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Verification of radio frequency pasteurization process for in-shell almonds

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ABSTRACT

Salmonella outbreaks related to consumption of raw almonds have forced nut industry to seek effective pasteurization processes. Radio frequency (RF) treatment is a potential pasteurization method for controlling *Salmonella* while maintaining product quality. *Escherichia coli* ATCC 25922 is nonpathogenic and can be used as a surrogate of pathogenic *Salmonella* when their thermo-tolerances are equivalent. Since thermal resistance of target pathogens can be reduced by raising water activity in dry samples, the aim of this study was to explore the application of RF treatments to control *E. coli* ATCC 25922 in pre-washed in-shell almonds without quality losses. A 6 kW, 27.12 MHz RF heating system was used to rapidly pasteurize 1.7 kg washed in-shell almonds together with hot air heating at 55 °C. To meet effective pasteurization requirements and maintain almond quality, the RF treatment protocol was obtained by using an electrode gap of 10.5 cm for pasteurization, 12 cm for drying, and followed by forced room air cooling in 3 cm thick samples. The results showed that the RF heating at the electrode gap of 10.5 cm for 1.5 min achieved 5-log reductions of *E. coli* ATCC 25922 and the followed RF drying for 8.5 min reduced the moisture content of almond shell back to 5.08% w.b. Kernel color, peroxide value, weight loss, fatty acid value and fatty acid composition of RF treated almonds met good quality standard used by almond industry. Therefore, RF treatments are an effective and rapid heating method to control *Salmonella* in pre-washed in-shell almonds.

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1. Introduction

Almonds are one of the most important nuts in the world. The global production of almonds was approximately 2.96 million tons in 2013, which are mainly contributed by the USA, Australia, Spain, Turkey, Italy and China (FAOSTAT, 2013). A major problem of almonds is *Salmonella Enteritidis* PT 30 contamination when almonds fall to the ground after mechanical shaking in harvesting orchards (Isaacs et al., 2005). Outbreaks of *Salmonella Enteritidis* PT 30 were associated with raw almonds occurred in USA and Canada in 2000–2001 and 2004 (CDC, 2004; Isaacs et al., 2005). Therefore, the US Department of Agriculture (USDA) regulations mandate that almonds must be subjected to pasteurization processes to reduce pathogens in a reasonably acceptable level prior to export (Anon, 2007).

Several methods have been used or are under development for almond pasteurization, including propylene oxide (PPO) fumigation (ABC, 2007), hot air (Yang et al., 2010), hot water (Harris et al., 2012), hot oil (Du et al., 2010), steam (Chang et al., 2010; Lee et al., 2006), infrared heating (Bingol et al., 2011; Brandl et al., 2008), and high hydrostatic pressure (Goodridge et al., 2006; Willford et al., 2008). However, almonds treated by PPO fumigation are banned in the European Union and many other countries (ABC, 2007). Hot air for almond pasteurization cannot meet the 4-log reduction of *Salmonella Enteritidis* PT 30 even with the temperature of 130 °C for 11.3 min (Yang et al., 2010). Hot water at 88 °C for 1.6–2.1 min provides at least 4-log reduction criteria of *Salmonella Enteritidis* PT 30 but with the almond skin loosening (Harris et al., 2012). Hot oil roasting meets the pasteurization requirements only for roasted almonds not for raw almonds (Du et al., 2010). Steam has been used for pasteurization of *Salmonella Enteritidis* PT 30 in almonds, however, the oxidative rancidities have not been reported (Chang et al., 2010; Lee et al., 2006). Small scale infrared heating with 60 min holding in hot air results in a 4-log reduction of *Salmonella Enteritidis* but is difficult to scale up for large volume industrial

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implementations (Bingol et al., 2011; Brandl et al., 2008). High hydrostatic pressure for almond pasteurization is only effective with high temperature and water activity, which makes it too expensive for large-scale commercial applications (Goodridge et al., 2006; Willford et al., 2008).

Radio frequency (RF) heating holds potential for pathogen control in agricultural commodities, since it can rapidly raise the temperature of agricultural commodities volumetrically and significantly reduce heating time to avoid the quality loss caused by slower heating rate in conventional thermal treatments (Hou et al., 2016; Marra et al., 2009). Several studies demonstrate that RF heating may provide more than 4 log reductions of target pathogens in agricultural commodities (Jeong and Kang, 2014; Kim et al., 2012; Schlisselberg et al., 2013). Up to now, however, there are little studies on determining the effective RF process parameters during heating, holding and cooling to validate the inactivation rate of the targeted pathogens in in-shell almonds.

Thermal resistance of pathogens is higher in a dry environment than in moist environment. For example, a 2% increase in moisture content in melted chocolate reduced the $D_{71°C}$ value of Salmonella from 20 to 4 h (Do Nascimento et al., 2012). $D_{62°C}$ value for *Salmonella* decreased from 875 min to 29 min when 0.1 increase in water activity in flour (Silva and Gibbs, 2012). $D_{68°C}$ value of *Salmonella* PT 30 decreased from 6.97 to 0.42 min after increasing 0.226 water activity in almonds (Villa-Rojas et al., 2013). Therefore, by raising water activity of commodities before RF treatments, the thermo-tolerance of pathogens could be reduced while the commodities quality may be maintained. Increasing water activity before RF treatment has been proposed to control *Salmonella Typhimurium* and *Escherichia coli* O157:H7 in small black and red pepper spice samples (Jeong and Kang, 2014) and *Salmonella Enteritidis* PT 30 in in-shell almonds without inoculation for validation studies (Gao et al., 2011).

Salmonella Enteritidis PT 30 cannot be directly used to conduct thermal pasteurization validation tests in food processing plants because of critical safety requirements for operators, the product and the processing environment. Using a surrogate microorganism is an alternative way to conduct microbial validation. *Escherichia coli* ATCC 25922 is nonpathogenic and has been recommended as a surrogate species of pathogenic *Salmonella* for validation of thermal pasteurization (Eblen et al., 2005). But the relative thermal resistance between *Escherichia coli* ATCC 25922 and *Salmonella Enteritidis* PT 30 needs to be identified in almonds under the required conditions.

The objectives of this study were (1) to select suitable soaking times to obtain the required moisture content of in-shell almonds, (2) to compare the thermal tolerance of *Escherichia coli* ATCC 25922 and *Salmonella Enteritidis* PT 30 in almonds, (3) to develop and validate a RF treatment protocol to achieve 5-log reduction of *Escherichia coli* ATCC 25922 in pre-washed in-shell almonds, and (4) to evaluate the almond quality after RF treatments and a storage period.

2. Materials and methods

2.1. Materials

Raw and dried in-shell almonds (Nonpareil) were purchased from Paramount Farming Company (Modesto, CA, USA). The dielectric constant and loss factor of almonds are 5.9 and 1.2 at 27.12 MHz and room temperature. The initial moisture contents of almond shells, kernels and whole samples were 5.58 ± 0.03%, 3.83 ± 0.02%, and 4.46 ± 0.03% w.b., respectively. The moisture contents (MC) was determined according to the AOAC Official Method 925.40. About 2–3 g flour samples randomly collected from a number of almonds were placed in aluminum dishes, and then dried at 105 °C under pressure ≤13.3 kPa in a vacuum oven (DZX-6020B, Nanrong Laboratory Equipment Co., Ltd., Shanghai, China) until it reached to a constant weight of samples. The weight loss was used to estimate moisture content. Water activity (Aw) of almond shell and kernel was determined by Aqua Lab water activity meter (Model 4TE, Decagon Devices, Inc., Pullman, WA, USA). About 5–6 g of almond flour samples were placed into a sample cup and then in the water activity meter to measure Aw under ambient temperature (25 °C). In-shell almond samples were sealed into polyethylene bags and stored at 4 ± 1 °C until testing. Before each test, the samples were placed in an incubator (BSC-150, Boxun Industry & Commerce Co., Ltd., Shanghai, China) for 12 h at 25 ± 0.5 °C for equilibrium.

2.2. RF and hot air heating systems

RF heating was conducted in a 6 kW, 27.12 MHz pilot-scale RF system (SO6B, Stray field International, Wokingham, U.K.) with a hot air system (Fig. 1). By moving the top electrode (40 cm × 83 cm), the electrode gap was changed, thus regulating the RF power. The hot air was provided through an air distribution box under the bottom electrode, and the air speed was 1.6 m/s inside the RF cavity measured at 2 cm above the bottom electrode by an anemometer (DT-8880, China Ever best Machinery Industry Co., Ltd., Shenzhen, China). The details about the RF and hot air systems could be found in Wang et al. (2010). To achieve a stable air temperature for sample surface heating, hot air was circulated in the empty RF cavity for 1 h prior to the combined RF and hot air treatment.

2.3. Preparation of cell suspension

The *Escherichia coli* ATCC 25922 strains were obtained from the College of Food Science and Engineering, Northwest A&F University (Yangling, China). Stock cultures were made in Luria-Bertani broth (LB: Beijing Land Bridge, Beijing, China) and stored at −20 °C with 15% (vol/vol) glycerol. Stock cultures were allowed to thaw at room temperature for 5 min. A loopful was then streaked onto LB agar and incubated at 37 °C for 24 h. A well separated colony (single colony) was streaked onto LB agar and incubated at 37 °C for 24 h. Then, a signal colony was transferred into 30 ml LB broth and also incubated for 24 h at 37 °C. Finally, 3 ml bacterial suspension was transferred to 300 ml LB broth and incubated again at 37 °C for 24 h. This culture was centrifuged three times for 10 min at 3500 rpm (SC-3610, Anhui USTC Zonkia Scientific Instruments Co., Ltd. China), and the pellet was washed with sterile physiologic saline. The cell population was adjusted to a level of 10^{10} CFU/ml and refrigerated (4 °C) for no more than 7 d before inoculation of almond samples (Read et al., 1968; Wesche et al., 2005).

2.4. Comparing thermal resistance of Salmonella PT 30 with E. coli ATCC 25922

Almond flour samples were adjusted to Aw value of 0.946 (moisture content value of 18% w.b.) and then sterilization by autoclaving at 101 °C for 10 min to avoid interference by mesophilic bacteria. Then almond flour samples (0.5–0.8 g) inoculated with 10 μl of *Salmonella Enteritidis* PT 30 suspension in the aluminum cells designed and manufactured by Washington State University (Chung et al., 2008). The cells were closed and left for 24 h to achieve moisture equilibrium at room temperature, and then heated at 68 °C using a water bath. All the procedures in this study were the same as those indicated by Villa-Rojas et al. (2013), which were used for comparison with the thermal resistance of *Salmonella*

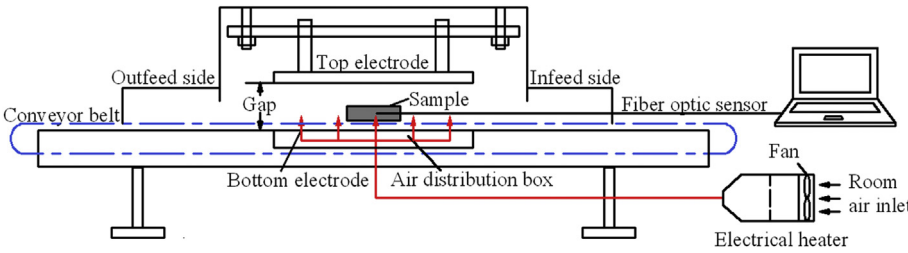

Fig. 1. Schematic view of the pilot-scale 6 kW, 27.12 MHz RF system showing the plate electrodes, conveyor belt, and the hot air system (Adapted from Wang et al., 2010).

PT 30. These tests were repeated twice and used to confirm that the *E. coli* ATCC 25922 could be used as a surrogate of *Salmonella* PT 30.

2.5. Design of the complete treatment protocol

2.5.1. Soaking time determination

Almond samples (200 g) were immersed in a plastic container equipped with 2000 ml tap water at room temperature. During immersion for 15, 30, 45, 60, 75, 90, 105 and 120 s, the almonds were agitated by hands with the clean gloves, and then placed in a single layer in ambient air at room temperature for 1 h to reach moisture equilibrium. Finally shell and kernel were separated manually. The determined soaking time could be used for industrial washing processes before RF treatments.

2.5.2. Determination of the electrode gap

To obtain a general relationship between different electrode gaps and the electric current (I, A), a plastic container with perforated screens on the side and bottom walls (30 cm × 22 cm × 6 cm) (Fig. 2) containing 1.7 kg soaked in-shell almonds was placed on the stationary conveyor belt between two electrodes without hot air heating. The anode current displayed on the screen of the RF system was used to calculate the output power (P, kW) of the RF system with a relationship ($P = 5 \times I$-1.5) provided by the manufacturer (Hou et al., 2014). The range of the electrode gap was selected from 10 to 19 cm with 1 cm interval. After setting the electrode gap, RF power was turned on, and the electric current was immediately recorded. Tests were repeated three times. Based on the measured electric current, four suitable electrode gaps of 10.5, 11.0, 11.5, and 12.0 cm were selected to further study RF heating, drying and the final treatment protocol development. The fiber-optic temperature sensor system (HQ-FTS-D120, Heqi Technologies Inc., Xian, China) with an accuracy of ±0.5 °C was used to measure sample temperatures by inserting the probe into almond kernels through a predrilled hole at five representative locations (A-E) (Fig. 2).

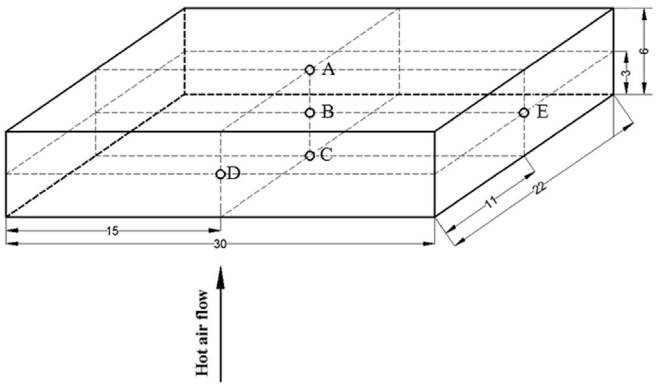

Fig. 2. Plastic container with five locations for sample temperature measurements (all dimensions are in cm).

2.5.3. Determination of RF heating parameters to control pathogens

Each prewashed almond sample (5 ± 0.05 g) was placed into a polyethylene bag (85 × 130 mm) and 100 μl of the inoculums were added. The bag was closed and then mixed thoroughly for 1 min. Inoculated prewashed almonds were spread onto filter paper and dried for 1 h inside a bio-safety hood (22 ± 2 °C). The dried inoculated almonds were wrapped up using paper (100 × 100 mm). Then three paper bags were placed around cold spot (A) of the plastic container, where was predetermined by preliminary tests. One paper bag was placed at room temperature served as an untreated control. With achieving a fast heating rate of more than 20 °C/min and similar heating rate obtained by Villa-Rojas et al. (2013), the electrode gap of 10.5 cm was used for RF treatments with circulated hot air at 55 °C to control *Escherichia coli* ATCC 25922 in almond samples. The container was taken out of the RF system every 0.5 min in the whole process of inactivation test. Each of the 3 paper bags in the container was taken out each time for evaluation. The almond samples in the container were immediately placed back into the RF cavity for further heating under the same conditions. Then the removed bag was placed in sealed plastic bag and put into ice water (≈4 °C, for at least 2 min) for cooling until further analysis was performed. RF treated in-shell almonds were poured into a dilution bottle with 95 ml of sterile physiologic saline to achieve a 100-fold dilution. Serial dilutions were performed and 0.1 ml of each one was plated onto LB agar and incubated at 37 °C for 24 h for the count of *Escherichia coli* ATCC 25922. This process was repeated twice. The RF heating time was determined based on the >4 log reduction of the targeted pathogens.

2.5.4. Drying time

The added MC in almond samples for effective pasteurization needed to be reduced back to the original level suitable for long storage. RF drying was conducted using an electrode gap of 12 cm with circulated hot air at 55 °C, which were selected based on achieving a relatively low and constant sample temperature. The container was taken out of the RF system every 1 min in the whole process of the drying test until the MC of almond shell reached about 5.08% w.b. The sample weight in the container was measured by an electronic balance (PTX-FA210, Huazhi Scientific Instrument, Co., Ltd. Fuzhou, China) with a sensitivity of 0.01 g. Each measurement took less than 1 min, and the samples in the container were placed back into the RF cavity for further drying under the same heating conditions. The drying tests were replicated twice.

2.5.5. Cooling method

Rapid cooling is necessary to avoid quality degradation after RF treatments. In-shell almond samples after drying with 6 and 3 cm depths in the plastic container were subjected to natural and forced ambient air cooling using an electric fan. The air velocities on the

sample surface were about 0.2 and 3.5 m/s for the natural and forced air cooling, which were measured by the anemometer. The temperature in the almond center was measured until it dropped to 30 °C. The best cooling method was further used to develop the treatment protocol for almond samples after RF heating and drying.

2.6. Almond quality analyses after processed with the developed RF treatment protocol

The quality of almonds was evaluated after processed with the developed RF treatment protocol and for an accelerated storage at 4 °C for 1 and 2 years. To shorten the duration of studies, the accelerated shelf life storage was conducted in the incubator set to 35 °C with 30% relative humidity for 10 and 20 d to simulate commercial storage at 4 °C for 1 and 2 years. The storage time at 35 °C was calculated based on a Q_{10} value of 3.4 for lipid oxidation (Taoukis et al., 1997) and was validated by real-time storage experiments (Wang et al., 2006). The quality index includes MC, peroxide values (PV), free fatty acid (FFA), fatty acid (FA) composition and color. The MC of in-shell almonds was measured using the AOAC Official Method 925.40. The PV and FFA were determined by official method Cd 8–53 and Ab 5–49. FA composition was determined by forming fatty acid methyl esters (FAME). Kernel skin, inner kernel and ground kernel color was measured using a computer vision system (CVS) as described in detail in Ling et al. (2015). Color images of treatment were captured and stored in the computer, and then analyzed by Adobe Photoshop CS3 (Adobe Systems Inc., USA). Finally, the color values (L, a, and b) obtained from Photoshop were converted to CIE LAB (L^*, $a^*$and b^*) values using the following formulas (Ha et al., 2013)

$$L^* = \frac{L}{2.5} \quad (1)$$

$$a^* = \frac{240}{255}a - 120 \quad (2)$$

$$b^* = \frac{240}{255}b - 120 \quad (3)$$

Results were expressed as means ± standard deviations (SDs) over 3 replicates. Differences were estimated by the analysis of variance followed by Tukey's test and considered significantly at $P \leq 0.05$. All statistical analyses were performed using the statistical software SPSS 17.0 version (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Thermal resistance of E. coli ATCC 25922 in almonds

$D_{68°C}$ value of *E. coli* ATCC 25922 in almonds at Aw of 0.946 was 0.95 ± 0.02 min with two replication tests. It was higher than $D_{68°C}$ value (0.42 ± 0.04 min) of *Salmonella Enteritidis* PT 30 in almonds under the same treatment condition (Villa-Rojas et al., 2013). This result indicated that *Escherichia coli* ATCC 25922 could be used as a surrogate of *Salmonella* PT 30 for validating thermal pasteurization tests in food processing. These data are in agreement with the results in previous study (Eblen et al., 2005).

3.2. Determination of soaking time

The moisture content (MC) of almond shell and kernel as a function of soaking time at 25 °C is shown in Fig. 3. The MC of almond shell increased rapidly from 5.58% w.b. to 41.6% w.b. after soaking 75 s, and maintained the same MC level in the shell from

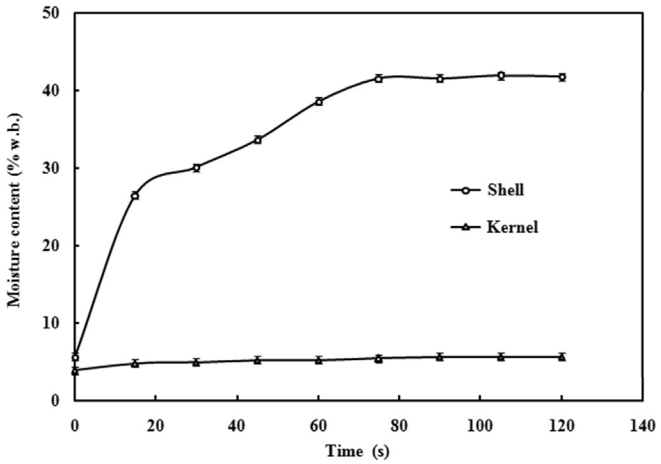

Fig. 3. Moisture content of almond (Nonpareil) shell and kernel as a function of soaking time at 25 °C.

75 s to 120 s. The MC of kernel increased slowly and remained below 5.61% w.b. even after soaking 120 s. Thus, 75 s was selected for almond soaking time, which was in the good agreement with the results obtained by Gao et al. (2011). The Aw values of almond kernels and shells were 0.492 (3.83% w.b.) and 0.528 (5.66% w.b.) at the beginning of soaking, and 0.576 (5.61% w.b.) and 0.989 (41.8% w.b.) at the end, respectively, which were in agreement with those reported by Villa-Rojas et al. (2013).

3.3. Electric current under different electrode gap

The relationship between electric current and electrode gap with and without 1.7 kg soaked in-shell almonds is shown in Fig. 4 without conveyor belt movement and hot air assisted heating. With in-shell almonds, electric current rapidly decreased from 0.99 A to 0.7 A when the electrode gap increased from 10 cm to 12 cm. Without almonds, the electric current was almost constant, around 0.31 A, which was not affected by the electrode gap changes. The same trends were also found by Hou et al. (2014) and Zhou et al. (2015). Thus, based on the measured electric current, four suitable electrode gaps of 10.5, 11.0, 11.5 and 12.0 cm were selected for further tests.

3.4. Pasteurization, drying and cooling protocol

The temperature-time history at the five locations in the

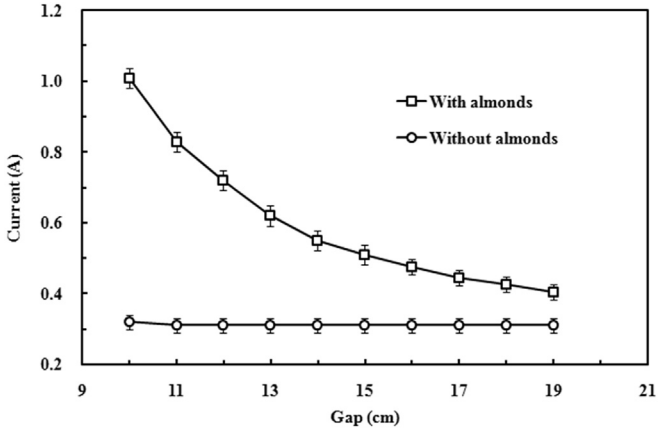

Fig. 4. Electric current of the radio frequency system as a function of electrode gap.

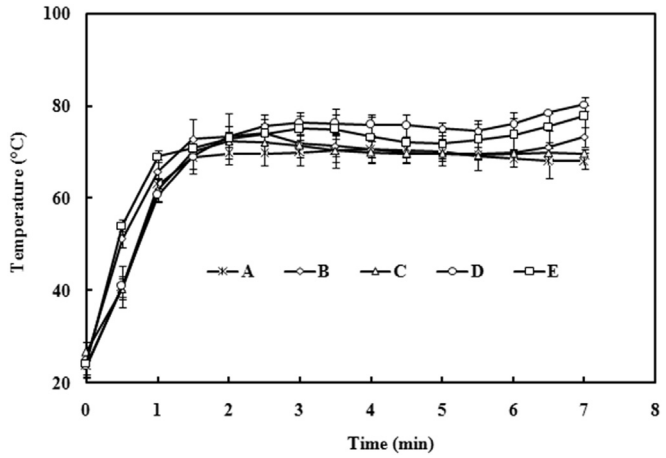

Fig. 5. Temperature-time history at the five locations in the container when subjected to 10.5 cm electrode gap in the RF system with 55 °C hot air heating.

container when subjected to 10.5 cm electrode gap in the RF system with 55 °C hot air heating is shown in Fig. 5. The cold spot in the container was observed to be at point A, where could be used as the location for inoculation samples. Mean and standard deviation values of prewashed in-shell almond temperatures over five locations in the container are shown in Fig. 6 when subjected to 10.5, 11.0, 11.5 and 12.0 cm electrode gaps in the RF system with 55 °C hot air heating. For the electrode gaps of 10.5, 11.0, 11.5 and 12.0 cm, the heating rate in the prewashed almonds was 23.6 °C/min, 15.3 °C/min, 8.8 °C/min, and 7.4 °C/min, respectively. To achieve a fast heating rate of more than 20 °C/min, the 10.5 cm electrode gap with 55 °C hot air heating was selected for pasteurization. Survival curves of *E. coli* ATCC 25922 in in-shell almonds are shown in Fig. 7. The reduction of *E. coli* ATCC 25922 in almonds decreased quickly as RF heating time increased. Only 1.5 min of RF heating was needed to achieve 5-log reduction of *E. coli* ATCC 25922 in in-shell almonds. This was shorter than what was required based on thermal inactivation $D_{68°C}$ value of *E. coli* ATCC 25922 in almonds at Aw of 0.946 (MC value of 18% w.b.) using the aluminum cells. This could be caused by higher moisture contents and temperatures in real RF heated samples, such as 27% w.b. MC (Fig. 8) and 70 °C (Fig. 6) even after 1.5 min RF heating, respectively. Higher MC of samples might reduce the thermo-resistance of pathogens and the higher temperature could reduce the heating time when achieving the same

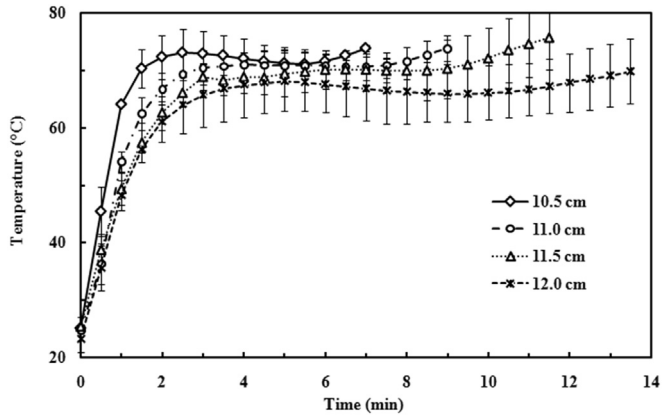

Fig. 6. Mean and standard deviation values of in-shell almond temperatures over five locations in the container when subjected to hot air and stationary RF heating with four electrode gaps under hot air surface heating at 55 °C.

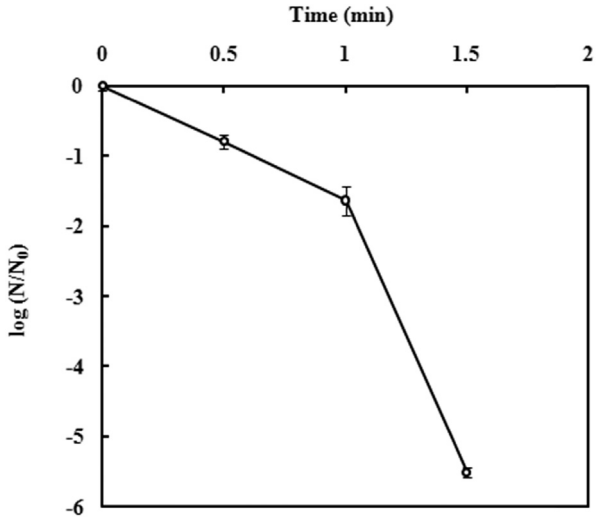

Fig. 7. Survival curves for *E. coli* ATCC 25922 in in-shell almonds after hot air assisted RF heating.

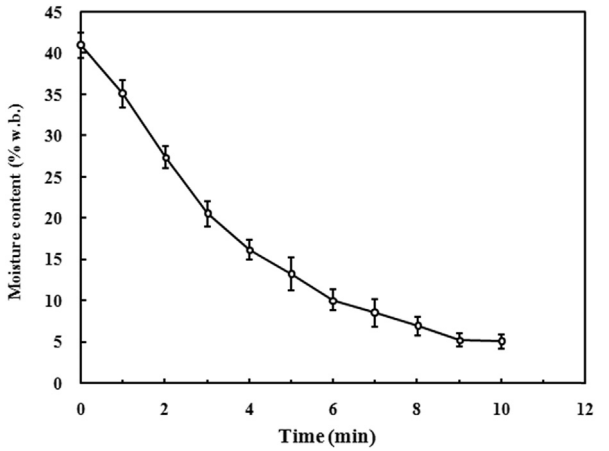

Fig. 8. Changes in moisture content (MC) of almond shell after hot air assisted RF heating and drying.

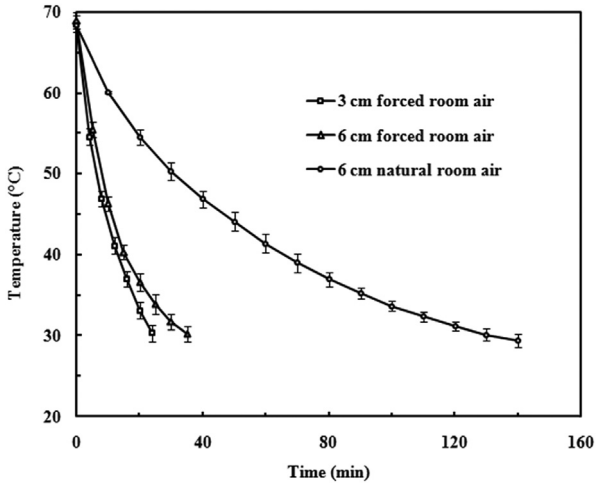

Fig. 9. Cooling curves of in-shell almonds in the sample center as a function of sample thickness under natural and forced room air cooling.

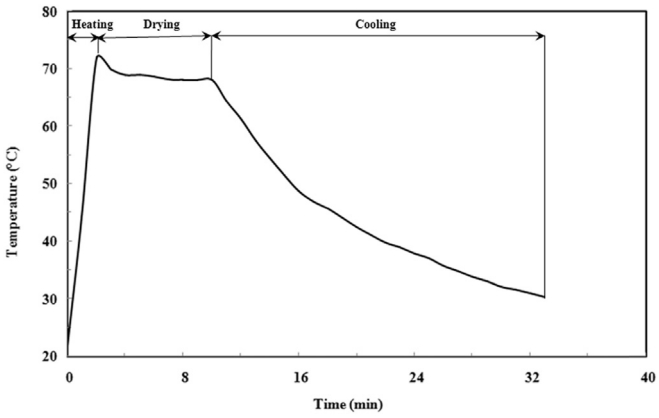

Fig. 10. Average temperature-time histories of the pre-washed in-shell almonds over five locations in the 6 cm deep container when subjected to hot air (55 °C) assisted RF treatments under the electrode gap of 10.5 cm for heating and 12 cm for drying followed by forced room air cooling in 3 cm thick samples.

Table 1
Changes in moisture content (MC, % w.b.) of control and radio frequency (RF) treated in-shell almonds during storage at 35 °C.

Storage time at 35 °C (d)	MC (% w.b.)	
	Control	RF treated
0	4.46 ± 0.02Aa*	4.43 ± 0.01Aa
10	4.43 ± 0.01Aa	4.41 ± 0.02Aa
20	4.42 ± 0.02Aa	4.40 ± 0.02Aa

*Mean values are not significantly different ($P > 0.05$) for the same capital letters within a row among the treatment, and for the same lower case letters within a column among the storage time.

inactivation level of pathogens, which were also observed in previous studies (Villa-Rojas et al., 2013; Jeong and Kang, 2014).

With the electrode gap of 12 cm assisted by 55 °C hot air heating, the temperature of almonds was around 68 °C, which was stable and the lowest one as compared with other electrode gaps (Fig. 6). Therefore, for maintaining the almond quality, the electrode gap of 12 cm was used for RF drying. Fig. 8 shows the MC of almond shell as a function of drying time under the combined RF and hot air heating. The MC gradually decreased with increasing heating time. It took totally 10 min for the almond shell's MC to reduce from 41.6 to 5.08% w.b., which was slightly lower than the original MC level for long storage. The rapid drying with 1.5 min RF pasteurization and 8.5 min RF drying was achieved since the vapor pressure gradient remained from the sample center towards the surface, resulting in a constant sample temperature when the heat loss from water evaporation was balanced by the input RF energy (Wang et al., 2014).

Fig. 9 shows the cooling curves in the almond center as influenced by the sample thickness and cooling methods. About 140 min were needed for the 6 cm deep in-shell almond to cool from 68 °C to 30 °C with natural room air. But the cooling time was largely reduced to about 24 min for forced air cooling of 3 cm deep samples to reach 30 °C due to fast heat conduction in thin layer and high heat transfer under forced convection. Thus, this cooling parameter was finally used for cooling processes.

Based on the above studies, the final RF treatment protocol was determined as follows: the electrode gap of 10.5 cm with 55 °C hot air for 1.5 min was used for pasteurizing 1.7 kg prewashed in-shell almonds, and 12 cm electrode gap assisted by 55 °C hot air for 8.5 min was used for drying followed by the forced room air cooling in 3 cm thick samples for 24 min. The typical sample temperature profile for this RF treatment protocol is shown in Fig. 10.

Table 2
Peroxide value and fatty acid (mean ± SD over three replicates) of in-shell almonds (Nonpareil) before and after RF treatments.

Storage time at 35 °C (d)	Peroxide value (meq/kg)		Fatty acid (%)	
	Control	RF treated	Control	RF treated
0	0.35 ± 0.02Aa*	0.40 ± 0.01Ba	0.18 ± 0.00Aa	0.25 ± 0.01Ba
10	0.41 ± 0.01Ab	0.48 ± 0.00Bb	0.24 ± 0.01Ab	0.30 ± 0.03Bb
20	0.51 ± 0.01Ac	0.53 ± 0.02Ac	0.29 ± 0.01Ac	0.36 ± 0.01Bc

*Different upper and lower case letters indicate that means are significantly different among treatments and storage time, respectively, at $P = 0.05$.

Table 3
Changes in the fatty acid composition of oil extracted from treated almond during storage at 35 °C.

Fatty acid (relative g/100 g)	Storage time (d)	Treatment	
		Control	RF treated
Palmitic acid (16:0)	0	5.95 ± 0.01Aa*	5.90 ± 0.04Aa
	10	5.94 ± 0.02Aa	5.96 ± 0.03Aa
	20	6.01 ± 0.01Ab	5.97 ± 0.03Aa
Palmitoleic acid (16:1)	0	0.41 ± 0.00Aa	0.41 ± 0.00Aa
	10	0.41 ± 0.00Aa	0.41 ± 0.00Aa
	20	0.42 ± 0.00Ab	0.41 ± 0.02Aa
Stearic acid (18:0)	0	1.25 ± 0.01Aa	1.25 ± 0.02Aa
	10	1.26 ± 0.01Aa	1.27 ± 0.02Aa
	20	1.26 ± 0.03Aa	1.26 ± 0.00Aa
Oleic acid (18:1)	0	68.40 ± 0.01Aa	68.43 ± 0.02Aa
	10	68.52 ± 0.02Ab	68.54 ± 0.03Ab
	20	68.51 ± 0.00Ab	68.55 ± 0.02Bb
Linoleic acid (18:2)	0	23.76 ± 0.00Aa	24.79 ± 0.03Aa
	10	23.76 ± 0.00Aa	23.76 ± 0.01Aa
	20	23.75 ± 0.01Aa	23.78 ± 0.02Aa

*Mean values are not significantly different ($P > 0.05$) for the same capital letters within a row among the treatment, and for the same lower case letters within a column among the storage time.

Table 4
Changes in color value of treated almonds during storage at 35 °C.

Color parameters		Treatment	Storage time (d)		
			0	10	20
Kernel skin color	L^*	Control	47.83 ± 4.42Aa*	46.19 ± 3.65Aa	45.95 ± 3.26Aa
		RF	45.38 ± 3.31Aa	44.06 ± 3.25Aa	46.44 ± 3.52Aa
	a^*	Control	16.60 ± 0.80Aa	16.70 ± 0.78Aa	17.82 ± 1.02Aa
		RF	16.56 ± 1.70Aa	17.29 ± 2.02Aa	18.74 ± 0.99Aa
	b^*	Control	37.91 ± 2.26Aa	36.01 ± 2.13Aa	34.80 ± 1.87Aa
		RF	35.16 ± 1.44Aa	35.50 ± 2.34Aa	33.81 ± 2.43Aa
Inner kernel color	L^*	Control	82.73 ± 0.95Aa	81.93 ± 0.85Aa	81.82 ± 0.65Aa
		RF	81.59 ± 1.46Aa	82.34 ± 0.86Aa	81.94 ± 0.97Aa
	a^*	Control	−2.03 ± 0.41Aa	−2.03 ± 0.46Aa	−1.83 ± 0.38Aa
		RF	−2.01 ± 0.22Aa	−2.01 ± 0.34Aa	−1.97 ± 0.33Aa
	b^*	Control	11.11 ± 1.61Aa	10.27 ± 2.21Aa	10.26 ± 2.14Aa
		RF	11.62 ± 2.05Aa	10.79 ± 2.25Aa	10.03 ± 1.95Aa
Ground kernel color	L^*	Control	69.47 ± 0.92Aa	68.96 ± 0.96Aa	68.93 ± 0.79Aa
		RF	69.45 ± 0.75Aa	68.70 ± 0.92Aa	69.91 ± 0.85Aa

*Mean values are not significantly different ($P > 0.05$) for the same capital letters within a row among the storage time, and for the same lower case letters within a column among the treatment.

3.5. Almond quality after RF treatment and storage

Table 1 shows the MC of control and RF treated in-shell almonds during storage for 0, 10 and 20 d at 35 °C. After RF treatments and during storage, the average MC of almonds had no significant difference among them ($P > 0.05$), indicating that the weight loss could be negligible after RF treatment and during storage period.

Table 2 shows mean peroxide values (PV) and free fatty acid (FFA) values of in-shell almonds before and after RF treatments when storage at 35 °C for 0, 10 and 20 d. The mean PV and FFA values of almonds increased with RF treatment and storage time due to unavoidable oxidation with high temperature and during the storage period. Since PV and FFA values remained within the marketable range (PV < 1.0 meq/kg and FFA < 0.6%) according to the nut industry standard (Gao et al., 2011), the quality of RF treated almond after storage at 35 °C for 20 d which simulating 2 years of real storage at 4 °C was still acceptable. Similar results were also observed in RF treated walnuts (Wang et al., 2007) and pistachio (Ling et al., 2015). The fatty acid (FA) composition determined in almonds was listed in Table 3. No significant difference ($P > 0.05$) in the five major FA compositions was observed between control and RF treated samples during storage at 35 °C for 10 d. Similar result was also obtained by Martínez et al. (2013) for almonds with storage at 60 °C ± 1 °C of 12 d.

The color values of kernel skin, inner kernel and ground kernel showed that there were no significant differences ($P > 0.05$) between control and RF treatments during the storage (Table 4). The *L*-values of kernel skin, inner kernel and ground kernel were above 45, 81 and 69, respectively, which meet the requirements for almond industry (Gao et al., 2011). The color values of almonds were not changed after the storage period, which were in agreement with those reported in previous studies (Ha et al., 2013; Jeong and Kang, 2014). Based on the above results, the major almond quality attributes were not affected by RF treatments and the required storage time. Therefore, RF treatment protocol may provide a practical and effective pasteurization and drying method for in-shell almonds.

4. Conclusions

Escherichia coli ATCC 25922 can be used as a nonpathogenic surrogate species of pathogenic *Salmonella* for validation of thermal pasteurization due to higher thermal tolerance in almonds. An effective RF pasteurization process for in-shell almonds was developed. In-shell almonds were soaked for 75 s and equilibrated for 1 h before RF treatments. Then hot air (55 °C) assisted RF treatments with the electrode gap of 10.5 cm for 1.5 min were used for pasteurization, and with the electrode gap of 12 cm for 8.5 min used for drying. After that, the RF treated almonds were cooled by forced room air in 3 cm deep layer. The results showed that the moisture content of almond shell was reduced back to 5.08% w.b, which was nearly the same as its initial moisture content. Almond quality parameters, such as MC, PV, FFA, FA compositions and color values, were not affected by the RF treatments even after the accelerated storage at 35 °C for 20 d. This RF process provides a practical, effective and environmentally friendly pasteurization and drying method for pre-washed in-shell almonds.

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