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Mathematical modeling and Monte Carlo simulation of thermal inactivation of non-proteolytic *Clostridium botulinum* spores during continuous microwave-assisted pasteurization☆

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ABSTRACT

The objective of this study was to develop a mathematical method to predict the internal temperature history of products exposed to a microwave-assisted pasteurization system and use Monte Carlo simulation to analyze the inactivation of the spores of *Clostridium botulinum* Types B in beef meatball trays and Type E in salmon fillet trays. With a target of 6 log-reduction in the spores, the simulation showed >98.8% and 99.1% of the processes will achieve a minimum of 5-log reductions of *C. botulinum* Type B in beef meatball trays and Type E in salmon fillet trays, respectively. Sensitivity analysis showed that the heating temperature, time, and product heating rate in the Microwave-Assisted Heating section and the heating temperature in the Pre-Heating section are four most critical factors affecting the accumulation of lethality. The results of this study may be used to guide the development of more effective thermal processes in microwave-assisted pasteurization systems.

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1. Introduction

Most raw food materials require some processing and preparation to inactivate or eliminate microbial contaminants prior to human consumption. Some foodborne pathogens, such as *Clostridium botulinum*, which can produce deadly neurotoxins and causes botulism (CDC, 1998; 2004), must be destroyed or inhibited in products such as minimally-heated, chilled foods that are packaged under reduced oxygen conditions before they can be distributed (Peck, 2006). Non-proteolytic *C. botulinum* (Types B and E), for example, is a potential hazard in minimally processed food products such as *sous-vide* products and other pasteurized and refrigerated products (Juneja et al., 1995). Non-proteolytic *C. botulinum* type E, a naturally occurring marine microorganism that can grow at refrigeration temperatures (Aberoumand, 2010), is often associated with various types of seafood. Its occurrence in fish and fishery products deserves a worldwide attention (FAO, 2001). In the U.S., most seafood-associated cases of *C. botulinum* infections are caused by *C. botulinum* Type E, which is the second most commonly reported bacterial pathogen causing seafood-associated outbreaks (Iwamoto et al., 2010). According to CDC (1998), 61% of the 67 outbreaks of botulism Type E reported from 1950 through 1996 have been traced to marine products (fish or marine mammals). Overall, an average of 28 cases of foodborne botulism is annually reported in the U.S. (CDC, 2006).

Foodborne botulism is rare. However, *C. botulinum* can present a serious public health hazard due to the severity of infections and the ability of non-proteolytic *C. botulinum* spores to germinate and grow at refrigerated temperatures (Grecz and Arvay, 1982). Food manufacturers must adopt intervention measures such as thermal processing to pasteurize products before they can be shipped to consumers. For *C. botulinum* Type E or non-proteolytic Types B and F in food, a 6D thermal process is required to prevent foodborne

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botulinum (FDA, 2011). Conventional thermal processing methods often use hot water or steam as the heating medium to inactivate foodborne pathogens and to extend the shelf-life of products. The long cooking time during conventional heating may lead to undesirable changes in product qualities. Microwave-assisted thermal processing may provide more uniform and rapid volumetric heating (Ohlsson, 1991; Ohlsson and Bengtsson, 2002; Tang et al., 2008). Microwave-assisted water immersion heating process has been used to sterilize sliced beef in 7-oz. trays (Tang et al., 2008). Microwave-assisted pasteurization system (MAPS) is a new pasteurization technology that uses microwave heating in combination with hot water immersion in producing higher quality ready-to-eat chilled prepared meals. This technology is being developed by Washington State University.

Many factors, including the initial temperature of a product, heating time and temperature, and heat transfer rates, may affect the total lethality in a thermal process. Monte Carlo simulation can be used to evaluate the uncertainties of food safety and quality estimations associated with process variations (Chotyakul et al., 2011) and to analyze the impact of input data variability on estimations of the equivalent constant temperature time for microbial inactivation during HTST and retort processing (Salgado et al., 2011). MAPS is a new continuous thermal processing technology. Therefore, the objective of this research is to develop a mathematical method to simulate and predict the internal temperature history of products processed in a prototype MAPS, and conduct a probabilistic analysis using Monte Carlo simulation to evaluate the effect of variations in process parameters on cumulative lethality during thermal processing. The goal of this research is to identify critical factors affecting the effectiveness of MAPS in inactivating the spores of *C. botulinum* in packaged foods.

2. Materials & methods

2.1. Brief description of MAPS

Microwave-assisted pasteurization is a new technology currently under development in Washington State University (WSU). It differs from a previous microwave-assisted pressurized sterilization system (Eves et al., 2004; Tang et al., 2008), and is designed for pasteurization of packaged foods at temperatures below the boiling point of water under atmospheric pressure. A more detailed description and documentation of MAPS is beyond the scope of this work. However, Fig. 1 illustrates a sketch diagram of a 15-kW, 915 MHz pilot-scale MAPS. Basically, this MAPS system can be divided into three operating sections. The first section is the Pre-Heating section, which is used to load and heat the packaged food products in a hot water tunnel to an elevated temperature. The second section is the microwave-assisted heating (MAH) section, in which a pulse of microwave energy is introduced to heat the products in combination with hot water immersion and stabilization in a tunnel to further increase the internal temperature to a target final heating temperature. The final section is the cooling section, where the pasteurized products are cooled. In a continuous process, the products are transported from one section to another by a conveyor. The residence time, or the transit time in each section is controlled by the speed of the conveyor. Water is used in each section for heat transfer. The heating and cooling water temperature, held constant in each section, is independently controlled. During a MAPS process, the products pass through each section sequentially for heating and cooling.

2.2. Products and microorganisms of concern

Two products were evaluated in this study. The first product was 10 oz. beef meatball in tomato sauce trays. The second product was 16 oz. salmon fillet in sauce trays. All products were vacuum-packaged prior to thermal processing. For beef meatball trays, non-proteolytic *C. botulinum* Types B spores were considered. For salmon fillet trays, *C. botulinum* Type E spores were evaluated. At the temperatures of interest (70–100 °C), there are no reported D and z values for *C. botulinum* Types B spores in beef. One study reported the D and z values of *C. botulinum* Types B and E spores in turkey slurry (Juneja et al., 1995). The calculated D_{90} and z values in turkey slurry are 1.05 min and 9.38 °C for *C. botulinum* Type B spores, and 0.60 min and 9.88 °C for *C. botulinum* Type E spores, respectively (Table 1). Since the spores of *C. botulinum* Type B were more heat-resistant than those of *C. botulinum* Type E in meat, *C. botulinum* Type B was chosen as the target microorganism for thermal processing in beef meatball trays. The D and z values of *C. botulinum* Type B in turkey slurry were used to represent the D and z values of the spores in beef in this study.

Similarly, there are no thermal resistance data for *C. botulinum* Type E spores in salmon fillets at temperatures between 70 and 100 °C. However, Gaze and Brown (1990) reported the thermal resistance of *C. botulinum* Type E spores in cod. The thermal resistance of *C. botulinum* Type E spores in cod reported by Gaze and Brown (1990) were higher than other values reported in the literature (Silva and Gibbs, 2010) and therefore, were chosen for this study. The calculated D_{90} and z values were 0.92 min and 8.18 °C for *C. botulinum* Type E spores in cod (Table 1).

2.3. Determination of heat transfer parameters

To evaluate the cumulative lethality of a product, it is necessary to use the temperature history at the lowest heating point, or cold spot in a package. The cold spot of the packages during heating was

Table 1
Thermal resistance of *C. botulinum* Type B and Type E spores.

Type/substrate	D_{90} (min)	z (°C)
B[a]/turkey	1.052	9.391
E[a]/turkey	0.599	9.881
E[b]/cod	0.921	8.177

[a] Determined from Juneja et al. (1995).
[b] Determined from Gaze and Brown (1990).

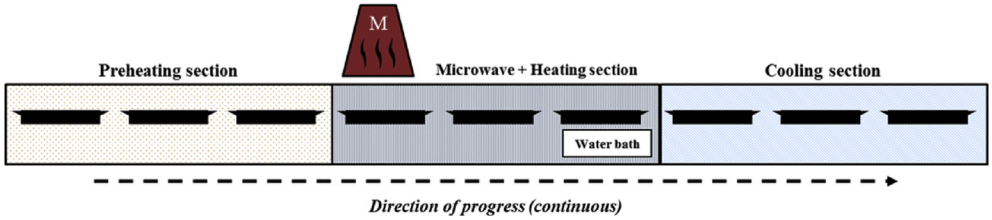

Fig. 1. Schematic diagram of microwave-assisted pasteurization system (MAPS).

determined using the methodology described by (Pandit et al., 2007; Tang and Liu, 2015). The cold spot temperature histories of products were obtained by recording the internal temperature of the products (Tang and Liu, 2012), and were used to determine the overall heat transfer rates during heating and cooling. Overall, the temperature curve in each section of the MAPS showed an exponential trend with an initial delay. Denoting t as the total time spent (or residence time, min) in each section (heating or cooling), and t_{lag} as the initial lag time (min), the temperature of the cold spot in each section can be described by

$$T = T_a + (T_i - T_a)e^{-k(t-t_{lag})}, for\ t > t_{lag}$$
$$T = T_i, for\ t \le t_{lag} \quad (1)$$

In Eq. (1), T is the internal temperature measured at the cold spot in a package during an MAPS process (°C); T_a is the ambient temperature of the heating or cooling medium (°C); T_i is the initial temperature of the product at each section of the pre-heating and cooling sections (°C); and k is the rate of heat transfer in the Pre-Heating, MAH, or cooling section (min^{-1}). Eq. (1) describes a heating or cooling process that the temperature of cold spot increases exponentially after an initial lag period. This equation is mathematically equivalent to the Ball formula (Eq. (2)) (Lund, 1975) during heating or cooling.

$$\frac{T_a - T}{T_a - T_i} = j_p e^{-\frac{2.303t}{f_p}} \quad (2)$$

In Eq. (2), the subscript p can be either h (heating) or c (cooling). The parameter f_p (min) is the heating rate factor (f_h) or cooling rate factor (f_c), and is equal to 2.303/k. The parameter j is a dimensionless heating (j_h) or cooling (j_c) lag factor, which is defined by Eq. (3). In Eq. (3), T_{ip} is the pseudo initial product temperature, which is determined by linearizing the heat penetration curve (heating or cooling). The parameter j_p (j_h or j_c) is a dimensionless parameter. It can be expressed as a function of heating/cooling rate (k) and lag time (t_{lag}) (Eq. (4)). Compared with j_p, t_{lag} is a parameter that can be directly estimated from Eq. (1) and is more intuitive to use. Therefore, Eq. (1) was used to simulate the heating and cooling temperature history in MAPS processes. To obtain the heat transfer parameters in each section (k and t_{lag}), the entire temperature history curve of each product was directly analyzed by nonlinear regression, performed using the nlin procedure in SAS® (Version 9.4, SAS Institute Inc., Cary, NC). The standard errors of each parameter were estimated by the nlin procedure. During thermal processing, the effect of mass of the package is reflected in k and t_{lag}.

$$j_p = \frac{T_a - T_{ip}}{T_a - T_i} \quad (3)$$

$$j_p = e^{k \times t_{lag}} \quad (4)$$

Thermal pasteurization studies were conducted to collect the product temperature histories for use to determine the k and t_{lag} values in each section. Table 2 lists two examples of processing conditions for collecting the temperature histories. Once the k and t_{lag} parameters of all three sections were obtained, the internal temperature histories were predicted using Eq. (1), which is a time-delayed exponential model for the internal temperature history in the products. These parameters were then validated using an arbitrarily chosen temperature history of a beef meat ball tray to verify the accuracy of Eq. (1).

2.4. Thermal process lethality

The predicted and measured temperature histories were used to calculate the cumulative lethality using the General method (Biglow et al., 1920). The cumulative lethality (or Log reduction, LRD) was calculated by

$$LRD = \frac{1}{D_{ref}} \int_0^t 10^{\frac{T - T_{ref}}{z}} dt \quad (5)$$

In Eq. (5), T is the cold spot temperature; D_{ref} is the D-value at the reference temperature T_{ref}, and z is a thermal resistance coefficient characterizing the effect of temperature on D values. The coefficient z is the increase in temperature needed to reduce the D value of a microorganism by 90%. T_{ref} was chosen at 90 °C.

2.5. Probabilistic process analysis – Monte Carlo simulation

The cumulative lethality achieved in a thermal process is affected by variations in the process parameters in each step of the MAPS. Once the heat transfer parameters were obtained and validated, Monte Carlo simulation (Sokolowski, 2010) was used to conduct a probabilistic analysis of the effect of the variations in the process parameters on the total lethality in the products (Chotyakul et al., 2011; Poschet et al., 2003; Salgado et al., 2011). In thermal processing of foods, the time and temperature used to kill *C. botulinum* spore are often precisely controlled to prevent inadequate cooking. Therefore, the objective of Monte Carlo simulation in this study was to evaluate the effect of small changes in the process parameters on the distribution of the total lethality that can be achieved during thermal processing. For Monte Carlo simulations, the goal of thermal pasteurization was set to achieve an average 6-log (6D) reduction in the spores of *C. botulinum* in the products. Table 3 lists the assumptions of the values and distributions in Monte Carlo simulation. During Monte Carlos simulation, the heat transfer parameters in Eq. (1) and the residence time in each section were treated as random variables.

The heat transfer parameters, including k and t_{lag} from each section, were used in combination with the process parameters listed in Table 3 during Monte Carlo simulation. Each heat transfer parameter (k and t_{lag}) was assumed to follow a normal distribution, with its mean equal to the value of each parameter and a standard deviation of 1% of the mean. The heating or cooling temperature in each section was also assumed to follow a normal distribution

Table 2
Processing parameters for Fig. 2.

Parameters	10 oz. beef meatball trays	16 oz. salmon fillet trays
Initial temperature (°C), product	4.97	20.49
Pre-heat time (min)	35.0	35.0
Pre-heat temperature (°C), hot water	61.0	61.1
MAH time (min)	14.33	11.93
MAH temperature (°C), hot water	93.0	93.0
Cooling temperature (°C), cooling water	23.0	23.0

Table 3
Distribution of processing parameters used to simulate the lethality of *Clostridium botulinum* at reference process conditions of the 10 oz. beef meatball with tomato sauce.

Parameters	Distribution/values	Sources/note
Initial temperature of products	Uniform(2, 8)[a]	Assumed values[c]
Preheating section		
Preheating temperature (°C)	Normal(61, 0.6)[b]	Measured values[d]
Preheating time (min)	Uniform(34.83, 35.17)	Assumed values
Microwave assisted heating section		
Heating temperature (°C)	Normal(93, 0.21)	Measured values
Heating time (min), beef	Uniform(15.08, 15.42)	Assumed values[e]
Heating time (min), salmon	Uniform(10.13, 10.47)	Assumed values[e]
Cooling section		
Cooling temperature (°C)	Normal(20, 0.9)	Assumed values
Cooling time (min)	Uniform(29.83, 30.17)	Assumed values

[a] Uniform(a, b): uniform distribution with values ranging from a and b.
[b] Normal distribution(a, b): normal distribution with mean a and standard deviation b.
[c] Assumed values: hypothetical values taken by assumption.
[d] Measured values: these values were obtained by experimental measurements.
[e] These values were assumed to achieve a 6 log-reduction in the spores of *C. botulinum*.

using measured values. The residence time (heating or cooling) in each section was assumed to follow a uniform distribution with a mean and ±10 s, i.e., the residence time may vary within a 20 s window. This was a reasonable assumption as the speed of the conveyor was properly controlled. The residence time in the MAH section was adjusted to achieve a 6 log-reduction of *C. botulinum* spores after thermal processing. The cooling temperature and time were changed to 20 °C and 30 min. The initial temperature of product was set to change in a wider range, following a uniform distribution between 2 and 8 °C. The assumption of uniform distribution allowed a parameter to take a random value between the lower and upper limits with equal opportunity.

Monte Carlo simulation started with generation of a random number for each parameter listed in Table 3 for an MAPS process. For each set of random numbers, a time-temperature history was calculated, which was used to calculate the total lethality achieved during cooking. This process was repeated multiple times and the distribution of the total lethality for the spores of *C. botulinum* Type B in beef meatball trays and Type E in salmon fillet trays was obtained. In this study, Monte Carlo simulation was conducted using a commercial software tool, @Rick 7.0 (Palisade Corporation, NY). The simulation was iterated 10,000 times, and the results were analyzed using the Latin Hypercube Sampling (LHS) procedure.

3. Results & discussion

3.1. Determination of heat transfer coefficients and calculation of thermal lethality

The nonlinear regression with Eq. (1) converged easily to minimize the sum of squared errors between the measured and predicted temperatures over of the entire temperature curve, producing estimates of the heat transfer parameters for each section (Table 4). The lag time in the MAH section for beef meatball trays was negative, which did not agree with the physical process. Therefore, a zero value was assigned in Table 4. The root mean square error (RMSE) of the predicted temperature curves was 0.76 °C for beef meatball trays and only 0.44 °C for salmon fillet trays. The pseudo-R^2 of the predicted temperature curve was >0.999 for both products. Since the Pre-Heating and cooling sections are governed by pure heat conduction, the heat transfer coefficients (k) in these two sections should be very close to each other, as this parameter is determined by the physical properties of the products. The results from nonlinear regression analysis showed the heating and cooling rates were indeed very close to each other in both products (Table 4).

Table 4
Estimates of heat transfer parameters in each section.

Parameter	Beef meatball	Salmon fillet
Pre-heating section		
K (min^{-1})	8.57e-2[a] (2.21e-4)[b]	0.191 (5.67e-4)
t_{lag} (min)	2.20 (2.04e-2)	1.72 (1.10e-2)
MAH section		
K (min^{-1})	0.128 (7.33e-4)	0.382 (2.16e-3)
t_{lag} (min)	0	1.51 (1.04e-2)
Cooling section		
K (min^{-1})	8.99e-2 (2.12e-4)	0.185 (5.84e-4)
t_{lag} (min)	1.94 (1.70e-2)	1.62 (7.97e-4)

[a] Estimate from nonlinear regression.
[b] Approximate standard error.

The product heating rate was 49.4% higher in the MAH section for 10 oz. beef meatball and 100% higher in 16 oz. salmon fillet trays, suggesting that microwave heating indeed enhanced the heat transfer in the MAH section of the MAPS. The difference in the k values of salmon fillet and beef meatball trays apparently resulted from the difference in product dimension (mainly thickness) and weight, composition and amount of sauces used, surface area, and dielectric properties of products.

With parameters in Table 3, the temperature history in each product could be predicted and used to calculate the lethality accumulated throughout the heating and cooling processes. For beef meatball, the total lethality calculated from measured temperature history was 4.51 log-reductions, while the total lethality calculated from predicted temperature history was 5.68 log-reductions. For salmon fillet, the total lethality calculated from measured temperature history was 7.97 log-reductions, while the total lethality calculated from predicted temperature history was 9.06 log-reductions. Therefore, the lethality calculated from predicted temperature histories was about 1 log-reduction higher than the lethality calculated from measured temperature histories. To accommodate for this difference, an additional 0.7 min of lag time was added to the MAH section for each product. After this adjustment, the lethality calculated from predicted temperature was 4.87 log-reductions for beef meatball and 7.94 log-reductions for salmon fillet. The difference in lethality between the predicted and measured temperature histories was below 0.4 log-reductions, which is reasonably accurate. With additional 0.7 min t_{lag} in the MAH section, Fig. 2 shows the comparison between the predicted and measured temperature histories for both products. After the adjustment in the lag time of the MAH section, the RMSE was increased to 1.6 °C for beef meatball and 1.4 °C for salmon fillet for the entire temperature curve. The increased RMSE was primarily caused by the increased difference between the predicted and measured temperature in the early stage of the MAH section in each product. However, the adjustment in the lag time was necessary to improve the accuracy in the estimation of the lethality in predicted temperature curves.

As a validation, the heat transfer parameters (k and lag time) estimated from beef meatball trays were used to simulate the temperature history of the same product, but with different heating times in each section and a different initial temperature. Fig. 3 shows the comparison of the measured and predicted temperature histories for this package. In Fig. 3, a 0.7 min lag time was also added to the MAH section. The RMSE of the predicted temperature curve was also 1.6 °C, suggesting the heat transfer parameters estimated using the proposed method (Eq. (1)) is reasonably

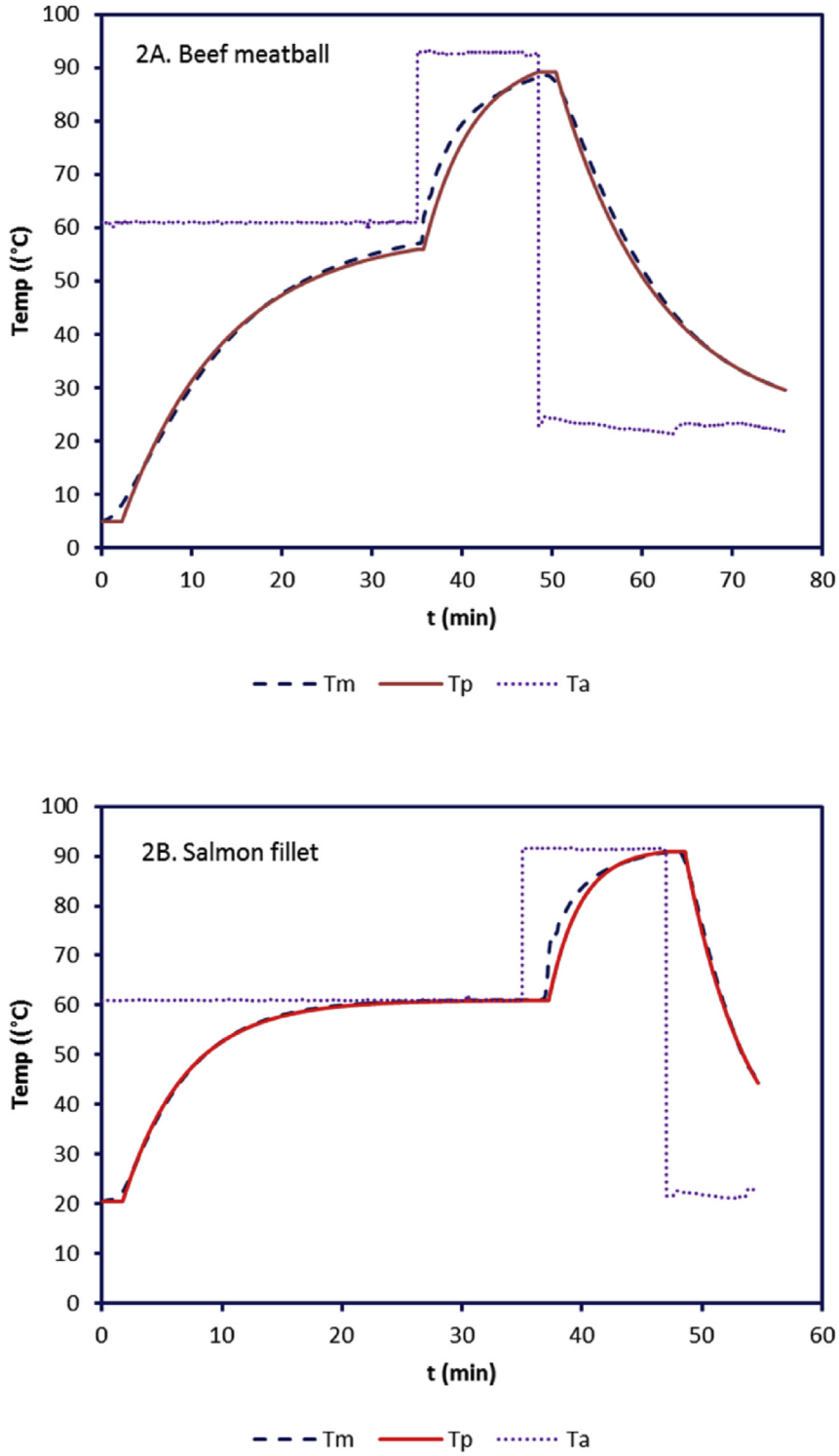

Fig. 2. The measured and predicted internal temperatures of 10 oz. beef meatball trays (A), and 16 oz. salmon fillet trays (B). T_m and T_p: measured and predicted temperature at cold spots. T_a: ambient temperature.

accurate.

Different amount of lethality is accumulated in each section (Fig. 4). The hot water temperature in the Pre-Heating section is relatively low and is not sufficient to inactivate the spores of *C. botulinum*. Therefore, a minimum amount of lethality (<0.1%) could be accumulated in the Pre-Heating section, which is not by design to achieve any meaningful lethality, but to condition the trays to an elevated temperature before MAH processing. In the MAH section, the temperature at the cold spot continues to increase and becomes lethal to the spores. Little lethality is accumulated at the early stage of the MAH section. However, the lethality begins to accumulate dramatically with increase in the product temperature. A significant amount of lethality is accumulated at the end of the MAH section. If the MAH section is sufficiently long, all spores could be

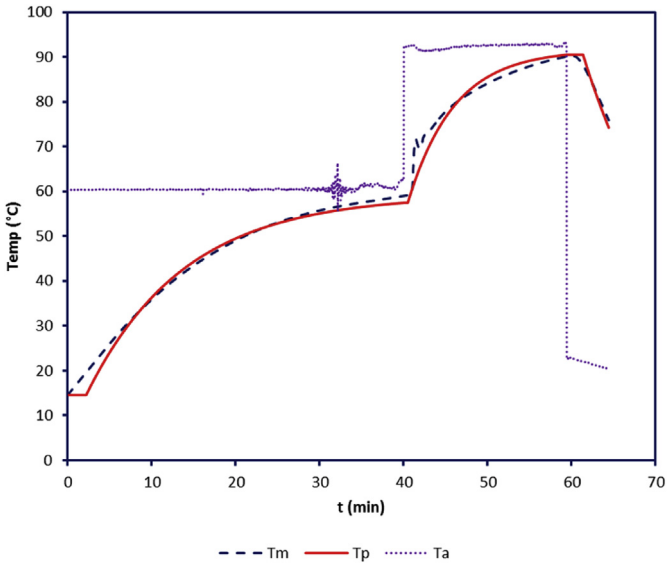

Fig. 3. Validation of temperature simulation models (beef meatball trays). T_m and T_p: measured and predicted temperature at cold spots. T_a: ambient temperature.

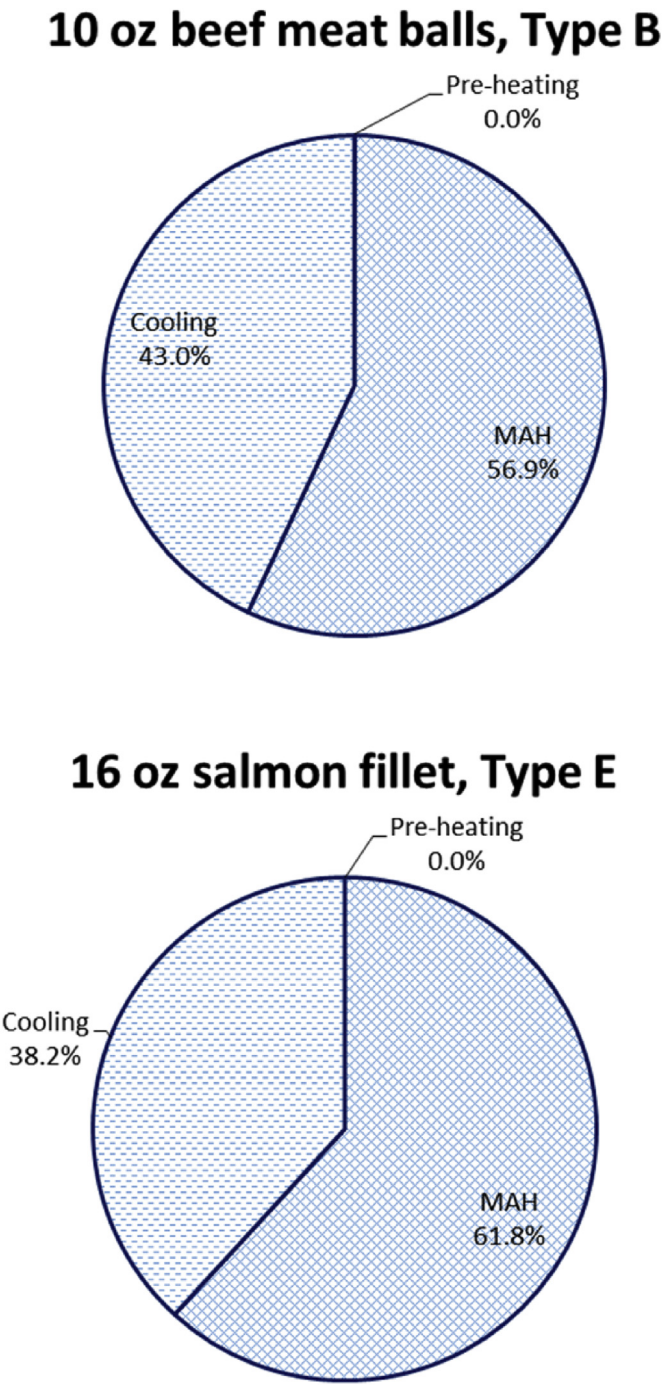

Fig. 4. Relative distribution of lethality in each section of MAPS using the temperature histories shown in Fig. 2.

inactivated. For beef meatball, about 57% of the total lethality could be achieved in the MAH section, while the remaining lethality is contributed from the cooling section. Similarly, about 62% of the total lethality could be accumulated in the MAH section for salmon fillet, while the remaining lethality comes from the cooling section. The lethality accumulated in the cooling section can be attributed to residual heat and the relatively high temperature in the products as they enter the cooling section. As a product leaves the MAH section, its internal temperature is still very high. Because it takes time (t_{lag}) for the product to respond to the change in the ambient temperature, additional lethality may be accumulated within this period. The results in Fig. 4 suggest that, while the majority of the thermal lethality can be achieved in the MAH section, the cooling section also contributes a significant portion to the total lethality. However, the relative contribution depends on the lethality accumulated in the MAH section. If more lethality is accumulated in the MAH section, the relative contribution from the cooling section may be reduced.

In a MAPS, the lethality achieved in the Pre-Heating section is basically negligible. While the MAH section contributes to the majority of the total lethality in these two examples, a significant portion of the lethality can be achieved in the cooling section. To obtain a higher quality product, it is possible to take advantage of the lethality accumulated in the cooling section, which may reduce the heating requirement in the MAH section and achieve the best quality possible in the processed produces. However, the decision to consider the lethality accumulated in the cooling section should be carefully evaluated to ensure sufficient safety margins.

3.2. Monte Carlos simulation of MAPS process

When a thermal process is established, a product is processed following a set of pre-determined conditions (residence time and heating temperature). In traditional thermal process for low acid foods in hermetically sealed containers, the most conservative condition is usually used. Although this approach may prevent botulism to the greatest extent, it may inevitably damage the quality of product. The products processed in the MAPS are not commercially sterile and require refrigeration during storage. Most of these products require heating prior to consumption. To produce the best quality of products treated in MAPS, it is not necessary to expose them to the most severe heating conditions required in processing commercially sterile products. To ensure adequate cooking, it is important to precisely control the residence time and the heating temperature in the Pre-Heating and MAH sections. However, even with the best engineering controls, the residence time and heating temperature at each section may fluctuate in a random manner, which may cause variations in the total lethality actually accumulated during thermal pasteurization using MAPS. In addition, the physical properties of food may also affect thermal processing. Monte Carlo simulation is a useful tool in identifying

the most critical factors contributing to the total lethality accumulated throughout the heating and cooling processes.

With an objective to achieve 6 log-reductions in the spores of *C. botulinum* Type B, the results of Monte Carlo simulation showed that the total lethality follows a normal distribution in 10 oz. beef meatball trays (Fig. 5A), with a mean of 5.72 and standard deviation of 0.33 in log-reductions of the spores. The minimum lethality is 4.54, and the maximum is 7.13 log-reductions. Under the conditions listed in Table 4, more than 98.8% of the process will achieve a minimum of a 5-log reduction of the spores of *C. botulinum* Type B in 10 oz. beef meatball trays. About 20.5% of the process will lead to a 6-log reduction in the spores.

For 16 oz. salmon fillet trays, the spores of *C. botulinum* Type E are the target microorganism. With a target to achieve a 6-log

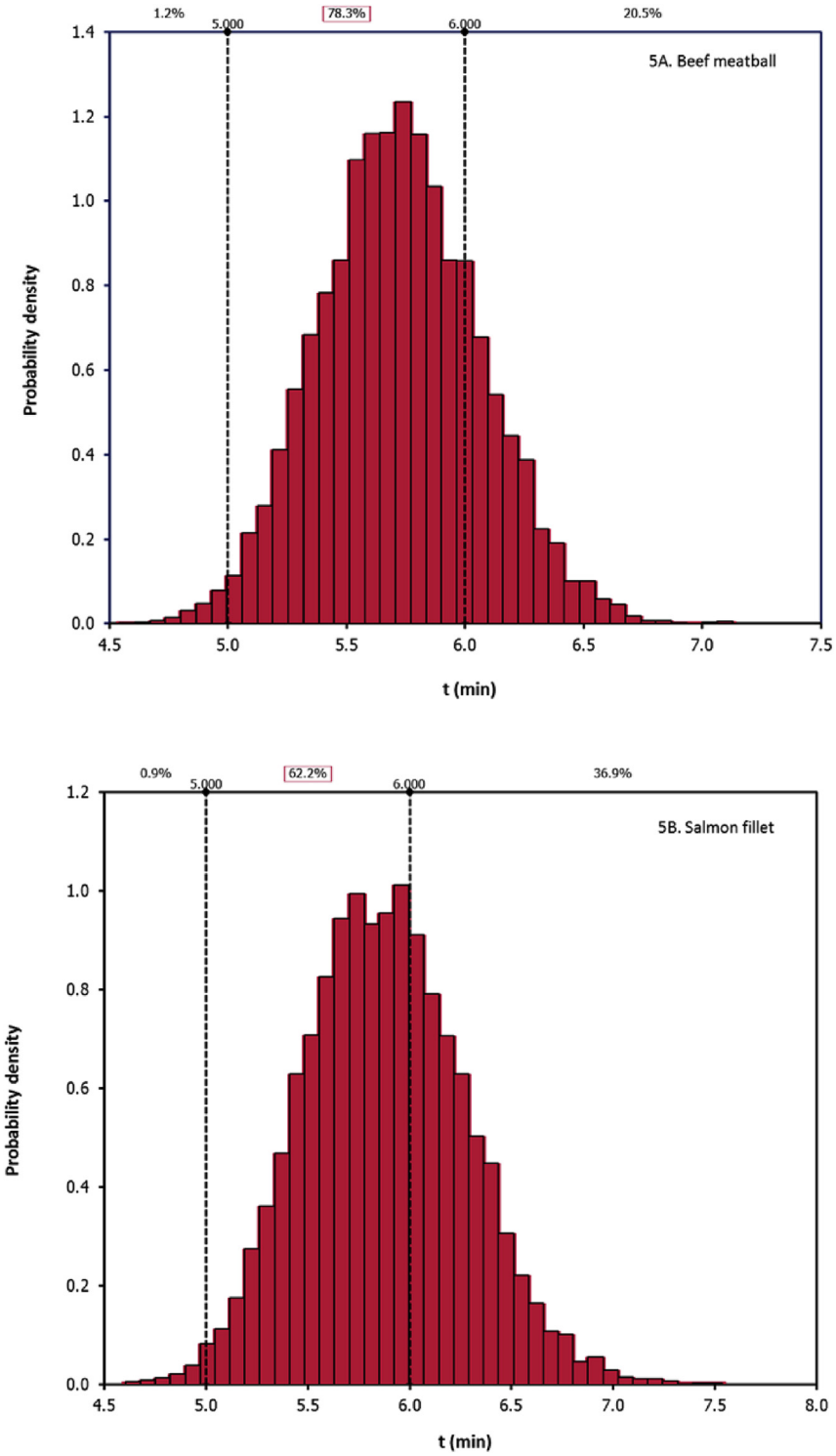

Fig. 5. Monte Carlo simulation of the total lethality achieved during microwave-assisted pasteurization. A) *C. botulinum* Type B spores in 10 oz. beef meatball trays; B) *C. botulinum* Type E spores in 16 oz. salmon fillet trays.

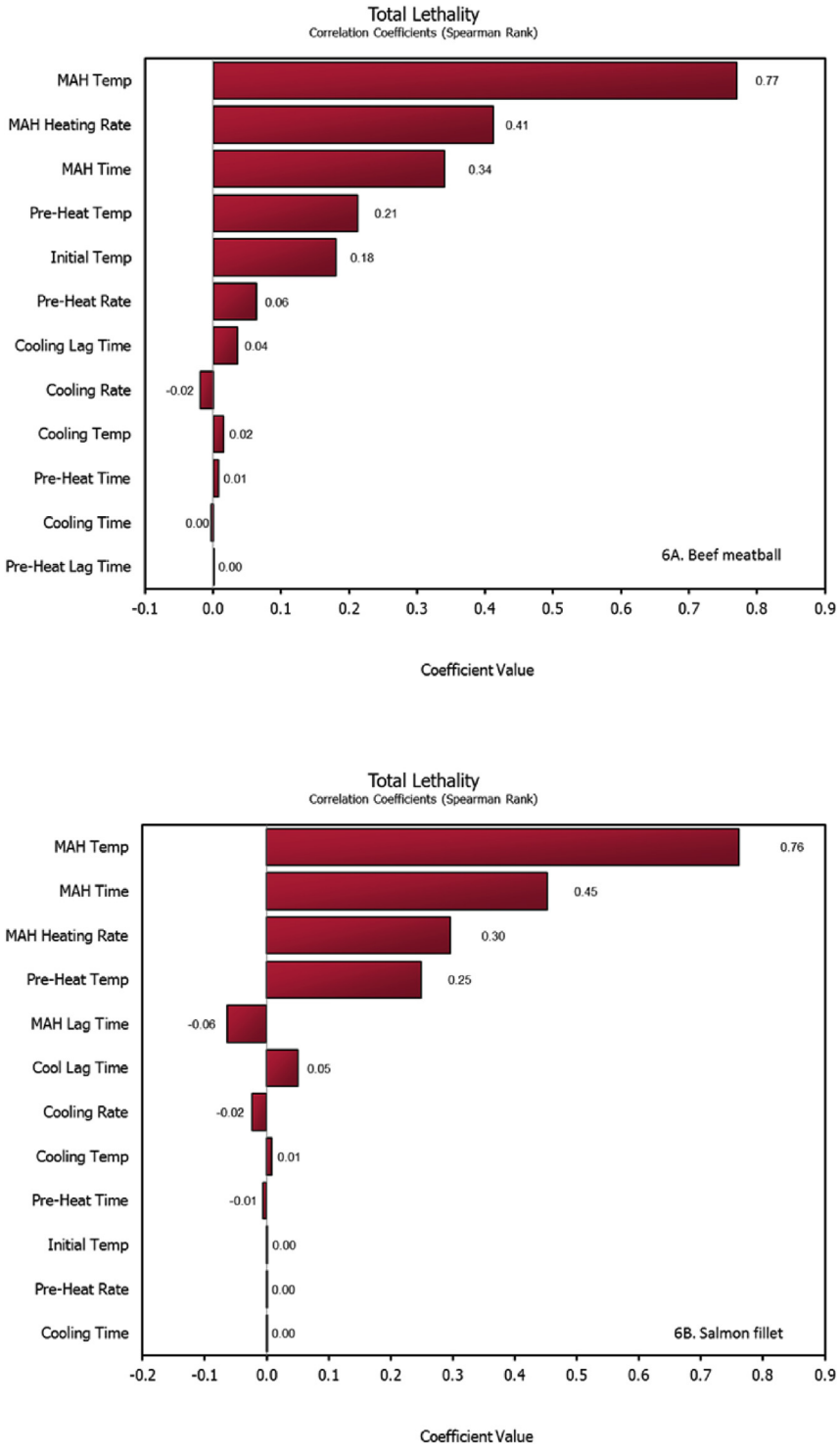

Fig. 6. Sensitivity analysis for effect of processing parameters on the total lethality of the spores of *C. botulinum* Type B in 10 oz. beef meatball trays (6A) and *C. botulinum* Type E in 16 oz. salmon fillet trays (6B).

reduction of the spores of *C. botulinum* Type E in this product, the results of Monte Carlo simulation showed that the total lethality also follows a normal distribution, with a mean and standard deviation of 5.87 and 0.39 in log-reductions of the spores, respectively (Fig. 5B). The minimum lethality achieved in this process is 4.60 log-reductions in the spores, while the maximum is 7.54 log-reductions. Overall, more than 99.1% of the processes achieve >5 log-reductions in the spores of *C. botulinum* Type E in 16 oz. salmon fillet trays, while 36.9% will acquire >6 log-reductions.

The results of Monte Carlo simulation show how the variations in the process parameters and heat transfer parameters can affect the total lethality accumulated in the products. Therefore, if a minimum lethality is required in the products, the thermal design should not be based on the average lethality. Instead, the process

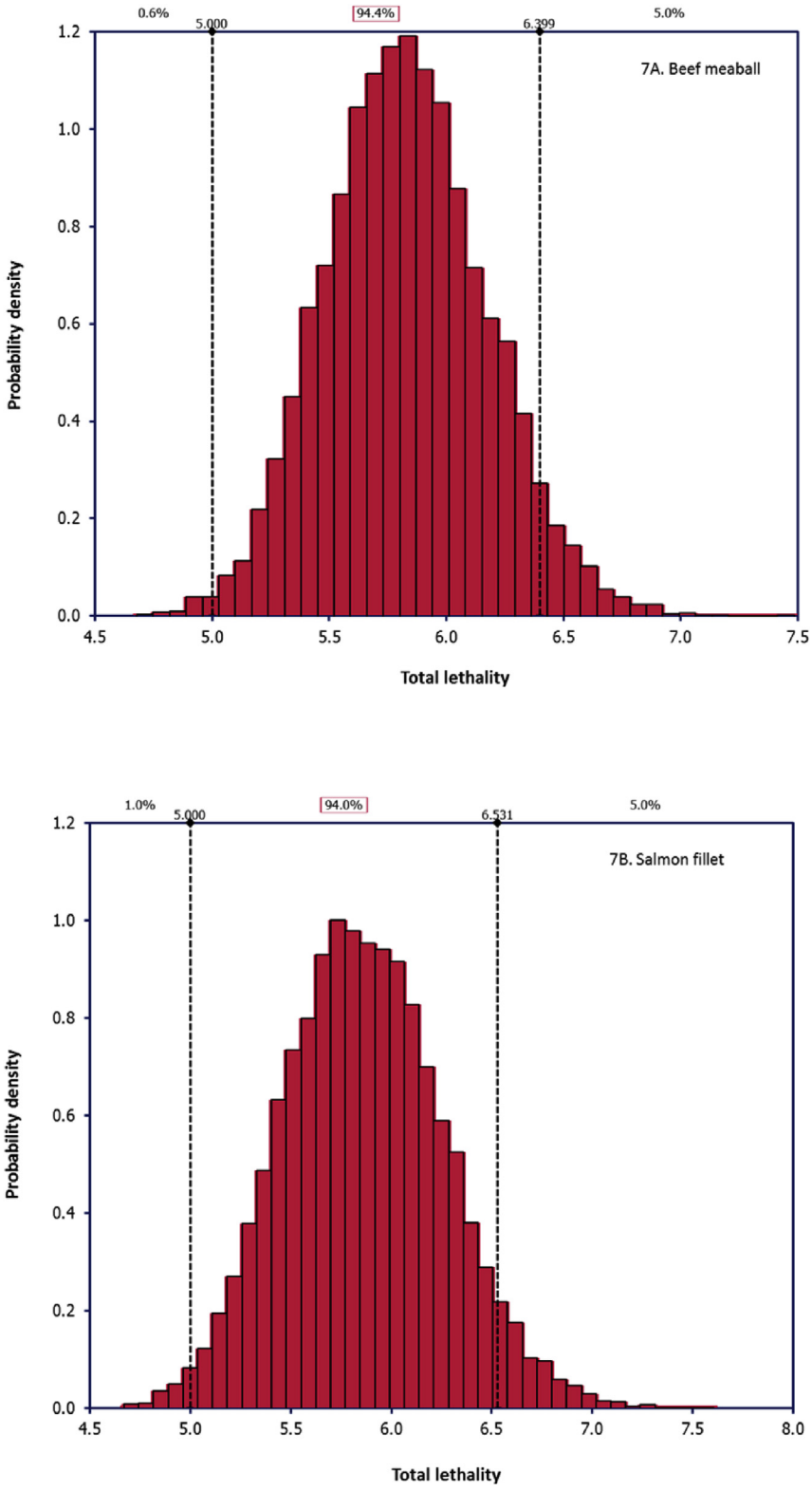

Fig. 7. Effect of cooling water temperature on the distribution of total lethality.

design should be based on the minimum lethality in the products. Monte Carlo simulation can be used to simulate the distribution of lethality and estimate the probability of products that receives a minimum lethality.

3.3. Sensitivity analysis

Although all processing parameters, including the initial temperature of product, lag time, residence time and temperature at

each section, and heat transfer coefficients of the products, may affect the total lethality accumulated in the final products, the relative contribution of each parameter may be different. Sensitivity analysis is a useful tool to reveal the contribution of each parameter to the final lethality achieved in a process. Fig. 6 shows the results of the sensitivity analysis, presented as the correlation between the total lethality and each of the process parameters.

Both plots in Fig. 6 clearly show that the heat treatment in the MAH section is the most critical section contributing to final lethality. This is not surprising, but it demonstrates that the Monte Carlo simulation can correctly capture the physical process of MAPS. Among the different process parameters in the MAH section, the heating temperature has the most significant effect on the total lethality, showing a correlation coefficient of 0.77 for beef meatball and 0.76 for salmon fillet. Therefore, increasing the hot water temperature during MAH is the most effective way to increase the lethality of the MAPS process.

The next two most influential factors are the heating time and heating rate in the MAH section. The order of these two parameters depends on products. The correlation coefficient between the total lethality and heating rate in the MAH section is 0.41 for beef meatball trays and 0.30 for salmon fillet trays, while the correlation coefficient between the total lethality and heating time in the MAH section is 0.34 in beef meatball and 0.45 in salmon fillet. Under the same temperature at the MAH section, longer residence time in this section allows the product to absorb more thermal energy, thus increasing the total lethality. The heating rate is related to the ability of the product absorbing the microwave energy and the ability to conduct heat. This parameter may be affected by thermal properties, dielectric properties, and dimension of the products. Therefore, any measures that can enhance heat conduction and absorption of microwave energy can increase the total lethality.

The fourth most influential factor is the hot water temperature in the Pre-Heating section. While minimal lethality is acquired in the Pre-Heating section, the product will leave this section with a higher temperature if the hot water temperature is higher before entering the MAH section. With a higher incoming temperature in the products, more lethality will be accumulated under the same heating temperature and time in the MAH section.

It is also interesting to note that the correlation coefficient between the lethality and cooling rate is negative in both products, which suggests that increasing the cooling rate has a negative impact on lethality. The correlation coefficient between the lethality and the lag time in the MAH section is also negative in salmon fillet trays, suggesting that a longer lag time would decrease the total lethality. A longer lag time in this section would delay the increase in the temperature of the product.

Interestingly, while the cooling section can contribute a significant portion to the final lethality, the changes in the cooling conditions (temperature and time) do not correlate well with the final lethality, suggesting that the final lethality is not sensitive to the variation in the cooling water temperature and time. One natural question arises as to what extent the changes in the cooling water temperature could impact the total lethality accumulated in the product. New Monte Carlo simulation was conducted to further evaluate the effect of cooling temperature on the total lethality. In the new Monte Carlo simulation, the cooling temperature was allowed to change in a wider rage, following a uniform distribution between 5 and 25 °C. Fig. 7 shows the results of the simulation under the new cooling conditions. Fig. 7A shows that the total lethality of spores of *C. botulinum* Type B in 10 oz. beef meatball trays follows a normal distribution, with a mean and standard deviation of 5.83 and 0.34 log-reductions of the spores, respectively. The minimum and maximum total lethalities are 4.68 and 7.49 log-reductions in the spores, respectively. These values are very close to the results obtained in Fig. 6A, in which the cooling water temperature is 20 ± 0.6 °C. Fig. 7B shows the distribution of total lethality of spores of *C. botulinum* Type E in 16 oz. salmon fillet trays cooled under new cooling conditions. As shown in Fig. 7B, the total lethality also follows a normal distribution, with a minimum, mean, and maximum log-reductions of 4.67, 5.86, and 7.61 log-reductions, respectively. These values are also very close to the results obtained in Fig. 6B, where the cooling water temperature is 20 ± 0.6 °C.

The results of the new Monte Carlo simulation further affirm that the variation in the cooling water temperature does not significantly alter the accumulation of the total lethality in a process. The correlation coefficient between the cooling temperature and final lethality is only 0.08 for beef meatball trays and 0.05 for salmon fillet trays. This result may have practical application for MAPS processes. Since the variation in the cooling water temperature does not significantly change the total lethality, it may not be necessary to precisely control the cooling water temperature during the cooling process. This may significantly simplify the engineering design and reduce the operational costs of the MAPS.

4. Conclusion

In this research, a simple mathematical method using a time-delayed exponential model (Eq. (1)), was developed to describe the time-temperature history of two different products processed in an MAPS. Two products, 10 oz. beef meatball trays and 16 oz. salmon fillet trays, were tested in this study. Nonlinear regression was used to derive the heat transfer parameters, including the heating/cooling rate and lag time in each section of the MAPS. The time-delayed exponential model could accurately simulate the time-temperature history of the entire process, with RMSE of nonlinear regression only 0.76 °C for beef meatball trays and 0.44 °C for salmon fillet trays. The pseudo-R^2 between the predicted and measured temperature curves were >0.999 for both products. To improve the accuracy in estimation of log reductions, an adjustment of 0.7 min in the lag time of microwave-assisted heating (MAH) section was used in the models. With this adjustment, the difference in the measured and calculated lethality was <0.4 log-reductions for two products. Based on computer simulation of the time-temperature history under the processing conditions for beef meatball and salmon fillet trays, the simulation results showed that the Pre-Heating section is insignificant in the inactivation of the spores, and 57–62% of the total lethality is accumulated in the MAH section, while the remaining lethality is contributed by the cooling section. While the relative contribution of the total lethality may be significantly affected the processing conditions in the MAP section, the simulation results suggested that the cooling section could contribute a significant portion of the lethality. It is may be beneficial to include the lethality accumulated in the cooling section during thermal process development to avoid overheating for products that are intended for refrigerated storage. However, such a decision should be carefully made to allow sufficient safety margins for food safety.

Monte Carlo simulation was conducted to evaluate the effect of processing parameters, including the initial temperature, heating or cooling temperature, residence time, lag time, and heat transfer coefficient on the accumulated total lethality. The results of Monte Carlo simulation showed that the total lethality of the products follows normal distributions. With a target lethality of 6 log-reductions, the simulation showed that more than 98.8% of the process could achieve a minimum of a 5-log reduction of the spores of *C, botulinum* Type B in 10 oz. beef meatball trays, and more than 99.1% of the processes achieve >5 log-reductions in the spores of *C. botulinum* Type E in 16 oz. salmon fillet trays.

The sensitivity analysis of Monte Carlo simulation showed that the MAH section is critical to the accumulation of the final lethality. The hot water temperature, heating time, and heating rate in the MAH section and the hot water temperature in the Pre-Heating section are top four most critical factors affecting the accumulation of lethality in the products. The sensitivity analysis also suggested that the variations in the cooling section do not significantly alter the total lethality.

In summary, this study developed a method to accurately simulate the temperature history of products during microwave-assisted pasteurization. This study also conducted a Monte Carlo simulation of the heating and cooling process, and identified factors most critical to the accumulation of the total lethality. The effect of product formulation and variation on thermal lethality should be investigated in the future. The results of the study may guide the development and optimization the MAPS and assist developing thermal processes that minimize overcooking.

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