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

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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\textsubscript{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 wide-spread 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., \textit{Escherichia coli}, and \textit{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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** Keywords:**
- Come up time
- Food safety
- Isothermal tests
- Kinetic model
- Surrogate

** ABSTRACT **

\textbf{Background:} Outbreaks of foodborne pathogens (including \textit{Salmonella} spp., \textit{Escherichia coli}, and \textit{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.

\textbf{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.

\textbf{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 \textit{Salmonella} in several LMFs.
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Kou, Zhang, and environmental conditions, such as relative humidity, and atmo-

dditions, such as heating temperature, heating rate, holding time, etc.,

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Table 1

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<tr>
<th>Acronyms</th>
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<td>CA-</td>
<td>Controlled atmosphere heating block system</td>
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<td>HBS</td>
<td></td>
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<tr>
<td>CCFH</td>
<td>Codex Committee on Food Hygiene</td>
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<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>CUT</td>
<td>Come up time</td>
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<td>FDA</td>
<td>US Food and Drug Administration</td>
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<td>FDE</td>
<td>Freeze-dried E. faecium NRRL B-2354</td>
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<tr>
<td>HBS</td>
<td>Heating block system</td>
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<tr>
<td>HTST</td>
<td>High-temperature short time</td>
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<tr>
<td>LCI</td>
<td>Lithium Chloride</td>
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<td>LMFs</td>
<td>Low-moisture foods</td>
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<tr>
<td>LTLT</td>
<td>Low-temperature long time</td>
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<tr>
<td>MC</td>
<td>Moisture content</td>
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<tr>
<td>PTD</td>
<td>Proportional-integral-derivative</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
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<td>RH</td>
<td>Relative humidity</td>
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<tr>
<td>RMSE</td>
<td>Root mean square error</td>
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<tr>
<td>TAC</td>
<td>Thermal-accelerated cell</td>
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<tr>
<td>TDT</td>
<td>Thermal-death-test</td>
</tr>
<tr>
<td>WSU</td>
<td>Washington State University</td>
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</table>

Symbols

- $A_1$ Initial population (CFU/g) of foodborne pathogens in the first phase
- $A_2$ Initial population (CFU/g) of foodborne pathogens in the second phase
- $A_f$ Accuracy factor parameter
- $A_{0c}$ Corrected Akaike information criterion
- $a_m$ Water activity
- $B_f$ Bias factor
- $D_T$ Thermal decimal time (min) at a given temperature $T$ (°C)
- $D_{of}$ Decimal time (min) at $T_{ref}$ (°C)
- $F$ Cumulative lethal time (min)
- $k$ Model parameter
- $m_s$ Molality of salt (mol/kg H$_2$O)
- $m_w$ 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)
- $T_c$ Inactivation rate accelerating temperature (°C)
- $T_{ref}$ Reference temperature (°C)
- $v$ 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)
- $Z_w$ Increments to achieve 1-log reduction of $D_{of}$
- $Z_T$ 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
- $\lambda_1$ Temperature-dependent survival rate parameters for the first phases
- $\lambda_2$ Temperature-dependent survival rate parameters for the second phases
- $\mu_0$ Initial mean temperatures (°C)
- $\rho_v$ Final mean temperatures (°C)
- $\sigma_0$ Initial standard deviations of temperatures (°C)
- $\phi$ Osmotic coefficients of LiCl

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 mathematical models to evaluate the influence of important parameters on thermal inactiva-
tion 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 food-
borne 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, $a_m$, 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 $a_m/MC$ 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-Cusick., 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 path-
ogens (such as Salmonella spp., Shiga toxin-producing Escherichia coli,

microorganism-related factors (strain, population of bacteria, and growth phase/condition, etc.), process-related factors (treatment condi-
tions, such as heating temperature, heating rate, holding time, etc., and environmental conditions, such as relative humidity, and atmos-
phere 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.
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 height, Fig. 2 b) was further designed at Washington State University. For example, Jin, Tang, and Zhu (2020) determined thermal resistance 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 Salmonella 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, aw, 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 aw of the selected food systems along with thermal inactivation tests (Ashammi 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 aw 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 aw 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 aw on inactivation parameters of foodborne bacteria, a novel thermal-aw 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 aw of the inoculated low moisture samples when heated from room temperature to high treatment temperatures. Specifically, the relative humidity (RH) in TAC I headspace over the samples is controlled by a Lithium Chloride (LiCl) solution. The aw of the solution can be calculated as the following equation (Gibbard & Scatchard, 1973):

\[
aw \approx \frac{RH}{100} = \exp(-\varphi M_w m_w)
\]

where \(\varphi\) 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 \(m_w\) (mol/kg H2O) is the molality of salt. The aw 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 aw 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 gradients (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 aw equilibrium time between LMFs and LiCl solutions from -14 min to <4 min by reducing sample height and increasing in sample diameter. The D90 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 equilibrium 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 aw levels (0.25, 0.45, 0.65, and 0.85, at room temperature) and reported exponential reductions of D90 °C values of S. Enteritidis PT 30 with increasing aw 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 in samples in...
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, see 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 \( D \) values were measured at room temperature.

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<th>Pathogens/Surrogates</th>
<th>Food categories</th>
<th>Thermal resistance data</th>
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<tr>
<td><strong>Closed system test methods</strong></td>
<td>Glass jar (6 cm diameter, 8 cm height)</td>
<td>S. Braenderup JWC-1808, S. Enteritidis ATCC BAA-1045, S. Saintpauly LHI-1311-1, and S. Paratyphi OSPHL 15092808070</td>
<td>Almond butter (( \alpha_a = 0.31-0.38 )), hazelnut butter (( \alpha_a = 0.36 )), Peanut butter (( \alpha_a = 0.38 ))</td>
<td>Boiling water bath at more than 90 (^\circ)C held for 30 min achieve 5 log CFU/g reductions</td>
<td>Tadapaneni et al. (2017)</td>
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<td></td>
<td>Metal plate</td>
<td>S. Tennessee and Oranienburg</td>
<td>Peanut butter (( \alpha_a = 0.33 ))</td>
<td>( D )-values for S. Tennessee and S. Oranienburg at 85 (^\circ)C were 11.95 ± 1.55 min and 12.83 ± 2.35 min, respectively</td>
<td>Keller et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Plastic stomacher bag</td>
<td>S. Tennessee K4643</td>
<td>Milk powder (( \alpha_a = 0.29 )), peanut butter (( \alpha_a = 0.55 ))</td>
<td>High precision oil bath (90 (^\circ)C) held for 10 min achieved 2- and 4-log reductions in milk powder and peanut butter, respectively</td>
<td>Li et al. (2014)</td>
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<tr>
<td></td>
<td>Sterile glass tube</td>
<td>E. coli P1 and E. faecium NRRL-B2354</td>
<td>Wheat flour (( \alpha_a = 0.29 ), and 0.55)</td>
<td>Heated at 82 (^\circ)C for 5 min achieved 1.7 log CFU/g and 6.0 log CFU/g reductions at ( \alpha_a ) of 0.29 and ( \alpha_a ) 0.55 levels, respectively</td>
<td>Daryasi et al. (2020)</td>
</tr>
<tr>
<td></td>
<td>TDT cell I</td>
<td>S. Enteritidis PT 30</td>
<td>Peanut butter (( \alpha_a = 0.04-0.45 )), Wheat flour (( \alpha_a = 0.45-0.80 ))</td>
<td>( D )-values of Salmonella were 17.0 ± 0.9 min and 6.9 ± 0.7 min peanut butter and wheat flour with ( \alpha_a ) of 0.45 at 20 (^\circ)C</td>
<td>Syamaladevi et al. (2016a)</td>
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<td></td>
<td>TDT cell I</td>
<td>L. monocytogenes NRRL B-57618 (1/2a), NRRL B-33053 (4b), and NRRL B-33466 (1/2b)</td>
<td>Coca powder (( \alpha_a = 0.30 ), 0.45, and 0.60)</td>
<td>( D )-values at 65–80 (^\circ)C at ( \alpha_a ) 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 ( \alpha_a ) of 0.30, 0.45, and 0.60 were 15.5, 15.9, and 14.9 (^\circ)C, respectively</td>
<td>Tsai, Taylor, et al. (2019)</td>
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<tr>
<td></td>
<td>TDT cell I</td>
<td>L. monocytogenes NRRL B-57618, NRRL B-33053, NRRL B-33466</td>
<td>Milk powder (( \alpha_a = 0.25 ), 0.30, and 0.45)</td>
<td>( D )-values at 70, 75, and 80 (^\circ)C and ( \alpha_a ) 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 ( \alpha_a ) of 0.25–0.45 were 14.6–16.0 (^\circ)C, respectively</td>
<td>Ballom, Tsai, Taylor, Tang, and Zha (2020)</td>
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<tr>
<td></td>
<td>TDT cell I</td>
<td>E. faecium NRRL B-2354</td>
<td>Wheat flour (( \alpha_a = 0.30 ), 0.45, and 0.60)</td>
<td>( D )-values at 70–80 (^\circ)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 (^\circ)C, respectively</td>
<td>Taylor et al. (2018)</td>
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<tr>
<td></td>
<td>TDT cell I</td>
<td>S. Enteritidis (G3, MD4, UK1, G2, MD9 and P97)</td>
<td>Wheat flour (( \alpha_a = 0.45 ))</td>
<td>( D )-values of biofilm former Salmonella (14.1 ± 0.6 min) were significantly (( p &lt; 0.05 )) higher than that of non-formers (6.0 ± 0.2 min)</td>
<td>Villa-Rojas, Zhu, Paul, et al. (2017)</td>
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<tr>
<td></td>
<td>TDT cell I</td>
<td>E. faecium NRRL B-2354, S. Agona, S. Montevideo, S. Tennessee, and S. Typhimurium</td>
<td>Paprika, white pepper and cumin powder (( \alpha_a = 0.45 ) ± 0.05 at, 25 (^\circ)C)</td>
<td>( D )-values of Salmonella and E. faecium were 1.2 and 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</td>
<td>Ozturk et al. (2020)</td>
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<td>TDT cell II</td>
<td>E. faecium NRRL B-2354, and S. Enteritidis PT30</td>
<td>Brown rice flour (( \alpha_a = 0.45 ))</td>
<td>The ( D )-values in the ( \alpha_a ) of 0.45 at 70, 75, 80, and 85 (^\circ)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</td>
<td>Jin and Tang (2019a)</td>
</tr>
<tr>
<td></td>
<td>TDT cell II</td>
<td>S. Enteritidis PT30</td>
<td>Soy protein powder (( \alpha_a = 0.13-0.82 ))</td>
<td>( D )-values at ( \alpha_a ) of 0.25–0.70 showed a semi-log relationship under each treatment temperature level from 70 to 99 (^\circ)C</td>
<td>Jin et al. (2020)</td>
</tr>
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<td></td>
<td>TDT cell (NRC, Lausanne, Switzerland)</td>
<td>E. coli O157:H7 Sakai, O157:H7 SEA13888, O157:H7 F902, and six major non-O157 serotypes (O26, O45, O103, O111, O121, and O145)</td>
<td>Chicken meat powder (( \alpha_a = 0.26 )), confectionery (( \alpha_a = 0.43 )), pet food dry mix (( \alpha_a = 0.55 )), seasoning (( \alpha_a = 0.63 ))</td>
<td>Heated temperatures to achieve 5-log reductions were 90.9, 92.7, 99.1, and 106.0 (^\circ)C for pet food, savory seasoning, confectionery formulations, and chicken meat powder, respectively</td>
<td>Daryasi et al. (2018)</td>
</tr>
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<td></td>
<td>Whirl-Pak bags put in sandwich magnetic copper plates</td>
<td>S. Tennessee S010 1P, S13952 (782), S13972 (784), FSL RB-5221, and E. faecium NRRL B-2354</td>
<td>Peanut paste (( \alpha_a = 0.60 ))</td>
<td>( D )-values of E. faecium (3.4 min) was significantly (( p &lt; 0.05 )) larger than that of Salmonella (1.1 min)</td>
<td>Enache et al. (2015)</td>
</tr>
</tbody>
</table>

**Controlled system test methods**

| Controlled relative | Thermal-\( \alpha_a \) cell (TAC) | Organic wheat flour (\( \alpha_a = 0.45 \) at 20 \(^\circ\)C, increased to 0.73 at 80 \(^\circ\)C) | Not provided | Tadapaneni et al. (2017) |

(continued on next page)
Table 3 (continued)

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<td>humidity (RH) methods</td>
<td>TAC I (RH 18%-72%)</td>
<td>S. Enteritidis PT30 and E. faecium NRRL B-2354</td>
<td>Inoculated SiO₂ (α₀ &lt; 0.1)</td>
<td>Dₐ₀, C-values of Salmonella and E. faecium exponentially increased with reduced α₀ levels. α₀-values (The change in α₀ needed to change Dₐ₀, C by 1 log) of S. Enteritidis and E. faecium were 0.31 and 0.28, respectively</td>
<td>Liu, Tang, et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>TAC II (RH 50%)</td>
<td>S. Enteritidis PT30</td>
<td>Wheat flour (α₀ = 0.45 at 20 °C)</td>
<td>D-value of Salmonella at 80 °C tested with TAC II (20.7 ± 1.1 min) was significantly higher (p &lt; 0.05) than the result obtained from TAC (7.5 ± 0.7 min)</td>
<td>Tadapaneni et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>TAC II (RH, 20%-80%)</td>
<td>S. Enteritidis PT30</td>
<td>Wheat flour, Almond flour, and Whey protein (α₀ = 0.25, 0.45, 0.60, and 0.80 at 20 °C)</td>
<td>D- and z-values of E. coli ATCC 25922 under the controlled atmosphere (2% O₂/20% CO₂) treatments were significantly (p &lt; 0.05) lower than those under the regular atmosphere treatments</td>
<td>Xu et al. (2019)</td>
</tr>
<tr>
<td>Controlled heating block system (HBS) methods</td>
<td>Controlled atmosphere heating block system (CA-HBS, Range of O₂/CO₂ concentrations 0-25%)</td>
<td>E. coli ATCC 25922</td>
<td>Almond powder (MC of 6% w.b.)</td>
<td></td>
<td>Cheng et al. (2017)</td>
</tr>
<tr>
<td></td>
<td>TDT-HBS (Maximum set-point temperature 120 °C, heating rates 0.1-13.3 °C/min)</td>
<td>E. coli ATCC 25922</td>
<td>Almonds powder (MC of 6.0% w.b.)</td>
<td>D-values were similar when heating rates above from 1 °C/min, but significantly increased at lower heating rates</td>
<td>Kou et al. (2016)</td>
</tr>
<tr>
<td></td>
<td>TDT-HBS (Set-point temperature 120 °C, heating rates 5 °C/min)</td>
<td>E. coli ATCC 25922</td>
<td>Almond powder (MC 6% of w.b.)</td>
<td>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</td>
<td>Cheng and Wang (2018)</td>
</tr>
<tr>
<td></td>
<td>TDT-HBS (62, 65, and 68 °C, heating rates 5 °C/min)</td>
<td>S. Typhimurium ATCC 50115, S. Braenderup H9812, and E. coli ATCC 25922</td>
<td>Red pepper powder (α₀ = 0.55, 0.76 and 0.86)</td>
<td>Log reduction levels with three given particle sizes (0.45-1.00, 0.20-0.45 and &lt; 0.20 mm) were 1.45, 1.67, 2.24 log CFU/g, respectively</td>
<td>Zhang, Zhang, et al. (2020)</td>
</tr>
<tr>
<td></td>
<td>TDT-HBS (56-70 °C, heating rates 5 °C/min)</td>
<td>S. H9812, E. coli ATCC 25922 and S. aureus ATCC 25923</td>
<td>Walnut shells (MC of 8.93%-18.10%)</td>
<td>D-value at 64 °C was 10.2 ± 0.7 min with α₀ at 0.59, but was reduced to 3.9 ± 0.2, 1.8 ± 0.17 and 0.6 ± 0.0 min at α₀ = 0.73, 0.84 and 0.93</td>
<td>Zhang et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>TDT Sandwich (70, 90, and 110 °C, heating rates 25, 50, and approximately 100 °C/min)</td>
<td>Not provided</td>
<td>Whole milk powder</td>
<td></td>
<td>Lau and Subbiah (2020)</td>
</tr>
</tbody>
</table>

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/infusion into samples leading to changes of the α₀ 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:

\[
\log \frac{N_t}{N_0} = -t / D_T
\]  
\[
z = \frac{T_2 - T_1}{\log D_{T_2} - \log D_{T_1}}
\]

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 upward and downward concavities (Cheng & Wang, 2018; Villa-Rojas et al., 2013). equation (4) is used to describe the Weibull model (Peleg, 2006):

\[
\log \frac{N_t}{N_0} = \log S(t) = -a(T)e^{b(T)}
\]  

where \(t\) (min) is a treatment time. \(S(t)\) is the momentary logarithmic
survival ratio. $T$ ($^\circ$C) is the treatment temperature. $\alpha(T)$ and $\beta(T)$ represent scale and shape parameters, which are temperature-dependent coefficients. When the shape parameter $\beta(T) < 1$, 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 $\beta(T) > 1$, 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 $\beta(T) = 1$, 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 ($^\circ$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:

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

where $A_1$ and $A_2$ are the initial populations of foodborne pathogens/surrogates in the first and second phases, respectively. $\lambda_1$ and $\lambda_2$ 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 $-\lambda_1$ to $-\lambda_2$ 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:

$$D(T, a_w) = D_{ref} \cdot 10^{a_w - a_{w,ref} \cdot Z_T}$$

$$Z_{aw} = \frac{a_{w,ref} - a_w}{\log D_1 - \log D_2}$$

where $T_{ref}$ is the reference temperature ($^\circ$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 $D_{aw}$ (Alshammari, Xu, Tang, Sabiani, & 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).
\[
\frac{d \log S(t)}{dt} = -\frac{\alpha(T(t))}{\beta(T(t))} \left( \frac{-\log S(t)}{\alpha(T(t))} \right)^{\alpha(T(t))/\beta(T(t))} \tag{8}
\]

In equation (8), temperature is a function of time, expressed as \( T(t) \). The parameter \( \beta \) 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 \( \alpha \) 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:

\[
\alpha(T) = \ln \left( 1 + \exp \left[ k(T - T_c) \right] \right) \tag{9}
\]

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

$$ R^2 = \frac{\sum (\text{predicted value} - \text{average})^2}{\sum (\text{measured value} - \text{average})^2} $$  \hspace{1cm} (10)

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

$$ A_f = 10 \sum \log(\text{predicted value} / \text{measured value}) / n $$  \hspace{1cm} (12)

$$ B_f = 10 \sum \log(\text{predicted value} / \text{measured value}) / n $$  \hspace{1cm} (13)

$$ AICc = n \ln \left( \frac{SS}{n} \right) + 2K + \frac{2K(K+1)}{n-K-1} $$  \hspace{1cm} (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):

$$ F = \int_0^T 10^{(T(t)-T_{ref})/z} \, dt $$  \hspace{1cm} (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
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 Enterococcus faecium 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 ($\lambda$) 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, et al., 2018) and in-shell walnuts (Zhang et al., 2019).

$$\lambda = \sqrt{\frac{\sigma^2 - \sigma_0^2}{\mu - \mu_0}}$$  \hspace{1cm} (16)

where $\sigma_0$ and $\sigma$ are initial and final standard deviations ($^\circ$C) of temperature in LMFs over RF heating time. $\mu_0$ and $\mu$ are initial and final mean temperatures ($^\circ$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.
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 non-pathogenic 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, Irnich, 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 NRR L-2354* has been mostly used as non-pathogenic surrogate for *Salmonella Enteritidis* PT30 in LMFs, such as almonds (ABC, 2007). Except for *E. faecium NRR L-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, 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. Novel RF heating has been successfully applied in pasteurization of LMFs, and shown great potential in industrial pasteurization processing. Heating uniformity, survival of pathogens/surrogates at cold spot, and strict safety requirements need to be considered when conducting RF microbial validation studies.
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. Sensory 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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References


Table 5

<table>
<thead>
<tr>
<th>Nonpathogenic surrogates used in RF treatments for foodborne pathogens in LMFS.</th>
<th>Surrogates</th>
<th>LMFS</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Agona, S. Montevideo, S. Tennessee, and S. Typhimurium</td>
<td><em>E. faecium</em> NRRL B-2354</td>
<td>Paprika, white pepper and cumin powder</td>
<td>The $D_{F_{-}}$ values of <em>E. faecium</em> were larger than that of Salmonella spp. in three given LMFS</td>
</tr>
<tr>
<td>S. Agona 447967, S. Reading Moff 180418, S. Tennessee K4643, S. Montevideo 488275, S. Mbandaka 698538</td>
<td><em>E. faecium</em> NRRL B-2354</td>
<td>Cumin seed</td>
<td><em>E. faecium</em> consistently showed lower log reductions than Salmonella spp. under RF treatments with same conditions</td>
</tr>
<tr>
<td>S. Agona 447967, S. Reading Moff 180418, S. Tennessee K4643, S. Montevideo 488275, S. Mbandaka 698538</td>
<td><em>E. faecium</em> NRRL B-2354</td>
<td>Egg white powder</td>
<td>Microbial Reduction (log CFU/g) of Salmonella spp. was significantly ($p &lt; 0.05$) larger than that of <em>E. faecium</em> at the same location</td>
</tr>
<tr>
<td>S. Agona 447967, S. Reading Moff 180418, S. Tennessee K4643, S. Montevideo 488275, S. Mbandaka 698538</td>
<td><em>E. faecium</em> NRRL B-2354</td>
<td>Ground black pepper</td>
<td>$D_{F_{-}}$ value of <em>E. coli</em> ATCC 25922 (7.0 ± 0.8 min) was larger than that of <em>S. Enteritidis</em> PT 30 (1.63 min) at the same $a_w$ level</td>
</tr>
<tr>
<td>S. Enteritidis PT 30</td>
<td><em>E. coli</em> ATCC 25922</td>
<td>Almond kernel</td>
<td>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 <em>S. Enteritidis</em> and <em>E. faecium</em>, respectively</td>
</tr>
<tr>
<td>S. Enteritidis PT 30</td>
<td><em>E. coli</em> ATCC 25922</td>
<td>In-shell almond</td>
<td>$D_{F_{-}}$ value of <em>E. coli</em> ATCC 25922 (9.95 ± 0.02 min) was larger than RF heated at 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 <em>S. Enteritidis</em> and <em>E. faecium</em>, respectively</td>
</tr>
<tr>
<td>S. Enteritidis PT 30</td>
<td><em>E. coli</em> ATCC 25922</td>
<td>Wheat flour</td>
<td>E. faecium had significantly higher ($p &lt; 0.05$) $D$-values compared to <em>S. Enteritidis</em> at three given temperatures</td>
</tr>
<tr>
<td>S. Enteritidis PT 30</td>
<td><em>E. coli</em> ATCC 25922</td>
<td>Corn flour</td>
<td>$D_{F_{-}}$ value of Freeze-dried <em>E. faecium</em> B-2354 (FDE) was 5.92 ± 0.39 min larger than at 4.3 ± 0.2 min for the liquid-form <em>S. enteritidis</em> PT 30 under the same treatment condition</td>
</tr>
<tr>
<td>S. Enteritidis PT 30</td>
<td><em>E. coli</em> ATCC 25922</td>
<td>Wheat flour</td>
<td>The average reductions of <em>S. aureus</em> ATCC 25923 were lower than those of Salmonella HB9182 under the same heating treatments</td>
</tr>
<tr>
<td>S. Enteritidis PT 30</td>
<td><em>E. coli</em> ATCC 25922</td>
<td>In-shell walnut</td>
<td>The average reductions of <em>S. aureus</em> ATCC 25923 were lower than those of Salmonella HB9182 under the same heating treatments</td>
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