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Inactivation kinetics for *Salmonella typhimurium* in red pepper powders treated by radio frequency heating

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ABSTRACT

Radio frequency (RF) dielectric heating has been investigated to inactivate pathogens in some low-moisture foods. This study was aimed to evaluate RF inactivation effects on *Salmonella typhimurium* in red pepper powders, by focusing on the influence of sample initial water activity (a_w) and applying Weibull model to describe the inactivation curves. The experimental results showed that RF heating rate increased when a_w was in the range of 0.57–0.71, but decreased after a_w reached to 0.71. During the come-up time of RF heating, 2–3 log reduction of the pathogen was achieved depending on a_w levels. Increasing initial a_w could first increased log reductions and then decreased the log reductions, optimum a_w level was 0.71 for RF inactivation of *Salmonella* in red pepper powders. For red pepper powders with a_w of 0.71, RF heating to 70 °C (come-up time was 110 s) with holding time over 60 s could achieve >5 log reduction of *S. typhimurium*. Weibull model well fitted the survival curves of the pathogen with goodness of fit (R^2 > 0.93, RMSE<0.29). Scale factor (b) of the model increased with treatment temperature increasing, while the shape factor (n) was independent on temperature. This study provided basic guideline for using RF heating to inactivate *Salmonella* in red pepper powders.

1. Introduction

Red pepper (*Capsicum annuum* L.) is extensively used worldwide as seasoning and natural food colorant due to its attractive color, unique taste and pungency. While spices including red pepper powders are nonperishable commodities because of their low moisture content, they are burdened with high level of microorganisms (Schweiggert, Carle, & Schieber, 2007). Many studies reported microbial contamination of spices at the point of retail (Banerjee & Sarkar, 2003; Hampikyan, Bingol, Colak, & Aydin, 2009; Harakudo et al., 2006; Moreira, Lourenção, Pinto, & Rall, 2009), including pathogenic bacteria *Salmonella*. Use of the contaminated spices can lead to severe food-borne illnesses, especially when the spices are utilized in ready-to-eat foods without further cooking (Little, Omotoye, & Mitchell, 2003).

Salmonella contamination of spices was identified as the cause of 95% U.S. food recalls associated with spices over the period of 1980–2000 (Vij, Ailes, Wolyniak, Angulo, & Klontz, 2006). Salmonellosis represents an important food-borne disease that continues to pose a major threat to human public health in worldwide (EFSA, 2010). In 1993, a nationwide outbreak of salmonellosis occurred in Germany due to contaminated paprika and paprika-powdered potato chips (Lehmacher, Bockemuhl, & Aleksic, 1995), and more recently (2009 and 2010), two *Salmonella* outbreaks related to spices occurred in the United States (Zweifel & Stephan, 2012). Therefore, microbial safety of spices is an emerging and high priority challenge in worldwide food industry.

Additionally, *Salmonella* is known to be extremely resistant to lethal treatments in low-moisture foods (Carrasco, Morales-Rueda, & García-Gimeno, 2012). Unfortunately, there is hardly any commercial pasteurization process across low-moisture food categories with reliable validation. Several inactivation methods, such as fumigation with ethylene oxide, irradiation, and steam treatment have been developed to reduce the microbial load of low-moisture foods (Lee et al., 2006). However, ethylene oxide is generally regarded as a carcinogen which was restricted and even banned in

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the European Union, and irradiation has lower consumer acceptance although it is allowed for decontamination of dried spices in numerous countries (Farkas, 2006; Schweiggert et al., 2007; Waje, Kim, Kim, Todoriki, & Kwon, 2008). Steam heating at 93 °C for 65 s can result in more than 4-log reduction of *Salmonella enteritis* on almond surfaces, but the increase of moisture content in steam treated almonds may affect almond oxidative rancidity and reduce product shelf-life (Lee et al., 2006). Hence, there is a need to develop innovative technologies that can not only reduce microbial contamination, but also maintain quality of the products.

Radio frequency (RF) is one kind of electromagnetic wave with frequency range of 3 kHz–300 MHz, and RF heating is a fast and volumetric heating technology that can generate heat inside the dielectric materials through molecular friction caused by ionic conduction and dipole rotation. RF heating holds great potential as an alternative pasteurization or sterilization method for low-moisture foods (Bermúdez-Aguirre & Corradini, 2012). Several studies have shown positive results, including RF inactivation of *Salmonella* typhimurium and *E. coli* O157:H7 in peanut butter cracker sandwiches (Ha, Kim, Ryu, & Kang, 2013) and black and red pepper spices (Kim, Sagong, Choi, Ryu, & Kang, 2012).

For conventional thermal process, log-linear kinetic model is commonly used to estimate the effectiveness of the thermal treatment, while for novel thermal processing methods, such as microwave, ohmic and RF heating, recent studies showed that inactivation of pathogenic bacteria does not follow the traditional log-linear kinetics, and microbial survival curves shows upward (tailing) or downward (shoulder) concavities (Bermúdez-Aguirre & Corradini, 2012). Weibull model has the ability to describe both kinds of survival curves as well as log-linear model, and thus has been widely applied to characterize the inactivation of *Salmonella* by thermal treatments (Leguérinel, Spegagne, Couvert, Coroller, & Mafart, 2007; Mattick, Legan, Humphrey, & Peleg, 2001; Takhar, Head, Hendrix, & Smith, 2009; van Boekel, 2002).

Even though a few studies had investigated RF inactivation effects on pathogenic bacteria in spices, including RF inactivation of red peppers (+16 mesh, −16 + 25 mesh, and −25 mesh) inoculated with *Salmonella Typhimurium* and *E. coli* O157: H7 and associated color changes (Kim et al., 2012), and the influence of moisture content on dielectric properties of powdered red pepper spice, RF heating rate and inactivation of foodborne pathogens (Jeong & Kang, 2014), very limited information could be found on initial water activity (a_w) effects on RF inactivation kinetics of pathogens in spices. Therefore, the objectives of this study were to (1) investigate the effect of RF treatments on *Salmonella* inactivation in red pepper powders and the influence of initial a_w of the sample, and (2) conduct inactivation kinetics study and develop inactivation model that can be used to describe the survival curves of *Salmonella* under RF treatments.

2. Materials and methods

2.1. RF heating system

RF heating unit (GJD-6A-27-JY, Huashi Jiyuan Co. Ltd., Hebei, China) with a maximum power of 12 kW at the frequency of 27.12 MHz was used in current study. The RF heating system has two parallel electrodes and an imbedded conveyor belt, vertical position of top electrode (75.0 × 55.0 cm^2) can be adjusted to achieve different RF heating rates. Fiber optic sensors (ThermAgile-RD Optsensor, Xi'an Heqiguangdian Co. Ltd., Shaanxi, China) connected with data acquisition system were used to measure real-time temperature in samples during RF heating. More information about this system could be found at Jiao, Zhu, Deng, and Zhao (2016).

2.2. Bacterial strains and cell suspension

Two strains of *S. typhimurium* (ATCC 11464, ATCC 11472) were selected in this study. The strains were originally from bacteria culture collection of American Type Culture Collection (ATCC). Stock cultures were prepared by mixing 0.7 mL of tryptic soy broth (TSB; Difco/BD, Sparks, MD) for 24 h, and culturing at 37 °C with 0.3 mL of 50% sterileglycerol and then storing at −80 °C. Working cultures were streaked onto tryptic soy agar (TSA; Difco/BD, Sparks, MD), incubated at 37 °C for 24 h, and stored at 4 °C.

2.3. Preparation of pathogen inocula

The strain of *S. typhimurium* was cultured in 100 mL of Luria-Bertani at 37 °C for 12 h, harvested by centrifugation at 5000×*g* for 20 min at 4 °C, and washed three times with buffered peptone water (BPW; Difco, Sparks, MD). Subsequently, suspended pellets of each strain of the two-pathogen species were combined to make culture cocktails. The cocktails with final concentration of 10^8–10^9 CFU/mL were used in subsequent experiments.

2.4. Sample preparation and inoculation

Red pepper powders were purchased from Deyoulin Food Co. Ltd. (Shaanxi, China) with initial moisture content of 6.8% (w.b.), which was determined by toluene distillation method. In order to study the influence of a_w on RF inactivation efficacy, four moisture levels of red pepper powders (11%, 14%, 18% and 21%) were selected with corresponding a_w of 0.57, 0.64, 0.71 and 0.74, respectively. Samples were inoculated in such a way to simultaneously obtain the desired moisture levels. Based on the initial and our target moisture contents, the pre-determined amount of admixture, including mixed culture cocktail and sterile distilled water were sprayed into certain amount of red pepper powders by an atomizer, then mixed by handheld agitator for 20 min, after that, transferred into hermetic bag and placed in a refrigerator with periodically mixing the powders to make the moisture and pathogens uniformly distribution and ready to use. The inoculated powders with 10^7–10^8 CFU/g *Salmonella* were weighted and filled in container for RF treatments.

2.5. RF treatments

The gap between the top and bottom electrode was fixed at 9.5 cm in order to obtain an appropriate heating rate based on our preliminary experiments. According to our previous study (Jiao, Deng, Zhong, Wang, & Zhao, 2015), for reducing the influence of non-uniform RF heating, a polypropylene (PP) plastic cuboid container (16.0 L × 10.5 W × 6.8 H cm^3) was used to hold un-inoculated red pepper powders, and a small rounded PP container (6.8 cm Dia. × 3.4 cm H) placed in the middle of it was used to put inoculated samples (Fig. 1). The samples within the container were placed on the center of the bottom electrode, and they were heated by RF energy to 50–90 °C and then holding for 0–180 s depending on different initial a_w levels which would affect thermal resistance of pathogens (Jeong & Kang, 2014). Each treatment was replicated at least three times, mean values and standard deviations were calculated in Microsoft Excel (v. 2015).

2.6. Microbial enumeration

Pepper samples (25 g) were diluted in 225 mL buffered peptone water (BPW) and homogenized for 5 min in a plastic homogeneous container. After homogenization, samples were diluted (1:10) in 0.1% peptone solution and plated on selective medium *Salmonella*