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Inactivation of *Salmonella* Enteritidis PT30 on black peppercorns in thermal treatments with controlled relative humidities

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ABSTRACT

Traditional method utilizes steam to pasteurize low-moisture ingredients like black peppercorns and almonds. Exposure to steam results in direct condensation on the product, unfavorable for a broader range of food ingredients such as dried herbs, fruits, and ground materials. Recent studies on the thermal inactivation of *Salmonella* in low-moisture foods suggest that the relative humidity in treatment chambers is an important factor, besides temperature, that determines the death rate of bacteria. Thus, thermal treatments with controlled high relative humidity can be an effective method to replace steam pasteurization. No condensation will occur when the products are preheated to above the dew-point temperature of the hot air in the treatment chamber, thus eliminating the need for post-treatment drying. To prove this concept, a special device was developed that preheated samples in a dry environment before exposing them to a controlled relative humidity (RH) at a high temperature. Using this device, the death rate of *Salmonella* Enteritidis PT30 (*S.* Enteritidis) in black peppercorns was determined at 80 °C and three different RH levels (60, 70, or 80 %) after the innoculated samples were heated to 78°C. The results indicate that the treatments at 80 °C and 80 % RH for 3 min, 70 % RH for 9 min, and 60 % RH for 25 min caused 5.4 ± 0.2, 6.2 ± 0.6, and 6.1 ± 1.0 log reductions, respectively. No condensation was observed on all of the treated samples. The moisture content (wet basis) of fully pasteurized (5-log reduction) black peppercorns at 60, 70, and 80 %RH reduced from 9.7 ± 0.4 % (untreated) to 8.7 ± 0.5 %, 9.2 ± 0.4 %, and 9.2 ± 0.2 %, respectively, indicating that post-drying is not required after the treatments. This study demonstrated the potential of using short-time high-RH treatments to control pathogens in low-moisture foods without the need for post-treatment drying.

1. Introduction

The food industry has recently started implementing effective pathogen control strategies to reduce the risk of contamination in low-moisture foods and ingredients (LMFs). Consequently, the estimated *Salmonella* prevalence (the ratio of samples positive for *Salmonella*) in spices, like basil and black pepper, has been reduced significantly between the point of entry to the United States and retail sales (FDA, 2017). In September 2007, the Almond Board of California (ABC) and the United States Department of Agriculture (USDA) made it mandatory for all almonds sold to consumers in North America to be pasteurized. Since then, no outbreaks have been attributed to California Almonds (Birmingham, 2018).

Three main methods have been found to be effective in pasteurizing LMFs: thermal processing, fumigation, and irradiation. Thermal processing includes wet heating, e.g., blanching and steam processing, and dry heating, e.g., dry roasting and oil roasting (Sanders & Calhoun, 2014). Fumigation utilizes chemical compounds such as propylene oxide (PPO), ethylene oxide (EtO), or chlorine dioxide (Verma et al., 2022). Irradiation can be accomplished using gamma rays (Song et al., 2014), X-rays (Jeong et al., 2012), or electron beams (Lung et al., 2015). However, the application of food irradiation process is limited to a few products due to customer concerns; fumigation is prohibited in European Union (Schweiggert et al., 2007), and it conflicts with the "organic" labeling protocol. Therefore, thermal processing is the best option for LMF pasteurization.

Regardless of the technologies used for food heating, e.g., conventional heating, infrared heating, or volumetric heating using radio-frequency or microwave power, the death of the bacteria is caused by irreversible alteration of critical cell components at elevated

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temperatures (Cebrián et al., 2017; Eliasson et al., 2015; FDA, 2000). The death rate of bacteria generally follows the first-order kinetics, and the moisture content in the bacteria cells plays a critical role (Yang, Cheng, et al., 2022; Yang, Wei, et al., 2022; Yang et al., 2020). Higher moisture content in the bacteria (vegetative cells and spores) leads to a faster reduction in the bacterial population (Murrell & Scott, 1966; Xie, Cheng, et al., 2021; Xie, Xu, et al., 2021). Due to the microscopic size of a bacterial cell, the moisture content in a cell changes rapidly under the control of the environment driven by the differences in water vapor pressure between the interior and the surrounding (Syamaladevi et al., 2016). For example, the moisture content of bacterial cells increases sharply in a steam treatment, reducing the bacterium's thermal resistance. This is why steam pasteurization is more efficient than dry roasting when the same temperature is employed. But a harsh steam treatment may cause clumping or caking of some materials (such as dried herbs and vegetables), and it always leads to condensate, which must be removed in post-treatment drying.

Within a food matrix, the moisture content of bacterial cells is controlled by the water activity of the food components (Yang et al., 2020; Yang, Wei, et al., 2022). While for the bacteria on the surfaces of peppercorns, nuts, or grains, the moisture content of bacteria is mainly determined by the humidity of the air (Liu et al., 2018; Xu et al., 2019). Thus, using humid hot air to treat LMFs can be more efficient in pathogen reduction compared to dry heating methods. Proper designs of thermal treatments with controlled relative humidity may also eliminate water condensation on the treated products. Attempts have been made to use moist heat for the pasteurization of *Salmonella* in black peppercorns in a dynamic system (Zhou et al., 2019). However, without knowing the relationship between temperature, humidity, and the death rate of bacteria, it is difficult for the food industry to design a valid and controllable process.

The objective of this study was to: 1) develop a test device that could preheat samples in a dry environment and provide an isothermal and isohumidity treatment for low-moisture ingredients, and 2) use the device to study the effect of controlled relative humidity at elevated temperatures on the death rate of *Salmonella* in a low-moisture ingredient. We selected black peppercorn as the food matrix for this study, considering that it was the most imported spice in the US (Nguyen et al., 2019), and the imported peppercorn has a high *Samonella* prevalence (6.7 %) according to FDA (2017).

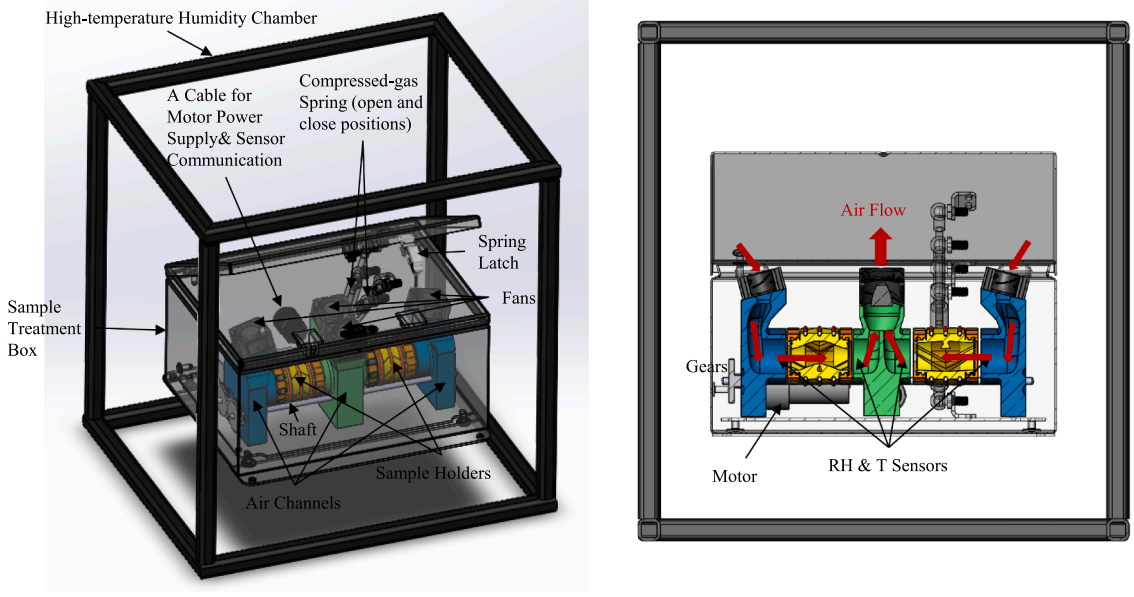
2. Material and methods

2.1. Design of the test device

The test device (Fig. 1a) consisted of a commercial high-temperature humidity chamber (HCP50, Memmert, Schwabach, Germany) (chamber), a customized humidifier (not shown in the figure), and a sample treatment box (box). The chamber (internal w × h × d: 400 × 425 × 330 mm) provided stable air condition at a controlled temperature of up to 90 °C and RH of up to 90 %. The chamber had a built-in humidifier, but it took about 40 min to raise the RH in the chamber. Thus, a customized steam humidifier was added to reduce the RH come-up time. The customized humidifier consisted of a glass tray containing deionized water (about 3 L) and an immersion water heater (1,500 W). The water heater was used to raise the RH in the chamber rapidly to 90 % (in 2 min) by generating steam. Then the water heater was turned off and allowed the chamber to bring down the RH and stabilize it at the target level. With this set-up, the time for RH in the chamber to reach a target level was reduced from over 30 min to less than 12 min.

The sample treatment box was designed to hold two identical samples during the tests. It consisted of two drum-shaped sample holders (each having a 25-ml internal volume), two rotary shafts (one labeled, the other hidden behind the sample holders in Fig. 1a), air circulation channels with fans (12 V, 0.16 A), and a lid attached to a compressed-gas spring and a spring latch to close and open the treatment box. Each sample holder has two screw caps installed with steel mesh screens to retain the samples while allowing passage of air. The air channels, sample holders, and caps were all 3-D printed with carbon nylon. In the tests, the air circulation channels were tightly connected with the sample holders. The shafts provided the rotational motion of the sample holders, while the air channels guided forced air to pass through the samples.

During a treatment (Fig. 1b), the sample holders rotated at ~37 rpm, driven by an electric motor. The inner walls of the sample holders were tapered toward the center with ribs that helped stir the sample to



(a) 3D Figure b. Front View

Fig. 1. Schematic representation of the 3D figure and front view of the test device that was used to study the thermal inactivation of *S.* Enteritidis in black peppercorns in isothermal and isohumidity conditions.

improve exposure to circulating air. The two fans on the two sides of the air channels (blue) were positioned downwards, while the two fans on the center channel (green) were positioned upwards so that air was forced to go through two sample holders in two separate routes (red arrows). Four temperature & humidity sensors (Honeywell Humid-Icon™ HIH8121, Morristown, NJ), each positioned by the air entrance or the exit of a sample holder, could reflect the temperature and RH conditions in each sample holder.

The sensors (Honeywell) in the sample treatment box and the built-in sensor (Rotronic Hygromer IN-1, Rotronic Instrument Corp, Hauppauge, NY 11788) in the humidity chamber were calibrated before the experiments. Temperature sensors were calibrated at 70, 80, and 90 °C with a resistive temperature detector (Omega HH376, Omega Engineering Inc., Stamford, CT). Humidity sensors were calibrated at 70, 80, and 90 % RH all at 80 °C with a factory-calibrated humidity sensor (Vaisala HMP5, Vaisala Oyj, Vantaa, Finland). Trial experiments were performed to analyze the temperature and humidity changes in the box and the chamber.

2.2. Sample preparation

Steam-sterilized black peppercorns were obtained from McCormick & Company, Inc. (Hunt Valley, MD). The samples were prepared following the procedures outlined in Fig. 2 for the thermal inactivation tests (solid lines) and moisture content change measurements (dashed lines).

Before the thermal inactivation tests, solid-state cultures of *Salmonella enterica* Enteritidis PT30 (ATCC BAA-1045) (*S.* Enteritidis) were prepared and inoculated on sterile black peppercorns. In brief, a colony of *S.* Enteritidis (strain was obtained from Dr. Linda Harris of University of California, Davis, CA) on a streak plate was incubated in two 9-ml trypticase soy broth (TSB, BD Difco, Franklin Lakes, NJ) with 0.6 % yeast extract (TSBYE) consecutively, at 37 °C each for 24 h. Then the culture was transferred for 1 ml to a 150 × 15 mm plate of trypticase soy agar (BD Difco) with 0.6 % yeast extract (BD Difco) (TSAYE) and incubated at 37 °C for 24 h. The culture on agar was harvested with 18 ml of buffered peptone water (BD Difco) (BPW), centrifuged at 3,000 × *g* for 15 min to get rid of the supernatant, and dissolved in 0.5 ml of BPW. The culture was mist sprayed on 50 g of sterile black peppercorns in a 500 ml glass jar. Each spray was followed with more than ten hand-shakings to give a uniform inoculation. The inoculated sample was transferred into a 150 × 15 mm petri-dish and equilibrated at 43 % RH for at least four days to have identical initial moisture content in the sample with a water activity of 0.43.

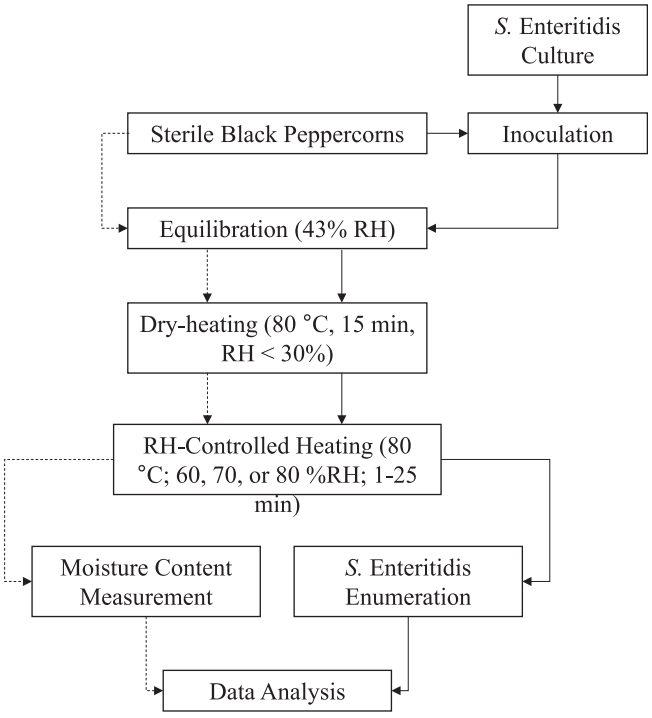


Fig. 2. Experimental flow diagram for the heat inactivation study and moisture content study.

To study the change of moisture content in black peppercorns caused by the humidity-controlled thermal treatments, with no inoculation step, 100 g of sterile black peppercorns were directly transferred into two 150 × 15 mm Petri-dishes (50 g each) and equilibrated at 43 % RH for more than four days before thermal treatments.

2.3. Humidity-controlled heat inactivation study of Salmonella in black peppercorns

Each test consisted of two steps: 1) preheating the inoculated samples to above the dew-point temperature of the humid air in the controlled RH chamber, and 2) treating the samples under controlled RH and temperature conditions. In the first step, the samples were heated by dry air in the closed box for 15 min while the air outside the box in the humidity chamber was conditioned to reach desired RH and temperature. We refer to this step as the dry heating step. In the second step, the lid of the box was lifted remotely by pulling a string tied to the spring latch that locked the lid to the box. When the box was open, the fans brought humid hot air from the chamber into the sample holders, so the samples were exposed to the humid air of controlled RH.

Prior to the above two steps, the treatment box (without the sample holders) and the chamber were warmed up to 80 °C without turning on the humidifier. Two 4-g samples were each added to a sample holder outside the humidity chamber, the chamber door was opened, and the two sample-holders were mounted on the shafts inside the box. The box was sealed, and the chamber door was closed to start the dry heating step. During the 15 min of dry heating, the fans and shaft were turned on, the RH in the chamber was brought up by turning on the customized humidifier, then stabilized at ~6 % above the target level (i.e., 66, 76, and 86 % corresponding to target levels 60, 70, or 80 % RH, respectively). When the lid of the treatment box was opened, the RH dropped to within ±1 % of the above target levels in less than 1 min. The customized humidifier was turned off, and the target RH levels were maintained through the rest of the tests. Duplicated samples were treated for different lengths of time in the humidity-controlled environment to determine the change in the surviving population of *S.* Enteritidis. The treatment times for each condition were determined from preliminary studies so that the longest treatment could result in more than 5 log reductions of *S.* Enteritidis. These treatment times were 0, 1, 13, and 25 min for 60 % RH, 0,1, 5, and 9 min for 70 % RH, and 0, 1, 2, and 3 min for 80 % RH.

Each treatment was terminated by opening the chamber door to release the humid air. After the thermal treatment, each sample (4 g) was vigorously washed with 36 g of BPW in a 50-ml centrifugal tube with 5-min vortexing for a 1:10 dilution. Then the diluted samples were subjected to serial dilutions and aerobic incubation (48 h, 37 °C) on TSAYE plates supplemented with 0.05 % ferric ammonium citrate (Sigma-Aldrich, St Louis, MO) and 0.03 % (w/v) sodium thiosulfate (Sigma-Aldrich). The colony-forming unit (CFU) of *S.* Enteritidis in 1 g of black peppercorns was calculated using the plate counts as the survival population. For replication of experiments, three batches of independently inoculated and equilibrated samples were tested on different days for each treatment RH level.

2.4. Study the change of MC in black peppercorns from treatment

The equilibrated black peppercorns (with no inoculum) were weighted into 4-g samples and divided into five groups (4 subsamples

per group) for different treatments at 80 °C: control (untreated), dry-heating (15 min), dry-heating (15 min) followed by treatment at 60 % RH for 25.6 min), dry-heating (15 min) followed by treatment at 70 % RH for 9.1 min, and dry-heating (15 min) followed by treatment at 80 % RH for 2.9 min. The treatment times were estimated to cause about a 5-log reduction of *S.* Enteritidis at each RH level, based on the results from the thermal inactivation tests described in Section 2.3. The moisture content of each sample was measured by the weight changes in ground black peppercorns from vacuum drying at 70 °C and absolute pressure of 100 mmHg for 6 h (Method: FSSAI 10.005:2021, Food Safety and Standards Authority of India, 2021). For each treatment condition, the experiment was repeated twice, each with two replicated samples.

2.5. Data analyses

The thermal death rates of *S.* Enteritidis were quantified with *D*-values (decimal reduction time), which were calculated from the surviving populations corresponding to different treatment times using the first-order model (Peleg, 2006):

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \tag{1}$$

where N and N_0 are the populations of *S.* Enteritidis (in log CFU/g) at time t (in min) and time t_0 (the start of sample exposure to the humid air), respectively. D, in min, is the decimal reduction time of the bacterial population at a given treatment temperature.

The effect of RH was quantified using Z_{RH} value, which indicates the amount of RH increase that would cause a tenfold (or one-log) reduction in the *D*-value (Eq. (2)) at a given treatment temperature. The concept of Z_{RH} was similar to the Z_{aw} value (Gaillard et al., 1998; Liu et al., 2018), but it is used to describe the effect of relative humidity in the treatment environment rather than the effect of the water activity (a_w) of the food matrix.

$$\log\left(\frac{D}{D_{ref}}\right) = -\frac{RH - RH_{ref}}{Z_{RH.}} \tag{2}$$

where D and D_{ref} are the *D*-values (min) corresponding to relative humidity levels (%), RH, and RH_{ref}. The unit of Z_{RH} is also in %.

The model fitting for Eqs. (1) & (2) was performed using the Linest function with Microsoft Excel 16.0. The goodness of fit was evaluated by the root mean squared error (RMSE). The means of *D*-values and moisture content were compared between different conditions using one-way ANOVA (performed with MATLAB R2022a) with a significant threshold of 0.05.

3. Results and discussion

3.1. Performance of the test device

Typical temperature and RH histories measured in the chamber and the sample treatment box are presented in Fig. 3. The target treatment temperature and RH for this test were 80 °C and 70 %; the corresponding dew point temperature was calculated using Arden Buck's equation to be 71.4 °C (Buck, 1981). The treatment box (without sample holder) and the chamber were preheated before the experiment. It took about 15 s to open the chamber door and mount the sample holders into the treatment box. During this period, the RH of the chamber dropped sharply. At t = 0 min, when the chamber door was closed, the RH was recorded as 10 %, and the temperature of the chamber and treatment box were 74 °C and 66 °C, respectively (see Fig. 3). During the initial 15 min of dry heating, the temperature in the box and the chamber climbed slowly to 80 and 78.6 °C, respectivley, and the RH in the chamber surged to about 88 % within 2 min with the help of a customized steam humidifier. Then the humidifier was turned off to allow the RH in the chamber to drop to the set level (76 %) under the control of the chamber. The box was sealed during the dry heating. The internal RH of the box went up to 31 %,

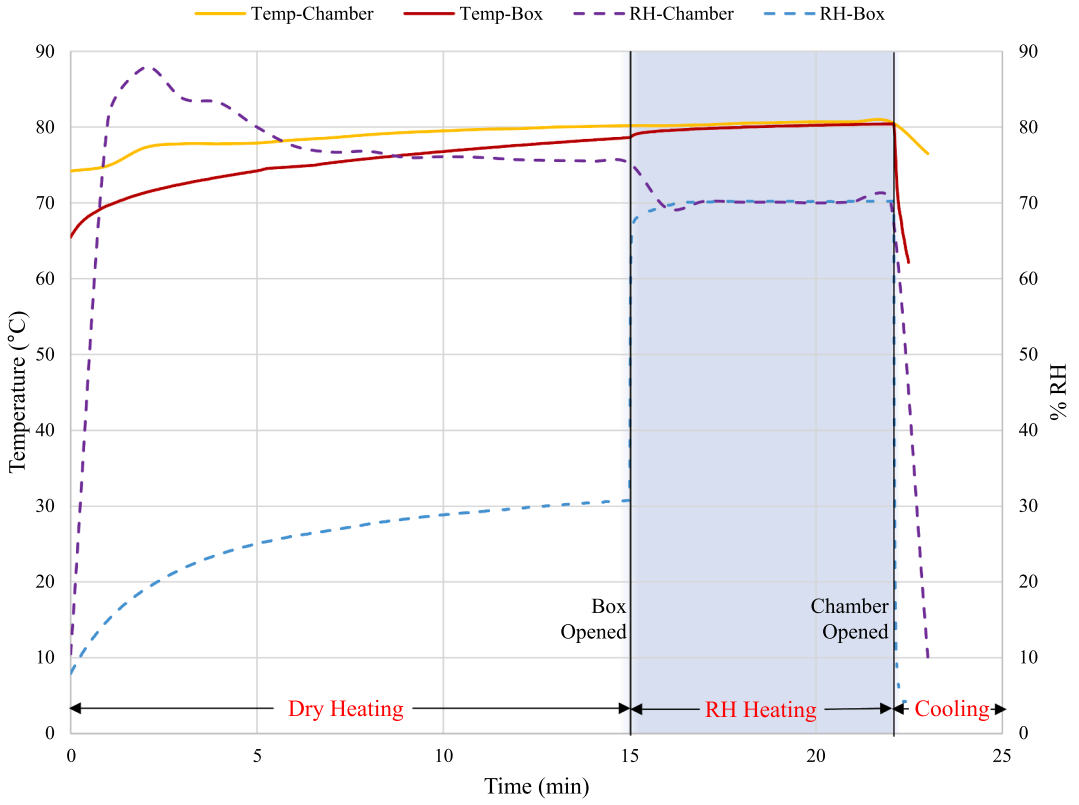


Fig. 3. An example of the temperature and RH history in the sample treatment box and in the high-temperature humidity chamber. The RH and T data were each captured by two sensors, one in the chamber and another in the box.

which might be contributed by the moisture equilibration between the sample and the air. The sample temperature increased from 23 to 78.6 °C, which was above the dew-point temperature of the air in the chamber. When the box lid was opened at 15 min, the humid air in the chamber was drawn into the sample holders by the fans (Fig. 1). Within 1 min, the temperature in the box went up from 77 to 80 °C, and the RH in the chamber and the box reached an equilibration of 70 %. In the particular case shown in Fig. 3, the two samples were treated at 70 % RH & 80 °C for 7 min, and then the chamber door was opened to stop the treatment. Both RH and temperature in the box and the chamber dropped sharply after the door was opened as the box started bringing the ambient air into the sample holder. After 3 min of air cooling, the samples were taken out of the treatment box, and each sample was kept in a closed 50 ml centrifugal tube before the next-step analysis.

3.2. Heat inactivation of Salmonella in black peppercorns

The recorded changes in the populations of *S.* Enteritidis in black peppercorns after 15 min dry heating and the subsequent treatments at three RH levels at 80 °C are presented in Fig. 4. The population of *S.* Enteritidis in the untreated samples at time 0 of the dry heating step was 8.72 ± 0.22 (log CFU/g). After 15 min of dry heating with a corresponding RH of less than 30 %, the population of *S.* Enteritidis in black peppercorns was 8.69 ± 0.20 (log CFU/g), which was not significantly different from that of the untreated sample. This result illustrated that dry heat treatments are ineffective in thermal inactivation of *Salmonella* in low moisture foods. This is because the thermal resistance (D-value) of *Salmonella* cells increases sharply when they are dehydrated (Liu et al., 2018; Xie, Cheng, et al., 2021; Yang et al., 2020). For example, it would take more than 64 min for a thermal treatment at 80 °C & 27 % RH to cause one log reduction of *S.* Enteritidis (Liu et al., 2018).

But once the sample was exposed to the high-humidity air at 80 °C and RH above 60 %, the inactivation of bacteria was instantaneously accelerated, most likely due to rehydration of the bacterial cells. As shown in Fig. 4, during the high RH heating, the population of *S.* Enteritidis (in log CFU/g) dropped sharply within the first minute and then decreased steadily with the treatment time. The sharper first-minute reduction of *Salmonella* in low-moisture foods have been reported in isothermal treatments (Villa-Rojas et al., 2017; Xu et al., 2019; Yang, Wei, et al., 2022). This was attributed to a vulnerable portion of bacteria in the inoculum and the experimental technique (Dufort et al., 2017). After 1 min, the reduction of *S.* Enteritidis followed a log-linear relationship with the treatment time at all three RH levels. The death rate of *S.* Enteritidis was higher under a higher RH which agrees with previous studies (Liu et al., 2018; Xu et al., 2019). The log reductions after treatments at 80 % RH for 3 min, 70 % RH for 9 min, and 60 % RH for 25 min were 5.4 ± 0.2, 6.2 ± 0.6, and 6.1 ± 1.0, respectively.

3.3. D and Z_{RH} values

The thermal death time for one-log reduction at 80 °C (D_{80C} value) and each relative humidity was calculated using Eq. (1) with the start of full exposure time (t_0) equal to 1 min. The Z_{RH} value was calculated using Eq. (2). The results are presented in Table 1. The RMSEs for Eq. (1) are 0.44, 0.72, and 0.51 (log CFU/g), respectively, for RH 60, 70, and 80

Table 1
Thermal death parameters of *S.* Enteritidis PT30 in black peppercorns as influenced by RH of the processing environment (n=3). Different grouping letters indicate significant differences between groups ($P < 0.05$).

RH (%)	D_{80C} (min)	95 % confidence Interval of D (min)	Time for 5-log reduction (95 % CI) (min)	Z_{RH} (%)
60 %	5.1 ± 1.1^{A}	4.03–6.33	25.6 (20.2–31.7)	21.3
70 %	1.8 ± 0.2^{B}	1.61–2.04	9.1 (8.1–10.2)	
80 %	0.58 ± 0.02^{C}	0.56–0.60	2.9 (2.8–3.0)	

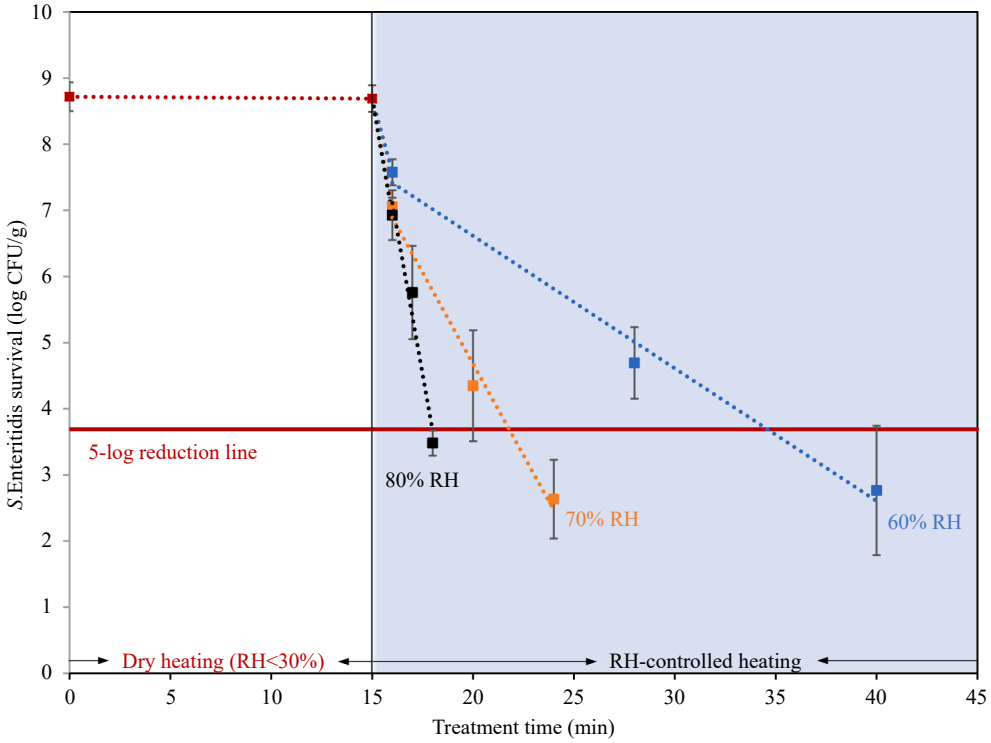


Fig. 4. Survival populations of *S.* Enteritidis PT 30 in black peppercorns at different steps of a thermal treatment combining 15-minute dry heating and RH-controlled heating at designated durations (n = 3). Prediction curves (dotted lines) were made by connecting dots (in dry heating and the first minute of RH-controlled heating) and fitting the first-order model (Eq. (1)) (in RH-controlled heating after the first minute).

%. The RMSE for Eq. (2) is 0.059 for log$D$ or 0.6 min for D. The RMSEs are within the expected range, indicating a good prediction of the model. According to this data, a higher RH in the treatment chamber contributed to a faster inactivation rate of *Salmonella* in black peppercorns. By increasing the RH from 60 % to 80 %, the treatment time for a 5-log reduction would reduce from 25.6 min to 2.9 min. Ideally, the treatment time can be further reduced by using a higher RH, like 90 %. But the dew-point temperature increases with RH (e.g., dew point temperature = 76 °C at 90 % RH and 80 °C dry-bulb temperature), which requires more precise temperature control in preheating to prevent condensation at the cold spots of the treatment chamber. On the other hand, based on the Z_{RH} value, D_{80C} for 50 % RH is estimated to be 15.2 min. That is, it would take about 76 min to achieve a 5-log reduction if 50 % RH were used in the treament. D_{80C} for 30 % RH is estimated to be 132 min. This explains why the 15 min preheating at RH below 30 % did not cause significant reduction of *Salmonella.*

3.4. Comparison with other low-moisture foods

The D_{80C}-values of *S.* Enteritidis obtained from this work are compared with the data for low moisture food materials from the literature in Fig. 5 on a semi-log plot against the RH at 80 °C. The prediction line (solid) obtained from linear regression of all the cited data (for low moisture foods) has a similar slope as the data obtained from this work which indicates a similar impact from the RH. The three data points from black peppercorns fell right on the lower 95 % prediction line, which suggests that the D-values from black peppercorns are in the lower range of prediction compared to the other studied food matrices. This can be attributed to the impact of antimicrobial content in the black pepper (Xie, Cheng, et al., 2021). This comparison suggests that the data from previous studies using high-temperature water activity of food matrices are comparable to the data from this study using controlled RH, which supports the theory that the moisture content in the bacterial cells is the fundamental factor to the thermal resistance of the organism no matter it was controlled by the water activity of the matrix or the RH in the surrounding atmosphere.

3.5. The change in moisture content

After the treatment and subsequent cooling, the surface water activity of black peppercorns was above 0.80 due to moisture absorption on the sample surfaces, but the interior remained dry. The water activity of the sample continues to drop during water activity measurement in a dew-poit chilled-mirror water activity meter (AquaLab, Meter Group, Inc., Pullman, WA), reflecting the removal of the surface moisture. Thus, it is more accurate to quantify the effect of the treatment based on overall sample moisture contents. Fig. 6 shows the moisture content (MC, wet basis) of black peppercorns from the control group after dry heating (15 min at 80 °C) and after pasteurization (for 5-log reduction of *S.* Enteritidis) using different RH levels (at 80 °C). The MC (wet basis) of the samples dropped from 9.7 ± 0.3 % to 8.4 ± 0.2 % after 15 min dry heating and then increased to 8.7 ± 0.5 %, 9.2 ± 0.4 %, and 9.2 ± 0.2 %, respectively, after the treatments at 60 % RH for 25.6 min, 70 % RH for 9.1 min, and 80 % RH for 2.9 min. That is, the 15 min dry heating caused a 1.3 % moisture loss, but the RH-controlled heating brought the MC back to within 0.5–1 % of the initial level. Statistically, the samples treated with 60 % RH for 25.6 min had a significantly lower moisture content compared to the control group. While the MC of samples treated with 70 % and 80 % RH for 9.1 and 2.9 min, respectively, was not significantly different from that of untreated samples (control). According to the American Spice Trade Association (2011), the moisture content of black peppercorns should be kept below 10.5 % (wet basis) to inhibit the growth of microorganisms. Therefore, the black peppercorns pasteurized in this study using RH-controlled moist heat at 80 °C and RH

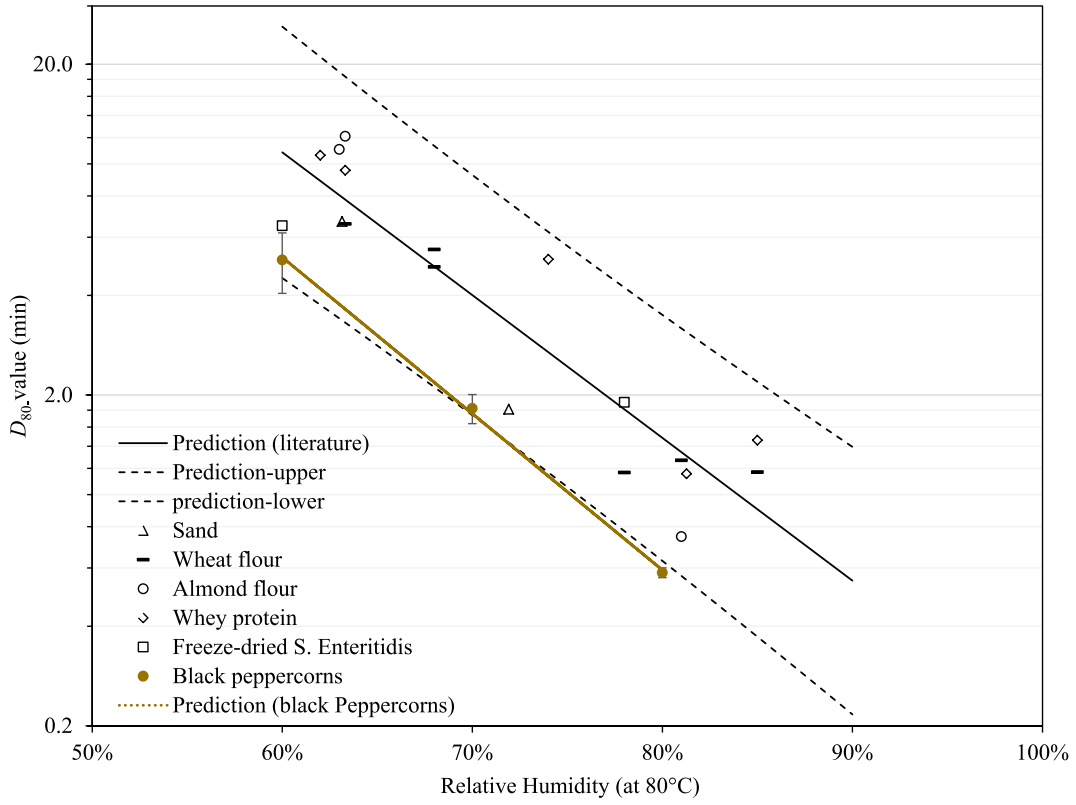


Fig. 5. Comparison of D-values of *S.* Enteritidis on different matrices at 80 °C and RH above 60 %. The prediction line with 95 % prediction interval was generated using data from the literature. The data for sand was from Liu et al. (2018); for wheat flour from Liu et al. (2018) and Xu et al. (2019); for almond flour and whey protein from Xu et al. (2019); for freeze-dried *S.* Enteritidis from Xie, Xu, et al. (2021). The data for black peppercorns was generated in this study (n=3).

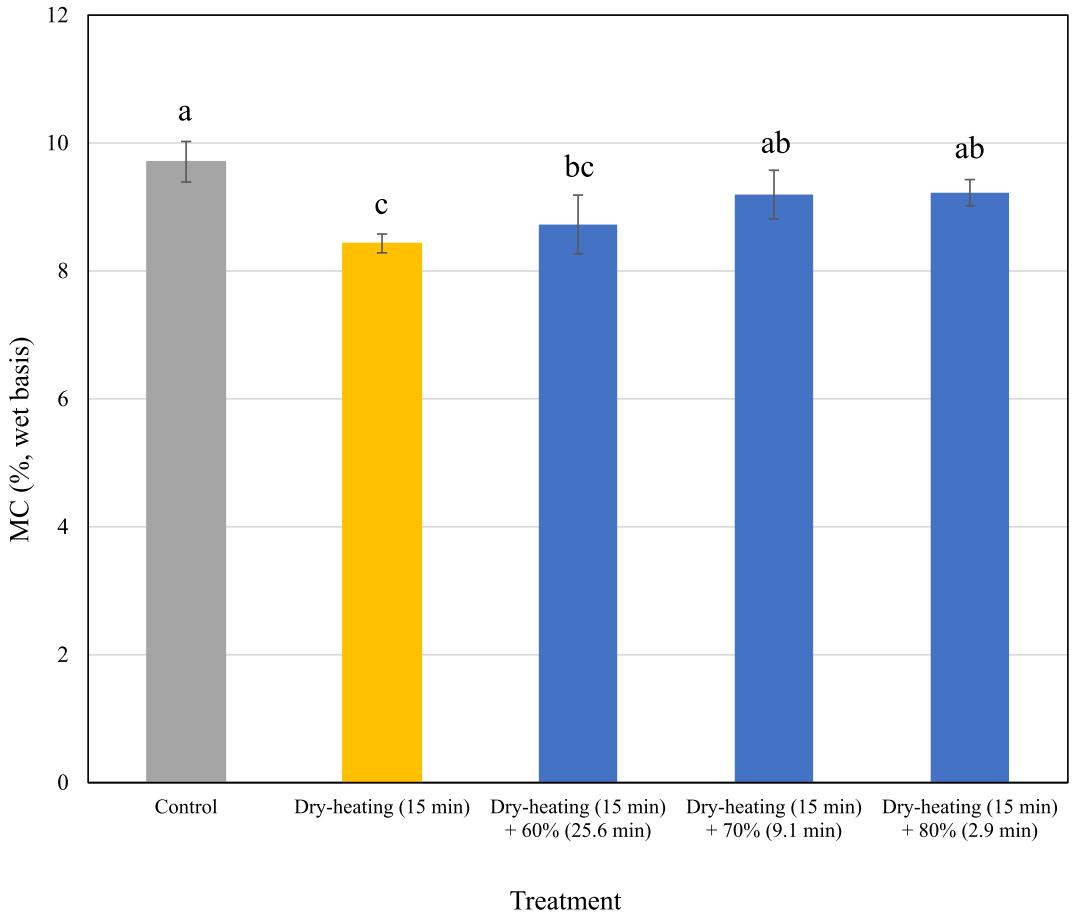


Fig. 6. The moisture content of black peppercorns without treatment (control, in grey), after 15 min of dry heating (yellow), and after three RH-controlled thermal treatments (80 °C) for 5-log reductions of *S.* Enteritidis (blue) (n = 4). Different letters indicate significant differences between groups ($P < 0.05$)

60–80 % should have an acceptable MC that is suitable for shipping and storage.

For industrial applications, treatments with different combinations of temperature and RH can be used to provide flexibility to preserve the quality of various food materials. The effect of temperature can be quantified using Z_T value which indicates the temperature increase that would cause a 10-fold reduction in D-value (Gaillard et al., 1998). A previous study suggests that Z_T value is RH-dependent or moisture content (of bacteria) dependent (Jin et al., 2020). The amount of moisture gained from the treatment is affected by the food particle properties (e.g., size, structure, hydrophilicity) and the treatment conditions (e.g., temperature, RH, and time). Depending on the product characteristics, a moisture equilibration step or a fast surface drying step might be needed. In the case of black peppercorns, the treated peppercorns do not require further drying, but an equilibration step may still be needed.

4. Conclusion

This study demonstrated the effectiveness of a controlled high RH thermal treatment method for control of *Salmonella* in low-moisture foods by using 80 °C and60, 70, and 80 % RH. The results indicate that the above treatment conditions can achieve a 5-log reduction of *S.* Enteritidis PT 30 in black peppercorns in a relatively short time. The study also showed that the first-order model could provide a good prediction to the thermal death of *S.* Enteritidis in black peppercorns, and the death rate was higher with a higher RH. The effect of RH can be modeled with a log-linear equation. With a dry preheating step to elevate the sample temperature before the high RH treatments, condensation can be avoided. The pasteurized samples can have an acceptable moisture content and thus do not need to be dried following the treatments. Overall, this study provided an alternative method for the food industry to control pathogens from low-moisture foods and ingredients. The new method is effective for *Salmonella* inactivation and prevents moisture condensation. The outcomes of the treatments are predictable when the temperature and RH are well controlled.

CRediT authorship contribution statement

Ren Yang: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Project administration. **Stephen P. Lombardo:** Methodology, Funding acquisition, Writing – review & editing. **William F. Conway:** Methodology, Writing – review & editing. **Juming Tang:** Conceptualization, Resources, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

American Spice Trade Association (2011). *Clean, safe spices: guidance from the American Spice Trade Association*. Washington, DC.

Birmingham, T. (2018). 10 Years, 0 Salmonella Outbreaks. *Food Safety* Tech. Available at: https://foodsafetytech.com/column/10-years-0-salmonella-outbreaks/. Accessed 03 Aug. 2021.

Buck, A. L. (1981). New equations for computing vapor pressure and enhancement factor. *Journal of Applied Meteorology, 20*, 1527–1532. https://doi.org/10.1175/1520-0450(1981)020%3C1527:NEFCVP%3E2.0.CO;2

Cebrián, G., Condón, S., & Mañas, P. (2017). Physiology of the inactivation of vegetative bacteria by thermal treatments: Mode of action, influence of environmental factors and inactivation kinetics. *Foods, 6*(12), 107. https://doi.org/10.3390/foods6120107

Dufort, E. L., Sogin, J., Etzel, M. R., & Ingham, B. H. (2017). Inactivation kinetics of pathogens during thermal processing in acidified broth and tomato purée (pH 4.5). *Journal of Food Protection, 80*(12), 2014–2021. https://doi.org/10.4315/0362-028X.JFP-17-147

Eliasson, L., Isaksson, S., Lövenklev, M., & Ahrné, L. (2015). A comparative study of infrared and microwave heating for microbial decontamination of paprika powder. *Frontiers in Microbiology, 6*, 1071. https://doi.org/10.3389/fmicb.2015.01071

Food and Drug Administration (2000). *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies*: A Report of the Institute of Food Technologists for the Food and Drug Administration of the US Department of Health and Human Services. Available at: https://www.fda.gov/files/food/published/Kinetics-of-Microbial-Inactivation-for-Alternative-Food-Processing-Technologies.pdf. Accessed 03 June 2022.

Food and Drug Administration (2017). Risk Profile: Pathogens and Filth in Spices. Available at: https://www.fda.gov/media/108126/download. Accessed 27 April 2022.

Food Safety and Standards Authority of India (2021). *Manual of Methods of Analysis of Foods: Spices Herbs and Condiments*. Available at: https://fssai.gov.in/upload/uploadfiles/files/Manual_Revised_Spices_Herbs_22_06_2021.pdf. Accessed 08 June 2022.

Gaillard, S., Leguérinel, I., & Mafart, P. (1998). Model for combined effects of temperature, pH and water activity on thermal inactivation of Bacillus cereus spores. *Journal of Food Science, 63*(5), 887–889. https://doi.org/10.1111/j.1365-2621.1998.tb17920.x

Jeong, S., Marks, B. P., Ryser, E. T., & Harte, J. B. (2012). The effect of X-ray irradiation on Salmonella inactivation and sensory quality of almonds and walnuts as a function of water activity. *International Journal of Food Microbiology, 153*(3), 365–371. https://doi.org/10.1016/j.ijfoodmicro.2011.11.028

Jin, Y., Tang, J., & Zhu, M. J. (2020). Water activity influence on the thermal resistance of Salmonella in soy protein powder at elevated temperatures. *Food Control, 113*, Article 107160. https://doi.org/10.1016/j.foodcont.2020.107160

Liu, S., Tang, J., Tadapaneni, R. K., Yang, R., & Zhu, M. (2018). Exponentially increased thermal resistance of *Salmonella* spp. and *Enterococcus faecium* at reduced water activity. *Applied and Environmental Microbiology, 84*(8). https://doi.org/10.1128/AEM.02742-17. e02742-17.

Lung, H. M., Cheng, Y. C., Chang, Y. H., Huang, H. W., Yang, B. B., & Wang, C. Y. (2015). Microbial decontamination of food by electron beam irradiation. *Trends in Food Science & Technology, 44*(1), 66–78.

Murrell, W. G., & Scott, W. J. (1966). The heat resistance of bacterial spores at various water activities. *Microbiology, 43*(3), 411–425. https://doi.org/10.1099/00221287-43-3-411

Nguyen, L., Duong, L. T., & Mentreddy, R. S. (2019). The US import demand for spices and herbs by differentiated sources. *Journal of Applied Research on Medicinal and Aromatic Plants, 12*, 13–20. https://doi.org/10.1016/j.jarmap.2018.12.001

Peleg, M. (2006). *Advanced quantitative microbiology for foods and biosystems: Models for predicting growth and inactivation*. Boca Raton, FL: CRC Press.

Sanders, T. H., & Calhoun, R. S. (2014). Effect of oil and dry roasting of peanuts at various temperatures and times on survival of *Salmonella* and *Enterococcus faecium*. *Peanut Science, 41*(2), 65–71. https://doi.org/10.3146/PS13-16.1

Schweiggert, U., Carle, R., & Schieber, A. (2007). Conventional and alternative processes for spice production–a review. *Trends in Food Science & Technology, 18*(5), 260–268. https://doi.org/10.1016/j.tifs.2007.01.005

Song, W. J., Sung, H. J., Kim, S. Y., Kim, K. P., Ryu, S., & Kang, D. H. (2014). Inactivation of Escherichia coli O157: H7 and Salmonella Typhimurium in black pepper and red pepper by gamma irradiation. *International Journal of Food Microbiology, 172*, 125–129. https://doi.org/10.1016/j.ijfoodmicro.2013.11.017

Syamaladevi, R. M., Tang, J., & Zhong, Q. (2016). Water diffusion from a bacterial cell in low-moisture foods. *Journal of Food Science, 81*(9). https://doi.org/10.1111/1750-3841.13412

Verma, T., Wei, X., Chaves, B. D., Howell, T., Jr, & Subbiah, J. (2022). Antimicrobial efficacy of gaseous chlorine dioxide for inactivation of Salmonella and Enterococcus faecium NRRL B-2354 on dried basil leaves. *LWT, 153*, Article 112488. https://doi.org/10.1016/j.lwt.2021.112488

Villa-Rojas, R., Zhu, M. J., Paul, N. C., Gray, P., Xu, J., Shah, D. H., & Tang, J. (2017). Biofilm forming Salmonella strains exhibit enhanced thermal resistance in wheat flour. *Food Control, 73*, 689–695. https://doi.org/10.1016/j.foodcont.2016.09.021

Xie, Y., Cheng, T., Wei, L., Zhu, M. J., Sablani, S. S., & Tang, J. (2021). Thermal inactivation of Salmonella Enteritidis PT30 in ground cinnamon as influenced by water activity and temperature. *Food Control, 124*, Article 107935. https://doi.org/10.1016/j.foodcont.2021.107935

Xie, Y., Xu, J., Yang, R., Alshammari, J., Zhu, M. J., Sablani, S., & Tang, J. (2021). Moisture content of bacterial cells determines thermal resistance of *Salmonella enterica* Serotype Enteritidis PT 30. *Applied and Environmental Microbiology, 87*(3). https://doi.org/10.1128/AEM.02194-20

Xu, J., Tang, J., Jin, Y., Song, J., Yang, R., Sablani, S. S., & Zhu, M. (2019). High temperature water activity as a key factor influencing survival of *Salmonella* Enteritidis PT30 in thermal processing. *Food Control, 98*, 520–528. https://doi.org/10.1016/j.foodcont.2018.11.054

Yang, R., Xu, J., Lombardo, S. P., Ganjyal, G. M., & Tang, J. (2020). Desiccation in oil protects bacteria in thermal processing. *Food Research International, 137*, Article 109519. https://doi.org/10.1016/j.foodres.2020.109519

Yang, R., Cheng, T., Hong, Y., Wei, L., & Tang, J. (2022). The effect of dry headspace on the thermal resistance of bacteria in peanut oil and peanut butter. *Food Control*. https://doi.org/10.1016/j.foodcont.2022.108851

Yang, R., Wei, L., Dai, J., & Tang, J. (2022). Thermal death kinetics of Salmonella Enteritidis PT30 in peanut butter as influenced by water activity. *Food Research International, 157*, Article 111288. https://doi.org/10.1016/j.foodres.2022.111288

Zhou, Z., Zuber, S., Campagnoli, M., Putallaz, T., Devlieghere, F., & Uyttendaele, M. (2019). Effect of mild steaming treatment on the inactivation of Salmonella, Listeria monocytogenes, Escherichia coli O157: H7 and their surrogates on black peppercorns. *Food Control, 106*, Article 106726. https://doi.org/10.1016/j.foodcont.2019.106726