Fate of *Listeria monocytogenes* in Cocoa Powder during Isothermal Inactivation

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Introduction

Low moisture foods and food ingredients have been increasingly implicated in foodborne outbreaks, as reflected in the infamous nationwide *Salmonella* outbreaks related to peanut butter and products and numerous other outbreaks involving almonds, chocolate, wheat flour and others [1]. *L. monocytogenes* is an important pathogen, causing serious foodborne disease in susceptible individuals. It is ubiquitous and persistent in a variety of food processing environments, can remain viable on low moisture foods [2] and dry environment for extended periods especially if organic material (soil) is present [3], has its ability to grow at refrigerated temperature, low infectious dose and high motility. It causes about 1600 illnesses annually in the United States with a mortality rate of ~ 16%. It was historically associated with ready-to-eat food outbreaks. Recent multistate outbreaks in cantaloupes (33 death, 1 stillbirth and 147 sick) and caramel apples (7 deaths and 35 cases) indicate that *L. monocytogenes* is a new emerging foodborne pathogen in fresh produces, highlighting the food safety risks of *Listeria* in different foods and commodity groups including low moisture foods. This was further highlighted by a recent Classic Hummus *Listeria* recall. Despite an increasing number of studies addressing *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 to the factors impacting their survival in low moisture food. Cocoa powder is an essential ingredient and widely incorporated in different disserts and drink and thus a possible source of *L. monocytogenes* contamination. Therefore, the objective of this study was to evaluate the thermal resistance of *L. monocytogenes* in cocoa powder and further investigate the impact of water activity (a_w) on its survival in cocoa powder.

Materials and Methods

Cocoa powder inoculation

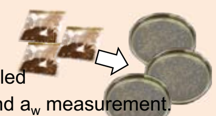
Inoculum were mixed with cocoa powder

Pre-equilibrated to target a_w

- Equilibrated in a chamber at target a_w for ~4 days
- a_w was measured by AquaLab series 3 (± 0.025 a_w)

Storage study

- Inoculated samples were aliquoted and sealed in moisture barrier bag and store at room temperature.
- At selected of time points, cocoa powders were sampled for survival enumeration and a_w measurement.



Heat intervention

- Samples were loaded to TDT test cells then subjected to thermal inactivation at a selected T.
- At each time point, remove 3 test cells and chilled in ice water immediately for survival enumeration.

Results

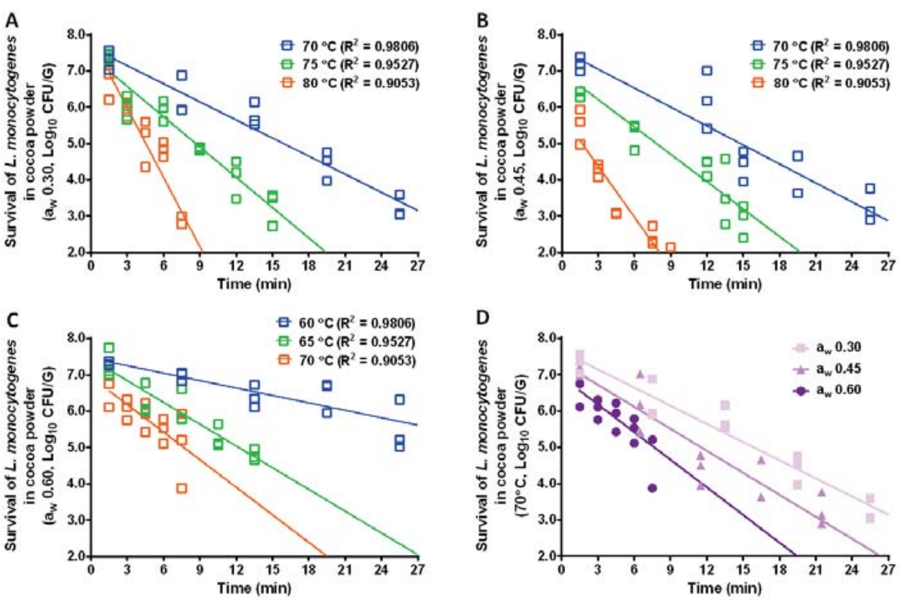


Figure 1. The representative death curve of *L. monocytogenes* at different temperatures in cocoa powder at 0.3 a_w (A), 0.45 a_w (B), 0.6 a_w (C). D. The comparison of 70 ˚C death curve at different a_w. Experiments were repeated independently three times with biological replication of 3.

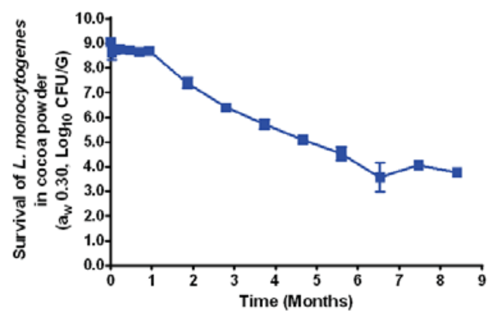


Figure 2. The survival of *L. monocytogenes* in cocoa powder under RT storage at 0.3 a_w. Mean+/-SEM, n=3.

Table 1. The heat inactivation kinetic data of *L. monocytogenes* inoculated cocoa powder at different a_w

		Linear Model				Weibull Model		
a_w	Temp (°C)	D-value (min)	95% PI upper limit for D-value (min)	RMSE (Log CFU/G)	z-value (°C)	k	α	RMSE (Log CFU/G)
0.30	70	6.95 ± 0.56	8.09	0.61	16.13 ± 2.54	0.07 ± 0.06	1.22 ± 0.26	0.62
	75	3.50 ± 0.18	3.85	0.46		0.60 ± 0.32	0.76 ± 0.16	0.45
	80	1.44 ± 0.12	1.67	0.92		0.05 ± 0.04	2.24 ± 0.38	0.86
0.45	70	4.82 ± 0.43	5.71	0.68	17.16 ± 1.83	1.29 ±1.13	0.49 ± 0.22	0.63
	75	2.64 ± 0.27	3.19	1.01		2.49 ± 3.20	0.41 ± 0.32	0.99
	80	1.00 ± 0.13	1.26	1.14		0.07 ± 0.10	2.45 ± 0.78	1.12
0.60	60	13.50 ± 2.63	18.81	0.82	16.01 ± 2.31	0.19 ± 0.37	0.73 ± 0.53	0.83
	65	5.50 ± 0.63	6.78	0.60		0.21 ± 0.26	0.95 ± 0.41	0.60
	70	3.36 ± 0.48	4.34	0.48		2.14± 5.32	0.34 ± 0.59	0.48

[a] k and α are the constant used in equation $Log(N) = Log(N_0) – kt^{α}$; [b] RMSE, root mean square error.
[c] Upper limit of the 95% prediction interval for the D-value was calculated from the regression line equation + 2RMSE

Conclusion

- *L. monocytogenes* is stable in cocoa powder;
- Demonstrated much higher thermal resistance in cocoa powder compared to the high moisture foods, which is impacted by a_w.
- Thermal resistance increased with the decreasing a_w.
- Data provide valuable information for industry to validate thermal processing for control of *L. monocytogenes* in low moisture foods.

Reference

[1] Beuchat et al., 2013. *J Food Prot*, **76:**150
[2] Kimber et al.,2012. *J Food Prot*, **75:**1394
[3] Vogel et al., 2010. *Int J Food Microbiol*, **140:**192.

Acknowledgements

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