Journal of Food Engineering 190 (2016) 61–71

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

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

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ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form
24 May 2016
Accepted 19 June 2016
Available online 23 June 2016

Keywords:
Microwave-assisted pasteurization system (MAPS)
Probabilistic modeling
Lethality
Clostridium botulinum spores
Monte Carlo simulation

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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http://dx.doi.org/10.1016/j.jfoodeng.2016.06.012
0260-8774/Published by Elsevier Ltd.

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).

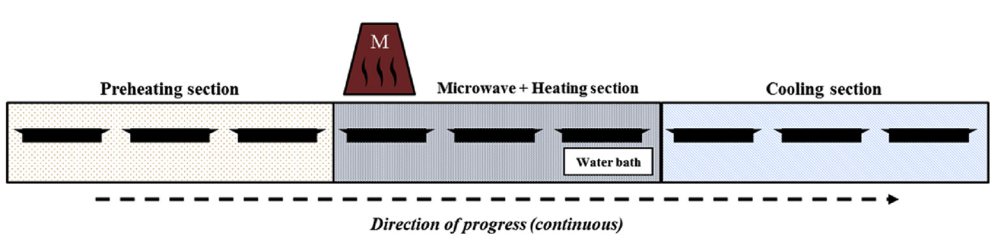

Fig. 1. Schematic diagram of microwave-assisted pasteurization system (MAPS).