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Methods to obtain thermal inactivation data for pathogen control in low-moisture foods

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ABSTRACT

Background: Outbreaks of foodborne pathogens (including *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes*, etc.) in low-moisture foods (LMFs), such as butter, cereal, cheese, dried vegetable, nut, and spice, are emerging concerns of food safety in recent decades. The food industry is in urgent need for reliable thermal inactivation data on these pathogens in a wide range of LMFs in order to develop effective pasteurization processes. Over the past ten years, research laboratories in academia have developed several effective test methods to obtain thermal inactivation data for LMFs. The data are generally fitted to thermal death models to obtain thermal processing parameters, which are then used in the design and validation of new thermal processes. An example of novel thermal processing technologies is the rapid and volumetric radio frequency (RF) heating, which has shown unique advantages over conventional heating in pasteurization of LMFs.

Scope and approach: Temperature and water activity are the key factors in determining thermal resistance of pathogens in LMFs. This review summarizes the recently published work on testing methods for thermal inactivation of bacteria, and discusses their unique features in controlling the key parameters, including temperature, and water activity (or moisture content) of the samples. This paper provides a systematic summary of the thermal inactivation models for pathogens in LMFs. It also illustrates the use of the thermal inactivation data and the predictive models to develop and validate pathogen control in LMFs using RF energy.

Key findings and conclusions: The closed and controlled system test methods are proven to be appropriate and effective approaches for obtaining thermal inactivation data of pathogens in LMFs. Key factors can be either constant or adjustable by using of the above experimental methods. Those data are used in developing and validating RF treatments for control of *Salmonella* in several LMFs.

1. Introduction

Low-moisture foods (LMFs, acronyms and symbols in the text can be found in Table 1) are defined as foods with water activity (a_w) no more than 0.85 by the Codex Committee on Food Hygiene (CCFH) and US Food and Drug Administration (FDA) (CCFH, 2015; FDA, 2015; Xu, Song, Tan, Villa-Rojas, & Tang, 2020). Many pathogen outbreaks and food recalls in recent years associated with LMFs have attracted widespread concerns from food companies, regulators, and consumers (Barba, Koubaa, Prado-Silva, Orlien, & Santana, 2017; Chitrakar, Zhang, & Adhikari, 2019). Table 2 summarizes the outbreaks caused recalls by foodborne pathogens associated with LMFs since 2015 reported by Centers for Disease Control and Prevention (CDC) and FDA. As shown in Table 2, main bacterial foodborne pathogens (including *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes*, etc.) in LMFs (including butter, cereal, cheese, dried vegetable, nut, and spice, etc.) result in many potential food safety outbreaks/accidents in USA, Canada, and other countries.

Thermal inactivation kinetic models of foodborne pathogens/surrogates are useful tools for developing and validating new thermal pasteurization processes (Peleg, 2006). But reliable thermal inactivation data are necessary in developing those models (Kou et al., 2016). Research indicated that the thermal inactivation data of pathogens in LMFs are dependent on multiple factors, including

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Table 1
Acronyms and Symbols for index.

Index		
Acronyms	CA-HBS	Controlled atmosphere heating block system
	CCFH	Codex Committee on Food Hygiene
	CDC	Centers for Disease Control and Prevention
	CUT	Come up time
	FDA	US Food and Drug Administration
	FDE	Freeze-dried *E. faecium* NRRL B-2354
	HBS	Heating block system
	HTST	High-temperature short time
	LiCl	Lithium Chloride
	LMFs	Low-moisture foods
	LTLT	Low-temperature long time
	MC	Moisture content
	PID	Proportional-integral-derivative
	RF	Radio frequency
	RH	Relative humidity
	RMSE	Root mean square error
	TAC	Thermal-a_w cell
	TDT	Thermal-death-test
	WSU	Washington State University
Symbols	A_1	Initial populations (CFU/g) of foodborne pathogens in the first phase
	A_2	Initial populations (CFU/g) of foodborne pathogens in the second phase
	A_f	Accuracy factor parameter
	$AICc$	Corrected Akaike information criterion
	a_w	Water activity
	B_f	Bias factor
	D_T	Thermal decimal time (min) at a given temperature T (°C)
	D_{ref}	Decimal time (min) at T_{ref} (°C)
	F	Cumulative lethal time (min)
	k	Model parameter
	m_s	Molality of salt (mol/kg H_2O)
	M_w	Molar mass of water (kg/mol)
	N_0	Initial population (CFU/g)
	N_t	Surviving population (CFU/g) at treatment time t (min)
	p	Statistical significance (At $p = 0.05$ level)
	R^2	Coefficient of determination
	t	Treatment time (min)
	T	Given temperature (°C)
	Tc	Inactivation rate accelerating temperature (°C)
	T_{ref}	Reference temperature (°C)
	ν	Number of ions formed when 1 mol of salt is dissolved in water
	z	Temperature (°C) increase required to result in 1-log reduction of D (min)
	Z_{aw}	$a_{w,ref}$ increments to achieve 1-log reduction of D_{ref}
	Z_T	Temperature (°C) increment to achieve 1-log reduction of D_T
	$\alpha\ (T)$	Scale parameters of Weibull model
	$\beta\ (T)$	Shape parameters of Weibull model
	$1/\delta$	Nonlinear rate
	λ_1	Temperature-dependent survival rate parameters for the first phases
	λ_2	Temperature-dependent survival rate parameters for the second phases
	μ_0	Initial mean temperatures (°C)
	μ_0	Final mean temperatures (°C)
	σ_0	Initial standard deviations of temperatures (°C)
	σ	Final standard deviations of temperatures (°C)
	Φ	Osmotic coefficients of LiCl

microorganism-related factors (strain, population of bacteria, and growth phase/condition, etc.), process-related factors (treatment conditions, such as heating temperature, heating rate, holding time, etc., and environmental conditions, such as relative humidity, and atmosphere condition, etc.), and product-related factors (composition, physical properties, moisture content (*MC*) of product, and pH, etc.) (Li, Kou, Zhang, & Wang, 2018; Syamaladevi, Tang, et al., 2016). Several experimental methods have recently been developed to study how some of these possible factors influence thermal resistance of foodborne pathogens/surrogates in LMFs.

Appling thermal inactivation data in microbial validation processing is a key step to bridge the gap between experimental methods and development of pathogen control strategies. Fig. 1 illustrates the general connections between thermal inactivation testing, model developments, and application of the model in developing and validating a new thermal treatment for pathogen control. As shown in Fig. 1, a prerequisite for an effective design of pathogen control treatments is to obtain the thermal inactivation data of target pathogens in LMFs using appropriate test methods. The systematic approach outlined in Fig. 1 is followed by several recent studies in developing and validating novel radio frequency (RF) treatments for pasteurization of LMFs (Liu, Ozturk, et al., 2018; Xu et al., 2018). RF heating is particularly suited for bulk LMFs when compared to traditional conduction and convection heating methods (Cheng et al., 2020; Jiao, Tang, Wang, & Koral, 2018).

The review seeks to provide the most recent studies on test methods and their applications on novel pathogen control technology using RF treatments. The objectives of this review were to 1) summarize and discuss about the testing methods for thermal inactivation kinetics of foodborne pathogens/surrogates in LMFs, 2) review mathematic models to evaluate the influence of important parameters on thermal inactivation of pathogens, 3) present case studies that used the thermal inactivation data and predictive models in developing and validating thermal treatment protocols based on RF energy for control of pathogens in LMFs, and 4) provide insights in future research directions to improve the performances of heating devices and methods for control of foodborne pathogens/surrogates.

2. Methods to obtain thermal inactivation data of pathogens in LMFs

2.1. Overview of experimental methods for LMFs

Several test methods have been developed to investigate the effect of key factors, such as temperature, time, *MC*, a_w, heating rate, sample composition, and surrounding atmosphere, on thermal inactivation kinetics of foodborne pathogens/surrogates in LMFs. These test methods can be divided into three general categories: closed, open, and controlled system methods. Traditional open system methods (such as open tubes, jars, or bottles) have many limitations used in LMFs, because the key factors a_w/*MC* of the inoculated samples cannot be maintained constant during heat treatments. Thus, Table 3 only summarizes the closed and controlled experimental methods, along with relevant heating devices and thermal inactivation data of foodborne pathogens/surrogates.

2.2. Closed system test methods

In closed systems, the *MCs* of food samples are maintained constantly during heat treatments. Therefore, sealed thermal-death-test (TDT) devices, such as sealed aluminum cells, glass jars, metal plates, plastic stomacher bags, polyethylene pouches, sterile glass tubes, etc., are commonly used to provide constant *MC* environment during isothermal inactivation experiments on a wide range of LMF, including almond butter (Wright, Minarsich, Daeschel, & Waite-Cusic., 2018), chia seed powder (Trimble, Frank, & Schaffner, 2020), milk powder (Li et al., 2014), and wheat flour (Daryaei et al., 2020).

Low-moisture foods, in particular the foods with porous structures, are poor media for heat transfer. Therefore, reducing the come up time (CUT) is an important consideration in the design or selection of appropriate testing methods. To overcome the long CUT, non-isothermal conditions in large tubes, and difficult unloading of food samples in narrow capillary tubes, a well-sealed aluminum TDT cell I (18 mm sample diameter and 4 mm height, Fig. 2a) was designed and evaluated by Chung, Birla, and Tang (2008). The TDT cell I has been widely used as a fundamental test apparatus to determine thermal resistances of pathogens (such as *Salmonella* spp., *Shiga* toxin-producing *Escherichia coli*,

and *Listeria monocytogenes*, etc.) in LMFs, as reported in Ozturk, Kong, and Singh (2020), Villa-Rojas, Zhu, Paul, et al. (2017), Xu et al. (2019), Taylor, Tsai, Rasco, Tang, and Zhu (2018) and Tsai, Ballom, et al. (2019).

An improved aluminum TDT cell II (31 mm sample diameter and 1 mm height, Fig. 2b) was further designed at Washington State University (WSU) to reduce the CUT by increasing the contact surface area and reducing in the height for better heat transfer (Jin & Tang, 2019a). The TDT cell II achieved accurate assessment of microbial inactivation by reducing CUT from 2.33 min (TDT cell I) to 0.67 min at target temperature of 85 °C in brown rice (Jin & Tang, 2019a). Because of the much shortened CUT, TDT cell II is suited for studying thermal resistance of bacteria at higher temperatures than TDT cell I or in food matrices containing antimicrobial compounds that sharply weakened the thermal resistance of bacteria. For example, Jin, Tang, and Zhu (2020) determined thermal resistsnce of *Salmonella* in soy protein powder at up to 99 °C, and Xie et al. (2021) used TDT cell II to study thermal resistance of *Samonella* in cinnamon powders.

In another method, two thin-layer metal plates were used by Keller et al. (2012) to reduce the CUT. The device provides a closed environment to explore the thermal resistance of *Salmonella* Tennessee and Oranienburg in peanut butter. Another good method is to seal inoculated dry samples in pouches and sandwich the samples between two copper plates with additional magnetic on both sides while heating in water bath. The aim of two sandwich magnetic plates is to hold the sample at uniform thickness and reduce the CUT (Enache et al., 2015).

When using the above methods, precaution is needed in preventing contamination of the inoculated samples due to leakage of the heating fluid oil/water bath. It is also important to recognize that water activity of food, a_w, often changes with temperature at fixed *MC* in closed systems (Liu, Rojas, Gray, Zhu, & Tang, 2018; Xu et al., 2019; Yang, Guan, Sicheng, Sablani, & Tang, 2020). It is, therefore, often necessary to measure changes of a_w of the selected food systems along with thermal inactivation tests (Ashammari et al., 2020; Jin et al., 2020; Yang et al., 2020b, 2021).

2.3. Controlled system test methods

2.3.1. Controlled relative humidity (RH) methods

The a_w of LMFs at elevated temperatures is reported as a critical factor influencing thermal resistance of foodborne pathogens/surrogates (Jin, Tang, & Sablani, 2019b; Syamaladevi, Tadapaneni, et al., 2016). However, the a_w of samples in TDT cells typically changes with temperature and cannot be maintained constant controlled with temperature increase during thermal kinetic tests. To study the direct effect of a_w on inactivation parameters of foodborne bacteria, a novel thermal-a_w cell (TAC I, 20 mm sample diameter, and 3 mm height, see Fig. 2c) is designed at WSU by Tadapaneni, Syamaladevi, Villa-Rojas, and Tang (2017). The advantage of TAC I is its ability to maintain a relatively stable a_w of the inoculated low moisture samples when heated from room temperature to high treatment temperatures. Specifcally, the relative humidity (*RH*) in TAC I headspace over the samples is controlled by a Lithium Chloride (*LiCl*) solution. The a_w of the solution can be calculated as the following equation (Gibbard & Scatchard, 1973):

$$a_w \approx \frac{\mathrm{RH}}{100} = \exp\left(-\varphi M_w v m_s\right) \quad (1)$$

where Φ is osmotic coefficients of *LiCl*, M_w (kg/mol) is the molar mass of water, v is the number of ions formed when 1 mol of salt is dissolved in water, and m_s (mol/kg H_2O) is the molality of salt. The a_w of a *LiCl* solution is relatively stable over a wide temperature range (Tadapaneni et al., 2017). TAC I has been used to study the effect of a_w on thermal resistance of *S.* Enteritidis PT 30 in wheat flour (Tadapaneni et al., 2017) and that of S. Enteritidis PT 30 and *Enterococcus faecium* on silicon dioxide gradules (Liu, Tang, Tadapaneni, Yang, & Zhu, 2018).

TAC II (50 mm sample diameter, 0.5 mm height, see Fig. 2d) was further improved by Tadapaneni, Xu, Yang, and Tang (2018). The TAC II reduces a_w equilibration time between LMFs and *LiCl* solutions from >14 min to <4 min by reducing sample height and increasing in sample diameter. The D_{80}-value of *S.* Enteritidis PT 30 in wheat flour (*RH* 50%) obtained in TAC II was 20.7 min, and had significant ($p < 0.05$) difference when compared to the results of 7.5 min observed by TAC I in Tadapaneni et al. (2017). TAC II has larger exposure area of *LiCl* solution and sample than that of TAC I, therefore, the TAC II achieves shorter equilibration time than TAC I. The results in TAC II would better reflect the true thermal resistance of *S.* Enteritidis PT 30 in wheat flour (Tadapaneni et al., 2018). Xu et al. (2019) used the TAC II to determine thermal resistance of *S.* Enteritidis PT 30 at four given a_w levels (0.25, 0.45, 0.65, and 0.85, at room temperature) and reported exponential reductions of $D_{80\ ^\circ C}$ values of *S.* Enteritidis PT 30 with increasing a_w of food samples. Yang, Xie, Lombardo, and Tang (2021) used TAC II to study the mechanisms for oil protection of bacteria in thin layer peanut oil.

2.3.2. Controlled heating block system (HBS) methods

The heating rates during the ramping period possibly affect thermal resistance of foodborne pathogens/surrogates (Chung, Wang, & Tang, 2007; Zhang, Kou, Zhang, Cheng, & Wang, 2018). However, heating rates of samples are difficult to control in water or oil baths. In addition, oil/water bath could cause liquid spillage/infiltration into samples in

Table 2
Foodborne pathogen outbreaks and recalls associated with LMFs since 2015.

Food category	Product	Pathogen	Country	No. affected	Sources
Butter	Cashew butter	Potential presence of *L. monocytogenes*	USA	0	FDA (2018a)
	Nut butter	Potential presence of *L. monocytogenes*	USA	0	FDA (2019a)
	Nut butter spreads	*S.* Paratyphi	USA	13	CDC (2015)
	Soynut butter	*E. coli* O157:H7	USA	32	CDC (2017)
	Sunflower butter	Potential presence of *L. monocytogenes*	USA	0	FDA (2018b)
Cereal	Flour	*E. coli* O26	USA	21	FDA (2019b)
	Flour	*E. coli* O121	USA	63	FDA (2017a)
	Flour	*E. coli* O121	Canada	30	PHAC (2017)
	Honey smacks cereal	*S.* Mbandaka	USA	135	CDC (2018a)
	Tahini	*S.* Concord	USA	6	CDC (2019)
Cheese	Soft cheese	*L. monocytogenes*	USA	8	FDA (2017b)
Dried vegetable	Dried-vegetable spice mixes	*S.* Enteritidis phage type 13a	Sweden	174	Jernberg et al., (2015)
	Potato chips	Potential presence of *Salmonella*	USA	0	FDA (2018c)
Nut	Dried coconut	*S.* Typhimurium	USA	14	CDC (2018b)
	Frozen shredded coconut	*S.* Newport	USA	27	FDA (2017c)
	Pistachios	*S.* Montevideo and S. Senftenberg	USA	11	CDC (2016)
Spice	Chili kits	Potential presence of *Salmonella*	USA	0	FDA (2018d)
	Fennel seed whole	Potential presence of *Salmonella*	USA	0	FDA (2019c)
	Ginger powder	Potential presence of *Salmonella*	USA	0	FDA (2017d)

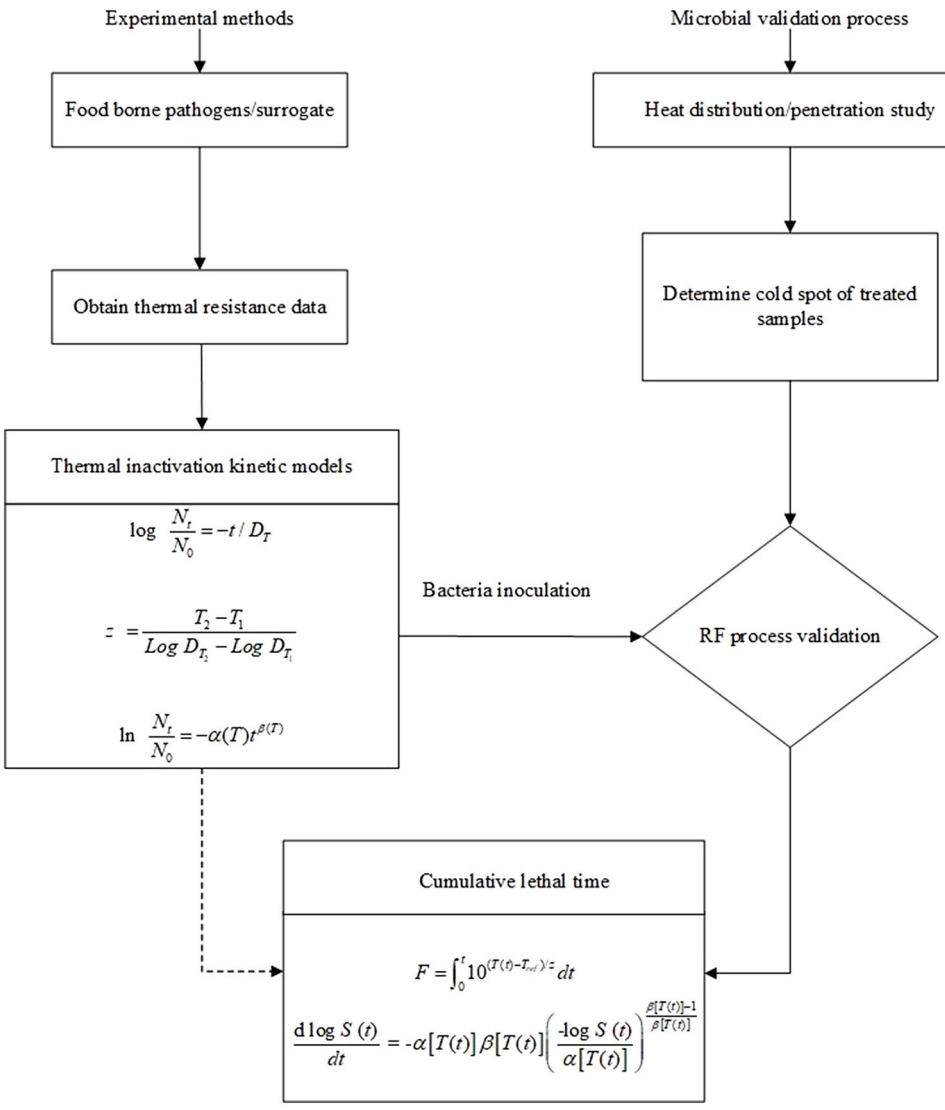


Fig. 1. Logical flow chart of this review paper.

pooled sealed containers. To overcome these disadvantages, dry heat sources are applied with heating pads/plates in the controlled heating block systems (Kou et al., 2016; Lau & Subbiah, 2020).

A TDT heating block system (TDT-HBS, see Fig. 3) was designed by Kou et al. (2016). The system is heated by eight heating pads (250 W each) in which the heating rates (0.1–13.3 °C/min) are regulated by two proportional-integral-derivative (PID) controllers. The performance of the TDT-HBS is evaluated on diverse solid and semi-solid food materials, such as egg white powder, peanut butter, and rice paste (Kou, Li, Hou, Cheng, et al., 2018). The experimental and simulation results showed that the TDT-HBS could be used as a useful and precise test apparatus for determining the thermal resistance of foodborne pathogens/surrogates in LMFs.

The TDT-HBS has been used to investigate thermal inactivation kinetics of foodborne pathogens/surrogates in almond powder (Cheng & Wang, 2018), peanut kernels (Zhang et al., 2018), walnut shells (Zhang et al., 2018), and red pepper powder (Zhang, Zhang, Cheng, Guan, & Wang, 2020). The TDT-HBS was also used as a traditional heat treatment to identify possible non-thermal effects of RF heating on pasteurization of foodborne pathogens/surrogates by simulating the same heating rate, heating temperature and heating uniformity under 27.12 MHz RF heating conditions (Kou, Li, Hou, Zhang, & Wang, 2018).

A controlled atmosphere heating block system (CA-HBS, as shown in Fig. 4) was developed to explore effects of gas concentrations, target temperatures, and heating rates on heat resistance parameters of *E. coli* ATCC 25922 in almond powder (Cheng, Li, Kou, & Wang, 2017). The results showed that the CA-HBS held the potential to be an efficient test apparatus to evaluate thermal inactivation of foodborne pathogens/surrogates under controlled atmosphere during heat treatments.

Fig. 5 shows a TDT Sandwich test device designed by Lau and Subbiah (2020) at University of Nebraska-Lincoln. The device can heat samples up to 140 °C at controllable heating rate ranges of 0.2 to approximately 100 °C/min in LMFs. The open sources of modular nature of TDT Sandwich allow multiple unites to be connected with computers, which is convenient to control and record test parameters. When using this method, inoculated samples in disposable aluminum pouches are sandwiched between two parallel rigid aluminum plates, which are heated similar to TDT-HBS. This arrangement makes it relatively easy to place and remove the samples.

In summary, closed tube, jar or bag methods might be convenient for liquid matrices, while TDT-sandwich methods are more convenient for solid/powder samples. When using TDT cells and thermal-a_w cells (TAC), commercially available oil/water baths are convenient heating devices to provide isothermal heating conditions. But it is difficult to control the heating rates of the samples during the temperature come-up time using oil and water baths. HBS allows precise control of heating

Table 3
Experimental methods for obtaining thermal inactivation data of foodborne pathogens/surrogates in LMFs, and the a_w values were measured at room temperature.

Experimental methods	Heating devices	Pathogens/Surrogates	Food categories	Thermal resistance data	References
Closed system test methods	Glass jar (6 cm diameter, 8 cm height)	*S.* Braenderup JWC-1808, *S.* Enteritidis ATCC BAA-1045, *S.* Saintpaul LJH-1311–1, and *S.* Paratyphi OSPHL 15092808070	Almond butter (a_w = 0.31–0.38), hazelnut butter (a_w = 0.36), Peanut butter (a_w = 0.38)	Boiling water bath at more than 90 °C held for 30 min achieve 5 log CFU/g reductions	Wright, Minarsich, Daeschel, & Waite-Cusic. (2018)
	Metal plate	*S.* Tennessee and Oranienburg	Peanut butter (a_w = 0.33)	*D-values* for *S.* Tennessee and *S.* Oranienburg at 85 °C were 11.95 ± 1.55 min and 12.83 ± 2.35 min, respectively	Keller et al. (2012)
	Plastic stomacher bag	*S.* Tennessee K4643	Milk powder (a_w = 0.29), peanut butter (a_w = 0.55)	High-precision oil bath (90 °C) held for 10 min achieved 2- and 4-log reductions in milk powder and peanut butter, respectively	Li et al. (2014)
	Sterile glass tube	*E. coli* P1 and *E. faecium NRRL-B2354*	Wheat flour (a_w = 0.29, and 0.55)	Heated at 82 °C for 5 min achieved 1.7 log CFU/g and 6.0 log CFU/g reductions at a_w of 0.29 and a_w 0.55 levels, respectively	Daryaei et al. (2020)
	TDT cell I	*S.* Enteritidis PT 30	Peanut butter (a_w = 0.04–0.45), Wheat flour (a_w = 0.45–0.80)	D_{80}-values of *Salmonella* were 17.0 ± 0.9 min and 6.9 ± 0.7 min peanut butter and wheat flour with a_w of 0.45 at 20 °C	Syamaladevi et al. (2016a)
	TDT cell I	*L. monocytogenes* NRRL B-57618 (1/2a), NRRL B-33053 (4b), and NRRL B-33466 (1/2b)	Cocoa powder (a_w = 0.30, 0.45, and 0.60)	*D-values* at 65–80 °C at a_w of 0.30, 0.45, and 0.60 were: 21.9–5.0, 7.3–1.8 min, and 9.1–2.0 min. The z-values at a_w of 0.30, 0.45, and 0.60 were 15.5, 15.9, and 14.9 °C, respectively	Tsai, Taylor, et al. (2019)
		L. monocytogenes NRRL B-57618, NRRL B-33053, NRRL B-33466	Milk powder (a_w = 0.25, 0.30, and 0.45)	*D-values* at 70, 75, and 80 °C and a_w of 0.25, 0.30, and 0.45 were 66.2–21.3, 33.5–9.4, and 14.6–4.3 min. The z-values at a_w of 0.25–0.45 were 14.6–16.0 °C, respectively	Ballom, Tsai, Taylor, Tang, and Zhu (2020)
	TDT cell I	*L. monocytogenes* NRRL B-57618 (1/2a), NRRL B-33053 (4b), and NRRL B-*33466* (1/2b)	Wheat flour (a_w = 0.30, 0.45, and 0.60)	For a_w of 0.60, 0.45, and 0.30, *D-values* at 70–80 °C were 1.6–16.9, 3.1–17.4, and 7.1–37.1 min, *z-values* were 9.9, 14.2, and 12.9 °C, respectively	Taylor et al. (2018)
	TDT cell I	*E. faecium* NRRL B-2354	Wheat flour (a_w 0.45 at, 20 °C)	*D-values* at 75, 80, and 85 °C were 14.3 ± 0.9 min, 5.9 ± 0.4 min, and 2.8 ± 0.2 min, respectively. The z-value was 13.1 °C	Xu et al. (2018)
	TDT cell I	*S.* Enteritidis (G3, MD4, UK1, G2, MD9 and P97)	Wheat flour (a_w = 0.45)	$D_{80\,°C}$-values of biofilm formers *Salmonella* (14.1 ± 0.6 min) were significantly ($p < 0.05$) higher than that of non-formers (6.0 ± 0.2 min)	Villa-Rojas, Zhu, Paul, et al. (2017)
	TDT cell I	*E. faecium* NRRL B-2354, *S.* Agona, *S.* Montevideo, *S.* Tennessee, *and S.* Typhimurium	Paprika, white pepper and cumin powder (a_w = 0.45 ± 0.05 at, 25 °C)	$D_{80°C}$-values of *Salmonella* and *E. faecium* were 1.2and 1.8 min in paprika, 2.9 and 5.3 min in white pepper, and 4.5 and 9.5 min in cumin powder, respectively	Ozturk et al. (2020)
	TDT cell II	*E. faecium* NRRL B-2354, and *S.* Enteritidis PT30	Brown rice flour (a_w = 0.45)	The *D-values* in the a_w of 0.45 at 70, 75, 80, and 85 °C were 42.7, 17.5, 8.1, and 2.3 min for *Salmonella*, and were 65.8, 22.3, 6.1, and 1.9 min for *E. faecium,* respectively	Jin and Tang (2019a)
	TDT cell II	*S.* Enteritidis PT30	Soy protein powder (a_w = 0.13–0.82)	*D-values* at a_w of 0.25–0.70 showed a semi-log relationship under each treatment temperature level from 70 to 99 °C	Jin et al. (2020)
	TDT cell (NRC, Lausanne, Switzerland)	*E. coli* O157:H7 Sakaia, O157:H7 SEA13B88, O157:H7 P092, and six major non-O157 serotypes (O26, O45, O103, O111, O121, and O145)	Chicken meat powder (a_w = 0.26), confectionery (a_w = 0.43), pet food dry mix (a_w = 0.55), seasoning (a_w = 0.63)	Heated temperatures to achieve 5-log reductions were 90.9, 92.7, 99.1, and 106.0 °C for pet food, savory seasoning, confectionery formulations, and chicken meat powder, respectively	Daryaei et al. (2018)
	Whirl-Pak bags put in sandwich magnetic copper plates	*S.* Tennessee 5010 H[b], S13952 (782), S13972 (784), FSL R8-5221, and *E. faecium* NRRL B-2354	Peanut paste (a_w = 0.60)	$D_{85\,°C}$-values of *E. faecium* (3.4 min) was significantly ($p < 0.05$) lager than that of *Salmonella* (1.1 min)	Enache et al. (2015)
Controlled system test methods					
Controlled relative	Thermal-a_w cell (TAC) I (*RH* 50%)	*S.* Enteritidis PT 30	Organic wheat flour (a_w = 0.45 at 20 °C, increased to 0.73 at 80 °C)	Not provided	Tadapaneni et al. (2017)

(*continued on next page*)

Table 3 (*continued*)

Experimental methods	Heating devices	Pathogens/Surrogates	Food categories	Thermal resistance data	References
humidity (*RH*) methods	TAC I (*RH* 18%–72%)	*S.* Enteritidis PT30 and *E. faecium* NRRL B-2354	Inoculated SiO_2 (a_w <0.1)	$D_{80\ ^\circ C}$-values of *Salmonella* and *E. faecium* exponentially increased with reduced a_w levels. z_{aw} -values (The change in a_w needed to change $D_{80^\circ C}$ by 1 log) of *S.* Enteritidis and *E. faecium* were 0.31 and 0.28, respectively	Liu, Tang, et al. (2018)
	TAC II (*RH* 50%)	*S.* Enteritidis PT30	Wheat flour (a_w = 0.45 at 20 °C)	*D-value* of *Salmonella* at 80 °C tested with TAC II (20.7 ± 1.1 min) was significantly higher ($p < 0.05$) than the result obtained from TAC (7.5 ± 0.7 min)	Tadapaneni et al. (2018)
	TAC II (*RH*, 20%–80%)	*S.* Enteritidis PT30	Wheat flour, Almond flour, and Whey protein (a_w = 0.25, 0.45, 0.60, and 0.80 at 20 °C)	*S.* Enteritidis PT30 in the three given powder products showed different *D-values* with the same initial a_w at 20 °C	Xu et al. (2019)
Controlled heating block system (HBS) methods	Controlled atmosphere heating block system (CA-HBS, Range of O_2/CO_2 concentrations 0–25%)	*E. coli* ATCC 25922	Almond powder (*MC* of 6% w.b.)	*D-* and *z*-values of *E. coli* ATCC 25922 under the controlled atmosphere (2% O_2/20% CO_2) treatments were significantly ($p < 0.05$) lower than those under the regular atmosphere treatments	Cheng et al. (2017)
	TDT-HBS (Maximum set-point temperature 120 °C, heating rates 0.1–13.3 °C/min)	*E. coli* ATCC 25922	Almonds powder (*MC* of 6.0% w.b.)	*D-values* were similar when heating rates above from 1 °C/min, but significantly increased at lower heating rates	Kou et al. (2016)
	TDT-HBS (Set-point temperature 120 °C, heating rates 5 °C/min)	*E. coli* ATCC 25922	Almond powder (*MC* 6% of w.b.)	Held for 50.4 min at 75 °C could reach at least 4-log reductions of *E. coli* ATCC 25922 after modified atmosphere packaging pre-storage	Cheng and Wang (2018)
	TDT-HBS (62, 65, and 68 °C, heating rates 5 °C/min)	*S.* Typhimurium ATCC 50115, *S.* Braederup H*9812, and E. coli* ATCC 25922	Red pepper powder (a_w = 0.55, 0.76 and 0.86)	Log reduction levels with three given particle sizes (0.45–1.00, 0.20–0.45 and < 0.20 mm) were 1.45, 1.67, 2.24 log CFU/g, respectively	Zhang, Zhang, et al. (2020)
	TDT-HBS (56–70 °C, heating rates 5 °C/min)	*S.* H9812, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923	Walnut shells (*MC* of 8.93%–18.10%)	*D-value* at 64 °C was 10.2 ± 0.7 min with a_w at 0.59, but was reduced to 3.9 ± 0.2, 1.8 ± 0.17 and 0. 6 ± 0.0 min at a_w = 0.73, 0.84 and 0.93	Zhang et al. (2018)
	TDT Sandwich (70, 90, and 110 °C, heating rates 25, 50, and approximately 100 °C/min)	Not provided	Whole milk powder	Not provided	Lau and Subbiah (2020)

rate of the samples between 0.2 and 100 °C/min by PID controllers. In addition, the dry heating of the HBS can avoid the potential liquid spillage/infiltration into samples leading to changes of the a_w of the inoculated samples, thus changing thermal resistance of the target bacteria (Lau & Subbiah, 2020).

In thermal inactivation tests on bacteria in LMFs, it is a challenging task to properly inoculate bacterial cultures to the samples without changing their physical and chemical characteristics. Precautions need to be taken so that the inoculated samples in the thermal inactivation tests truly reflect the products in industrial thermal processes. This is different from thermal inactivation tests for pathogens in high moisture foods. Proper methods for bacteria inoculations are reviewed in Xu et al. (2021), and Xie et al. (2021).

3. Thermal inactivation kinetic models of foodborne pathogens/surrogates in LMFs

3.1. Primary model

3.1.1. First-order kinetic model

The first-order kinetic models are widely used in predicting inactivation of pathogens/surrogates under isothermal conditions (Peleg, 2006; Villa-Rojas et al., 2013), which are normally described by following equations:

$$\log \frac{N_t}{N_0} = -t \Big/ D_T \tag{2}$$

$$z = \frac{T_2 - T_1}{Log\ D_{T_1} - Log\ D_{T_2}} \tag{3}$$

where N_0 and N_t (CFU/g) are the initial population and the surviving population of pathogens/surrogates after a treatment time t (min), respectively. Thermal decimal time D_T (min) (the time required to cause 90% reduction in bacterial population) at a given temperature T (°C) can be calculated from equation (2). The *z-value* is the temperature (°C) increment required to result in 1-log reduction of *D-values*. It can be calculated from equation (3) or obtained by the −1/slope of the linear fitting curve of regression equation (3) (Li, Kou, Zhang, & Wang, 2018).

3.1.2. Weibull model

The Weibull model can be used to describe inactivation curves of pathogens/surrogates under isothermal conditions by nonlinear upwards and downward concavities (Cheng &, Wang, 2018; Villa-Rojas et al., 2013). equation (4) is used to describe the Weibull model (Peleg, 2006):

$$\log \frac{N_t}{N_0} = \log S(t) = -\alpha(T)t^{\beta(T)} \tag{4}$$

where t (min) is a treatment time. $S\ (t)$ is the momentary logarithmic

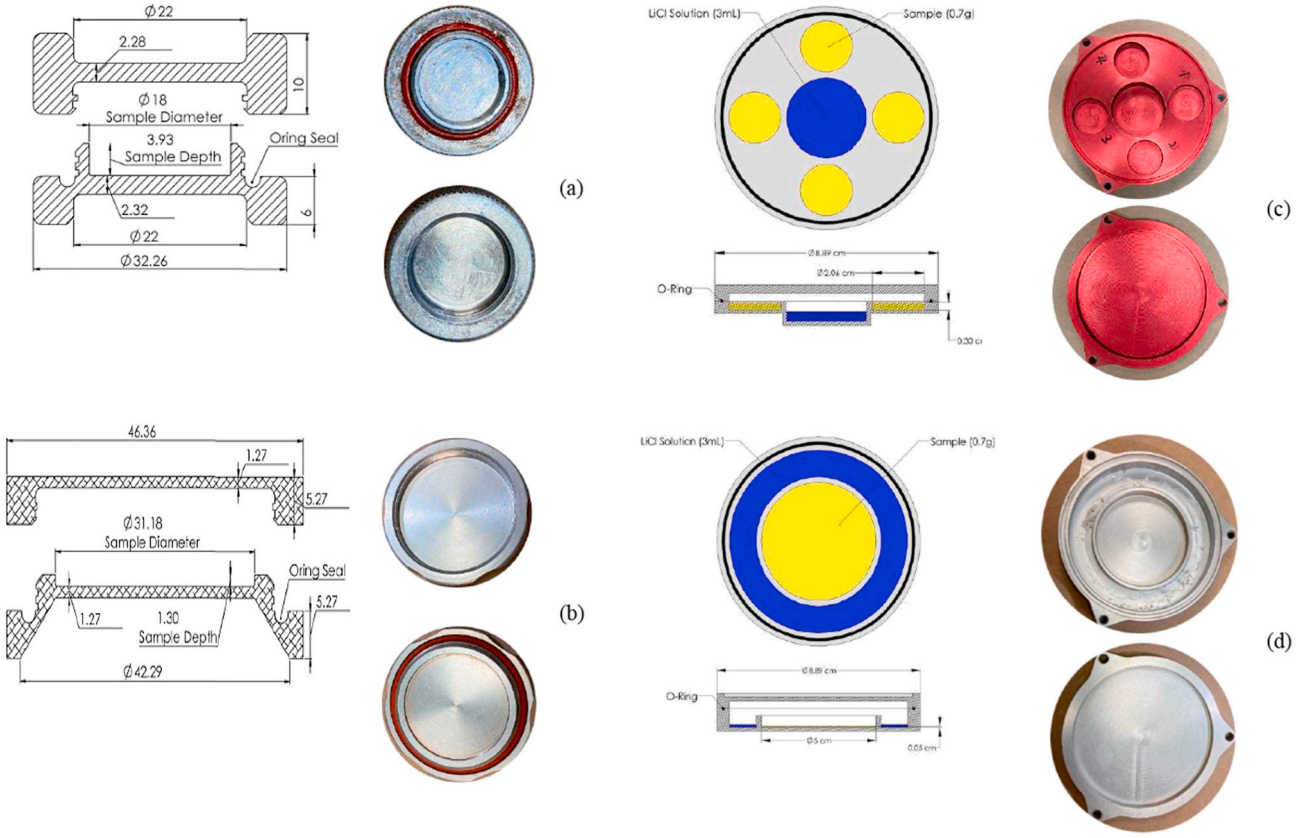


Fig. 2. Schematic and physical diagrams of TDT cell I (a), II (b), and TAC I (c), II (d) for LMFs (Adapted from Chung et al., 2008; Jin & Tang, 2019a; Tadapaneni et al., 2017, 2018).

survival ratio. T (°C) is the treatment temperature. α (T) and β (T) represent scale and shape parameters, which are temperature-dependent coefficients. When the shape parameter β $(T) < 1$, the Weibull description curve has an upward concavity, namely, the logarithmic curve with a "tail". The tail may attribute to heat resistance of pathogens variations in different life stage or potential heat adaptation of pathogens (Juneja, 2002). When β $(T) > 1$, the Weibull description curve has a downward concavity, and the logarithmic curve has a "shoulder". The presence of shoulder may result from poor heat transfer of heating devices and within the samples, leading to a relatively long time for the samples to reach the treatment temperature. It may also be caused by several sub-populations in the inoculated samples, certain microbial populations become increasingly easy to be inactivated (Awuah, Ramaswamy, & Economides, 2007). When β $(T) = 1$, equation (4) can be written as $\log N_t/N_0 = -\alpha$ (T) t, which is a linear semi-log survival curve (Boekel, 2002). Therefore, the first order model could be considered as a special case of the Weibull model. The Weibull model also could be used to predict the momentary microbial population N_t (CFU/g) by equation (4) at given exposure time t (min) and heating temperature (°C) (Li, Kou, Zhang, & Wang, 2018).

3.1.3. Biphasic model

Biphasic model can be used for describing broken curves (Humpheson, Adams, Anderson, & Cole, 1998), which have two different inactivation phases. Biphasic model contains three temperature-dependent parameters: first, second inactivation rates, and the phase transfer time (Corradini, Normand, & Peleg, 2007). The biphasic model has the following form:

$$N(t) = A_1 e^{-\lambda_1 t_1} + A_2 e^{-\lambda_2 (t - t_1)} \quad (5)$$

where A_1 and A_2 are the initial populations of foodborne pathogens/surrogates in the first and second phases, respectively. λ_1 and λ_2 are the temperature-dependent survival rate parameters for the first and second phases, respectively. t_1 is the critical time at which the inactivation rate changes from the first phase to the second phase (Stone, Chapman, & Lovell, 2009). The inactivation curves obtained are biphasic from inactivation rate -λ_1 to -λ_2 after critical time t_1, because some potential factors lead to lethality variations of foodborne pathogens/surrogates.

3.2. Secondary model

3.2.1. Bigelow model

According to Bigelow (1921), a secondary model was modified by Mafart, Couvert, Gaillard, and Leguerinel (2002) and Smith, Hildebrandt, Casulli, Dolan, and Marks (2016) to related D values to treatment temperatures:

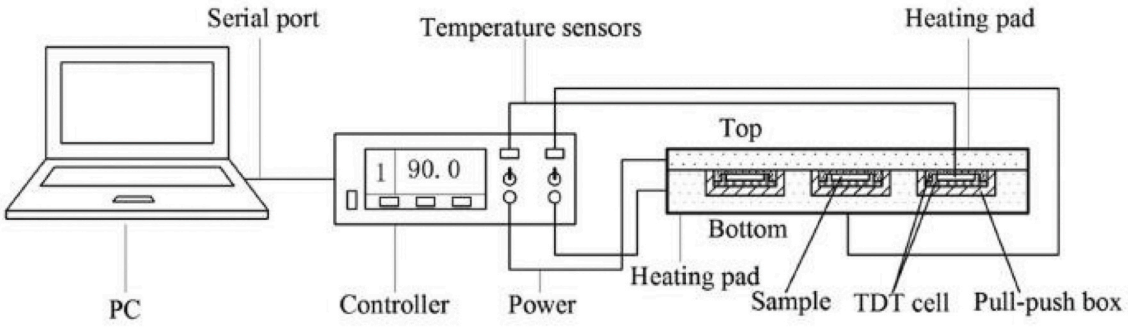
$$D(T,\ a_w) = D_{ref} \cdot 10^{\frac{a_{wref} - a_w}{z_{a_w}}} \cdot 10^{\frac{T_{ref} - T}{z_T}} \quad (6)$$

$$z_{a_w} = -\frac{a_{w_1} - a_{w_2}}{\log D_1 - \log D_2} \quad (7)$$

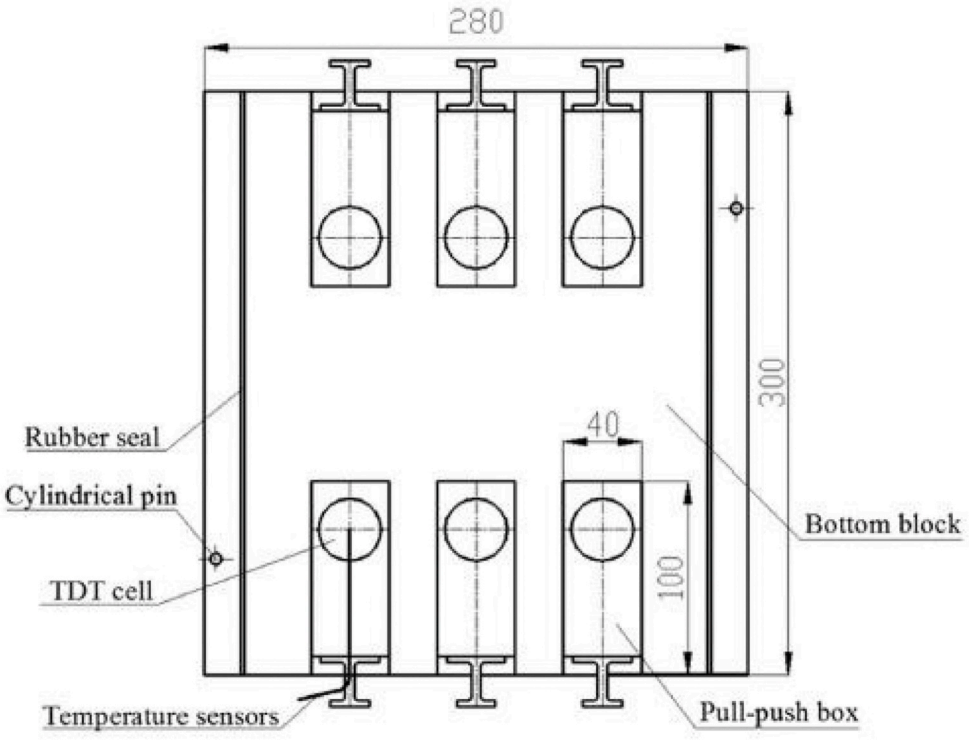
where T_{ref} is the reference temperature (°C), D_{ref} is the decimal time (min) at T_{ref}. $a_{w,ref}$ is the water activity at T_{ref}. Z_T is the temperature increment to achieve 1-log reduction of D_T, and Z_{aw} is the $a_{w,ref}$ increments to achieve 1-log reduction of D_{ref} (Alshammari, Xu, Tang, Sablani, & Zhu, 2020).

3.2.2. Weibullian log-logistic model

For dynamic conditions (such as in the temperature ramping stage), differential Weibull model could be expressed by the following equation based on equation (4) (Mendes-Oliveira, Deering, Martin-Gonzalez, & Campanella, 2020).



(a)



(b)

Fig. 3. Schematic diagram of (a) the TDT heating block system, and (b) top view after removing the top block (All dimensions are in mm and adapted from Kou et al., 2016).

$$\frac{\mathrm{d}\log S\cdot(t)}{dt} = -\alpha[T(t)]\beta[T(t)]\left(\frac{-\log S\cdot(t)}{\alpha[T(t)]}\right)^{\frac{\beta[T(t)]-1}{\beta[T(t)]}} \quad (8)$$

In equation (8), temperature is a function of time, expressed as $T(t)$. The parameter β was often considered as constant or adjusted to empirical equations case by case. For instance, $\beta = 1$ is in linear survival curve. Under non-isothermal conditions, the parameter α is referred to the rate of killing. The Weibull model can be used to estimate the momentary logarithmic survival ratio of target pathogens during dynamic heating processes when momentary temperature and time are given (Li et al., 2018). The Weibullian log-logistic model (Corradini & Peleg, 2003) is used by Zhang, Zhang, et al. (2020) to describe the relationship between processing temperature history and the rates of thermal inactivation. The Weibullian log-logistic model has the following form:

$$\alpha(T) = \ln\{\ 1 + \exp[k(T - T_c)\ ]\ \} \quad (9)$$

where T_c is the inactivation rate accelerating temperature (°C), α is the nonlinear killing rate, and k is the model parameter. When $T > T_c$, k (T - T_c) determines the nonlinear killing rate of α. When $T < T_c$, the exp [k (T - T_c)] tends to zero. Since ln (1) = 0, therefore α tends to zero. The Weibullian log-logistic equations are used as a secondary model to calculate T_c, and k values on thermal resistance of *S.* Enteritidis PT 30 in brown rice powder (Jin & Tang, 2019a).

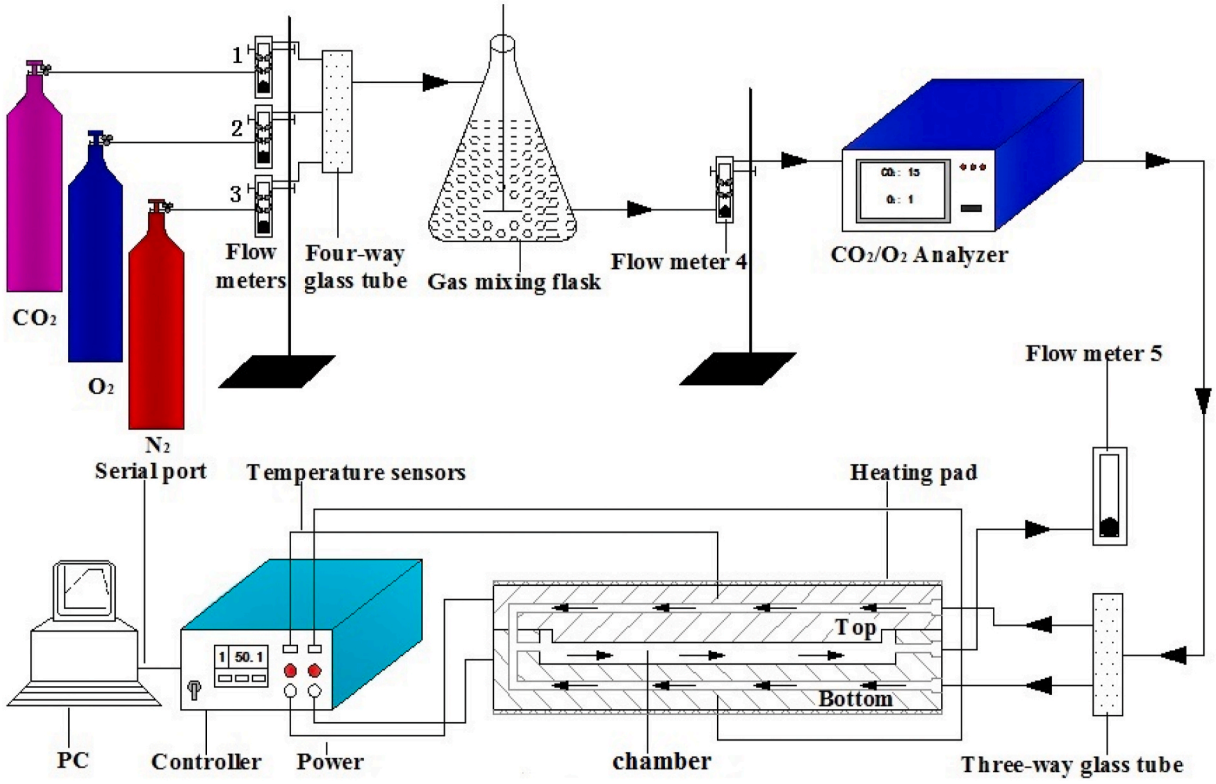


Fig. 4. Diagram of controlled atmosphere/heating block systems (adapted from Cheng et al., 2017).

3.3. Model evaluations and applications

3.3.1. Model evaluations

The accuracy of models can be evaluated by the coefficient of determination (R^2), root mean square error (*RMSE*), accuracy factor parameter (A_f), bias factor (B_f), and corrected Akaike information criterion (*AICc*) (Li, Kou, Zhang, & Wang, 2018; Smith et al., 2016) as follows:

$$R^2 = \frac{\sum_i (\text{predicted value} - \text{average})^2}{\sum_i (\text{measured value} - \text{average})^2} \tag{10}$$

$$RMSE = \sqrt{\frac{\sum (\text{measured value} - \text{predicted value})^2}{n-1}} \tag{11}$$

$$A_f = 10^{\frac{\sum |\log(\text{predicted value}/\text{measured value})|}{n}} \tag{12}$$

$$B_f = 10^{\frac{\sum \log(\text{predicted value}/\text{measured value})}{n}} \tag{13}$$

$$AICc = n \ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \tag{14}$$

where n is the number of measured data, K is the number of estimated parameters plus 1, and SS is the sum of squares of the residuals.

R^2 is used to evaluate how well the measured values fit with the predicted values. Higher R^2 (close to 1) means higher accuracy of the predicted model (Cheng & Wang, 2018; Nagelkerke, 1991). *RMSE* shows the average deviation between measured and predicted values. The lower *RMSE* values indicate a better fitting to the model (Huang, Tian, Gai, & Wang, 2012). The percentage discrepancy of measured and predicted values is evaluated by A_f and B_f. A_f cannot be negative. If A_f is closer to zero, it means a better fit (Baranyi, Pin, & Ross, 1999). *AICc* was used as a criterion for model selection, and lower *AICc* value means the model fitting the data better. Compared with R^2, *AICc* could choose models with reasonable model parameter freedoms, thus avoiding over-fitting (Smith et al., 2016).

3.3.2. Model applications

In primary models, *D*- and *z-values* are traditionally used to describe the foodborne pathogens/surrogates population evolution as a function of heating time and temperature (Zhang et al., 2018). An important application of primary models is to calculate the cumulative lethal time *F-value* (min) of a thermal processing by the following equation (Tang, Ikediala, Wang, Hansen, & Cavalieri, 2000; Xu et al., 2018):

$$F = \int_0^t 10^{\left(T(t) - T_{ref}\right)/z} dt \tag{15}$$

Secondary models could be modified from primary models by using second-order response surface regression and used for predicting inactivation curves under isothermal and non-isothermal conditions (Li, Kou, Zhang, & Wang, 2018). The modified Bigelow model has been effectively used to explore the effects of temperature and a_w on thermal inactivation of foodborne pathogens/surrogates in almond kernels (Villa-Rojas et al., 2013), soy-protein powder (Jin et al., 2020), and wheat flour (Liu, Tang, et al., 2018; Smith et al., 2016).

4. Applying thermal resistance data in RF microbial validation processing for LMFs

As shown in Fig. 1, after obtaining thermal inactivation data of target pathogens using different testing methods, microbial validation may be a critical step for developing effective pathogen control technologies (Wei, Lau, Reddy, & Subbiah, 2020). Rapid and volumetric RF heating has advantages over conventional conduction or convection heating techniques for bulk LMFs, which typically have low thermal diffusivities (Ling, Cheng, & Wang, 2020). Microwaves can also be used for heating LMFs. But compared to microwave heating, RF heating has greater

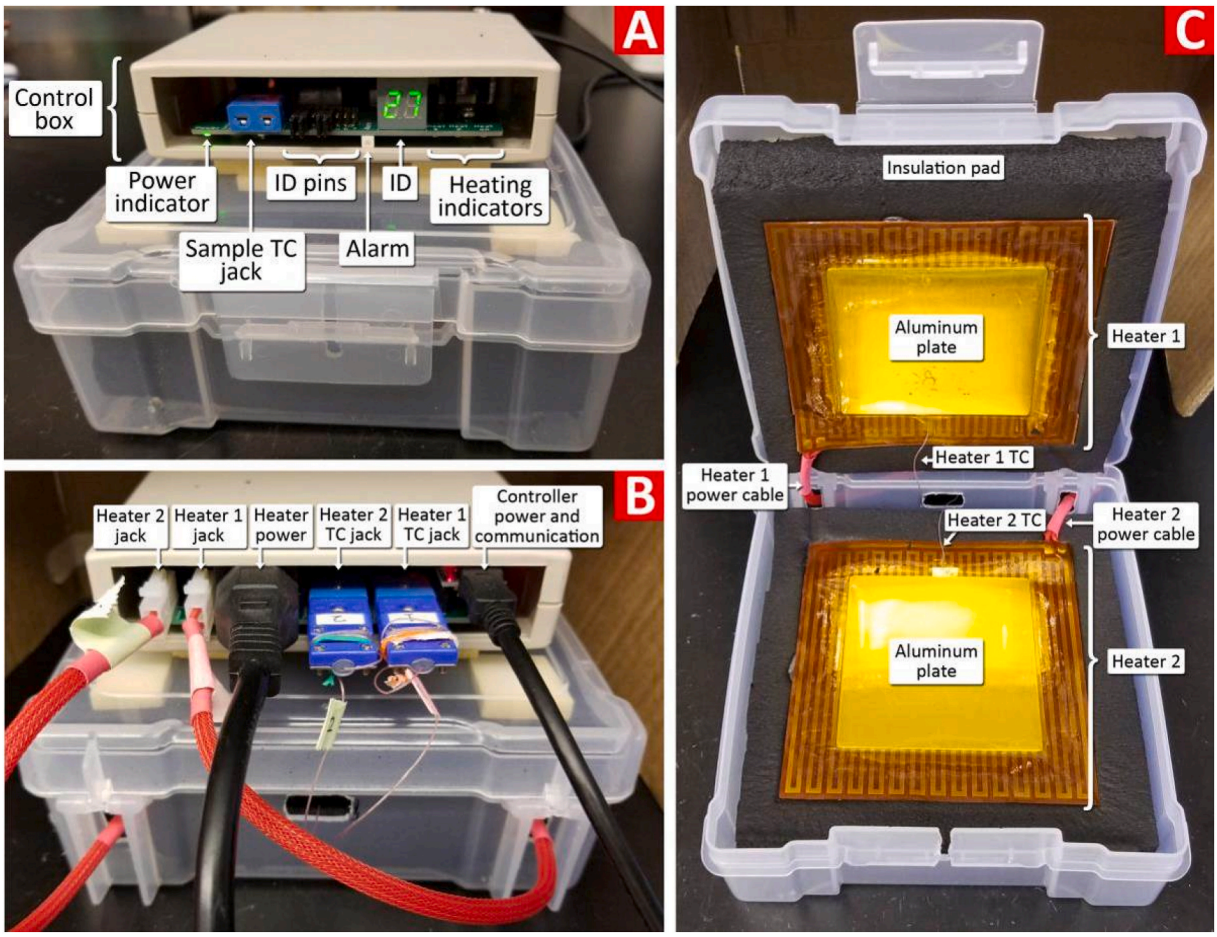


Fig. 5. Annotated views of the TDT Sandwich from the (A) front, (B) back, and (C) inside (Adapted from Lau & Subbiah, 2020).

penetration depth in large dimensions and bulk LMFs (Chen, Wei, Irmak, Chaves, & Subbiah, 2019). Thus, most recently published studies on novel thermal processes for pathogen control in LMFs have been focused on RF heating (Chen et al., 2021; Liu, Ozturk, et al., 2018; Xu et al, 2018, 2020b). In those studies, the thermal inactivation data for Salmonella and/or its surrogate Enterococcis faeciu were used to develop and/or validate RF treatments for wheat flours and hazelnuts. The process conditions are summarized in Table 4. A 6 kW, 27.12 MHz pilot-scale RF device (SO6B, Strayfield International, Wokingham, U.K.) has been widely used in microbial validation process in LMFs.

The successful industry application of RF pasteurization processes relies on heating uniformity in RF treated samples, confirmation studies of the target pathogen inactivation at cold spot, and final microbial validation of RF treatment protocols.

4.1. RF heating uniformity in LMFs

Non-uniformity of RF heating is still a major problem for LMFs (Ling et al., 2020). Many factors (such as uneven distribution of electromagnetic field, different food compositions, removal of moisture, etc.) could affect RF heating uniformity (Huang, Marra, Subbiah, & Wang, 2018). Heating uniformity index (λ) as shown in equation (16), was proposed in Wang, Yue, Chen, and Tang (2008), and has been used for evaluating RF heating uniformity in many LMFs, such as almond kernels (Li, Kou, Hou, Ling, & Wang, 2018) and in-shell walnuts (Zhang et al., 2019).

$$\lambda = \frac{\sqrt{\sigma^2 - \sigma_0^2}}{\mu - \mu_0} \tag{16}$$

where σ_0 and σ are initial and final standard deviations (°C) of temperature in LMFs over RF heating time. μ_0 and μ are initial and final mean temperatures (°C) of LMFs, respectively.

Several experiment and simulation methods are used to improve RF heating uniformity, such as moving samples with conveyor (Chen, Huang, Wang, Li, & Wang, 2016; Zhou & Wang, 2019), placing sample in a five-layer container (Li, Kou, Hou, et al., 2018), and surrounding container with a similar dielectric constant sheets to samples (Huang, Zhu, Yan, & Wang, 2015). RF heating uniformity was evaluated for the microbial validation processing in cumin powder (Ozturk et al., 2020), powder infant formula milk (Zhang, Zhang, et al., 2020), and wheat flour (Xu, Yang, et al., 2020).

4.2. Survival of foodborne pathogens/surrogates at cold spot

Cold spot is defined as the critical control point, where had the lowest temperature after RF treatments (Cheng & Wang, 2019). Due to different RF treatment conditions and LMFs characteristics, the location of cold spot could be located in different places (Li, Kou, Cheng, Zheng, & Wang, 2017). The temperature-time history measurements and computer simulation model might help identify the cold spot for different RF treatment conditions (Villa-Rojas, Zhu, Marks, & Tang, 2017). Computer simulation using COMSOL Multiphysics has been used to simulate the location of the cold spot during RF heating process for dry soybeans and wheat samples (Chen et al., 2016; Huang et al., 2015). For experimental methods, thermocouple sensors, fiber-optic sensor system, and infrared temperature measurement system are often used for cold spot determination. According to temperature-time history curves obtained by the experimental methods, cold spot location could be determined for experimental methods or validated for simulation

Table 4
Microbial validation of foodborne pathogens/surrogates in LMFs using RF energy at 27.12 MHz.

LMFs	Pathogens/surrogates	Containers	RF heating powers and target temperatures	References
Almond kernels (*MC* 8% w. b.)	*E. coli* ATCC 25922	Five-layer container (30 × 22 × 10 cm^3)	6 kW, 75 °C	Cheng and Wang (2019)
Almond kernels (*MC* 10.01% w.b.)	*E. coli* ATCC 25922	Five-layer container (25 × 15 × 10 cm^3)	6 kW, 72 °C	Li, Kou, Hou, et al. (2018)
Black/red pepper spice	*S. Typhimurium* (ATCC 19586/43174/DT 104) and E. *coli* O157:H7 (ATCC35150/43889/43890)	Glass beakers (7.5 cm in diameter and 10.0 cm deep)	19 kW, 62, 67, and 70 °C	Kim, Sagong, Choi, Ryu, and Kang (2012)
Corn flour (a_w 0.45 ± 0.05)	*S.* Enteritidis PT 30/*E. faecium* NRRL B-2354	(PEI) container (70 × 240 × 300 mm^3)	6 kW, 75, 80, and 85 °C	Ozturk et al. (2019)
Cumin seeds (*MC* 10.2% w. b.)	*Salmonella* spp., *E. faecium* NRRL B-2354	Polyethylene pouch (8 × 6 × 1 cm^3)	6 kW, 102 °C	Chen et al. (2019)
Egg white powder	*Salmonella* spp., *E. faecium* NRRL B-2354	Polyethylene bag (40 × 30 × 2 mm^3)	6 kW, 80 °C	Wei et al. (2020)
In-shell almonds (*MC* 5.1%–41.6% w.b.)	*E. coli* ATCC 25922	Plastic container (30 × 22 × 6 cm^3)	6 kW, 72 °C	Li et al. (2017)
Inshell hazelnuts ($a_{w\text{-}shell}$ 0.91 ± 0.01, $a_{w\text{-}kernel}$ 0.93 ± 0.01)	*Salmonella* spp., *E. faecium* NRRL B-2354	Laminated paper tray (16.5 × 13 × 4 cm^3)	6 kW, 65–90 °C	Chen et al. (2021)
In-shell walnut (15.0% w.b.)	*Staphylococcus aureus* ATCC 25923	Plastic container (37 × 30 × 6 cm^3)	6 kW, 70 °C	Zhang et al. (2019)
Powdered infant formula milk (a_w 0.20 ± 0.02 at 25 °C)	*Cronobacter sakazakii* (ATCC 29544)	Polyethylene pouch (70 × 65 × 5 mm^3)	6 kW, 65 °C	Lin, Subbiah, Chen, Verma, and Liu (2020)
Powdered infant formula milk (a_w 0.20–0.40 at 25 °C)	*Cronobacter sakazakii* (ATCC 29544)	Cylindrical petri dishes (35 mm diameter and 12 mm high)	6 kW, 55, 60, 65, and 70 °C	Zhang, Zhang, et al. (2020)
Powdered red and black pepper spices (*MC* 10.1%–30.5% d.b.)	*E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), and *S.* Typhimurium (ATCC 19585, ATCC 43971, ATCC 700408)	Polypropylene jar, 6.4 cm in diameter and 10.8 cm deep	9 kW, 90 °C	Jeong and Kang (2014)
Red pepper powders (a_w 0.57–0.71)	*S.* Typhimurium (ATCC 11464, ATCC 11472)	Polypropylene plastic cuboid container (16.0 × 10.5 × 6.8 cm^3)	12 kW, 70 °C	Hu, Zhao, Hayouka, Wang, and Jiao (2018)
Wheat flour (a_w 0.25–0.65)	*S.* Enteritidis PT 30 and *E. faecium* NRL B-235	PET and PEI plastic box on different platforms (tripod, glass, Petri dish)	0.5 kW, 75 °C	Villa-Rojas, Zhu, Marks, and Tang (2017)
Wheat flour (a_w 0.45 ± 0.02)	*S.* Enteritidis PT 30 and *E. faecium* NRRL B-2354	Rectangular PEI container (30 × 24 × 7 cm^3)	6 kW, 75, 80, and 85 °C	Liu et al. (2018a)
Wheat flour (a_w 0.45 ± 0.02 at 25 °C)	*E. faecium* NRRL B-2354	Rectangular PEI container (30 × 24 × 7 cm^3)	6 kW, 75, 80, and 85 °C	Xu et al. (2018a)
Wheat flour (a_w 0.45 ± 0.02 at 22 °C)	*E. faecium* NRRL B-2354	Three-layer PEI container (25 × 15 × 7 cm^3)	6 kW, 80 °C	Xu, Yang, Jin, Barnett, and Tang (2020)

methods. Microbial challenge studies in pilot scale RF pasteurization processes by inoculating target pathogens at cold spot as the worst-case scenario, where had the least lethality, could ensure the food safety in LMFs (Cheng et al., 2020; Xu, Yang, et al., 2020).

4.3. Using surrogates in RF microbial validation processing

Pathogenic *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes,* and *Cronobacter sakazakii* should not be directly used in RF treatments due to strict safety requirements for operators, products, and processing environments (Niebuhr, Laury, Acuff, & Dickson, 2008). Since most food safety labs and food processing pilot-plants belong to biosafety level 1 lab, they cannot be utilized to conduct validation tests with pathogenic bacteria due to potential cross-contaminations and biosafety concerns (Wilson, 2009, pp. 21–112). Therefore, similar or higher thermal resistance nonpathogenic bacteria have been used as a surrogate in RF microbial validation processing for LMFs, such as almond (Cheng & Wang, 2019; Cheng et al., 2020; Li et al., 2017), corn flour (Ozturk et al., 2019), cumin seed (Chen et al., 2019; Chen, Irmak, Chaves, & Subbiah, 2020), in-shell walnut (Zhang et al., 2019), paprika, white pepper and cumin powder (Ozturk et al., 2020), and wheat flour (Liu, Ozturk, et al., 2018; Xu et al., 2018; Xu, Ren, Jin, Barnett, & Tang, 2020). As shown in Table 5, *E. faecium* NRRL B-2354 has been mostly used as non-pathogenic surrogate for *Salmonella* Enteritidis PT30 in LMFs, such as almonds (ABC, 2007). Except for *E. faecium* NRRL B-2354, other potential surrogates, such as *E. coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923, have been successfully used as surrogates for validation of RF pasteurization processes in LMFs.

5. Conclusions

Temperature, *MC*, a_w, and heating rate could be critical factors for thermal resistance of pathogens in LMFs. Closed system and controlled system test methods can make those factors constant or adjustable. These systems are effective to obtain reliable thermal inactivation data of target pathogens.

Thermal inactivation kinetic models have been proved to be critical tools for predicting and control of foodborne pathogens/surrogates in LMFs. Based on the first order kinetic model under isothermal treatment, differential *Weibullian models* and cumulative lethal value models could be used to validate the inactivation rate under non-isothermal conditions.

Novel RF heating has been successfully applied in pasteurization of LMFs, and shown great potential in industrial pasteurization processing for LMFs. Heating uniformity, survival of pathogens/surrogates at cold spot, and strict safety requirements need to be considered when conducting RF microbial challenge studies.

6. Challenges and future trends

Due to complicated factors in real microbial survival environment, more controllable test methods should be developed to simulate the real-temperature and relative humidity environment during ramping, holding, and cooling stage.

Compared to first kinetic models, secondary models contain a wider range of model parameters. However, secondary models have limited applications in real pasteurization processing. In future research, a

Table 5
Nonpathogenic surrogates used in RF treatments for foodborne pathogens in LMFs.

Foodborne pathogens	Surrogates	LMFs	Remarks	Sources
S. Agona, *S.* Montevideo, *S.* Tennessee, and *S.* Typhimurium	*E. faecium* NRRL B-2354	Paprika, white pepper and cumin powder	The $D_{80^\circ C}$ values of *E. faecium* were larger than that of *Salmonella* spp. in three given LMFs	Ozturk et al. (2020)
S. Agona 447967, *S.* Reading Moff 180418, *S.* Tennessee K4643, *S.* Montevideo 488275, *S.* Mbandaka 698538	*E. faecium* NRRL B-2354	Cumin seed	*E. faecium* consistently showed lower log reductions than *Salmonella* spp. under RF treatments with same conditions	Chen (2020)
S. Agona 447967, *S.* Reading Moff 180418, *S.* Tennessee K4643, *S.* Monte video 488275, *S.* Mbandaka 698538	*E. faecium* NRRL B-2354	Egg white powder	Microbial Reduction (log CFU/g) of *Salmonella* spp. was significantly ($p < 0.05$) larger than that of *E. faecium* at the same location	Wei et al. (2020)
S. Agona 447967, *S.* Reading Moff 180418, *S.* Tennessee K4643, *S.* Monte video 488275, *S.* Mbandaka 698538	*E. faecium* NRRL B-2354	Ground black pepper	130 s continuous RF pasteurization process achieved 5.98 log CFU/g reduction for *Salmonella* spp. and 3.89 log CFU/g for *E. faecium*, respectively	Wei, Lau, Stratton, Irmakc, & Subbiah (2019)
S. Enteritidis PT 30	*E. coli* ATCC 25922	Almond kernel	$D_{80^\circ C}$ value of *E. coli* ATCC 25922 (7.0 ± 0.8 min) was larger than *S.* Enteritidis PT 30 (1.63 min) at the same a_w level	Cheng et al. (2020)
S. Enteritidis PT 30	*E. coli* ATCC 25922	In-shell almond	$D_{68^\circ C}$ value of *E. coli* ATCC 25922 (0.95 ± 0.02 min) was larger than *S.* Enteritidis PT 30 (0.42 ± 0.04 min) at $a_w = 0.946$	Li et al. (2017)
S. Enteritidis PT 30	*E. faecium* NRRL B-2354	Corn flour	RF heated to 85 °C, held for 10 min, and then stored at −20 °C for 48 h, achieved 6.59 ± 0.21 log and 4.79 ± 0.17 log reductions for *S.* Enteritidis and *E. faecium*, respectively	Ozturk et al. (2019)
S. Enteritidis PT 30	*E. faecium* NRRL B-2354	Wheat flour	5 and 3 log reductions were reached after RF heated for 8.5 min at $a_w = 0.25$ for *Salmonella* and *E. faecium*, respectively	Villa-Rojas, Zhu, Marks, and Tang (2017)
S. Enteritidis PT 30	*E. faecium* NRRL B-2354	Wheat flour	*E. faecium* had significantly higher ($p < 0.05$) *D-values* compared to *S.* Enteritidis at three given temperatures	Liu, Ozturk, et al. (2018)
S. Enteritidis PT 30	*E. faecium* NRRL B-2354	Wheat flour	$D_{80^\circ C}$*-value* of Freeze-dried *E. faecium* NRRL B-2354 (FDE) was 5.92 ± 0.39 min larger than that of 4.3 ± 0.2 min for the liquid-form *S.* enteritidis PT 30 under the same treatment condition	Xu et al. (2018a)
S. H9812	*Staphylococcus aureus* ATCC 25923	In-shell walnut	The average reductions of *S. aureus* ATCC 25923 were lower than those of *Salmonella* H9812 under the same heating treatments	Zhang et al. (2019)

comprehensive model could be developed to integrate the microbial inactivation kinetics into the Multiphysics heat and mass transfer model to predict thermal inactivation of pathogens under real microbial environment (Chen, 2020).

Another challenge is that thermal resistance parameters are obtained at either constant MC or a_w levels. When subjected to RF heating in open system, the MC and a_w of food samples may change. The normal *F-value* model may not provide reliable lethality prediction due to thermal resistance changes at different MC and a_w conditions. Chen (2020) proposed to use the thermal inactivation data at final a_w of the food products after RF treatment in *F-value* model to give a conservative prediction. In future studies, a comprehensive *F-value* model should involve a_w condition models during the whole processing.

For further studies, the large material variations are also a challenge for pathogen control in real LMFs. Synergetic consideration of low-temperature long time (LTLT) and high-temperature short time (HTST) processes are needed to ensure food safety and balance food quality at the same time. RF treatments have unique advantages for control pathogens in LMFs, and its combination with conventional heating methods can normally improve processing efficiency. Therefore, further research may need to focus on applying combined technologies in control of pathogens in LMFs, such as combined using rapid RF heating during ramping, and stationary conventional oven heating during holding to achieve desired lethality. In addition, novel rapid cooling technology should also be considered to maintain food quality of LMFs.

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