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Pasteurization process development for controlling *Salmonella* in in-shell almonds using radio frequency energy

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ABSTRACT

Radio frequency (RF) treatment holds potential as a pasteurization method to control *Salmonella* in almonds without causing a substantial loss of product quality. Thermal resistance of *Salmonella* can be reduced by increasing water activity, thus a soaking process was designed prior to RF treatments. A pilot-scale 27 MHz, 6 kW RF heating system was used to rapidly heat 1.7 kg washed in-shell almonds with hot air heating at 55 °C. To achieve appropriate heating rate, constant drying temperature and short time cooling, the RF treatment protocol was obtained using an electrode gap of 13 cm for heating, 14 cm for drying, and followed by forced room air cooling of 5-cm thick samples. The results showed that almond temperatures above 75 °C at 23% moisture contents for 2–4 min RF heating could meet the requirements to achieve 5-log reduction of *Salmonella*. The RF treatment process for 20 min reduced the moisture content to 5.7% w.b. Peroxide value, fatty acids values and kernel colors of the RF treated almonds met good quality standard used by nut industry.

1. Introduction

Almonds are one of the most representative nuts for food safety concerns. A major problem of raw almonds is cross contamination of *Salmonella enterica* serovar Enteritidis, which is most likely to occur prior to processing. *Salmonella enteritidis* is an important foodborne pathogen in eggs and poultry, causing diarrhea, fever, nausea, or vomiting (D'Aoust, 1977; Doyle and Mazzotta, 2000). Although nut-related outbreaks are relatively rare, recent highly publicized incidents involving peanuts and pistachios have resulted in bankruptcy for one of the largest peanut processors in the US, and a loss of millions of dollars to the peanut and pistachio industries (UPI, 2009; Wahba and Chasan, 2009). Two incidents of *S. enteritidis* due to the consumption of raw almonds occurred in the USA and Canada in 2001 and 2004 (CDC, 2004). Both epidemiological and environmental investigations confirm whole natural almonds as the source of contamination by a rare strain, *S. enteritidis* PT 30, which occurs when almonds fall to the ground after mechanical shaking during harvest (Isaacs et al., 2005). Starting from 2007, therefore, the US Department of Agriculture (USDA) mandated pasteurization of raw almonds prior to export.

Several potential treatment methods are under development for in-shell almonds, including propylene oxide fumigation, high pressure, steam and infrared heating (ABC, 2007; Goodridge et al., 2006; Lee et al., 2006). These methods might reduce *S. enteritidis* population on almond surfaces (ABC, 2007). However, propylene oxide treatment of foods is banned in the European Union and many other countries. Its chemical effects on food include gene mutation, DNA strand breaks, and neoplastic cell transformation. The substance is classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (Cornucopia Institute, 2007). High hydrostatic pressure for almond pasteurization is only effective with temperatures at 50 °C or higher and high water activity. This process is too expensive for large scale commercial applications (Goodridge et al., 2006). Steam heating at 93 °C for 65 s can result in more than 4-log reduction of *S. enteritidis* on almond surfaces. But the increase of moisture content in steam treated almonds may reduce shelf life, and effects of steam on almond oxidative rancidities have not been reported (Lee et al., 2006). Small scale experiments with a dozen almond kernels have shown that infrared heating with 60 min holding in hot air resulted in a 4-log reduction of *S. enteritidis* (Brandl et al., 2008). But since each individual kernel needs to be fully exposed to infrared radiation, this method may be difficult to scale up for large volume commercial applications.

Radio frequency (RF) heating holds potential for pathogen control in agricultural commodities. RF energy can directly interact with agricultural commodities to rapidly raise the temperature of a whole treated bed in an industrial system (Tang et al., 2000). A

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major advantage of RF treatments over microwave energy in bulk materials is deeper penetration depth, providing better heating uniformity in almonds (Wang et al., 2003). Many studies have explored the possibility of using RF energy to inactivate pathogens in high-moisture foods (Luechapattanaporn et al., 2005) and for pest control (Lagunas-Solar et al., 2007; Nelson and Payne, 1982; Wang et al., 2007a,b). But there have been no reported studies on RF control of *S. enteritidis* in dry almonds.

Thermal resistance of *S. enteritidis* is several orders of magnitude higher in a dry environment than in a moist environment. The decimal reduction time (*D*-value) defined as the time required to reduce a microbial population by 90% at a constant temperature, is commonly used to characterize the thermal resistance of microorganisms. For example, average *D*-values for some *Salmonella* strains are around 1.3 min or 0.6 min at 57 °C or 60 °C in liquid eggs (Doyle and Mazzotta, 2000; Jin et al., 2008) but reach hours between 70 and 80 °C in chocolate or peanut butter (Robinson et al., 2000), and are extremely large (>10 h at 76.6 °C) in dried milk powders (McDonough and Hargrove, 1968). This makes the control of *S. enteritidis* in dry almonds more challenging. In addition, high temperature and long exposure time to control *S. enteritidis* in a dry environment could be harmful to almond quality. But the thermal resistance of *S. enteritidis* can be sharply reduced by adding moisture in the product. $D_{62°C}$ values for *S. weltevereden* in wheat flour are reduced from 875 to 29 min after increasing water activity (a_w) from 0.4 to 0.5 (Archer et al., 1998). Preliminary results in our laboratory showed that $D_{73°C}$ values of *Salmonella* PT 30 were reduced from 75 to 0.25 min when the almond shell moisture contents increased from 6% to 18% w.b. It should be practical to increase moisture content and, therefore, water activity on the almond surfaces before RF treatments to reduce the thermotolerance of *S. enteritidis*.

The objectives of this study were (1) to select the suitable soaking times to increase the moisture contents to the required levels in almond shell and kernel, and establish the moisture absorption and desorption isotherms to determine moisture content relations using water activity, (2) to develop a RF treatment protocol to achieve 5-log reduction of *S. enteritidis* PT 30, and (3) to evaluate the almond quality after RF treatments and storage.

2. Materials and methods

2.1. Materials and determination of soaking time

The in-shell almonds (*Nonpareil*) were obtained from the Almond Board of California, Modesto, CA, USA. The almonds had an initial moisture content of 5.7% w.b. in-shell and 3.2% w.b. in kernel with a peroxide value (PV) of 0.24 meq/kg, a free fatty acid (FA) value of 0.11% and color parameters of 58.8, 12.6 and 47.3 for *L*, *a* and *b* in the hunter scale, respectively.

For soaking time tests, the almond samples (200 g) were immersed in 2000 mL tap water in a plastic container at room temperature. The tap water could be pretreated to avoid any impact of chlorine on the survival of targeted *Salmonella*. The almonds were agitated by hands protected with clean gloves for 15, 30, 45, 60, 75, and 90 s. The determined soaking times with agitation could be integrated into industrial washing processes before RF treatments. After soaking, the almond samples were conditioned in a single layer in ambient air for 1 h at room temperature to reach moisture equilibrium. After that, shell and kernel were separated manually before grinding with a coffee grinder (ID557, Mr. Coffee, Guangzhou, China). The powder passed through a No. 18 mesh (16 Tyler) was used to determine the moisture content of the shell and kernel in a vacuum oven at 100 °C and 75–85 kPa for 1 h using a modified standard oven method described by Ca 2d-25 of the American Oil Chemists Society (AOCS, 1997a). Triplicates were used for every soaking time.

2.2. Moisture isotherms during absorption and desorption of almond shell

Moisture isotherms can be used to estimate moisture content using rapid water activity measurements. Completely dried and ground shells (5 g) were used to determine moisture absorption isotherms by adding distilled water at 1% intervals to prepare the relatively low moisture samples (1–6% w.b.) and 3% intervals to prepare samples with moisture contents between 6% and 34% w.b. The moisture-adjusted samples were placed in closed containers, and stored at 4 °C for 2 days for the moisture to distribute uniformly throughout the samples. Prior to the moisture measurements, the samples were equilibrated to room temperature and then were used to determine the corresponding moisture contents and a_w using an AquaLab water activity meter (Series 3, Decagon devices, Inc., Pullman, WA, USA) with an accuracy of ±0.003 a_w. For desorption tests of the pre-washed and ground shells, 20 samples were dried in the oven at 100 °C without using vacuum for different period of time to obtain appropriate moisture levels. The dried shell samples (2 g) were used to determine the moisture content and water activity. The measurements were conducted in triplicate.

2.3. RF and hot air heating systems

A 6 kW, 27 MHz pilot-scale RF system (COMBI6-S, Strayfield International, Wokingham, UK) with a hot air system (5.6 kW) was used to treat in-shell almonds (Fig. 1). The mechanism of the RF system was based on the free running oscillator, which was discussed in detail in Wang et al. (2001). The configuration of the RF and hot air systems was also described in Wang et al. (2010). The gap between the two plate electrodes was adjusted to change RF power coupled into the samples. Hot air flow was forced from the holes on the bottom electrode to the almond samples through perforated screens on the side and bottom walls of a plastic container (25.5 cm × 15 cm × 10 cm) (Fig. 2). The samples were heated in RF systems with stationary conditions.

2.4. Temperature profiles of almonds during RF heating and drying

The hot air assisted RF treatments developed in this study included three steps: (1) RF heating of the in-shell almonds with the increased shell (34% w.b.) and kernel (4.4% w.b.) moisture contents after soaking for 75 s to inactivate the target *S. enteritidis* PT 30, (2) RF drying to reduce the moisture contents, and (3) forced room air cooling to reduce thermal damages to the product quality. To achieve 5-log reduction of the *S. enteritidis*, the minimum almond temperature of 73 °C held for 1.5 min should be applied for the moisture contents of more than 18% w.b. based on $D_{73°C}$-value (0.25 min) of the *S. enteritidis* PT 30 obtained from our preliminary results.

In-shell almond samples (1.7 kg) previously washed at room temperature for 75 s followed by ambient air conditioning on a single layer for 1 h were placed in the container (Fig. 2) to obtain temperature profiles during RF heating. The sample temperatures at five representative locations (Fig. 2) in the container were measured using a fiber optic temperature measurement system (UMI, FISO Technologies Inc., Sainte-Foy, Quebec, Canada). The sensor was inserted into the kernel through a predrilled hole. An appropriate electrode gap was used to achieve a fast heating rate of more than 10 °C/min. During drying, a constant sample temperature was maintained through a balance effect of RF heating and circulation air cooling while the sample's moisture content was steadily reduced to reach the target level over relatively short period. Based on the preliminary tests, the RF treatments using an electrode gap of 13 cm with circulated hot air at 55 °C or an electrode gap

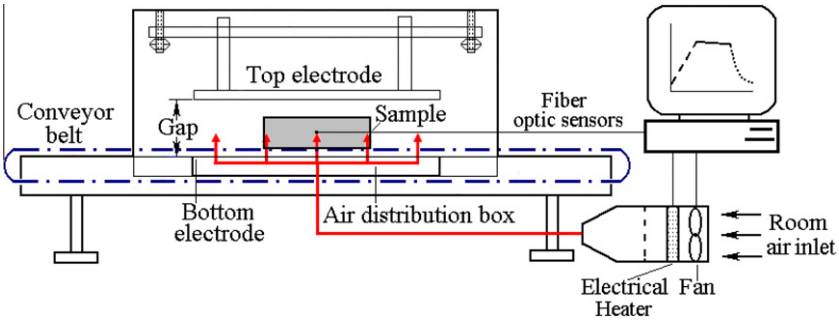

Fig. 1. Schematic view of the pilot-scale 6 kW, 27.12 MHz radio frequency (RF) unit showing the two-pair plate electrodes, conveyor belt and the hot air system (Wang et al., 2010).

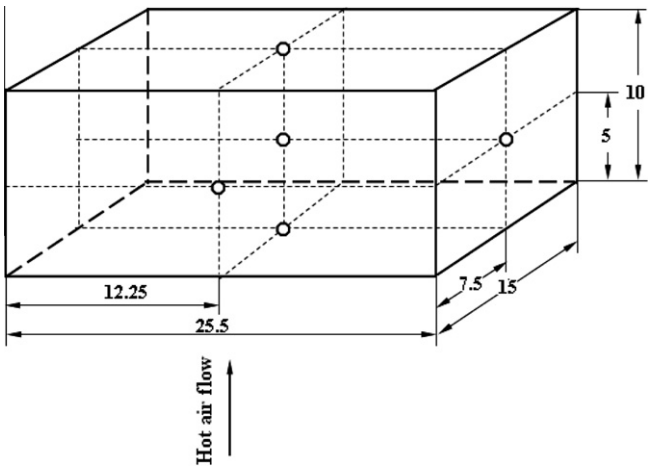

Fig. 2. The plastic container with five locations (○) for sample temperature measurements (all dimensions are in cm).

of 14 cm with circulated hot air at 55 and 64 °C were selected to obtain proper heating rates and final temperatures. The air velocity in the RF cavity was measured by a rotating vane anemometer (LCA 6000, AIRFLOW Instrumentation, Buckinghamshire, UK). The sample temperatures were measured during RF treatments over whole heating and drying periods. Average and standard deviation values of RF treated sample temperatures at the five locations were compared to determine the suitable electrode gap and hot air temperatures during heating and drying.

2.5. Determination of cooling methods

Rapid cooling is necessary to minimize quality degradation after RF treatments. In-shell almond samples heated to about 73 °C by hot air with 10 and 5 cm depths in the plastic container were subjected to natural and forced ambient air cooling. The cooling test was conducted by placing the container in the ambient air and with a cross airflow driven by a fan. The measured air velocities on the sample surface were about 0.2 and 1.0 m/s for the natural and forced air cooling, respectively. The temperature in the sample 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.

2.6. Drying curve

After developing the treatment protocol, the drying curve was obtained for in-shell almonds during the RF heating and drying described above. After soaking, the initial moisture content of the almond shell was about 34% w.b. The weight loss of the almond samples during RF treatments was determined at 0, 2.4, 3.9, 8,

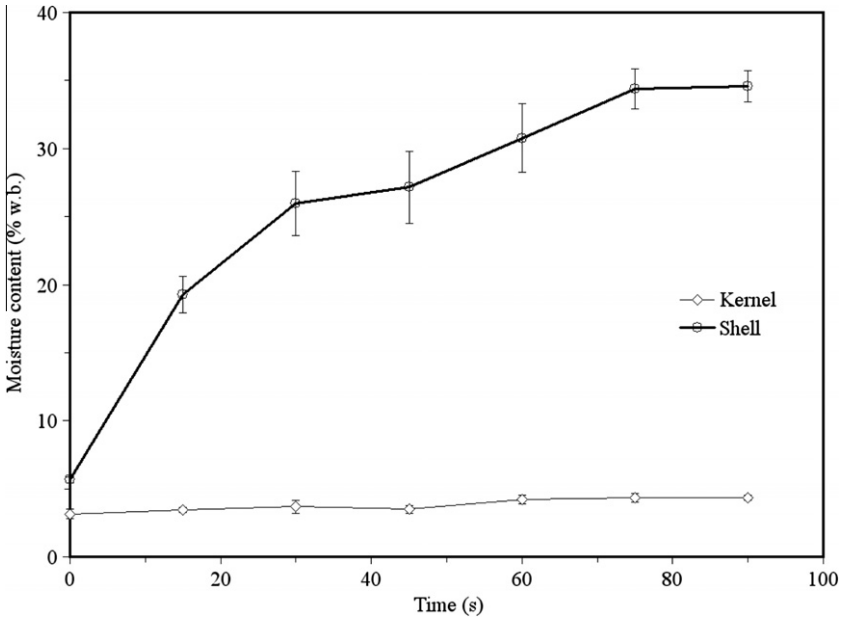

Fig. 3. The moisture content of almond (*Nonpareil*) shell and kernel as a function of soaking time at 24 °C.

12, 16, and 19 min, and used to estimate the moisture content of the almond shell. Each treatment was replicated thrice.

2.7. Quality evaluations

Quality attributes of almond samples were evaluated taken before and after RF treatments, which were able to achieve 5-log reduction of *S. enteritidis* PT 30 in in-shell almonds. The quality indexes include PV, FA and color. These are the three most important quality indicators for possible lipid oxidation at elevated temperatures (Buranasompob et al., 2007; Wang et al., 2002). To shorten duration of the studies, the accelerated shelf life tests were conducted in an incubator 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, respectively. The equivalent 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 confirmed by real-time storage experiments (Wang et al., 2006). The PV and FA values were determined using methods Cd 8b-90 and Ca 5a-40 of the American Oil Chemists Society (AOCS, 1997b, 2003) and followed the detailed measurement procedures described in Wang et al. (2001).

3Almond kernel color was measured with a computer vision system (CVS) as described in detail in Wang et al. (2010). Color images of 30 almond kernel's skin and core color per treatment were captured and stored in the computer using Adobe Photoshop CS (Adobe Systems Inc., USA). These color values were then converted to Hunter *L* (darkness), *a* (green–red), and *b* (blue–yellow) parameters. The changes in kernel's skin and core color were analyzed for *L*, *a*, *b* values, and *L* values, respectively. Mean values and standard deviations were calculated from three replicates for RF treated product quality parameters. The mean values were compared and separated using least significant (LSD) *t*-test using the variance procedure (Microsoft Office Excel 2003) at a significant level of $p = 0.05$.

3. Results and analyses

3.1. Determination of soaking time

Fig. 3 shows the moisture content of almond shell and kernel as a function of soaking time at 24 °C. The moisture content of the almond shell increased rapidly from the initial value of 5.7% w.b. to final 34.4% w.b. for a soaking time up to 75 s. There was no change of moisture contents in the shell between 75 and 90 s. The moisture content of kernels slightly increased with the soaking time, but remained below 4.4% w.b. over 90 min soaking time. Thus, the soaking time of 75 s was selected for the almond shells and kernels to achieve the moisture content of about 34.4% and 4.4% w.b., respectively.

3.2. Relationship between moisture content and water activity of almond shells

Fig. 4 shows moisture absorption and desorption isotherms of almond shells at 24 °C. The moisture content increased slowly at low levels (e.g. <5%) but more sharply at high levels (e.g. >15%) with the water activity. The absorption curve was almost overlapped with the desorption one at $a_w < 0.85$. This is similar to the typical trend of isotherms for Shokufeh almonds observed by Pahlevanzadeh and Yazdani (2005). The isotherm relationship was used to estimate the moisture contents of almond shell samples by measuring the water activity. In this way, the moisture measurement time was reduced from 1 h to 5 s.

3.3. Cooling profiles

Fig. 5 shows cooling curves of in-shell almonds in the sample center as a function of sample thickness in natural and forced room air cooling. About 150 min were needed for 10 cm deep in-shell almond samples to cool from 73 to 30 °C in natural room air. The cooling time decreased sharply with reducing sample thickness and when introducing forced air. It took only 12 min for forced air cooling of 5-cm deep samples to reach 30 °C, which could be used for cooling after RF treatments.

3.4. Treatment protocol developments

Fig. 6 shows the mean and standard deviation values of washed in-shell almond temperatures over five locations in the container when subjected to hot air and stationary RF heating with two electrode gaps and air temperatures. The smaller gap resulted in faster heating because of larger applied RF power. For the electrode gap

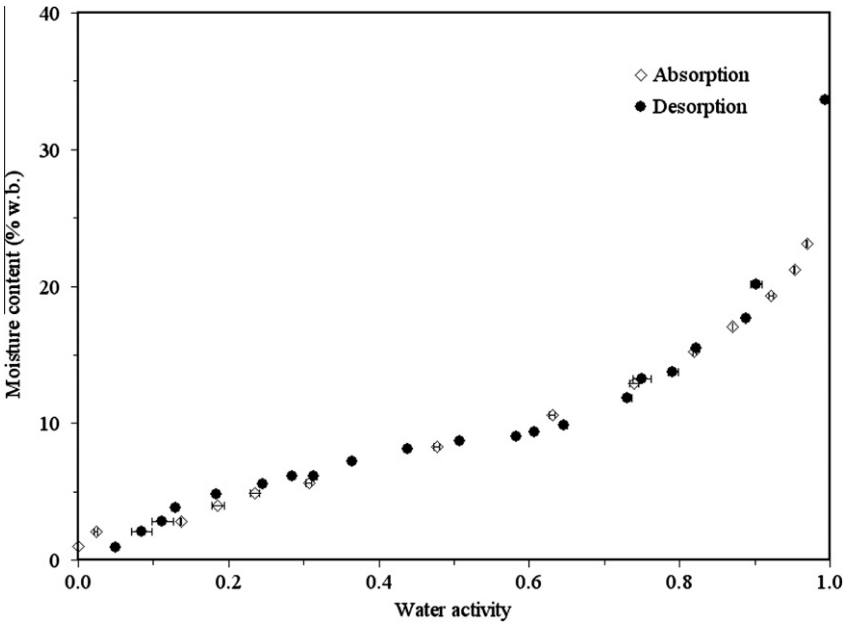

Fig. 4. Moisture absorption and desorption isotherms of almond shells at 24 °C.

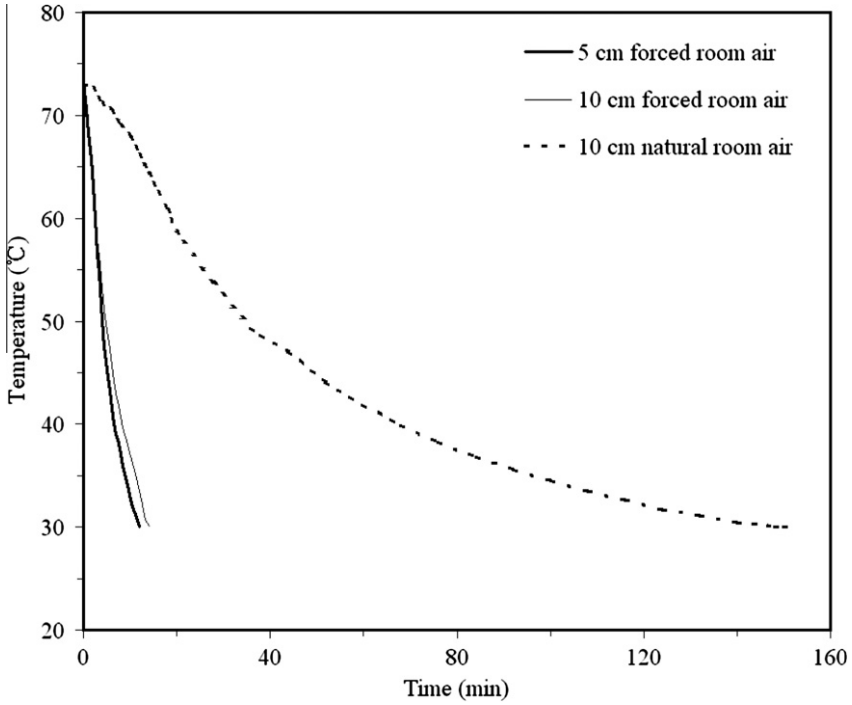

Fig. 5. Cooling curves of in-shell almond in the sample center as a function of sample thickness under natural and forced room air cooling.

of 13 cm, the heating rate of the washed almonds reached 23.3 °C/min within the first 2.4 min of RF heating with an average sample temperature of about 86 °C. For the electrode gap of 14 cm, the heating rate for 55 °C hot air was slightly larger than that for 64 °C hot air, probably caused by different air velocities for these hot air temperatures. After the come-up time, the sample temperature was maintained at a fairly constant value as the absorbed RF power was balanced by the latent heat of water evaporation. The average sample temperature (75.5 °C) in RF treatments at 55 °C hot air was lower than that (81.4 °C) at 64 °C hot air heating due to heat convection. To obtain the required 5-log reduction of *Salmonella*, the RF heating for 3.6 min with the electrode gap of 13 cm and hot air heating at 55 °C was selected first, followed by increasing the gap to 14 cm and maintaining the hot air temperature of 55 °C to maintain low sample temperatures (75.5 °C) and avoid almond quality degradation during drying. This combined RF heating with hot air at 55 °C treatments during 19 min could meet the treatment requirements both for pasteurization and drying.

Fig. 7 shows typical temperature–time histories of the washed in-shell almonds at five different locations in the 10 cm thick container when subjected to hot air (55 °C) assisted

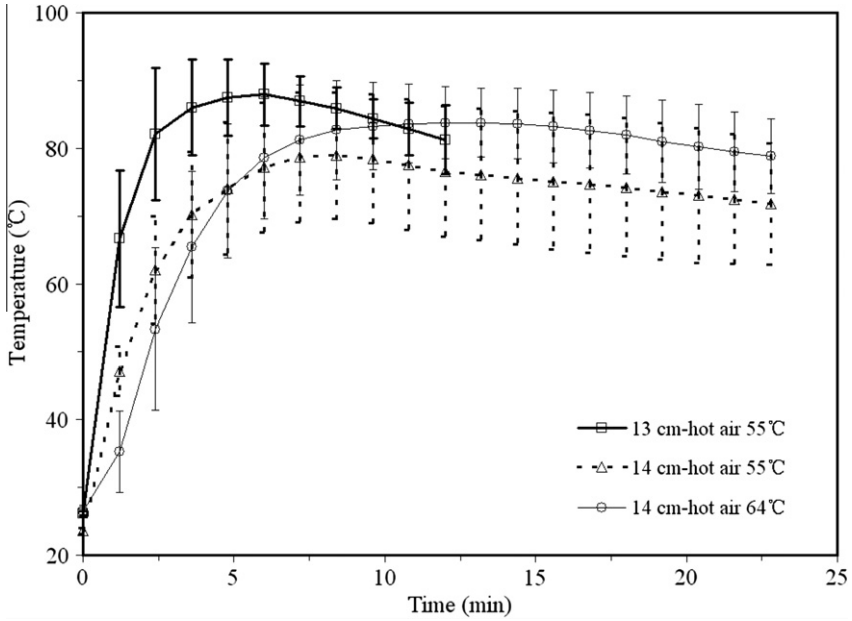

Fig. 6. The mean and standard deviation values of in-shell almond temperatures over five locations in the container when subjected to hot air and stationary RF heating with two electrode gaps and air temperatures as influenced by the heating time.

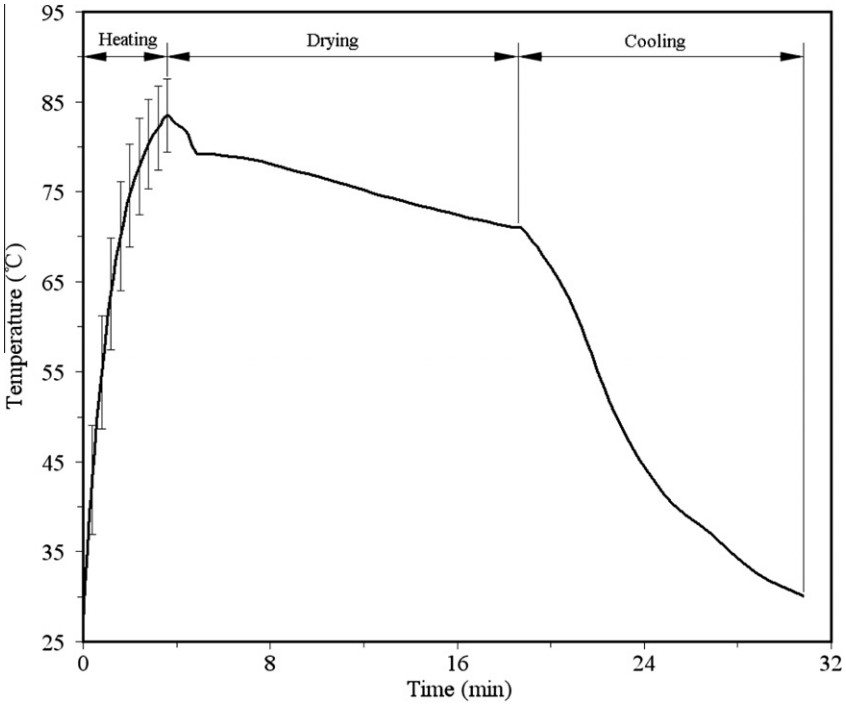

Fig. 7. Average temperature–time histories of the washed in-shell almonds over five locations in the 10 cm deep container when subjected to hot air (55 °C) assisted RF treatments under the electrode gap of 13 cm for heating and 14 cm for drying followed by forced room air cooling in 5 cm thick samples.

RF treatments under the electrode gap of 13 cm for pasteurization, 14 cm for drying, and followed by forced room air cooling of 5 cm thick samples. It took about 4 min for the sample temperature to reach the average temperature of 83 °C in RF heating. During RF heating period between 2 and 4 min, the minimum sample temperature was more than 75 °C (Fig. 7) and the moisture content remained above 23% w.b (Fig. 8). According to preliminary $D_{73°C}$-value (0.25 min) for *S. enteritidis* PT30 in 18% w.b. almond shell samples, this treatment protocol should provide enough safety margin to achieve 5-log reduction of the target *Salmonella*.

In RF drying, the sample temperature was maintained between 80 and 70 °C (Fig. 7), which resulted in a reduction of the moisture content to 5.7% w.b. after 19 min of exposure to both RF field and hot air drying (Fig. 8). This complete process shown in Fig. 7 was used as the treatment protocol.

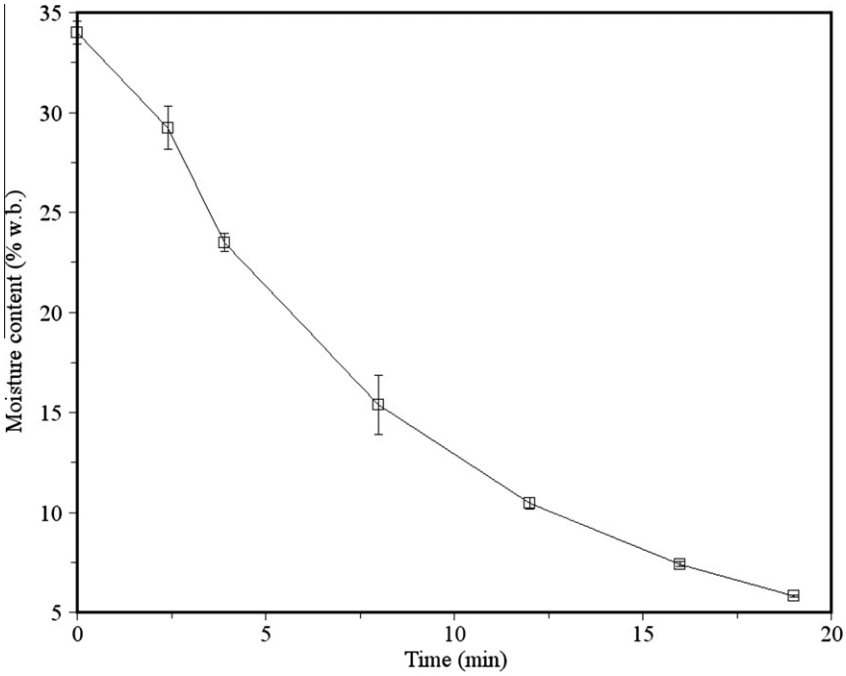

Fig. 8. Drying curve of in-shell almonds after hot air assisted RF heating and drying with forced room air cooling defined in Fig. 7.

Table 1
Storage quality parameters (mean ± SD over three replicates) of in-shell almonds (*Nonpareil*) before and after RF treatments.

Storage time at 35 °C (days)[a]	Peroxide value (meq/kg)[b]		Fatty acid (%)[b]		Kernel skin color						Kernel core color (*L*-value)	
					L[c]		*a*		*b*			
	Control	RF treated	Control	RF treated	Control	RF treated	Control	RF treated	Control	RF treated	Control	RF treated
0	0.24 ± 0.03aA[d]	0.39 ± 0.00bA	0.11 ± 0.05aA	0.15 ± 0.03aA	58.76 ± 2.95	59.89 ± 2.48	12.57 ± 1.44	14.58 ± 1.21	47.29 ± 1.50	47.39 ± 1.94	87.68 ± 1.32	87.90 ± 1.39
10	0.38 ± 0.01aB	0.45 ± 0.01bB	0.13 ± 0.03aA	0.25 ± 0.02bB	59.72 ± 3.39	59.08 ± 4.46	13.55 ± 2.11	14.33 ± 2.52	48.79 ± 1.69	48.13 ± 2.24	86.37 ± 1.20	89.62 ± 0.95
20	0.47 ± 0.02aC	0.49 ± 0.01aC	0.20 ± 0.01aB	0.27 ± 0.00bB	61.54 ± 2.81	55.49 ± 3.85	13.51 ± 2.12	14.46 ± 2.29	49.67 ± 2.01	46.49 ± 1.78	88.53 ± 1.18	86.24 ± 1.06

[a] 10 and 20 days at 35 °C to simulate 1 and 2 years storage at 4 °C, respectively.
[b] Accepted PV and FA values for good quality are less than 1.0 meq/kg and 0.6%, respectively.
[c] *L*-value (lightness): 0 = black and 100 = white; good quality ⩾40.
[d] Different lower and upper case letters indicate that means are significantly different among treatments and storage time, respectively, at $p = 0.05$.

3.5. Almond quality

Table 1 summarizes the results of almond quality evaluations after the samples were processed by the treatment protocol shown in Fig. 7. Mean PV and FA values significantly increased with the storage time at 35 °C for both control and treated almonds ($p < 0.05$) due to unavoidable oxidation during the storage period. Similar results were also observed in RF treated walnuts (Wang et al., 2007b). The PV and FA values of in-shell almonds were affected significantly by RF treatments ($p < 0.05$) except for PV stored at 35 °C for 20 days ($p > 0.05$) and FA values stored at 35 °C for 0 days ($p > 0.05$). But the RF treated almond quality stored at 4 °C for 2 year was still acceptable, since the final PV and FA values during accelerated storage at 35 °C for up to 20 days remained within the marketable range (PV < 1.0 meq/kg and FA < 0.6%) according to the nut industry standard.

The *L*-, *a*- and *b*-values of kernel skin color and core color (*L*-values) did not show a clear trend after the RF treatments and during the elevated storage temperature used for accelerated shelf life studies (Table 1). There were no significant differences ($p > 0.05$) for all color values between controls and RF treatments for the different storage times. *L*-values of kernel skin and core colors were above 58 and 86, respectively after the RF treatments stored at 35 °C for 20 days, which simulated two-year storage at 4 °C. These values were still much higher than 40 required by the almond industry.

4. Conclusions

To develop an effective pasteurization process for in-shell almonds, appropriate soaking time was determined to achieve the required moisture contents based on the absorption and desorption isotherms. The hot air (55 °C) assisted RF treatments consisted of heating and drying processes followed by cooling. The RF heating process of soaked almonds was conducted under the plate electrode gap of 13 cm to achieve the average sample temperature of above 75 °C and the moisture content of more than 23% w.b. for 2–4 min. The RF drying process with the electrode gap of 14 cm and hot air heating at 55 °C resulted in a stable sample temperature between 70 and 80 °C and reduced the moisture content to 5.7% w.b. The RF treated samples were cooled by forced room air in 5 cm deep layer to avoid further quality loss and increase the throughputs. Almond quality parameters were not affected by the RF treatments even after the accelerated storage for 20 days at 35 °C simulating 2 years of real storage at 4 °C. The RF process should provide a practical and effective pasteurization method for in in-shell almonds while maintaining product quality. Future research is needed to confirm the treatment efficacy using inoculated almonds.

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