treatment temperature 80 °C.

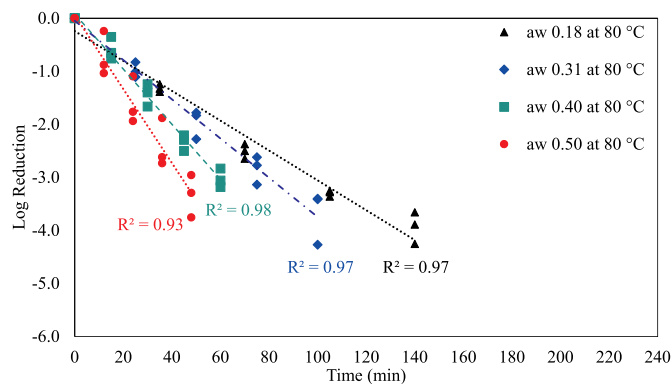


Fig. 6. Representative survival curves of *Salmonella* Enteritidis PT30 in honey powder at four a_w levels at treatment temperature 80 °C.

counts (CFU/mL) of survivors. The plates were incubated at 37 °C for 48 h and the black colonies were counted as *Salmonella*. All the tests were conducted in three biological replicates and two independent TAC cells runs.

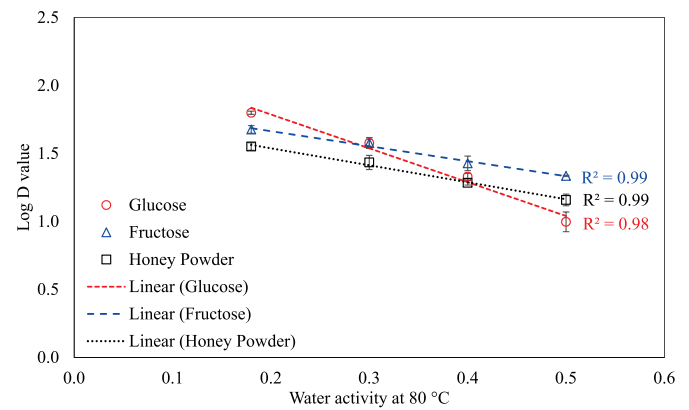


Fig. 7. The log D -values of *Salmonella* Enteritidis PT30 in glucose powder, fructose powder, and honey powder at four a_w levels. Error bar indicated standard deviation. The data points were from the average of three biological and two independent replicates.

2.7. Statistical analysis

Based on the log-linear-regression survival curves of *S. Enteritidis* PT30, the D -values at 80 °C were calculated by taking the inverse of the slopes of each replicate curve. The average of the slopes for the three biological replicates of each treatment condition was calculated to generate the D -value. The thermal inactivation data were analyzed using USDA Integrated Predictive modeling program (IPMP) (Huang, 2014). Differences between D -values among samples were tested using one-way ANOVA in Minitab Express version 1.5.2 (Minitab, LLC, PA).

The Z_{a_w} -value of *S. Enteritidis* PT30 explains the degree of water activity change to 1-log D -value in difference at the same treatment. The value of Z_{a_w} can be calculated from the following equation (Xu et al., 2019):

$$Z_{a_w} = -\frac{a_{w1} - a_{w2}}{\log D_1 - \log D_2} \quad (1)$$

where a_{w1} and a_{w2} are water activities measured at two temperatures, and $\log D_1$ and D_2 are D -values of *S. Enteritidis* PT30 at the corresponding temperatures.

Table 2

Parameter estimates of inactivation log linear model of *Salmonella* Enteritidis PT30.

Sample	a_w 25 °C	a_w 80 °C (predicated)	Experimental			R^2
			D -value (min)	$D_{80^\circ C}$ (min)	RMSE (log CFU)	
Glucose	0.13	0.18	63.0 ± 1.7 ^{a A}	63.0 ± 5.4	0.41	0.93
	0.24	0.31	37.9 ± 3.5 ^{b A}	37.7 ± 1.5	0.17	0.97
	0.34	0.40	21.4 ± 1.2 ^{c B}	21.3 ± 1.0	0.23	0.97
	0.45	0.50	10.0 ± 1.7 ^{d C}	9.7 ± 0.7	0.39	0.92
Fructose	0.13	0.18	47.6 ± 3.1 ^{a B}	47.4 ± 2.3	0.26	0.98
	0.24	0.31	37.6 ± 2.6 ^{b A}	37.6 ± 1.7	0.19	0.98
	0.34	0.40	26.9 ± 3.4 ^{c A}	26.6 ± 2.0	0.36	0.97
	0.45	0.50	21.6 ± 0.2 ^{c A}	21.6 ± 1.4	0.33	0.98
Honey powder	0.13	0.18	35.6 ± 2.3 ^{a C}	35.5 ± 2.3	0.35	0.97
	0.24	0.31	27.3 ± 3.2 ^{b B}	27.0 ± 1.6	0.30	0.97
	0.34	0.40	19.6 ± 0.7 ^{c B}	19.3 ± 1.8	0.39	0.98
	0.45	0.50	14.4 ± 1.4 ^{c B}	14.4 ± 1.1	0.35	0.93

^{a-d} Lowercase letters indicate the significant differences between the D -values of each sample at selected water activities ($p < 0.05$).

^{A-C} Uppercase letter indicate the significant differences between D -values of glucose, fructose and honey powder at selected water activities ($p < 0.05$); Data are represented as mean ± standard errors. RMSE: root mean squared error.

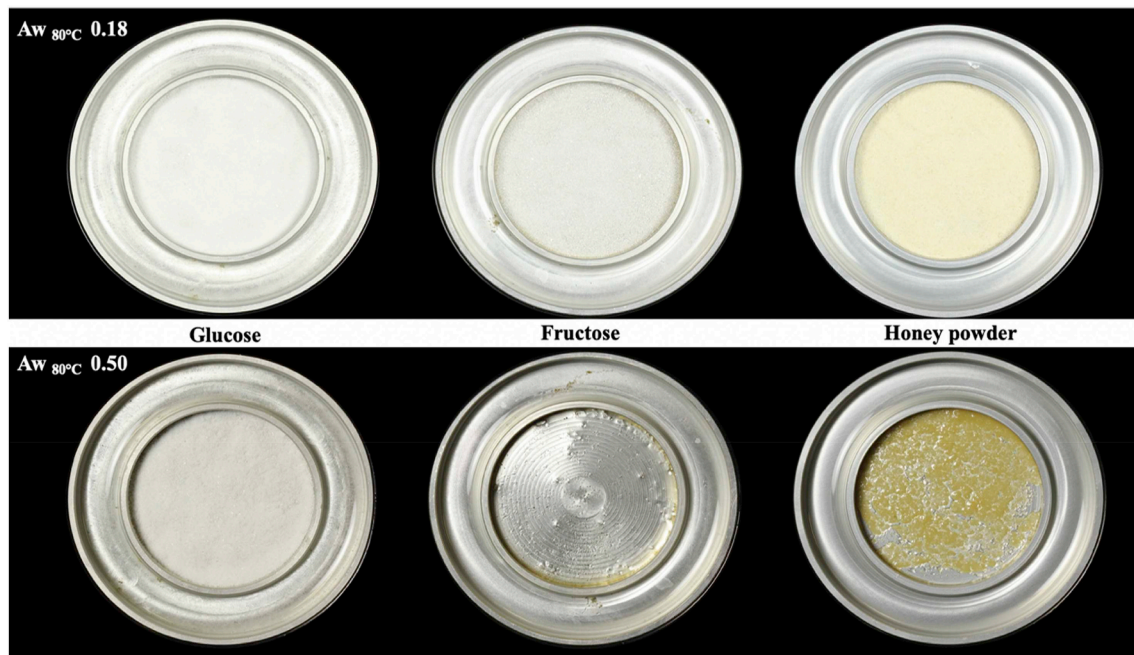


Fig. 8. State change of glucose, fructose, and honey powder samples in TAC cells after 200 min at 80 °C for a_w 0.18 and 0.50, respectively.

3. Results and discussion

3.1. Samples characterization

As shown in Table 1, glucose and fructose powder have a similar chemical formula, molecular weight, and initial water activity; while they have different melting point and glass transition temperature (T_g). The honey powder has 96.4% of carbohydrates with a ratio of 1.2:1 in fructose and glucose.

The variation in the physical structures of foods may affect the thermal susceptibility of microorganisms (Liu, Snoeyenbos, & Carlson, 1969; and Syamaladevi et al., 2016). Thus, particle size of powdered sugars was analyzed. The three samples had different size distributions in a range between 125 and 425 μm as shown in Fig. 3. For glucose, 38.0% of the total sample was in the range between 180 and 250 μm . For fructose, 45.5% of the total sample was in the range of particle size between 300 and 425 μm . Honey powder was the finest compared to glucose and fructose, the peak of the total sample was 33.5% collected in particle size less than 125 μm . Sugar type in the order of particle size from small to large was honey powder, glucose, fructose.

3.2. Thermal inactivation kinetics of *Salmonella*

The population level of *S. Enteritidis* PT30 in SiO_2 inoculated samples were 8.61 log CUF/g. The survival curves of *S. Enteritidis* PT30 at 80 °C with different a_w levels (a_w 0.18, 0.31, 0.40, and 0.50 estimated at 80 °C) in D-glucose, D-fructose, and honey powder are shown in Figs. 4–6, respectively. The survival curves of all samples were fitted well with the log linear model (R^2 , 0.92 to 0.98) as shown in Table 2 and Figs. 4–6. In all cases, the survival of *Salmonella* decreased logarithmically with treatment times. Similar trends were observed on survival of *Salmonella* in peanut butter spread (Burnett, Gehm, Weissinger, & Beuchat, 2000), survival of *Salmonella enterica* Serovar Enteritidis in tryptone soy agar (TSA) with NaCl 4, 6, and 8% (Kieboom et al., 2006); and survival of *Salmonella enterica* serovar Enteritidis PT4 and *Salmonella enterica* serovar Typhimurium DT104 in chicken broth and skimmed milk (Mattick et al., 2000). Our recent research studies reported that the heat resistance of *Salmonella* increases as a_w decreases (Jin & Tang, 2019; Taylor, Tsai, Rasco, Tang, & Zhu, 2018; Villa-Rojas

et al., 2017; and; Xu et al., 2019).

In all the three samples when the a_w at treatment temperature decreased, the log D -values of *S. Enteritidis* PT30 increased linearly as shown in Fig. 7. This trend was also observed similar to the typical inactivation kinetics in previous studies associated with the thermal resistance of *Salmonella* in other low-moisture matrices, such as silicon dioxide (Liu et al., 2018), wheat flour (Liu, Rojas, Gray, Zhu, & Tang, 2018), all-purpose wheat flour and peanut butter (Liu et al., 2018; and Syamaladevi et al., 2016), and nonfat milk powder (Liu, Xu, Xie, Zhu, & Tang, 2019).

3.3. Variation of *Salmonella* D -values at a_w 80 °C in different samples

The D -value of *S. Enteritidis* PT30 in glucose (63.0 ± 1.7 min) was significantly greater than those in fructose and honey powder (47.6 ± 3.1 and 35.6 ± 2.3 min) at a_w 0.18. However, at a_w 0.31, there was no significant ($p > 0.05$) difference in the D -values between glucose (37.9 ± 3.5 min) and fructose (37.8 ± 2.6), but the D -value of *Salmonella* in honey powder was significantly ($p < 0.05$) smaller (27.3 ± 3.2 min). It seems that *Salmonella* in honey powder is more heat sensitive in most cases, which might be caused by the presence of antimicrobial components, such as hydrogen peroxide and proteinaceous (Mandal, DebMandal, Pal, & Saha, 2010; and; Stagos et al., 2018).

At a_w 0.40 measured at 80 °C, the D -value of *S. Enteritidis* PT30 in fructose (26.9 ± 3.4) was significantly ($p < 0.05$) higher than in glucose (21.9 ± 1.2 min) and honey powder (19.3 ± 0.7 min). There was no significant ($p > 0.05$) difference between the D -value of *S. Enteritidis* PT30 in glucose and honey powder. Similarly, at a_w 0.50, the D -value of *S. Enteritidis* PT30 in fructose (21.6 ± 0.2 min) was significantly ($p < 0.05$) higher than honey powder and glucose (14.4 ± 1.4 and 10.0 ± 1.7 min), respectively.

At water activities 0.40 and 0.50, there were opposite results and inactivation behaviors from water activities at 0.18 and 0.31 among the three different samples. Physical change occurred in fructose and honey powder during the thermal treatments between a_w 0.40 and 0.50. At a_w 0.50, fructose powder and honey powder started melting during thermal treatment, while glucose powder did not melt and remained in the powder form (Fig. 8). The melted samples appeared to have a certain level of protection that enhanced the thermal resistance of *S.*

Enteritidis PT30 cells.

Z_{a_w} -value of *S. Enteritidis* PT30 calculated from Eq. (1) were 0.40, 0.91, and 0.80 for glucose, fructose and honey powder, respectively. Z_{a_w} (0.40) in glucose was close to the value (0.31) determined from the same strain in silicon dioxide (Liu et al., 2018), and 0.32 in wheat flour, almond flour, and whey protein (Xu et al., 2019). Z_{a_w} -values of *S. Enteritidis* PT30 in fructose (0.91) and honey powder (0.80) were extremely high compared to that in glucose and low sugar food matrices. The difference in Z_{a_w} values (the sensitive of thermal resistance of bacteria in terms of a_w change) between glucose versus fructose and honey powder is highly associated with the phase change happened during thermal treatment. Fructose and honey powder melted when a_w went beyond 0.3 at 80 °C, and thus, the melted sugar might cover the bacteria cells, limiting migration of moisture from the headspace into the bacterial cells. Further systematic studies are needed to explain the influence of sugar phase change on the thermal resistance of bacteria.

4. Conclusion

The *D*-values of *S. Enteritidis* PT30 in high sugar-low a_w products decreased with increased a_w , following a semi-log linear trend, during the thermal processing. At low a_w ranges (0.18, 0.31), the *D*-values of *S. Enteritidis* PT30 in glucose powder were higher than those in fructose and honey powder, while above a_w 0.31, *D*-values of *S. Enteritidis* PT30 in fructose became higher than those in glucose and honey powder. Also, these results showed that the physical change is important and might influence the thermal resistance of *Salmonella*. Future studies are recommended to quantify the physical change and its effect on the thermal resistance of *Salmonella* in other high sugar products. The results from this study provides an understanding of the thermal inactivation kinetics of *Salmonella* in high-sugar and low-moisture products as influenced by changes in water activity during thermal treatments. The information may be useful in designing thermal processes for sugar or sugar-rich products.

CRedit authorship contribution statement

Jaza Alshammari: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Jie Xu:** Methodology, Writing - review & editing. **Juming Tang:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing - review & editing. **Shyam Sablani:** Visualization, Writing - review & editing. **Mei-Jun Zhu:** Visualization, Writing - review & editing.

Declaration of competing interest

The authors declare there is no conflict of interest in this research.

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