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# Influence of radio frequency treatment on in-shell walnut quality and *Staphylococcus aureus* ATCC 25923 survival

Lihui Zhang[a], James G. Lyng[b], Ruzhen Xu[a], Shuang Zhang[a], Xu Zhou[a], Shaojin Wang[a,c,*]

[a] College of Mechanical and Electronic Engineering, Northwest A&F University, Yangling, 712100, China
[b] Institute of Food and Health, University College Dublin, Belfield, Dublin 4, Ireland
[c] Washington State University, Department of Biological Systems Engineering, Pullman, WA, 99164-6120, USA

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ABSTRACT

Radio frequency (RF) treatment is considered as a potential method for eliminating food-borne pathogens from low moisture foods. In this study, a treatment protocol for pasteurizing in-shell walnuts (with 15.01% w.b. of moisture) was developed using a 6 kW, 27.12 MHz RF oven with the objective of maintaining product quality. The uniformity of RF heating was improved by combining with hot air and also product mixing and holding during treatment. The optimized RF treatment protocol for in-shell walnuts involved pre-heating between an electrode gap of 16.0 cm, followed by drying for 40 min between an electrode gap of 19.0 cm, the process finished with forced air cooling of the walnuts in a single layer. This RF treatment produced in excess of a 4-log reduction of *Staphylococcus aureus* ATCC while differences in kernel color were not significant between control and RF treated walnuts during accelerated storage. Peroxide values of RF treated samples increased by more than 1 meq/kg during storage but showed no significant differences to controls while fatty acid values of both the control and RF treated samples were below 0.6% during storage. The moisture content and water activities of walnut shells and kernels of control and RF treated samples initially decreased and then tended to stabilize during accelerated storage. During storage the population of *S. aureus* ATCC 25923 in RF treated samples was gradually reduced to below the detection limit. Therefore, RF treatments could be considered as an effective method to control pathogens on in-shell walnuts.

## 1. Introduction

Raw walnuts are increasing in popularity among consumers due to their high nutritional values. However, bacterial pathogens have been isolated from walnuts in a number of outbreaks or in recalls associated with tree nuts or nut components added to foods (Harris, Palumbo, Beuchat, & Danyluk. 2016; Palumbo, Beuchat, Danyluk, & Harris, 2016). In addition, *Salmonella* and *E. coli* have been detected in walnuts in some sampling surveys (Davidson, Frelka, Yang, Jones, & Harris, 2015; Little, Rawal, de Pinna, & McLauchlin, 2010; Zhang et al., 2017). Although outbreaks originating from in-shell walnuts are less reported, *Salmonella* has been detected on in-shell walnuts (CFIA, 2012; 2017). Also, in a previous study performed by the current group, the genus *Staphylococcus* was isolated from in-shell walnut surfaces (Zhang & Wang, 2017). Contaminants on walnut shells can survive for prolonged periods and then transfer to the kernels during harvest and other processing steps, particularly where the shells are compromised (Blessington, Theofel, Mitcham, & Harris, 2013; Frelka & Harris, 2015). Furthermore, microbial levels were found to increase on kernels during hulling even without shell damage (Frelka, Blessington, & Harris, 2012). Frelka and Harris (2015) reported no effective reduction of microorganisms on walnut shells after both hot air drying and antimicrobial spray treatment during postharvest handling. Thus, an efficient method for pasteurizing in-shell walnuts to reduce the target pathogen population to an acceptable level is needed prior to consumption.

Still viewed as a relatively novel thermal treatment, radio frequency (RF) energy can raise the temperature of whole samples with high efficiency and deep penetration depths and has been widely used for the pasteurization of agricultural products. RF heating can maintain product quality while meeting the pasteurization requirements (≥4 log reductions) especially for low moisture products, such as corn (Zheng, Zhang, & Wang, 2017), and almonds (Gao, Tang, Villa-Rojas, Wang, & Wang, 2011; Jeong, Baik, & Kang, 2017; Li, Kou, Cheng, Zheng, & Wang, 2017a), chestnuts (Hou, Kou, Li, & Wang, 2018), seeds (Jiao, Zhong, & Deng, 2016), peppers (Hu, Zhao, Hayouka, Wang, & Jiao, 2018; Jeong & Kang, 2014), and wheat germ (Ling, Ouyang, & Wang,

* Corresponding author. College of Mechanical and Electronic Engineering, Northwest A&F University, Yangling, 712100, China.
*E-mail address:* shaojinwang@nwsuaf.edu.cn (S. Wang).

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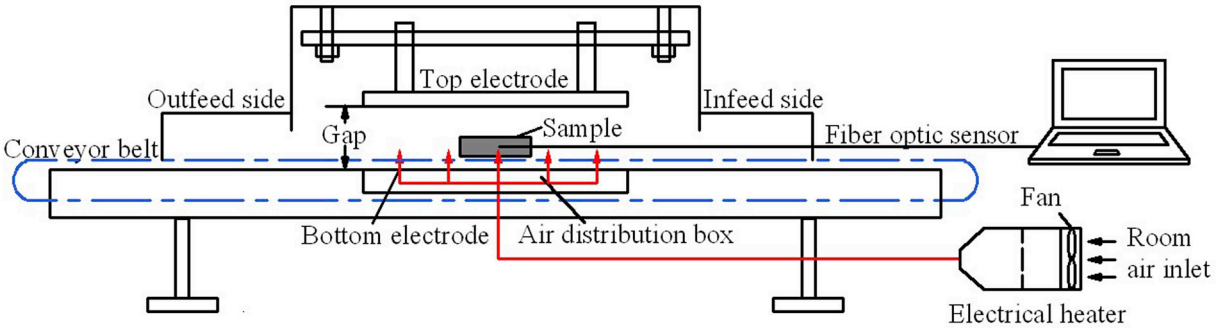


**Fig. 1.** Schematic view of the pilot-scale 6 kW, 27.12 MHz RF system (Adapted from Wang et al., 2010).

2019). Several studies have explored the use of RF energy for disinfestation and drying of in-shell walnuts (Wang, Monzon, Johnson, Mitcham, & Tang, 2007a, 2007b; Mitcham et al., 2004; Zhang, Zheng, Zhou, Huang, & Wang, 2016; Zhou, Gao, Mitcham, & Wang, 2017). Zhou et al. (2017) has showed that hot air-assisted RF drying is more efficient and rapid compared with conventional hot air drying. Simultaneously, the quality of in-shell walnuts was not affected after RF treatment and during accelerated storage. However, few studies focused on the feasibility of pasteurizing in-shell walnuts using RF energy. Although mold in the RF treated bread was not recovered during storage (Liu et al., 2011), there is still a lack of information on survival of bacterial pathogens in RF treated walnuts during storage.

*S. aureus* ATCC 25923 is a suitable surrogate microorganism for pasteurization validation in food processing (Zhang, Kou, Zhang, Cheng, & Wang, 2018). Moreover, water activity ($a_w$) has been confirmed as a crucial factor affecting the thermal resistance of *S. aureus* ATCC 25923 on walnut shells. $D_{64}$ values can be reduced from 10.21 ( ± 0.65) min to 0.56 ( ± 0.04) min as walnut $a_w$ value increases from 0.586 to 0.931. Thus, moisture content of in-shell walnuts should be raised before RF treatment to reduce the thermal resistance of pathogens and reduce the risk of loss in product quality. However, the moisture variation among walnuts would result in non-uniform RF heating (Mitcham et al., 2004; Wang et al., 2007b). In addition, there are many other non-product factors that have also been shown to affect RF heating uniformity (Huang, Marra, Subbiah, & Wang, 2018). Therefore, RF heating uniformity is an important factor to consider during the development of effective RF treatments for the control of pathogens on in-shell walnuts.

The objectives of this study on in-shell walnuts were (1) to select appropriate RF heating parameters for controlling pathogens, (2) to improve the RF heating uniformity (3) to establish and validate a RF treatment protocol for achieving more than 4-log reduction of *S. aureus* ATCC 25923 and (4) to evaluate the walnut quality and the survival of *S. aureus* ATCC 25923 on in-shell walnuts during an accelerated storage following RF treatments.

## 2. Materials and methods

### 2.1. Materials

Raw in-shell walnuts (*Juglans regia* L.) which had been stored for at least three months were purchased from a local market in Yangling, Shaanxi, China. Before the experiment, in-shell walnuts were pre-screened based on the categories described by Frelka (2013) and Frelka and Harris (2015), and intact samples were selected and stored in polyethylene bags at 4 °C. The shells and kernels of ten randomly collected in-shell walnuts were separated and then milled in a blender (FLB-100, 50–300 mesh, Philip Bo Food Machinery Corp, Shanghai, China). The moisture content (MC) of these walnut shells, kernels and whole samples were found to be 12.48 ± 0.06%, 5.15 ± 0.02%, and 8.50 ± 0.03% (w.b.), respectively, measured using a moisture analyzer (HE53, Mettler-Toledo, Shanghai, China). Samples which were treated with this moisture content were designated as native moisture content (NMC) samples.

### 2.2. Sample preparation

In addition to the NMC samples, a series of adjusted moisture content (AMC) samples were prepared. This adjustment was performed in order to reduce the thermal resistance of the target pathogens and in an effort to maintain product quality following heating (Gao et al., 2011; Li et al., 2017a). AMC in-shell walnut samples had their moisture content elevated by adding pre-calculated quantities of distilled water (Zheng et al., 2017) to the walnuts which were sealed in polyethylene bags and placed at 4 °C for 7-days to equilibrate. Walnuts were shaken twice daily to ensure uniformity of moisture distribution. Finally, the AMC in-shell walnuts were then removed from the packs and maintained for 12 h at room temperature (20 ± 1 °C) to further equilibrate prior to each test. The target MC of the AMC walnut shells was 18.00% w.b. which was chosen based on the thermal death kinetics of *S. aureus* ATCC 25923 from our previous study (Ling, Tang, Kong, Mitcham, & Wang, 2015; Zhang et al., 2018). The attained MCs were 18.05% ( ± 0.32), 11.67% ( ± 0.57), and 15.01% ( ± 0.28) w.b. for the shells, kernels and the whole in-shell walnuts, respectively.

### 2.3. Hot air-assisted RF heating system

The hot air-assisted RF heating system (Fig. 1) used in the present study consisted of a 6 kW, 27.12 MHz pilot-scale free running oscillator (SO6B, Strayfield International, Wokingham, U.K.) and a hot air system consisting of a 6-kW electric heater (Wang, Tiwari, Jiao, Johnson, & Tang, 2010). Adjustments to RF power delivery to the samples were made by adjusting the top electrode. A forced hot air flow was provided by the air distribution box under the bottom electrode to maintain sample surface temperatures.

### 2.4. Selection of electrode gap

The AMC in-shell walnuts (2.08 ± 0.10 kg) and the NMC samples (1.85 ± 0.06 kg) were each placed in two layers in polyethylene containers (30 cm × 37 cm × 9 cm) (Fig. 2). The general relationship between the electrode gap and electric current (*I*, A) was obtained by placing the samples on the stationary conveyor belt between the two electrodes. The electrode gaps ranged from 10.0 cm to 19.0 cm and were adjusted at 1.0 cm intervals according to the method of Zhou, Ling, Zheng, Zhang, and Wang (2015). Three electrode gaps were selected for further heating rate tests based on the electric current values displayed on the screen of the RF system. These gaps for the preconditioned in-shell walnuts were selected based on a target heating rate (4–6 °C/min). A six-channel fiber-optic temperature sensor system (HQ-FTS-D120, Heqi Technologies Inc., Xian, China) was used to measure the sample temperatures. The probes were inserted into

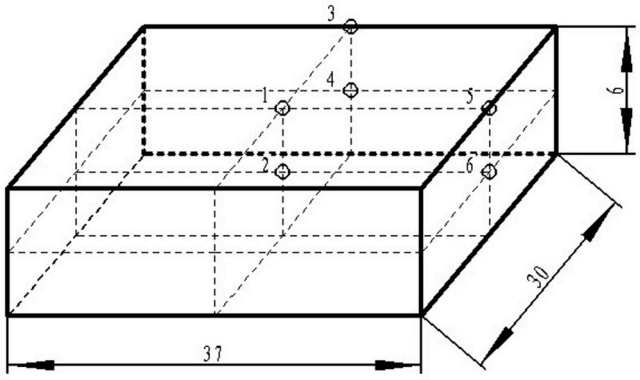


**Fig. 2.** Locations (1–6) in the plastic container for in-shell walnut temperature measurements during RF treatment (all dimensions are in cm).

walnut kernels at six representative locations (Fig. 2) through predrilled holes. The temperature of the samples was recorded continuously until the samples reached 70 °C.

### 2.5. Heating uniformity tests

Many studies reported that RF heating uniformity was improved when combined with forced hot air, product movement, mixing and so on (Hou, Ling, & Wang, 2014; Huang, Zhu, Yan, & Wang, 2015; Ling, Hou, Li, & Wang, 2016; Wang et al., 2007b; Zheng, Zhang, Zhou, & Wang, 2016; Zhou et al., 2015). In this study, the uniformity index (λ) defined as the ratio of standard deviation increase to the average temperature increase (Wang, Yue, Chen, & Tang, 2008) was determined and compared under the following conditions: (1) RF pre-heating to 70 °C; (2) Hot air assisted RF pre-heating to 70 °C with samples mixed on three occasions during pre-heating; (3) Hot air assisted RF pre-heating to 70 °C with samples mixing on 3 occasions followed by holding at 70 °C in hot air for 40 min. The smaller the uniformity index the greater the RF heating uniformity. Based on the above studies, the total pre-heating time was 9 min with the selected heating rate of 5 °C/min and three mixings were performed after one third of total RF pre-heating time had elapsed (i.e. 3, 6 and 9 min). For mixing, the RF system was turned off, the container containing the samples was removed and samples were mixed by hand for < 1 min before being returned to the RF cavity for the next phase of pre-heating and mixing. The samples were divided into two layers by nylon gauze (300 meshes/inch) after the third mixing to easily map the surface temperatures. The surface temperatures of two layers were mapped using a thermal imaging camera (DM63, Zhejiang Dali Technology Co., Ltd, Hangzhou, China). Each test was repeated in triplicate.

### 2.6. Determination of RF treatment protocol to control pathogens

#### 2.6.1. Preparation of cell suspension

*S. aureus* ATCC 25923 was chosen for validation of the RF treatment protocol based on the outcomes of our previous study (Zhang et al., 2018). The cell suspensions of the strain were obtained according to the methods of Li et al. (2017a) and Li, Shi, Ling, Cheng, Huang, & Wang (2017b). The final cell populations were adjusted to a level of $10^9$–$10^{10}$ CFU/mL and stored in phosphate buffer saline (PBS, pH7.2) at 4 °C prior to the experiment.

#### 2.6.2. Inoculation

Before inoculation, both of the NMC and AMC in-shell walnuts were exposed to UV light for 1 h to reduce any background natural microbial loads already present on the surfaces. A slightly modified version of the method of Uesugi, Danyluk, and Harris (2006) was then used for inoculating the in-shell walnuts. The inoculum (20 mL) was added to a sterile polyethylene bag with 400 g in-shell walnuts following which the samples were shaken by hand for 5 min. The inoculated walnuts were spread onto two layers of filter paper and then dried for 1 h and 24 h to select the most suitable drying time under ambient conditions (20 ± 1 °C with 25–35% relative humidity). Simultaneously, the MC and water activity ($a_w$) of inoculated walnut shells and kernels were analyzed before and after drying. After drying, the inoculated samples were placed into nylon mesh bags (4 cm × 6 cm, 300 mesh/inch) for RF treatments. For health and safety reasons, the samples used for measuring MC and $a_w$ were inoculated with the cell suspension sterilized at 121 °C for 20 min. An Aqua Lab water activity meter (Model 4 TE, Decagon Devices, Inc., Pullman, WA, USA) was used to measure $a_w$ of samples.

#### 2.6.3. Treatment protocol development

Based on the above studies, the electrode gap was fixed at 16.0 cm, hot air temperature was adjusted to 70 °C and samples were mixed on 3 occasions during RF pre-heating (as previously described). After elevating the samples to the target temperature, a suitable electrode gap was adjusted to hold a target temperature of 70 °C for drying in the RF cavity. The inoculated samples which were removed at set time intervals were immediately placed in sealed plastic bags and immersed in ice water (< 4 °C for at least 2 min) for cooling prior to enumeration (Li et al., 2017a).

A rapid cooling method is necessary to avoid quality degradation after hot air assisted RF treatments. According to several previous studies (Li et al., 2017a; Wang et al., 2010; Zheng et al., 2016), the in-shell walnut samples after RF treatments in a single layer were subjected to forced ambient air cooling (3.5 m/s) provided by an electric fan. The center temperature of in-shell walnuts was measured by the fiber-optic temperature sensor system until it dropped to about 30 °C. This process was repeated in duplicate.

#### 2.6.4. Enumeration method

After RF heating treatments, individual in-shell walnuts were added to 10 mL phosphate buffer saline (PBS, pH7.2) supplemented with 0.1% Tween 80 in sterile flasks. Then, the suspension was serially diluted 10-fold in sterile PBS, and 0.1 mL of the appropriate diluent was spread onto LB agar. All plates were incubated at 37 °C for 24 h. For in-shell walnuts, the calculated CFU per milliliter of plated solution multiplied by 10 mL (the volume of diluent per each nut) which was considered to be equivalent to the CFU recovered per individual in-shell nut (Blessington et al., 2013; Frelka, Davidson, & Harris, 2016). The lower limit of detection (LOD) was 10 CFU/nut. Enrichment was needed when the results were expected to be below the LOD. According to the method of Blessington, Mitcham, and Harris (2012), 1 mL of the 10 mL diluent was added to 50 mL of LB broth and incubated at 37 °C for 24 h or 48 h. Then 0.1 mL or 10 μL of the enrichment broth was streaked onto the *Staphylococcus* chromogenic medium (LB: Beijing AOBOX, Beijing, China) and incubated at 37 °C for 24 h or 48 h. When counts were positive after enrichment, it was assumed the value equaled the LOD. When enrichment results were negative, the counts were assigned a value of 0 log CFU/nut.

### 2.7. Drying curve

Following treatments, it was necessary to restore the MC of AMC in-shell walnuts to their original levels for long term storage. A drying curve was obtained for in-shell walnuts during the RF drying protocol described above. Three walnuts were placed in a nylon mesh bag (4 cm × 6 cm, 300 mesh/inch). Six bags were prepared and each bag was placed in one of the six locations shown in Fig. 2. During drying, each of the six bags were removed at 2-min intervals to measure the sample weight and immediately returned to the RF cavity for further RF drying until the MC of the AMC in-shell walnuts were reduced to their initial value. This process was repeated in duplicate.

### 2.8. Storage experiment and quality analyses

#### 2.8.1. Storage conditions

The accelerated storage test was performed in an incubator (HWS-150, Shanghai Senxin Instrument Co., Ltd., Shanghai, China) at 35 °C and 30% relative humidity (RH) for 10 and 20 days to simulate commercial storage at 4 °C for 1 and 2 years (Wang, Monzon, Johnson, Mitcham, & Tang, 2007a). Meanwhile, in another incubator, a higher RH condition (65%) at 35 °C was set to evaluate the effect of humidity on survival of *S. aureus* ATCC 25923 on in-shell walnuts during storage.

#### 2.8.2. Quality analyses

After RF treatments, the quality of NMC samples and RF treated AMC samples was evaluated immediately post treatment and after the accelerated storage test. Fatty acid (FA), peroxide values (PV) and the color value of walnut kernels were evaluated. The FA and PV values were determined by official methods of Ca 5a-40 and Cd 8–53 (AOCS, 1998a, b), which were also described by Wang et al. (2001). The color value was measured with a computer vision system (CVS), which was described in detail by Hou et al. (2014) and Ling et al. (2016).

#### 2.8.3. Survival of S. aureus ATCC 25923 on in-shell walnuts

As *S. aureus* ATCC 25923 could still be detected on in-shell walnuts after RF drying so it was necessary to investigate the survival of *S. aureus* ATCC 25923 during storage. Two parallel RF treatments were performed using separately prepared inoculum for the storage test. In addition, two controls were used in this study: AMC control represented inoculated AMC samples with moisture content restored to its original level of 15.01% w.b. while NMC control represented the inoculated NMC samples. Thus, there were two RF dried samples and four controls (i.e. two replicates of AMC control and NMC control) under each storage condition. The controls and RF dried samples were then packed in polyethylene plastic woven bags and stored as previously described. To evaluate the survival of *S. aureus* ATCC 25923 on in-shell walnuts during storage, two individual walnuts were removed from each of these samples each day during the storage. The enumeration method used on these samples was as described above and the *Staphylococcus* chromogenic medium was used to differentiate the microorganisms infected during storage. About 100 g of the controls inoculated with sterilized cell suspension and RF treated in-shell walnuts were removed to measure the MC and $a_w$ of shells and kernels every two days during the storage using the method described above.

### 2.9. Statistical analysis

The mean values and standard deviations were calculated from two or three replicates for each treatment. Differences ($P \leq 0.05$) were estimated by analysis of variance followed by Tukey's pairwise comparison test. All statistical analyses were performed using the statistical software SPSS 17.0 version (SPSS Inc., Chicago, IL, USA).

## 3. Results and discussion

### 3.1. Electric current under different electrode gap

Fig. 3 shows the relationship between electric current and electrode gap when the container was empty or was filled with in-shell walnuts. The electric current was relatively stable when an empty container was used and the electrode gap was changed. When walnuts were added to the container, the electric current gradually decreased as the electrode gap increased from 10.0 cm to 19.0 cm (Fig. 3). Similar trends were found in other studies (Li et al., 2017a; Ling et al., 2016; Zhang et al., 2016; Zhou et al., 2015). Based on these results, three electrode gaps (12.0, 12.5 and 13.0 cm were chosen for the NMC samples and while 14.0, 15.0 and 16.0 cm spacing's were chosen for AMC samples were selected for further RF heating tests.

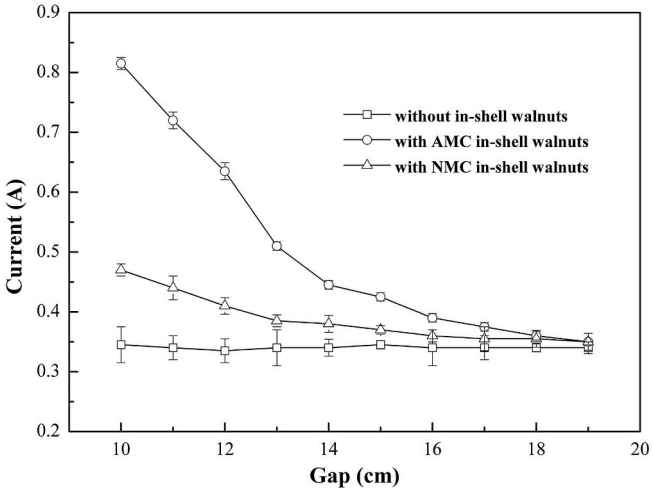


**Fig. 3.** Electric current of the radio frequency system as a function of electrode gap.

### 3.2. Determination of electrode gap

Fig. 4 shows the average and standard deviation for walnut temperatures at the six locations in the container placed between the three selected electrode gaps during RF heating. The heating rate increased with decreasing electrode gap. However, more rapid RF heating adversely affected the heating uniformity. To obtain a better balance between throughput and heating uniformity, the heating rate was chosen as 5 °C/min. The electrode gaps of 16.0 and 12.5 cm for the adjusted samples and control were chosen to achieve a suitable heating rate of 5.14 ( ± 0.75) and 4.95 ( ± 0.54) °C/min, respectively, used for further heating uniformity tests.

### 3.3. Heating uniformity in RF treated walnuts

The initial surface temperature distribution of in-shell walnuts was relatively uniform before RF treatment. Table 1 shows a detailed comparison of the temperature distribution and heating uniformity index values in top and bottom layers for NMC and AMC samples after RF heating under various conditions. The average surface temperatures of the walnut samples were obtained by the collecting individual data points from the thermal images. The average surface temperatures in the walnuts in the top layer were higher than those in the bottom layer. The results were similar to those reported by Hou et al. (2014) but differed from those found for corns (Zheng et al., 2016) and pistachios (Ling et al., 2016), where the surface temperatures in bottom layer were found to be higher than those in top layer. The samples in the bottom layer with lower temperatures could be due to the larger voids among walnuts and surface contact with the bottom wall of the container, which could cause more heat loss to ambient air.

The moisture content (MC) of the samples had an effect on the RF heating uniformity. Wang et al. (2007b) found that the washed walnuts had a higher heating uniformity index. In addition, Zheng et al. (2016) also reported that the heating uniformity index was larger in corn samples with higher MC. Similar trends were found in our study (Table 1). The difference was possibly related to the dielectric properties of walnuts and electrode gaps (Tiwari, Wang, Tang, & Birla, 2011).

A combination of forced hot air and mixings improved the RF heating uniformity of in-shell walnuts based on the lower uniformity index value as shown in Table 1. The heating uniformity index values were larger than those for corns (Zheng et al., 2016), chestnuts (Hou et al., 2014) and pistachios (Ling et al., 2016) but were similar to those of walnuts (Wang et al., 2007b). The heating uniformity index values were evaluated after 40 min drying in the RF cavity (Fig. 5) and the

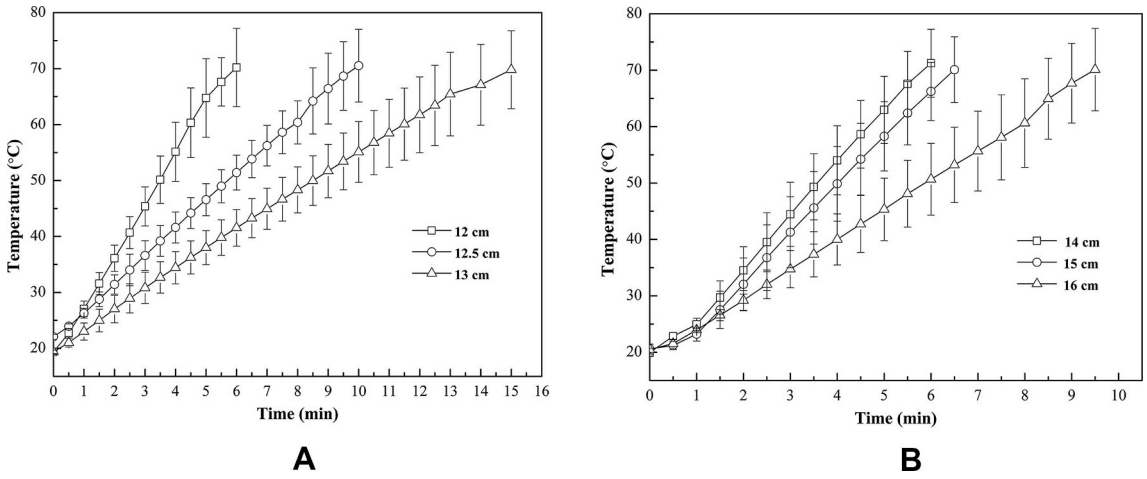


**Fig. 4.** Average temperature-time histories of the RF heated in-shell walnuts over six locations in the container with different electrode gaps (a, NMC in-shell walnuts; b, AMC samples with moisture content of 15.01% w.b.).

values were further reduced. This could be probably caused by the decreased temperature differences between samples due to heat conduction. Therefore, it was adequate to include hot air, mixing and holding in the integrated RF treatments.

### 3.4. Treatment protocol developments

The change of AMC in-shell walnut MC under the combined RF and hot air drying is shown in Fig. 5. It took almost 40 min to reduce the MC of the whole in-shell walnuts to less than 8% w.b., which was the target required for long-term storage (Zhang et al., 2016; Zhou et al., 2017). Therefore, the drying time was fixed at 40 min as illustrated in the drying curve (Fig. 5). The time for the temperature of single layer samples to cool from 70 to 30 °C was about 14 min with forced ambient air. Based on the above studies, the final RF treatment protocol for pasteurization was determined as follows. The electrode gap of 16.0 cm was used for heating 2.08 kg in-shell walnuts in an RF system with 70 °C hot air with samples mixed on three occasions during pre-heating. After RF pre-heating, the electrode gap was adjusted to 19.0 cm and the RF system was continuously turned on for 30 s and then turned off for 1 min to hold the target temperature of in-shell walnuts at 70 °C for 40 min in the RF cavity, then followed by about 14 min forced room air (at 20 °C and 3.5 m/s) cooling in single-layer samples to drop the temperature of samples to about 30 °C. Fig. 5 shows the sample temperature profile of the specific RF processing protocol.

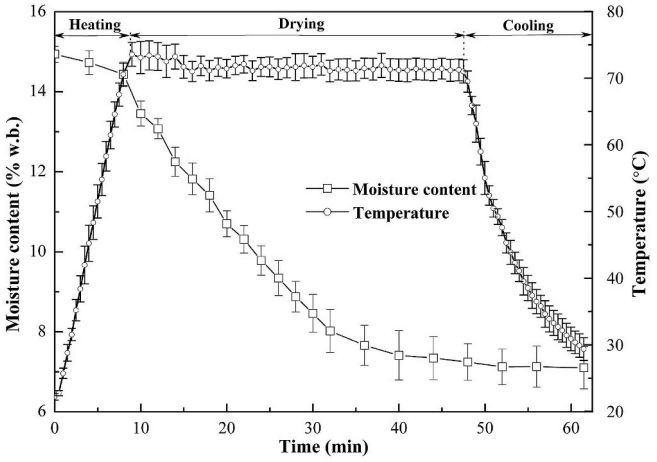


**Fig. 5.** Average temperature-time histories and changes in moisture content of the hot air (70 °C) assisted RF treated AMC in-shell walnuts (15.01% w.b.) over six locations under the electrode gap of 16.0 cm for pre-heating and 19.0 cm for drying followed by forced room air cooling in single layer samples.

### 3.5. Changes in moisture content (MC) and water activity ($a_w$) of walnut shells and kernels after inoculation

Table 2 lists the influence of inoculation on MC and $a_w$ in walnut shells and kernels. Both MC and $a_w$ of the shells and kernels were very similar after equilibration for 1 h. After drying for 24 h, however, the MC and $a_w$ of shells were reduced by nearly 3% w.b. and 0.1, respectively. These results were different from those observed by Blessington et al. (2013), which showed a slightly change in MC (< 0.05%) and $a_w$ (< 0.01) values after drying for 24 h. This could be caused by the different initial MC and $a_w$. Finally, the drying time after inoculation was chosen as 1 h at ambient conditions.

**Table 1**
A comparison of the temperature and heating uniformity index (mean ± SD over 3 replicates) of AMC and NMC in-shell walnut samples after radio frequency (RF) heating under a variety of conditions.

| Layers | RF pre-heating to 70 °C | | RF pre-heating to 70 °C (with hot air and 3 mixings) | | RF pre-heating to 70 °C (with hot air and 3 mixings) followed by 40 min holding | |
|---|---|---|---|---|---|---|
| | NMC | AMC | NMC | AMC | NMC | AMC |
| *Temperature (°C)* | | | | | | |
| Top | 71.11 ± 0.66 | 70.85 ± 1.33 | 70.72 ± 0.93 | 71.33 ± 1.43 | 71.34 ± 1.08 | 72.44 ± 0.60 |
| Bottom | 68.25 ± 1.46 | 68.68 ± 1.65 | 68.61 ± 0.87 | 69.21 ± 0.84 | 70.44 ± 0.71 | 70.67 ± 0.74 |
| *Heating uniformity index (λ)* | | | | | | |
| Top | 0.117 ± 0.003 | 0.145 ± 0.011 | 0.074 ± 0.017 | 0.086 ± 0.009 | 0.069 ± 0.004 | 0.078 ± 0.017 |
| Bottom | 0.150 ± 0.019 | 0.179 ± 0.009 | 0.081 ± 0.011 | 0.104 ± 0.003 | 0.074 ± 0.010 | 0.080 ± 0.012 |

**Table 2**
Moisture content and water activity (mean ± SD over 3 replicates) of AMC walnut shells and kernels after being inoculated and equilibrated for 1 or 24 h at room temperature.

| | Moisture content (%) | | | Water activity | | |
|---|---|---|---|---|---|---|
| | AMC [#] | Equilibrium (1 h) | Equilibrium (24 h) | AMC | Equilibrium (1 h) | Equilibrium (24 h) |
| Shell | 18.05 ± 0.32a[&] | 17.91 ± 0.16a | 15.37 ± 0.19b | 0.948 ± 0.002a | 0.945 ± 0.005a | 0.836 ± 0.008b |
| Kernel | 11.67 ± 0.57a | 11.56 ± 0.28a | 11.52 ± 0.22a | 0.954 ± 0.001a | 0.949 ± 0.002a | 0.933 ± 0.005b |

[#] The MC of the NMC in-shell walnuts was adjusted to 15.01% w.b. by adding distilled water.
[&] Mean values are not significantly different ($P > 0.05$) for the same low case letter within a row among treatments.

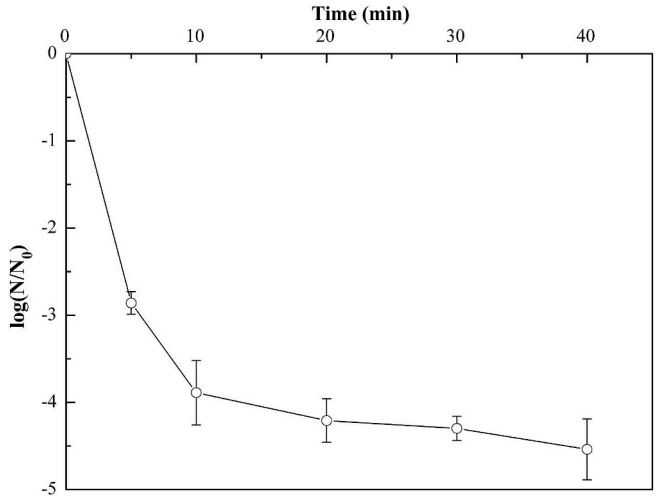


**Fig. 6.** Survival curves for *S. aureus* ATCC 25923 on AMC in-shell walnuts with the moisture content of 15.01% w.b. after hot air assisted RF heating ($N$ and $N_0$, CFU/nut, are the populations at each sampling point and the time zero, respectively).

### 3.6. Verification of RF pasteurization treatment for S. aureus ATCC 25923

Fig. 6 shows the survival curve of *S. aureus* ATCC 25923 on AMC in-shell walnuts after RF treatment. It took 10 min of holding time to reach almost 4-log reduction of *S. aureus* ATCC 25923 on in-shell walnuts. During remaining 30 min of the drying time, the reductions were less than 1-log. This phenomenon could be due to the higher MC at first, which reduced the heat resistance of *S. aureus* ATCC 25923 (Fig. 5). In our study, the shells' MC was reduced from about 18% w.b. (Table 2) to almost 9% w.b. (Fig. 7a, c). While the time to achieve at least 4-log reduction was longer than that obtained from the thermal death kinetics of *S. aureus* ATCC 25923 in walnut shells, in which the *D*-value of walnut shells with MC of 8.93% w.b. was 2.52 min at 70 °C (Zhang et al., 2018). This could be caused by the uneven temperature distribution in in-shell walnuts during RF treatments. Similar phenomena were found in corn samples even under the optimized RF processing protocol (Zheng et al., 2017).

### 3.7. Quality evaluation after RF treatment protocol and storage

Table 3 shows the quality results of NMC samples and RF treated AMC in-shell walnuts during the accelerated shelf-life storage. The mean FA and PV values after RF treated (0 day) samples were slightly higher than those of NMC control but within the acceptable range (PV < 1.0 meq/kg and FA < 0.6%) required by nut industry. The similar changes in PV and FA values after RF treatments were also observed in in-shell walnuts by Zhou et al. (2017). After 20 days of storage, the PV results of both NMC control and RF treated samples were higher than 1.0 meq/kg, which was considered to be rancid. These results were different from those of fresh walnuts reported by Wang et al. (2006), Wang et al. (2007a) and Zhou et al. (2017). This phenomenon could be mainly caused by the high initial PV value of stored walnuts. Similarly, Mitcham et al. (2004) also reported that the PV values exceeded 1 meq/kg due to the higher initial values during the accelerated storage. The FA values of both the NMC control and RF dried samples were below 0.6% even after the 20 days of accelerated storage.

There were no significant differences in $L^*$ values of ground kernel color after RF drying and storage. However, the $L^*$ values of the samples were lower compared to previous studies (Wang et al., 2007a; Zhang et al., 2016), which could be due to different walnut varieties or the pre-treatment conditions applied to in-shell walnuts.

### 3.8. Changes of in-shell walnuts' MC during storage

Fig. 7 shows the change in MC and $a_w$ of walnut shells and kernels during storage. The MC and $a_w$ of controls and RF treated samples (Fig. 7a and b) decreased rapidly during the first four days of accelerated storage (35 °C, 30% RH), especially for samples with higher initial MC. This phenomenon was different from that of corn (Zheng et al., 2016), rice (Zhou et al., 2015) and pistachio (Ling et al., 2016) with slight changes during storage. This could be caused by the difference in water content between the environment and the samples. When similar samples were held under storage conditions of 35 °C and 65% RH, the MC and $a_w$ presented different trends over the first four days (Fig. 7c and d). The MC of both the shells and kernels for AMC control samples lost almost 5–6% w.b. at an RH of 65%. Similar trends were found in chestnut shells and kernels after 8 days of accelerated storage (Hou et al., 2014). However, the final MC and $a_w$ values of shells and kernels under these two storage conditions gradually tended to stabilize as storage time became more prolonged.

### 3.9. Survival of S. aureus ATCC 25923 on in-shell walnuts during storage

Fig. 8 shows the survival curves of *S. aureus* ATCC 25923 on control and RF treated samples during storage. A rapid initial decline for the NMC control and AMC control samples was observed when stored at 35 °C with 30% RH conditions (Fig. 8a). At the end of the storage, at least one of the four NMC control samples was below the LOD (1 log/nut). The results were positive after enrichment. The rapid decline in microbial numbers for AMC control and NMC control samples could be due to the moisture loss observed over the first four days of storage (Fig. 7a). Blessington et al. (2013) also found a higher rate of decline in pathogens on in-shell walnuts stored under 23–25 °C and 25–35% RH conditions.

The magnitude of the reduction was lower for AMC control samples (about 3-log reductions) as the samples stored under condition of 35 °C and 65% RH, which could be due to MC changes (Fig. 7c). However, the microbial reduction in NMC control samples stored under these conditions was up to 5-log (Fig. 8b). This phenomenon was similar to that reported by Frelka et al. (2016), where pathogen reductions of up to 5-log were noted even under storage conditions of 10 °C and 65% RH. Thus, this difference in the degree of microbial reduction during storage was mainly due to the lower initial MC value of the NMC samples compared to AMC samples. In addition, the survival of pathogens on

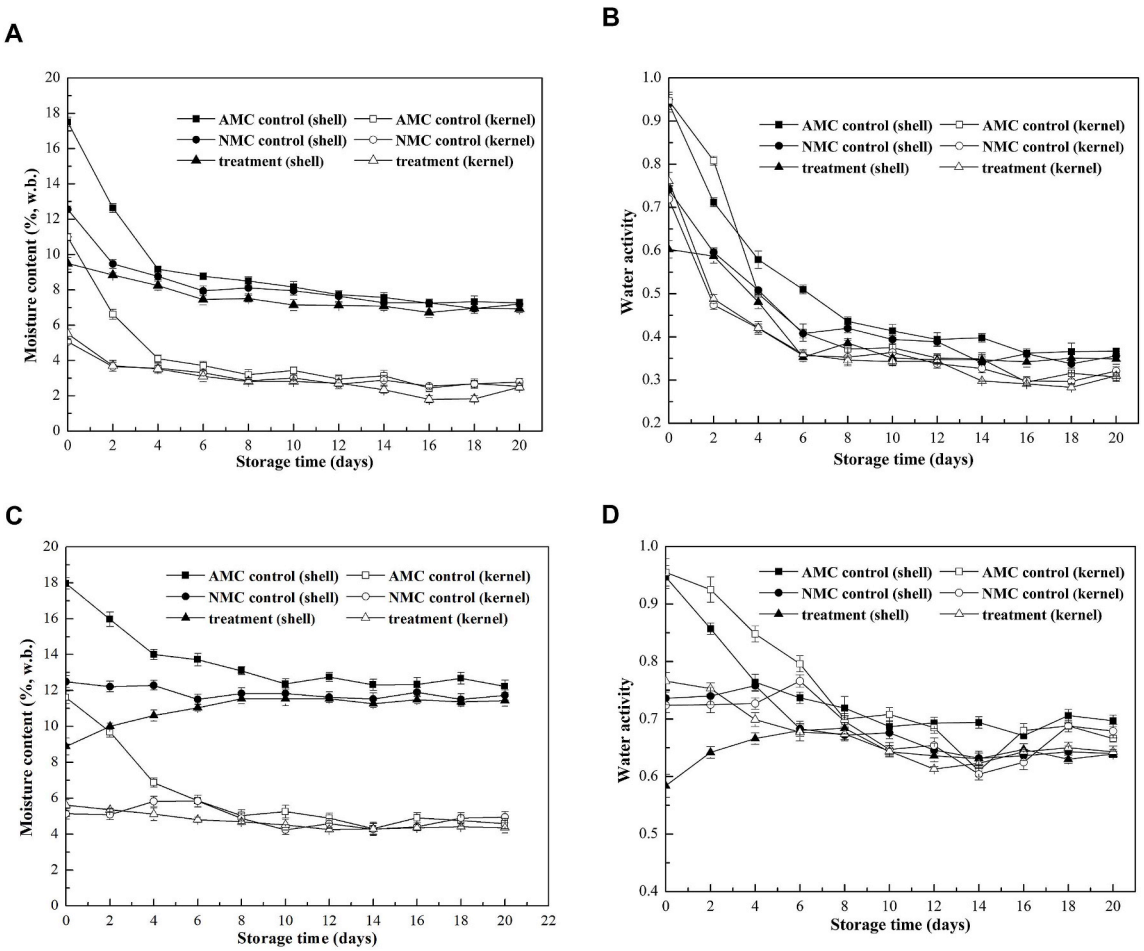


**Fig. 7.** Changes in moisture content and water activity of walnut shells and kernels (a and b, 30% RH; c and d, 65% RH) at 35 °C during storage after hot air assisted RF heating. AMC control represented inoculated AMC samples with moisture content of 15.01% w.b. while NMC control represented the NMC samples which were inoculated.

tree nuts was also affected by the storage temperature, which caused rapid reduction of pathogens (Blessington et al., 2012; Blessington et al., 2013; Brar, Proano, Friedrich, Harris, & Danyluk, 2015; Uesugi et al., 2006).

The RF treated walnuts also presented a rapid decline and all the four samples gradually reduced to below the LOD (1 log/nut) under storage conditions of 35 °C and 30% RH. By contrast the load population of *S. aureus* ATCC 25923 reduced more slowly under 35 °C and 65% RH storage conditions. Moreover, there was no growth trend of *S. aureus* ATCC 25923 after RF treatment during the storage. The results were similar to those observed by Liu et al. (2011). The rapid reduction of *S. aureus* ATCC 25923 in both control and RF treated samples could be also affected by the lack of nutrients except for the moisture loss. Thus the mechanism of this phenomenon remains to be further studied.

## 4. Conclusion

An effective RF pasteurization processing was developed for in-shell walnuts. An optimal heating rate of 5.14 ( ± 0.75) °C/min with the electrode gap of 16.0 cm was used for pre-heating, followed by a 40 min drying phase with the electrode gap adjusted to 19.0 cm, followed by forced cooling in a single layer under ambient conditions. RF treatments were capable of inducing at least a 4-log reduction in the population of *S. aureus* ATCC 25923. The moisture content of AMCC in-

**Table 3**
The storage quality characteristics (means ± SD over 2 replicates) of NMC control samples and hot air-assisted RF dehydrated AMC samples.

| Storage time at 35 °C (days) | Peroxide value (meq/kg) # | | Fatty acid (%) # | | Kernel color (*L*-value) & | |
|---|---|---|---|---|---|---|
| | Control | RF dried | Control | RF dried | Control | RF dried |
| 0 | 0.31 ± 0.03Aa* | 0.69 ± 0.08Ba | 0.11 ± 0.05Aa | 0.14 ± 0.02Ba | 48.66 ± 2.58Aa | 46.51 ± 3.02Aa |
| 10 | 0.58 ± 0.08Aa | 0.66 ± 0.16Aa | 0.21 ± 0.05Aa | 0.25 ± 0.04Ab | 44.05 ± 3.26Aa | 44.82 ± 2.78Aa |
| 20 | 1.14 ± 0.20Ab | 1.38 ± 0.24Ab | 0.20 ± 0.02Aa | 0.26 ± 0.06Bb | 42.15 ± 1.96Aa | 40.36 ± 1.95Aa |

# Peroxide value and fatty acid values for good quality are less than 1.0 meq/kg and 0.6%, respectively.
& *L*-value for good quality is more than 40.
* Mean values are not significantly different ($P > 0.05$) for the same capital letter within a row between the treatment and the same low case letter within a column among the storage time.

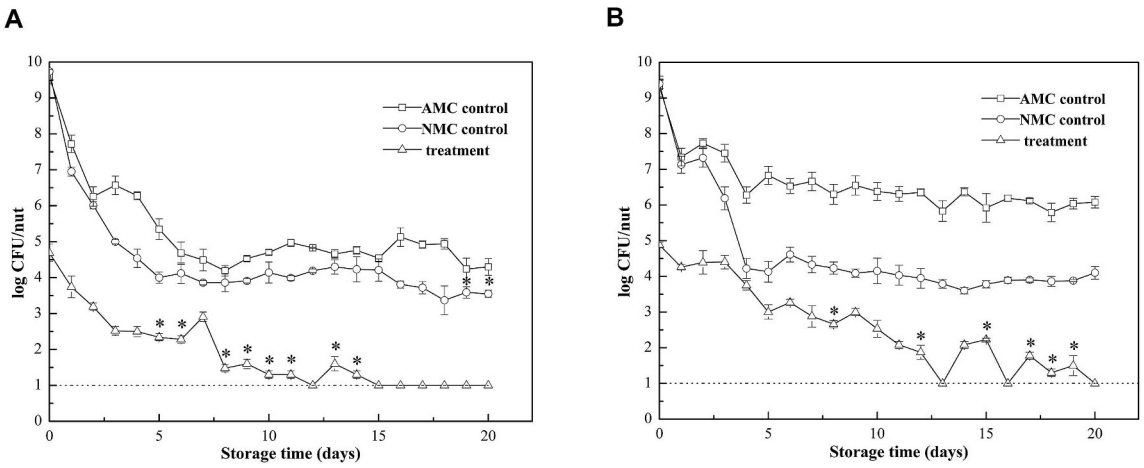


**Fig. 8.** Survival curves of *S. aureus* ATCC 25923 on in-shell walnuts (a, 30% RH; b, 65% RH) at 35 °C during storage after hot air assisted RF heating. *indicates at least one replicate was below the LOD (1 log CFU/nut). AMC control represented inoculated AMC samples with moisture contents of 15.01% w.b. while NMC controls represented the NMC samples which were inoculated.

shell walnuts was reduced back to almost 8% after the RF drying, which was close to their initial moisture content level. The difference in both PV and FA values between NMC control and RF treated samples was not significant during the accelerated storage for 0 and 10 days but significant only for storage at 20 days. In addition, the color values were not affected by the RF treatments and during the accelerated storage. Moreover, there was no growth of *S. aureus* ATCC 25923 on in-shell walnuts after RF treatments during the storage period. This hot air assisted RF process may provide a practical and effective pasteurization method for in-shell walnuts.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2019.03.030.

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