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Effect of water activity and heating rate on *Staphylococcus aureus* heat resistance in walnut shells

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ABSTRACT

Water activity (a_w) and heating rate have shown important effects on the thermo-tolerance of pathogens in low moisture foods during thermal treatments. In this study, three strains were selected to compare the heat resistance in walnut shell powder and finally the most heat resistant *S. aureus* ATCC 25923 was chosen to investigate the influence of a_w and heating rate using a heating block system (HBS). The results showed that *S. aureus* ATCC 25923 became more thermo-tolerant at lower a_w. The *D*-values of *S. aureus* ATCC 25923 increased with decreasing water activity and heating rates (< 1 °C/min). A significant increase in heat resistance of *S. aureus* ATCC 25923 in walnut shell powder was observed only for the heating rates of 0.2 and 0.5 °C/min but not at 1, 5 and 10 °C/min. There was a rapid reduction of *S. aureus* ATCC 25923 at elevated temperatures from 26 to 56 °C at a heating rate of 0.1 °C/min. The inactivation under non-isothermal conditions was better fitted by Weibull distribution (R^2 = 0.97 to 0.99) than first-order kinetics (R^2 = 0.88 to 0.98). These results suggest that an appropriate increase in moisture content of in-shell walnuts and heating rate during thermal process can improve the inactivation efficiency of pathogens in low moisture foods.

1. Introduction

Low-moisture foods are commonly considered as safe and storable products because of their low water activity (a_w < 0.6). Most microorganisms do not grow but can survive in food with this level of water activity. When the surrounding conditions become suitable, the microorganisms present in low moisture foods could result in serious food safety issues. Many outbreaks of food-borne illness have been linked to low moisture foods, such as nuts and nut products (Cavallaro et al., 2011; CDCP, 2014, 2016; Chang et al., 2013; Keady, 2004; Wittenberger and Dohlman, 2010), pepper (Gieraltowski et al., 2013), infant formula (Rodriguez-Urrego et al., 2010), etc. Among these foodborne pathogens, *Salmonella*, *E. coli* O157:H7, and *Staphylococcus aureus* have been found to be the most prevalent species in/on low-moisture foods (Beuchat et al., 2011, 2013). Consequently, the microbial safety in low water activity products has emerged as a high priority challenge across the food industry.

As a major kind of low moisture foods, in-shell tree nuts have been a main carrier of pathogens as reflected in many outbreaks and recalls (CDCP, 2011, 2016; Palumbo et al., 2016). Some studies have reported that the pathogens on in-shell nuts can survive for extended periods and migrate to the kernels through hulls and shells in wet conditions (Blessington et al., 2014; Danyluk et al., 2008; Frelka et al., 2016; Uesugi and Harris, 2006). Among these different tree nuts, the safety of walnuts has aroused much attention recently. Except for the recalls of walnuts, several surveys have also isolated *Salmonella* and *E. coli* from walnuts (CFIA, 2012; Davidson et al., 2015; Little et al., 2010). In addition, it has been reported that *Enterobacteriaceae* is the dominant family on in-shell walnut surface. *Staphylococcus aureus*, as an opportunistic pathogen, can also be isolated on the surface of in-shell walnuts (Zhang and Wang, 2017). Moreover, the microorganisms are mainly attached to the shells of walnuts because they can provide some levels of protection for kernels from contamination (Frelka et al., 2012; Frelka, 2013; Frelka and Harris, 2015). All these observations show that it is necessary to eliminate the risk of pathogens on in-shell walnuts.

Pasteurization is a thermal treatment as proposed by Louis Pasteur since early 1860's and one of the most common bactericidal treatments. Effective heat treatments, such as hot air, steam, infrared, ohmic, microwave and radio frequency heating, are dependent on essential knowledge about the inactivation temperature and heating time of the targeted pathogens (Lee et al., 2016). To obtain reliable validation guidelines, it is important to conduct the heat inactivation kinetics of these pathogens on the in-shell walnuts.

However, the heat resistance of target pathogens to pasteurization

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processes is a critical factor that should be considered in developing reliable inactivation models and process validation protocols. The thermal resistance of pathogens in low moisture foods is affected by many factors, such as the food-related factors (a_w, pH, and ingredients of food), temperature, and microbiological factors (Cebrian et al., 2010; Chen and Ganzle, 2016; Doyle and Mazzotta, 2000; He et al., 2011; Li et al., 2014; Syamaladevi et al., 2016b). Among these factors, a_w of foods is considered as one of the most influencing factors (Syamaladevi et al., 2016b). The influence of these factors has been usually studied under isothermal heating conditions. But the heat inactivation of pathogens in food industry needs to take non-isothermal processing stages into consideration in practical applications. Furthermore, the effect of heating rates on microbial heat resistance has been reported and the results show that the thermal resistance of microorganisms may be enhanced when the heating rate is reduced to certain low levels (Conesa et al., 2009; Hassani et al., 2005; Huertas et al., 2015, 2016; Kou et al., 2016). Therefore, systematic studies are needed on developing a complex relationship between the inactivation rate of the target pathogen and the sample water activity with heating rates.

It is important to select a device to study the influence of a_w and heating rates on thermo-tolerance of pathogens in walnut shells. A thermal death time (TDT) heating block system (HBS) designed by Kou et al. (2016) has been used for studying thermal inactivation of pathogens. Based on its advantages, the TDT HBS is suitable for studying thermal death kinetics of microorganisms in liquid, semi-solid and solid foods. This system has been successfully applied to study the influence of heating rates on the thermo-tolerance of *E. coli* ATCC25922 in mashed potato and almond powder (Cheng et al., 2017; Kou et al., 2016).

The objectives of this study were using the TDT HBS (1) to select the most heat resistant strain among the given three strains by comparing the log reduction in walnut shell powder when subjected to three temperature-time combinations, (2) to study the influence of different a_w and heating rates on heat resistance of the most heat resistant strain in walnut shell powder, and (3) to determine a suitable mathematical model for properly describing inactivation curves.

2. Materials and methods

2.1. Sample preparation

Walnuts (*Juglans regia* L.) were purchased from a local market in Yangling, Shaanxi, China. After removing the kernels, the shells of walnuts were milled in a blender (FLB-100, 220 V, 50–300 mesh, Philip Bo Food Machinery Corp, Shanghai, China), and then the powder passed through an 18 mesh/in. sieve (< 1.0 mm). The original moisture content of walnut shell powder was determined by a moisture analyzer (HE53, Mettler-Toledo, Shanghai, China). The shell powder at the initial moisture content was adjusted to different moisture content levels by adding calculated amounts of distilled water. The adjusted samples were conditioned in closed containers at 4 °C for at least 3 days for equilibrium and then were sterilized at 105 °C for 10 min (Cheng et al., 2017; Villa-Rojas et al., 2013). The water activity (a_w) of the samples was measured by Aqua Lab water activity meter (Model 4TE, Decagon Devices, Inc., Pullman, WA, USA).

2.2. Heating block system

A heating block system (HBS) consisted of a heating unit, a data acquisition/control unit, and a computer (Fig. 1a) was used for heat treatments. The HBS system could regulate the heating rate from 0.1 to 13.3 °C/min. The set-point temperature, heating rate and holding time of the HBS were controlled by the Visual Basic software via a solid-state relay. The temperatures of the top and bottom blocks, and samples were monitored by calibrated type-T thermocouples (TMQSS-020-6, Omega Engineering Ltd., Stamford, CT, USA) inserted through sensor paths. Two proportional-integral-derivative (PID) controllers (I32, Omega Engineering, Inc., Stamford, CT, USA) were used to regulate the two block surface temperatures. The detailed information of the HBS could be found in the previous study (Kou et al., 2016). During the heat treatment in this study, six TDT cells were placed in the pull-push boxes (Fig. 1b), including five cells with inoculated samples and one temperature monitor cell with non-inoculated samples (Fig. 1c). All the cells were preheated to a fixed temperature (26 ± 1 °C) and then subjected to heat treatment under predetermined conditions. The cell taken out when the sample temperatures reached the target set-point was defined as time zero sample and the rest cells were removed at the set time intervals. All the test cells were immediately immersed in an ice-water bath for cooling (< 4 °C for at least 2 min) after removed from the HBS.

2.3. Heat treatments

2.3.1. Preparation of cell suspension and inoculation

Salmonella H9812, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were chosen as the representative strains on walnut shells. The cell suspensions of these three strains were obtained according to the previous study described (Li et al., 2017). The final cell populations were adjusted to a level of 10^{10} CFU/mL and stored in phosphate buffer saline (PBS, pH 7.2) at 4 °C before the experiment.

The six TDT cells were fully filled with powder samples (0.38 to 0.42 g) without any headspace. Then five of the cells were inoculated with 20 μL of cell suspensions to achieve an initial population between 10^8 and 10^9 CFU/g. After closing up, all the cells were left for 1 h at room temperature to achieve moisture equilibrium.

2.3.2. Selection of the most heat resistant strain

Three temperature and time combinations: 56 °C + 15.0 min, 60 °C + 4.0 min and 64 °C + 1.5 min were selected to determine the most heat resistant strain. The heating rate was set to 5 °C/min and the matrix was chosen as the shell powder with 18.10% wet basis (w.b.). These three strains as described above were used to compare the thermal resistance in the walnut shell powder using the HBS.

2.3.3. Thermal inactivation kinetics

The inactivation kinetics of the most heat resistant strain was further characterized in the HBS. The preconditioned samples with moisture contents of 8.93, 12.09, 14.87 and 18.10% (w.b.) were used and the corresponding a_w values were 0.586, 0.726, 0.838, and 0.931, respectively. Three different temperatures at each moisture content level were selected for heat treatment in the HBS.

2.3.4. Effect of heating rates on heat tolerance of the most heat resistant strain

The heating rates of 0.1 °C/min, 0.2 °C/min, 0.5 °C/min, 1 °C/min, 5 °C/min and 10 °C/min were chosen to investigate the influence on thermo-tolerance of the most heat resistant strain. The samples with 18.10% (w.b.) moisture content were used as the matrix and the target temperature was set at 56 °C. When the heating rate was 0.1 °C/min, the cells with inoculated samples were accurately preheated to 26 ± 0.2 °C. Then the cells were taken out at 1 h time interval during the heating process. The other steps were the same as previously described.

2.4. Bacterial enumeration

After heating treatments, the samples were mixed with 10 mL PBS in sterile flasks. Then, the mixture was 10-fold serially diluted in 0.9 mL of sterile PBS, and 0.1 mL of the diluent was spread onto LB agar. 1 mL original homogenized samples were spread onto LB agar when the populations of surviving cells were below the detection limit (< 10 CFU/100 μL). All plates were incubated at 37 °C for 24 h for enumeration.