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Thermal resistance of Listeria monocytogenes in natural unsweetened cocoa powder under different water activity

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ABSTRACT

Listeria monocytogenes can survive in dry conditions for long periods. Despite an increasing research studying *Salmonella* inactivation in low-moisture foods, there is a general lack of knowledge related to *L. monocytogenes* inactivation in low-moisture foods during thermal processing and the factors impacting their survival in these products. Cocoa powder is an essential and widely incorporated ingredient in many desserts and drinks that do not need thermal processing. This study evaluated the thermal resistance of *L. monocytogenes* in cocoa powder and investigated the impact of water activity (a_w) on its survival in cocoa powder. Natural unsweetened cocoa powder was inoculated with a 3-strain *L. monocytogenes* cocktail (~9.0 Log_{10} CFU/g), equilibrated to a_w 0.30, 0.45 or 0.60 at 22 °C and subjected to isothermal treatments. Survivors were enumerated to obtain thermal-inactivation parameters. *L. monocytogenes* population was stable in cocoa powder (a_w 0.30) over the first month of storage, then decreased gradually but remained detectable after 12-month storage at 22 °C. Thermal inactivation of *L. monocytogenes* in cocoa powder at target a_w and different temperatures showed a log-linear trend. Heat resistance of *L. monocytogenes* is a_w-dependent with the highest resistance at a_w 0.30. The range of D-values (in min) at 70, 75 and 80 °C at a_w 0.30. and 0.45, respectively, were: 21.9–5.0 and 7.3–1.8. The range of D-values (in min) at 65, 70 and 75 °C at a_w 0.60 was 9.1–2.0. The z-value at a_w 0.30, 0.45, and 0.60 was 15.5, 15.9, and 14.9 °C, respectively. In summary, *L. monocytogenes* can survive in cocoa powder stored at 22 °C for an extended time. Thermal resistance of *L. monocytogenes* adapted to low a_w cocoa was conversely related to a_w. This study provides valuable information for the food industry to develop thermal inactivation strategies to control *L. monocytogenes* in cocoa powder.

1. Introduction

Low water activity (a_w) foods (La_wF) and food ingredients have been increasingly implicated in foodborne outbreaks (Beuchat et al., 2013), as reflected in numerous *Salmonella* outbreaks involving peanut butter and peanut products (CDC, 2009), almonds (CDC, 2004), chocolate (Werber et al., 2005), powdered milk (CDC, 1993) and cereal (CDC, 1998), as well as a recent Shiga-toxin producing *E. coli* outbreak related to flour (Crowe et al., 2017). Listeriosis, a rare but deadly disease, has the one of the highest mortalities among all foodborne illnesses (Marder et al., 2017) and has historically been associated with ready-to-eat food outbreaks (Zhu, Du, Cordray, & Ahn, 2005). Recent multistate *Listeria monocytogenes* outbreaks in cantaloupes (CDC, 2011) and caramel apples (FDA, 2014) indicate that *L. monocytogenes* is an emerging foodborne pathogen in fresh produce, highlighting the food safety risks of *Listeria* in different foods and commodity groups including La_wF. This risk was further emphasized by recent Classic Hummus (FDA, 2015) and frozen biscuit dough (FDA, 2017) recalls due to potential *L. monocytogenes* contamination.

L. monocytogenes remains viable in low-a_w almond kernels or shelled pistachios stored at 4 °C and −19 °C for more than one year (Kimber, Kaur, Wang, Danyluk, & Harris, 2012). It is stable in chicken meat powder, pet foods and confectioneries during 3-week storage at 16 °C (Rachon, Peñaloza, & Gibbs, 2016), and was able to survive in chocolate-peanut spread and peanut butter during 6-month 20 °C storage at 0.33 or 0.65 a_w (Kenney & Beuchat, 2004). Our recent study reported only 2.5 Log reduction of *L. monocytogenes* in wheat flour (0.31 a_w) during 210 days of storage at room temperature (Taylor, Tsai, Rasco, Tang, & Zhu, 2018). Concordantly, *L. monocytogenes* can survive in dry environments for prolonged periods, especially if organic material (soil) is present (Vogel, Hansen, Mordhorst, & Gram, 2010).

Thermal sterilization and pasteurization have been effective for

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pathogen control in high-a_w foods. However, thermal control of foodborne pathogens in La_wF presents a challenge to the food industry. Pathogens such as *Salmonella* in La_wF often exhibit increased tolerance to thermal and other treatments that are lethal under high-a_w conditions (Archer, Jervis, Bird, & Gaze, 1998; Liu, Rojas, Gray, Yang, Zhu, & Tang, 2018a; Liu, Tang, Tadapaneni, Yang, & Zhu, 2018; Smith, Hildebrandt, Casulli, Dolan, & Marks, 2016; Villa-Rojas et al., 2013). Their thermal resistance in LawF is also influenced by pathogen strain, food matrix, a_w and micro-environments of La_wF (Beuchat et al., 2013; Koseki, Nakamura, & Shiina, 2015; Li et al., 2014; Rachon et al., 2016; Syamaladevi et al., 2016; Tiganitas, Zeaki, Gounadaki, Drosinos, & Skandamis, 2009; Vogel et al., 2010). Research on high-solid egg mixes showed that lower a_w dramatically increases *L. monocytogenes* resistance to thermal lethal treatments, and that *L. monocytogenes* is more heat resistant than *Salmonella* at low a_w (Li, Sheldon, & Ball, 2005). In culinary seasoning (a_w 0.66) and pet food (a_w 0.65), *L. monocytogenes* showed a similar heat resistance compared to *Salmonella*, while in confectioneries (a_w 0.57) and chicken meat powder (a_w 0.38), *Salmonella* was much more heat resistant than *L. monocytogenes* (Rachon et al., 2016). Studies on *Salmonella* indicate a_w plays a critical role in enhanced desiccation stability (Beuchat, Mann, Kelly, & Ortega, 2017) and thermal stability of *Salmonella* in La_wF (He et al., 2013; Liu, Rojas, et al., 2018; Smith et al., 2016; Villa-Rojas et al., 2013). Similar phenomena were also observed for *L. monocytogenes* in wheat flour (Taylor et al., 2018).

Cocoa powder is an essential and widely incorporated ingredient in many desserts and snacks, such as candy bars, chocolates, dairy-based confections, and spreads. *L. monocytogenes* might be introduced during cocoa powder processing and transportation. Multiple *Salmonella* strains were implicated in chocolate outbreaks worldwide (Gill et al., 1983; Kapperud et al., 1990; Werber et al., 2005), clearly demonstrating a need to evaluate and validate thermal processing of cocoa powder against potential *L. monocytogenes* contamination. The objective of this study was to evaluate the desiccation stability and thermal resistance of *L. monocytogenes* in cocoa powder equilibrated to target a_w, as well as investigate impacts of a_w on its thermal survival in cocoa powder.

2. Materials and methods

2.1. Bacteria strains and lawn preparation

L. monocytogenes outbreak strains NRRL B-57618 (1/2a) and NRRL B-33053 (4b) and one processing plant isolate, NRRL B-33466 (1/2b), were obtained from the culture collection of the National Center for Agricultural Utilization Research (NRRL), USDA Agricultural Research service (Peoria, IL) and used to prepare a 3-strain cocktail inoculum. Bacterial strains were maintained at −80 °C in trypticase soy broth (TSB, Becton, Dickinson and Company, Sparks, MD) supplied with 0.6% Yeast Extract (Fisher Scientific, Pittsburgh, PA) (TSBYE) and 20% (v/v) glycerol. Each *L. monocytogenes* strain was twice activated individually in TSBYE at 35 ± 2 °C for 24 h, statically. Twice-activated *L. monocytogenes* was plated on TSAYE (TSBYE with 1.5% agar) and incubated at 35 ± 2 °C for 24 h. Bacterial lawn of each strain was collected from TSAYE using sterile phosphate-buffered saline (PBS, pH 7.4), then centrifuged at 8000 × *g*, 4 °C for 15 min. The resulting pellet was resuspended in sterile PBS to achieve ~1×10^{11} CFU/mL, which was mixed at equal volume to prepare 3-strain cocktail for further inoculation. The population of the inoculum was confirmed by enumeration.

2.2. Cocoa powder inoculation and water activity (a_w) equilibration

Natural unsweetened cocoa powder (Hershey Company) was purchased from a local grocery store. The proximate analysis composition of the purchased cocoa powder is shown in Fig. 1. A portion of cocoa powder was classified into different particle sizes through a set of screens (60, 80, 100, and 120 Mesh) (model 78–700, Fieldmaster, Science First, Yulee, FL, USA) into five size categories (< 125 to > 250 μm) with the majority in the range of 125–177 μm (Fig. 1).

2.3. Cocoa powder inoculation and water activity (a_w) equilibration

Forty grams of cocoa powder was inoculated with 400 μL of a 3-strain *L. monocytogenes* cocktail to achieve 10^{8-9} CFU/g cocoa powder in a 13.5 oz. stomacher bag (Fischer Scientific), then hand-mixed vigorously until homogenized. Background flora of cocoa powder were detected by plating appropriate serial dilutions on TSAYE and then incubating at 35 ± 2 °C for 24 h.

The above inoculated cocoa powder was divided into two 150 mm Petri dishes (Fisher Scientific), placed in a custom-designed a_w-equilibration chamber (Michigan State University) (Smith et al., 2016) set at target a_w (0.30, 0.45, and 0.60) and equilibrated for a minimum of 4 days at 22 °C (room temperature, RT) to target a_w. These a_w values present a typical range for low moisture foods. The a_w of the respective cocoa powder after equilibration was measured in triplicate at RT with an Aquameter (Aqualab Series 3, Decagon Devices, Inc., Pullman, WA). Samples were used for thermal inactivation after reaching the target a_w ± 0.02.

The population of *L. monocytogenes* in inoculated cocoa powder was enumerated right after inoculation and 4 days post-equilibration. One gram of inoculated cocoa powder was mixed with 9.0 ml sterile PBS, homogenized for 2 min at 220 rpm in a stomacher (Seward Stomacher® Circulator 400, Worthing, UK), then 10-fold serially diluted in sterile PBS. The appropriate serial dilutions were plated in duplicate onto TSAYE and incubated at 35 ± 2 °C for 48 h.

2.4. Thermal inactivation of L. monocytogenes in cocoa powder

After 4-day equilibration at the target a_w, inoculated and equilibrated cocoa powder (0.50 ± 0.02 g) was loaded into aluminum thermal death treatment (TDT) cells designed by Washington State University with a cavity capacity of one ml (Chung, Birla, & Tang, 2008). The loaded TDT cells were subjected to isothermal treatment (70, 75, and 80 °C for a_w 0.30 and 0.45; 60, 65 and 70 °C for a_w 0.60) by immersion in a pre-heated ethylene glycol bath (Isotemp Heat Bath Circulator®, Model 5150 H24, Fisher Scientific). The treatment temperatures were selected based on preliminary tests to yield desired levels of thermal inactivation to the target bacteria while not causing visible quality degradation in the cocoa powder samples. The temperature of glycol bath was calibrated by Omega Precision RTD temperature recorder (OM-CP-RTDTemp2000, Omega Engineering Inc., Norwalk, CT). TDT test cells with T-type thermocouples at the geometrical center were used to measure heat penetration and come-up time (CUT), which is the time needed to reach within 0.5 °C of the target temperature. The thermocouple was attached to a digital thermometer and time-temperature history was recorded in triplicate. The resulting CUT was 1.5 min, with timing of heat treatment starting directly afterwards. For cocoa powder at the selected a_w and heat treatment temperature, triplicate samples were collected at 5 sampling points: 0 min (actually 1.5 min, in consideration of CUT), and four others that varied based on a_w and temperature. TDT cells were withdrawn for each sampling point and immediately cooled in an ice-water bath for 2.0 min. All thermal inactivation tests were repeated three times independently.

2.5. Enumeration of L. monocytogenes survivors in cocoa powder

Heat-treated cocoa powder was transferred from each TDT test cell to a Whirl-Pak® bag (Nasco, Ft. Atkinson, WI) and diluted 1:10 with sterile PBS, then homogenized for 2 min at 220 rpm in a stomacher (Seward Stomacher® Circulator 400). The recovered *L. monocytogenes*

Component	Percentage (%)
Carbohydrate	57.36 ± 0.68
Moisture	4.00 ± 0.62
Protein	25.91 ± 0.09
Fat	6.50 ± 0.48
Ash	6.22 ± 0.03

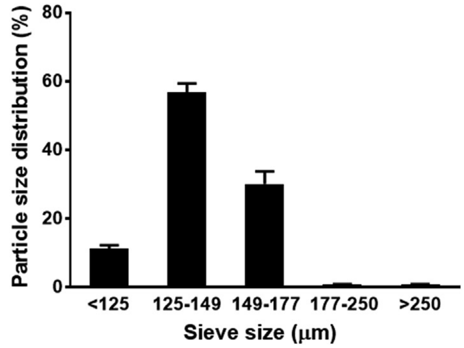

Fig. 1. The proximate analysis composition and particle size distribution of natural unsweetened cocoa powder. Mean ± SEM, n = 3.

suspensions were 10-fold serially diluted. The appropriated dilutions were plated on TSAYE in duplicate, then incubated at 35 ± 2 °C for 48 h.

2.6. Survival of *L. monocytogenes* in cocoa powder during storage

Inoculated cocoa powders were prepared and equilibrated as described above. Post-equilibrated at a_w 0.30 ± 0.03, a typical a_w of cocoa powder under environmental a_w, inoculated cocoa powder was aliquot at 2.0 g per bag (Whirl-Pak®, Nasco, Ft. Atkinson, WI), sealed in a moisture barrier bag (Dri-Shield 3000®, Desco Industries, Inc), then stored at RT (22 °C) for up to 12 months. Survival of *L. monocytogenes* in cocoa powder was analyzed bi-weekly or monthly per the above described method over a one-year period, with three replicates per sampling point. At each sampling, a_w of samples inside each moisture barrier bag was measured with an Aquameter. The storage study was repeated twice independently.

2.7. D-value and z-value estimation

The first-order kinetic model/log-linear model (Equation (1)) was used for analysis and comparison of the thermal inactivation curve (Peleg, 2006).

$$Log\left(\frac{N}{N_0}\right) = -t/D \tag{1}$$

where N_0 is the initial bacteria population, N is the bacteria population at time (t); t is the time of the isothermal treatment (min) after the come-up time to the specified treatment temperature; D is the time in min required to reduce the microbial population by 90% at a selected temperature (°C).

D-value, thermal resistance in log-linear model, was estimated from the thermal inactivation curve using a log-linear regression analysis and is reported in min. The z-values were determined from the regression of log D-value versus temperature and were calculated as z = slope^{-1} for the linear trend lines. Data were analyzed through the Integrated Pathogen Modeling Program (IPMP) (Huang, 2014).

The goodness-of-fit of the models was quantified by the root mean square error (RMSE) obtained from IPMP, accuracy factor (A_f) (Equation (2)) and bias factor (B_f) (Equation (3)) (Baranyi, Pin, & Ross, 1999):

$$A_f = 10^{\frac{\sum_1^n \left| \lg \frac{Log\left(\frac{N}{N0}\right)_{pred}}{Log\left(\frac{N}{N0}\right)_{data}} \right|}{n}} \tag{2}$$

$$B_f = 10^{\frac{\sum_1^n \lg \frac{Log\left(\frac{N}{N0}\right)_{pred}}{Log\left(\frac{N}{N0}\right)_{data}}}{n}} \tag{3}$$

where Log $(N/N_0)_{pred}$ is the predicted log reduction from IPMP, Log $(N/N_0)_{data}$ is the measured reduction of bacteria during treatment, and n is the total number of observations. The smaller the A_f and B_f value, the more effective is the model fitness (Baranyi et al., 1999).

3. Results

3.1. *L. monocytogenes* survival in cocoa powder during long-term storage

L. monocytogenes population at ~8.7 log CFU/g inoculation level remained detectable in cocoa powder of a_w 0.3 over one-year storage at RT (Fig. 2). After an initial 0.4 log reduction of *L. monocytogenes* in

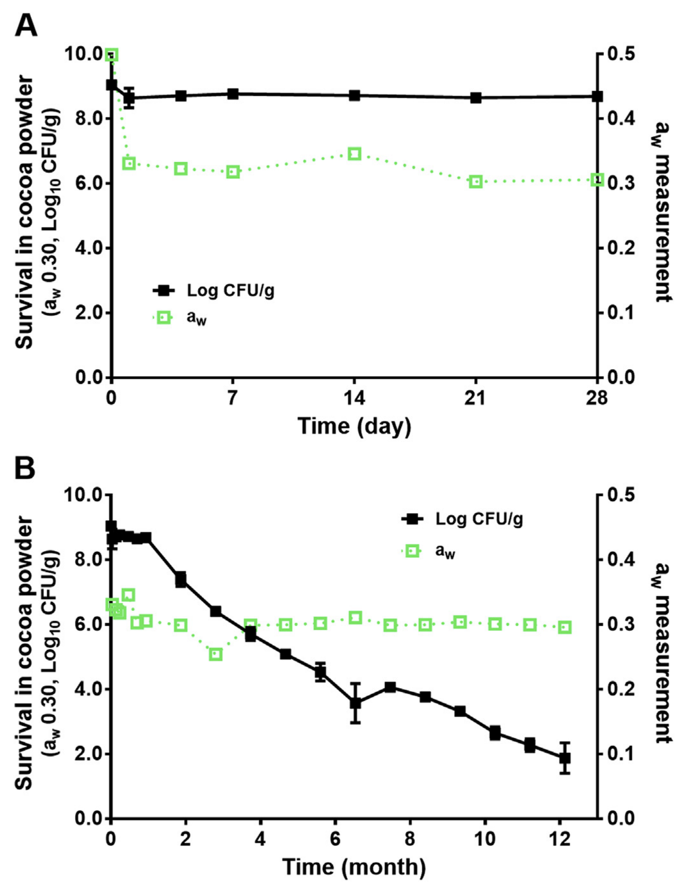

Fig. 2. Survival of *L. monocytogenes* in a_w 0.30 cocoa powder during 12-month storage at 22 °C. A. Enumeration over 1-month storage; B. Enumeration over 12-month storage. Solid line (black) shows the population of *L. monocytogenes*, while dashed line (green) represents a_w of cocoa powder over storage. Mean ± SEM, n = 3. Experiments were repeated independently twice. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Thermal inactivation parameters of *L. monocytogenes* in cocoa powder of different water activity.

a_w	Temp (°C)	D-value (min)	RMSE (Log CFU/g)	z-value (°C)	A_f	B_f
0.30	70	21.9 ± 1.5	0.54	15.5 ± 1.7	2.60	1.00
	75	11.0 ± 0.5	0.30		1.71	1.00
	80	5.0 ± 0.4	0.45		2.12	1.00
0.45	70	7.3 ± 0.4	0.48	15.9 ± 1.3	2.20	1.00
	75	3.4 ± 0.2	0.59		3.09	1.00
	80	1.8 ± 0.2	0.55		2.76	1.00
0.60	65	9.1 ± 0.8	0.56	14.9 ± 0.9	2.91	1.00
	70	4.2 ± 0.4	0.48		2.36	1.00
	75	2.0 ± 0.2	0.65		3.12	1.00

The D- and z-values are the means of three independent trials, expressed as mean ± SEM. RMSE: the root mean square error; A_f: accuracy factor; B_f: bias factor; a_w: water activity of cocoa powder samples measured at 22 °C.

cocoa powder 24 h post-inoculation, *L. monocytogenes* population remained stable during the first 4 weeks of storage (Fig. 2A), then decreased gradually, with ~7.0 log CFU/g reduction over 12 months of storage at RT (Fig. 2B).

3.2. Fitness of thermal kinetics model

The thermal inactivation data of *L. monocytogenes* in cocoa powder of different a_w were analyzed by log-linear, and the fitness of model was selected according to the root mean square error (RMSE), A_f, and B_f. Ideally, A_f and B_f would be 1 in the predictive model, but every variable would increase the value of A_f and B_f; normally, smaller A_f values and less deviation of B_f from 1 indicate a better fit of the model (Baranyi et al., 1999). RMSEs and A_f values from log-linear ranged from 0.30 to 0.65, and 1.71 to 3.12, respectively, while all B_f values were 1.0 (Table 1), indicating log-linear model has a good fitness to our data. Therefore, the log-linear model was used to report thermal inactivation parameters of *L. monocytogenes* in cocoa powder of different a_w (Table 1).

3.3. Thermal inactivation of *L. monocytogenes* in cocoa powder

At each inactivation temperature, the thermal inactivation curves show a log-linear trend for cocoa powder, meaning the inactivation rates were constant (R^2 > 0.9, Fig. 3). Representative thermal inactivation curves of *L. monocytogenes* in cocoa powder at a_w 0.30 (0.31 ± 0.02), 0.45 (0.45 ± 0.01) and 0.60 (0.60 ± 0.01) are shown in Fig. 3.

The D-values of *L. monocytogenes* in cocoa powder samples preconditioned to a_w 0.30 at room temperature were 21.9 ± 1.5 min for 70 °C, 11.0 ± 0.5 min for 75 °C and 5.0 ± 0.4 min for 80 °C. The D values were reduced to 7.3 ± 0.4 min for 70 °C, 3.4 ± 0.2 min for 75 °C, and 1.8 ± 0.2 min for 80 °C when the inoculated samples were conditioned to a_w 0.45 before thermal treatments (Table 1). The D-values in a_w 0.60 samples were 9.1 ± 0.8, 4.2 ± 0.4, and 2.0 ± 0.2 min for 65, 70, and 75 °C treatments, respectively (Table 1). The D_{70}-and D_{75}-values of *L. monocytogenes* in a_w 0.30, 0.45, and 0.60 samples decreased with increased a_w, with the highest D_{70}-and D_{75}-values at a_w 0.30 (Table 1). The D-values of *L. monocytogenes* at 80 °C were higher in a_w 0.30 than those at a_w 0.45. The z-values at a_w 0.30, 0.45 and 0.60 were 15.5 ± 1.7, 15.9 ± 1.3, and 14.9 ± 0.9 °C, respectively (Table 1 and Fig. 4).

4. Discussion

Cocoa powder is a prominent ingredient in many foods, such as

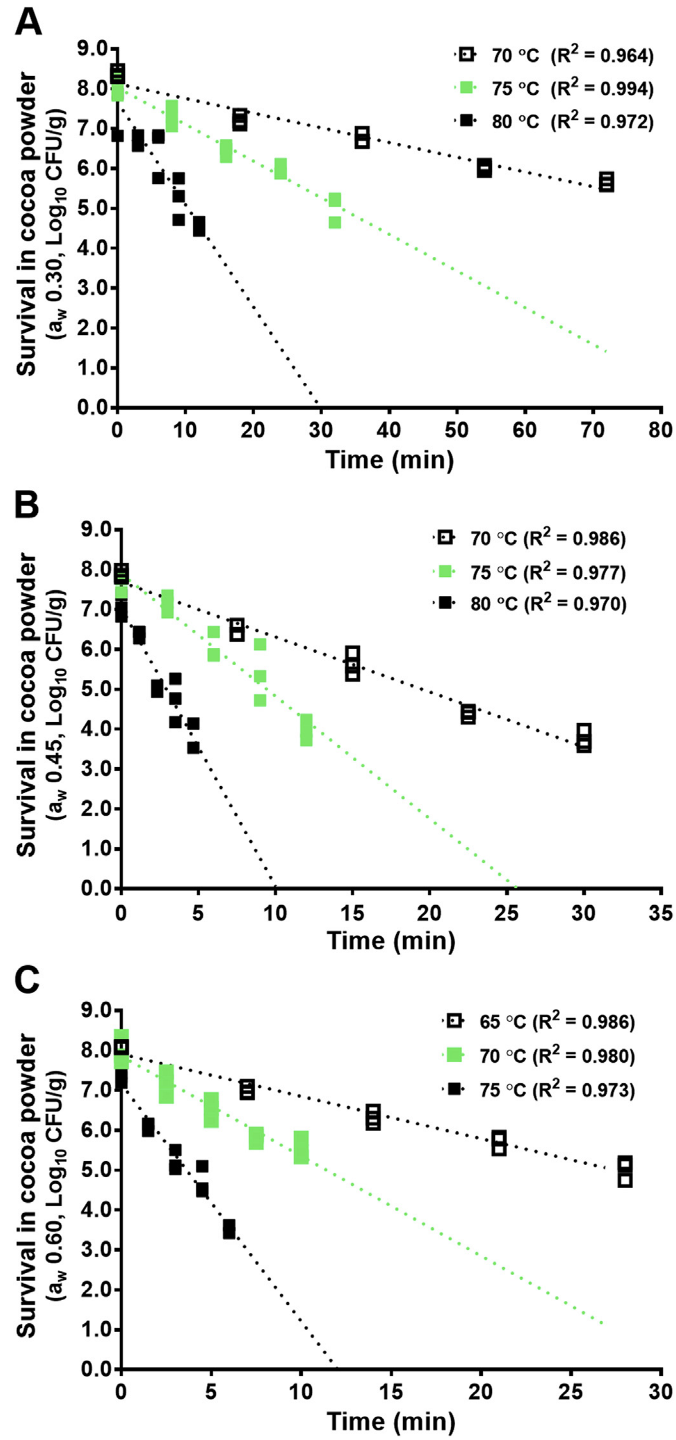

Fig. 3. Representative thermal inactivation curves of *L. monocytogenes* in cocoa powder equilibrated to the target a_w at different temperatures. A. a_w 0.30; B. a_w 0.45; C. a_w 0.60. For each heat treatment of the selected a_w, triplicate samples were collected at 5 sampling points: 0 min (actually 1.5 min, in consideration of CUT), and four others that varied based on a_w and temperature. Experiments were independently repeated thrice.

candy bars, cake frosting, cake mixes, and various drinks. *L. monocytogenes* has been reported to survive for more than a year in La_wF (Kimber et al., 2012). It has also shown similar or higher heat resistance in some matrices, such as solid egg mixes (Li et al., 2005), culinary seasoning and pet food (Rachon et al., 2016) and marinated chicken breasts (Karyotis, Skandamis, & Juneja, 2017), compared to *Salmonella*, which is normally considered the most heat resistant foodborne

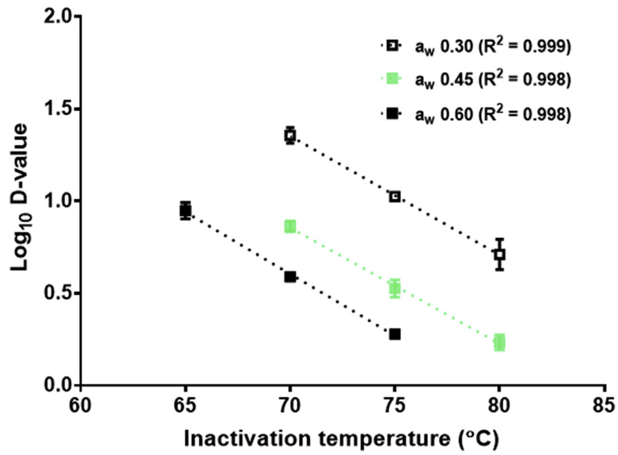

Fig. 4. Log D (decimal reduction time to achieve 90% population reduction at the selected temperature) values of *L. monocytogenes* in a_w 0.30, 0.45 and 0.60 cocoa powder at different temperature. The thermal inactivation tests were conducted three times independently.

pathogen in La$_w$F. In contrast to increasing studies assessing *Salmonella* thermal resistance in relation to La$_w$F, sparse information is available regarding *L. monocytogenes* thermal resistance in La$_w$F. In this study, we evaluated the fate of *L. monocytogenes* in cocoa powder during storage as well as thermal treatment, a most common food processing method. The influence of a_w on the thermal resistance of *L. monocytogenes* in cocoa powder was further examined. Data from the current study indicated that 30 min of thermal treatment at 80 °C should provide more than 6-log reduction of *L. monocytogenes* in cocoa powder with room temperature a_w of 0.30, a typical a_w of cocoa powder stored at ambient environment.

4.1. *L. monocytogenes survived in cocoa powder for 12 months*

In cocoa powder (a_w 0.30), *L. monocytogenes* remained stable during the first month of RT storage. Consistent with our finding, in confectionery, culinary, chicken meat powder, and pet food, *L. monocytogenes* populations were not significantly reduced during 3-week storage at RT (Rachon et al., 2016). *L. monocytogenes* population in a_w 0.30 cocoa powder decreased gradually during prolonged storage at RT, and 7-month RT storage resulted in ∼4 log reduction, which is higher than reduction observed in wheat flour with the same a_w and inoculation level, where 7-month RT storage only led to 2-log reduction (Taylor et al., 2018). The observed higher reduction rate of *L. monocytogenes* in cocoa powder might be due to different food matrices or antimicrobial compounds in cocoa powder. Cocoa suspension at 5.0% has antimicrobial effects against *S.* Typhimurium (Busta & Speck, 1968), and cocoa powder extract has antimicrobial effects against *S.* Abony, generic *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis* (Todorovic, Milenkovic, Vidovic, Todorovic, & Sobajic, 2017). In our study, *L. monocytogenes* remained detectable in cocoa powder over 12-month RT storage with a total of ∼7 log reduction. Concordantly, *L. monocytogenes* inoculated to almonds kernels or in-shell pistachios were detectable after 12-month storage at 24 °C, but with no appreciable decrease in population in either almonds or pistachios over a 12-month refrigerated storage (Kimber et al., 2012). These data highlight safety concerns associated with desiccation resistance of *L. monocytogenes* in La$_w$F.

4.2. *Thermal resistance of L. monocytogenes in cocoa powder*

L. monocytogenes had a D_{80}-value about 2–5 min in cocoa powder at a_w 0.30–0.45, which is similar to published D_{80}-values in other food matrices (Rachon et al., 2016). The mean D_{80}-values of *L. monocytogenes* in confectionery (a_w 0.57), culinary seasoning (a_w 0.66), chicken meat powder (a_w 0.38) and pet food (a_w 0.65) were 0.9, 1.8, 0.6, and 2.0 min, respectively (Rachon et al., 2016). Given that different food matrices with variable a_w were used, D-values published in the aforementioned study (Rachon et al., 2016) were not directly comparable to D-values of *L. monocytogenes* in cocoa powder obtained in this study. Using cocoa powder as a matrix and with controlled a_w, this study showed an inverse relationship between D-values at respective treatment temperature and a_w measured at room temperature. This negative relationship between a_w and D-values at the selected temperature was also observed in *L. monocytogenes* in wheat flour (Taylor et al., 2018). D_{80}-values of *L. monocytogenes* in wheat flour of a_w 0.30 and 0.45 were 3.1 and 7.1 min, respectively (Taylor et al., 2018), indicating that *L. monocytogenes* had a higher thermal resistance in wheat flour than that in cocoa powder. The difference could result from antimicrobial components in cocoa powder (Busta & Speck, 1968; Todorovic et al., 2017). Fat content is known to affect heat resistance of foodborne pathogens in foods; in general, higher fat content enhances microbial heat resistance. Heat resistance of *S.* Typhimurium (Juneja & Eblen, 2000) and *L. monocytogenes* (Fain et al., 1991) in beef increased with higher fat levels. However, cocoa powder has a higher fat content compared to wheat flour (3.3%) (Liu, Rojas, et al., 2018), indicating that factors other than fat contribute to a higher heat resistance in wheat flour. In addition, the a_w of a food system is subjected to changes during thermal processing, and the degree of change varies among food matrices (Tadapaneni, Yang, Carter, & Tang, 2017). Thus, a_w at treatment temperature might also contribute to the observed different thermal resistance, which warrants further studies.

4.3. *Influence of a_w on thermal resistance of L. monocytogenes*

In general, lower a_w is associated with the enhanced thermal resistance of microorganisms in La$_w$F (Archer et al., 1998; He et al., 2013; Lang et al., 2017; Liu, Tang, et al., 2018b; Smith et al., 2016; Villa-Rojas et al., 2013). At a given temperature, the increased bacterial thermal resistance or D-values of *Salmonella* at reduced a_w was reported in different food matrices, including almond kernels (Villa-Rojas et al., 2013), peanut butter (He et al., 2013), wheat flour (Smith et al., 2016), milk powder (Lang et al., 2017), silicon dioxide granules (Liu, Tang, et al., 2018b), and cocoa powder (Tsai et al., 2019). In this study, both D_{70}-and D_{75}-in cocoa powder decreased when a_w increased from 0.30 to 0.45 and 0.60, which indicates thermal resistance of *L. monocytogenes* in cocoa powder increases with reduced a_w. Concordantly, D_{70}-, D_{75}-and D_{80}-values of *L. monocytogenes* in wheat flour increased when a_w reduced from 0.60 to 0.30 (Taylor et al., 2018). Similarly, the D_{60}-values of *L. monocytogenes* in sucrose solution increased from 2.0 to 8.4 min when the a_w decreased from 0.98 to 0.90 (Sumner, Sandros, Harmon, Scott, & Bernard, 1991). Nonetheless, compared to *Salmonella*, thermal resistance of *L. monocytogenes* in cocoa powder was lower (Tsai et al., 2019). At the a_w 0.30, D_{70}-, D_{75}-and D_{80}-of *Salmonella* was about 46.2, 20.5 and 11.5 min, respectively (Tsai et al., 2019), which are about twice of those for *L. monocytogenes* at the corresponding inactivation temperature observed in this study.

The z-values of *L. monocytogenes* in cocoa powder at different a_w were similar, ranged from 14.9 to 15.9 °C. Similar phenomena were also found in *L. monocytogenes* (Taylor et al., 2018) and *Salmonella* in wheat flour (Smith et al., 2016) and cocoa powder (Tsai et al., 2019). There is very little information on *L. monocytogenes* behaviors and corresponding z-values in La$_w$F. The z-values of *L. monocytogenes* obtained from various foods and media ranged from 3.9 to 29.3 °C (Doyle, Mazzotta, Wang, Wiseman, & Scott, 2001; Monu, Valladares, D'Souza, & Davidson, 2015; van Asselt & Zwietering, 2006), with the lowest in tryptose phosphate broth (Golden, Beuchat, & Brackett, 1988) and the highest in cured sausage (Roering, Wierzba, Ihnot, & Luchansky, 1998). The z-values of *L. monocytogenes* in cocoa powder are within the reported z-value range obtained in various foods.

5. Conclusion

L. monocytogenes was able to survive in cocoa powder for a prolonged period and remained detectable over 12-month RT storage. Thermal resistance of *L. monocytogenes* in cocoa powder is affected by a_w and is conversely related to a_w of samples. These data broaden the horizon for research into the behavior of *L. monocytogenes* in La$_w$F and provide technical information and reference points for food processors to validate thermal processing and to develop other thermal intervention strategies for the control of *L. monocytogenes* in cocoa powder and other La$_w$F matrices.

Conflicts of interest

The authors have no known conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2019.03.006.

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