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Industrial-scale radio frequency treatments for insect control in walnuts II: Insect mortality and product quality

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Abstract

This paper reports on the second part of a scaling-up study investigating the technical feasibility of using radio frequency (RF) energy in commercial postharvest insect control in in-shell walnuts as an alternative to chemical fumigation. A large-scale treatment for conveyorized walnuts was designed based on a 25 kW, 27 MHz RF system to achieve an average walnut surface temperature of 60 °C, and minimum temperature of 52 °C, for 5 min. The treatment caused 100% mortality of fifth-instar navel orangeworm larvae, the most heat tolerant target pest, in both unwashed and air-dried walnuts, and was effective over a relatively wide range of walnut moisture contents (3–7.5%). Walnut quality was not affected by the RF treatments; kernel color, peroxide values and fatty acid values of treated walnuts were similar to untreated controls after 20 days at 35 °C simulating 2 years of storage under commercial conditions at 4 °C. The RF treatment slightly reduced the moisture content of the walnuts, especially the shells. The RF treatment developed in this study should also control codling moth, Indianmeal moth and red flour beetle in in-shell walnuts. This treatment will provide an effective and environmentally friendly phytosanitary treatment technology for the walnut industry.

Keywords: Navel orangeworm; Moisture; Quarantine; Radio frequency; Storage

1. Introduction

Field and stored-product insect pests, such as the codling moth (*Cydia pomonella* [L.]), navel orangeworm (*Amyelois transitella* [Walker]), Indianmeal moth (*Plodia interpunctella* [Hübner]) and red flour beetle (*Tribolium castaneum* [Herbst]), cause major economic losses during production, storage and marketing of walnuts (*Juglans regia* L.). These pests reduce product quality through feeding damage and by contaminating the product with webbing, cast skins and frass. Codling moth and navel orangeworm larvae are field pests that may also be present in processed nuts. Codling moth is regulated by both Japan and Korea as a quarantine pest, and navel orangeworm is a phytosanitary concern for Australian and European markets. The Indianmeal moth and red flour beetle, two common pests of stored nuts, are the insects most often responsible for consumer complaints (Johnson et al., 2004). Walnuts infested with these pests are not easily detected by external inspection, causing many processors and regulatory agencies to require phytosanitary treatments of product. Walnut processors normally use chemical fumigants to disinfest product of these pests, but recent regulatory restrictions on the most commonly used fumigant, methyl bromide, has generated interest in alternative disinfestation methods (UNEP, 1995).

Heating with radio frequency (RF) energy has been proposed as a potential alternative to chemical fumigation for postharvest control of insects infesting agricultural products (Tang et al., 2000). A laboratory scale RF system was used to evaluate the potential of RF heat treatments to control insect pests in walnuts (Wang et al., 2001, 2002c). Wang et al. (2007) studied heating uniformity of walnuts in a 25 kW industrial-scale RF system in an attempt to scale-up treatment protocols developed from laboratory tests. To further evaluate the 25 kW RF system, it is necessary to determine its efficacy in disinfesting walnuts of insect pests and to evaluate the effect of the treatments on product quality.

Developing a successful thermal treatment relies on a thorough knowledge of the thermal death kinetics of the targeted

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insects. Several experimental methods have been reported for characterizing the effect of thermal treatments on insect mortality (Yokoyama et al., 1991; Neven, 1994; Thomas and Shellie, 2000), of which direct hot water bath immersion is the most commonly used method (Hayes et al., 1984; Jang, 1986; Thomas and Mangan, 1997). A heating block system developed by Washington State University (Ikediala et al., 2000) eliminates the effect of heat transfer on the intrinsic thermal death kinetics of insect pests and is able to generate highly repeatable results for any given set of conditions (Wang et al., 2002b). Using the heating block system, parameters for effective RF treatments were determined and confirmed in laboratory RF units using insect-infested walnuts (Wang et al., 2002c; Mitcham et al., 2004). The thermal death kinetics of various life stages of codling moth, Indianmeal moth, navel orangeworm and red flour beetle have been reported elsewhere (Wang et al., 2002a,b; Johnson et al., 2003, 2004). At temperatures over 50 °C we found that the fifth-instar navel orangeworm larva is the most heat resistant life stage and species among these four insects. Consequently, efficacious treatments developed for fifth-instar navel orangeworm that provide product temperatures of 50 °C or above should control the other three walnut pests.

Commercially viable RF phytosanitary treatments for walnuts must also retain product quality. Because of the potential of walnut kernels to undergo rapid oxidative and hydrolytic rancidity at elevated temperatures, the main quality parameters of concern include peroxide values (PV, meq/kg), fatty acids (FA, % oleic) and kernel color. Peroxides are products from the primary oxidation of unsaturated fatty acids in walnut oils, while hydrolytic rancidity results in the release of fatty acids (Buranasompob et al., 2003, 2007). According to the industry standard (Diamond Walnut Growers, Inc., Stockton, CA), good quality walnuts should have a PV < 1.0 meq/kg and a FA < 0.6%. Buranasompob et al. (2003) reported that heating shelled walnut kernels with 60 °C hot air for up to 10 min did not increase rancidity compared to untreated walnuts. Mitcham et al. (2004) observed that final kernel temperatures around 75 °C did not alter walnut quality after laboratory RF treatments. Although these laboratory studies indicate that short term exposure to RF should not affect product quality, the effect of industrial-scale RF treatments on quality must be evaluated because these treatments may result in more variability in product temperature.

The objective of this study was to determine the potential of RF phytosanitary treatments for in-shell walnuts under commercial conditions by evaluating treatment efficacy and the effect on moisture content and product quality of an industrial-scale RF treatment system.

2. Materials and methods

2.1. RF treatment procedure

The typical commercial walnut process begins with harvest and proceeds through hull removal, heated air dehydration, fumigation, sizing, washing/bleaching, static air drying, packaging and storing (Fig. 1). We studied the use of RF treatments in two possible places in the process: (1) after receiving and before washing/bleaching; (2) after static air drying and before packaging (Fig. 1). The first option fits in the same location in the handling system as methyl bromide fumigation, disinfesting product as it enters the processing facility. At this point, any proposed RF treatment must be suitable for walnuts with a relatively wide range of moisture content as the product may come from different dehydrators. In the second option where RF treatment occurs after air drying, moisture content of the walnuts at the start of RF treatment would be more uniform. In addition, the second option would serve to reduce the amount of time and energy used to dry washed nuts because RF heating might remove some moisture from the treated walnuts. This option should also require less RF energy than the first option because of the high initial walnut temperatures (37–41 °C) found after air drying.

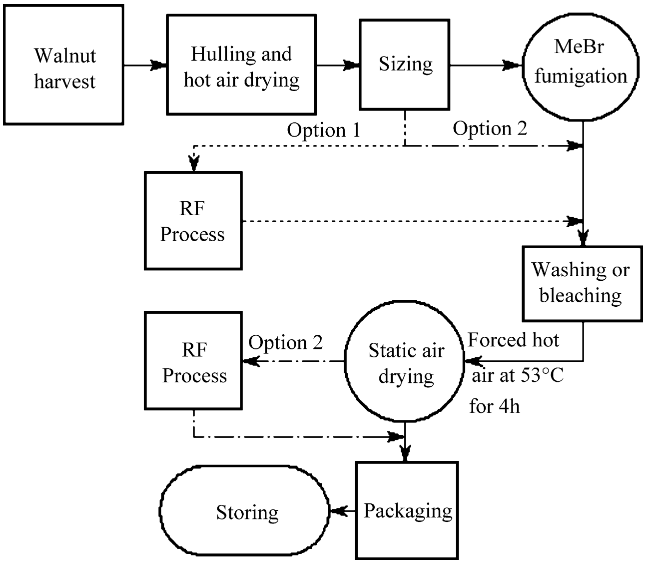

Fig. 1. Two options for placement of RF treatment in place of fumigation treatment to control pests in in-shell walnuts during industrial processes.

The efficacy tests were conducted in a 27.12 MHz industrial-scale, 25 kW RF system (Model, S025/T, Strayfield International Limited, Wokingham, UK) at a large processing plant owned by Diamond Walnut Growers, Inc., in Stockton, CA. A detailed description of the RF system is provided in Wang et al. (2007). Before the tests, walnut samples were collected either from trucks bringing new product to the plant (unwashed nuts) or from the processing line immediately after air drying (air-dried nuts). During RF treatments, walnuts were held in high-density polyethylene containers (0.6 m × 0.4 m × 0.22 m) with perforated bottoms and side walls. Each container was evenly filled with 11 kg of in-shell walnuts (about 800 walnuts) and placed on the conveyor belt for treatment. Before treatment, the hot air portion of the RF system ran for 2 h to ensure an air temperature of at least 60 °C in the RF cavity. Based on our previous study (Wang et al., 2007), we used an electrode gap of 280 mm, a conveyor belt speed of 57 m/h, and one thorough mixing of the nuts between two consecutive RF exposures to meet the treatment requirements needed for 100% mortality of fifth-instar navel orangeworm. To achieve less than 100% insect mortality, two

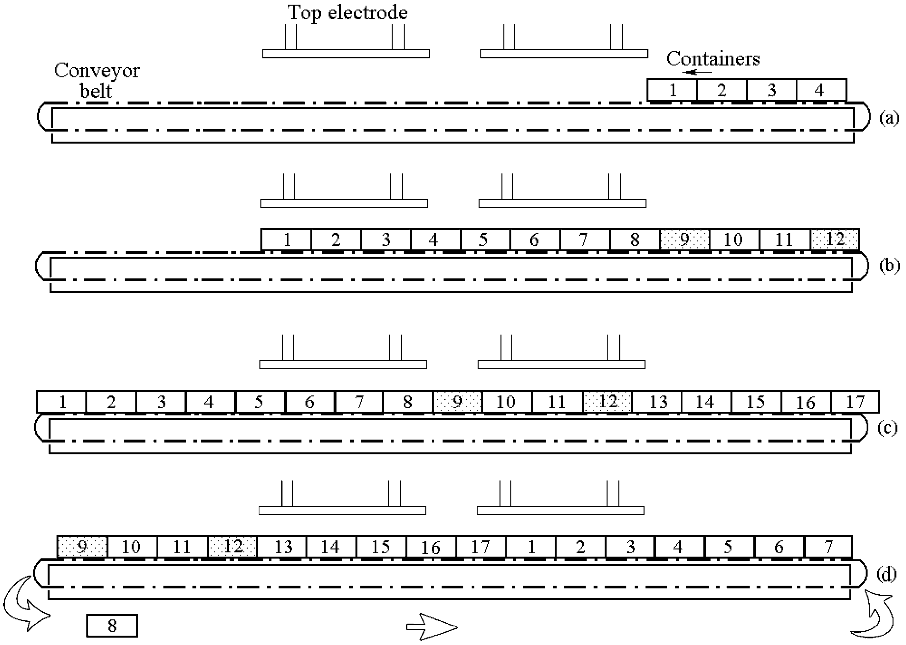

Fig. 2. Arrangement of containers during RF treatments: (a) beginning of treatment, (b) point where current is stabilized, (c) RF cavity fully loaded, and (d) containers placed back in RF system to finish treatment. Only containers 9 and 12 (shaded) were used for efficacy and quality evaluations.

tests were also done with an electrode gap of 285 mm, thereby reducing the power and final walnut temperatures to confirm the baseline of an optimum treatment.

For each treatment, 17 containers of walnuts were used to simulate a continuous operation with the RF system. Before the first container entered the RF cavity (Fig. 2a), the electrical current was about 0.41 A. The current then increased as containers moved into the system and eventually stabilized after the first container reached the far edge of the second pair of electrodes. At this point (Fig. 2b), the first eight containers were completely under the two top electrodes. In order to avoid the influence of the transient condition, none of the first eight containers of walnuts were used for efficacy and quality evaluations. Instead, containers #9 and #12 were selected for evaluation. As containers completed the passage through the RF cavity, they were returned to the front of the RF system and placed on the conveyor belt for a second pass (Fig. 2c and d). This whole process was considered a treatment run.

Before each treatment run, about 2 kg of untreated walnuts were randomly collected for initial moisture content determinations and quality evaluations. The walnut surface temperatures for the two selected containers (#9 and #12) were mapped with a thermal imaging camera (Thermal CAM$^{\text{TM}}$ SC-3000, N. Billerica, MA, USA) before RF treatments. Each thermal image took less than 1 s. Ten walnuts were also randomly selected from each of the containers for kernel temperature measurements before RF treatments using two thin Type-T thermocouple thermometers (Model 91100-20, Cole-Parmer Instrument Company, Vernon Hill, IL, USA) having an accuracy of ±0.2 °C and 0.8 s response time. For each replicate, the initial walnut surface and kernel temperatures were averaged across containers and treatment runs and reported as control temperatures.

After the first pass through the RF cavity, the walnuts in containers #9 and #12 were quickly mixed through a riffle-type sample splitter (SP-1, Gilson Company, Inc., Ohio, USA). The splitter divided the treated nuts into two approximately equal portions which were then poured back to the container. The containers were placed back on the conveyor belt for the second pass through the RF cavity. Immediately after the RF treatment was completed, the surface temperatures of the two selected containers (#9 and #12) were mapped with the thermal imaging camera. Another 10 walnuts were randomly selected from each of the selected containers (#9 and #12) for kernel temperature measurements as described above. This temperature measurement process took about 5 min which was considered as the exposure time at the final temperatures needed to provide adequate lethality to the insects in the infested walnuts. Our previous studies showed that walnut kernel temperature dropped only 1 °C during this 5 min period (Wang et al., 2002c). After that, infested walnuts were removed for insect mortality evaluation and about 2 kg of un-infested walnuts were collected from each container for final moisture content measurements and quality analysis.

2.2. Infestation procedure and mortality evaluation

Fifth-instar navel orangeworms, reared at the USDA-ARS San Joaquin Valley Agricultural Sciences Center (SJVASC), Parlier, CA, were brought to Diamond Walnut Growers, Inc., Stockton, CA on the morning of each test day. One larva was placed in each walnut through a 4 mm hole drilled in the shell. Each hole was closed with a white polyethylene plug to prevent the insects escaping from the walnuts. Because the plugs were hollow, there was concern that test larvae might use them as a refuge to escape the hot nutmeat. Consequently, the plugs

Table 1
Mortality (mean ± S.D., %) of fifth-instar navel orangeworm, final kernel ($n = 10$) and surface ($n = 45{,}056$) temperatures in control and RF treated walnuts

Unwashed walnuts (option 1)

Replicates	Runs	Containers	Kernel temperatures (°C)	Surface temperatures (°C)	Mortality (%)
Rep 1		Control	25.8 ± 0.7	26.4 ± 1.6	0
	1	#9	60.0 ± 5.2	63.4 ± 2.8	100
		#12	58.3 ± 3.4	63.5 ± 3.3	100
	2	#9	57.4 ± 2.0	64.3 ± 2.8	100
		#12	58.5 ± 4.7	64.0 ± 2.6	100
Rep 2		Control	23.7 ± 0.5	23.0 ± 0.5	1.1
	1	#9	57.5 ± 1.9	62.3 ± 2.9	100
		#12	58.2 ± 1.9	62.4 ± 3.1	100
	2	#9	57.4 ± 2.2	61.8 ± 3.1	100
		#12	58.5 ± 1.6	63.8 ± 3.5	100
Rep 3		Control	23.6 ± 0.9	23.9 ± 0.5	0
	1	#9	54.8 ± 2.1	58.6[a]	100
		#12	56.7 ± 2.3	59.6	100
	2	#9	57.0 ± 2.8	58.6	100
		#12	55.3 ± 2.3	60.1	100
Hot air-dried walnuts (option 2)					
Rep 1		Control	27.1 ± 1.1	26.7 ± 0.6	1
	1	#9	59.0 ± 2.3	67.2 ± 5.0	100
		#12	61.4 ± 5.9	68.4 ± 6.2	100
	2	#9	59.2 ± 3.0	64.5 ± 3.3	100
		#12	59.0 ± 2.4	66.0 ± 4.0	100
Rep 2		Control	27.7 ± 1.1	27.4 ± 0.6	0
	1	#9	58.9 ± 1.7	60.8[a]	100
		#12	59.8 ± 5.8	61.9	100
	2	#9	55.2 ± 1.8	58.8	100
		#12	56.6 ± 1.8	60.0	100

[a] Only mean surface temperatures were obtained.

were filled with plasticine, which did not heat in the RF field. Both unwashed walnuts collected before fumigation and air-dried walnuts collected before packaging were used in separate tests. Each replicate consisted of 75 infested walnuts for each of the two treatment containers (#9 and #12), and two runs made on the same day under the same test conditions. Efficacy tests with unwashed walnuts were done three times (900 test larvae), while tests with the air-dried walnuts were done twice (600 test larvae), for a total of 1500 test larvae. We also set aside 100 infested walnuts as unheated controls for each replicate.

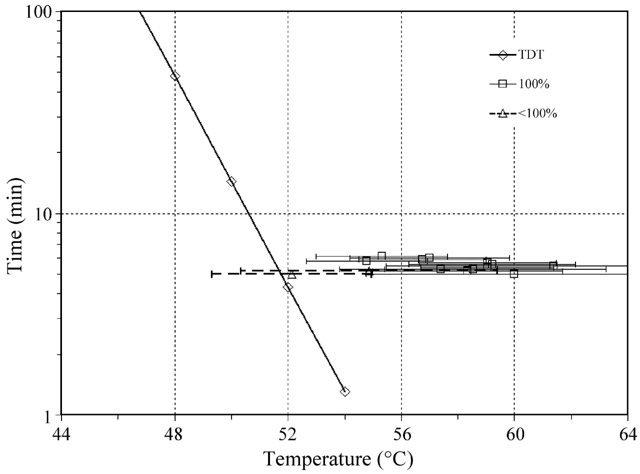

Fig. 3. Final walnut kernel temperatures (mean ± S.D.) after RF treatments related to insect mortality as determined by thermal-death–time (TDT) curve for complete kill of 600 fifth-instar navel orangeworm larvae obtained in a heating block system (Wang et al., 2002b).

Before treatment, each of the two treatment containers (#9 and #12) was prepared by mixing infested and uninfested nuts with the sample splitter. Each treatment container held 75 infested nuts and enough uninfested nuts to make a total of 11 kg (about 800 nuts). This represented an artificial infestation level of 9.4%, well above the 5% action level for insect damaged nuts (USDA-AMS, 1997) and 16 times the natural infestation level (0.55%) for codling moth in walnuts found by Vail et al. (1993). At the end of each treatment, all infested walnuts were taken back to USDA-ARS, Parlier, CA and held at 25 °C, 60% RH with a 14:10 (L:D) h photoperiod for 1 day before examination. All infested walnuts were cracked open for examination. Insects were considered dead if no movement was observed. Mortality was calculated as the percentage of dead insects relative to total treated insects for each treatment.

2.3. *Walnut quality analyses*

Walnut samples taken before and after RF treatments from each run per treatment day were transferred directly to the Diamond Walnut Growers Quality Assurance Laboratory where PV,

Table 2
Moisture contents (mean ± S.D., % w.b.) of the kernel, shell and whole walnuts in unwashed ($n = 3$) and air-dried ($n = 2$) samples before and after RF treatments

Walnut sample type	Kernel		Shell		Whole nut	
	Control	RF treated	Control	RF treated	Control	RF treated
Unwashed	3.2 ± 0.1a[a]	3.0 ± 0.1b	7.2 ± 0.4a	6.3 ± 0.3b	4.7 ± 0.4a	4.3 ± 0.3b
Air dried	3.3 ± 0.2a	3.0 ± 0.1b	7.4 ± 0.3a	6.4 ± 0.3b	4.9 ± 0.2a	4.3 ± 0.3b

[a] Different letters for each walnut fraction indicate that means are significantly different ($P < 0.05$) between the control and RF treated walnuts.

Table 3
Storage quality characteristics (mean ± S.D.) of unwashed ($n = 3$) and air-dried ($n = 2$) in-shell walnuts before and after RF treatments

Storage time at 35 °C (days)[a]	Peroxide value[b] (meq/kg)		Fatty acid[b] (%)		Kernel color (*L*-value)[c]	
	Control	RF treated	Control	RF treated	Control	RF treated
Unwashed walnuts						
0	0.04 ± 0.03	0.18 ± 0.29	0.15 ± 0.07	0.19 ± 0.06	–	–
10	0.32 ± 0.21	0.21 ± 0.03	0.23 ± 0.07	0.24 ± 0.04	47.46 ± 1.04	46.55 ± 1.29
20	0.71 ± 0.41	0.86 ± 0.26	0.20 ± 0.02	0.20 ± 0.05	46.80 ± 0.97	45.76 ± 1.00
Hot air-dried walnuts						
0	0.15 ± 0.11	0.09 ± 0.11	0.42 ± 0.36	0.17 ± 0.12	–	–
10	0.41 ± 0.13a[d]	0.75 ± 0.08b	0.17 ± 0.07	0.26 ± 0.07	46.30 ± 3.14	46.77 ± 2.77
20	0.74 ± 0.30	0.82 ± 0.28	0.23 ± 0.02	0.22 ± 0.08	44.98 ± 2.46	44.94 ± 2.42

[a] 10 and 20 days at 35 °C simulate 1 and 2 years storage at 4 °C.
[b] Accepted peroxide value and fatty acid 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 letters indicate that means are significantly different ($P < 0.05$) between the control and RF treated walnuts.

FA and color, the quality indexes that are most likely to change at elevated temperatures, were evaluated by lab personnel. The effect of storage time on PV and FA changes were also evaluated in accelerated shelf life tests. In those tests, in-shell walnuts were stored 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 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 8-53 and Ca 5a-40 of the American Oil Chemists Society (AOCS, 1998a,b). Detailed measurement procedures and calculation of PV and FA values described in Wang et al. (2001). Walnut kernel color was measured with a colorimeter (Model CM-2002, Minolta Corp., Ramsey, NJ) calibrated to a standard white reflective plate, and expressed in Hunter scale parameters. The change in kernel color was only analyzed for *L*-values that represent the light–dark spectrum with a range from 0 (black) to 100 (white). Color measurements were made for samples held under accelerated storage at 35 °C for 10 and 20 days. Due to technical problems, color measurements were not taken from samples immediately after RF treatment (0 days).

2.4. Walnut moisture content measurement and statistical analyses

Walnut samples taken before and after RF treatments were cracked manually. The shells and kernels were separated. Shell, kernel and whole walnut samples were ground into meal using a RAS mill (Romer Labs, Inc., Union, MO) for measurement with a moisture meter (GAC, 2000, Dickey-John Cooperation, Auburn, IL) pre-calibrated against the standard oven method (AOAC, 1990).

The initial (control) and final moisture content and quality parameters following RF treatment of the walnut samples from each of the two runs per day were averaged within each RF treatment date and considered as a replicate. Mean values and standard deviations were calculated from replicates for each RF treatment. The mean values were compared using the SAS analysis of variance (ANOVA) procedure (SAS Institute, 1989). Where there were significant differences ($P \leq 0.05$), means were separated using least significant difference (LSD) *t*-test (SAS Institute, 1989).

3. Results and analyses

3.1. Insect mortality

Table 1 shows insect mortality and final kernel and surface temperatures in unwashed and air-dried in-shell walnuts after RF treatments. The low mortality of controls (≤1%) indicated that the effects of transport and handling were negligible and the mortality data for samples after RF treatments required no correction. Final average shell and kernel temperatures were ≥55 °C due to RF heating, resulting in 100% insect morality for all RF treatments. It should be noted that shell temperatures were 2–8 °C higher than kernel temperatures, probably because of the higher moisture content in shells (Table 2). That is, RF energy was absorbed more in materials with higher moisture. Although variation in moisture content was the single most important contributor to non-uniform RF heating, the RF treatment developed in this study was effective for the moisture content variations found in unwashed kernels (3.1–3.3%), unwashed shells (6.6–7.7%), air-dried kernel (3.1–3.4%) and air-dried shells (7.1–7.7%).

The efficacy results agreed with the thermal death time (TDT) curves of fifth-instar navel orangeworm obtained by the heating block system (Wang et al., 2002b). The TDT curve showed that 5 min exposure to 52 °C or 1 min exposure to 54 °C should result in 100% mortality of a sample size of 600 insects (Fig. 3). In the current study, complete kill of test insects was observed when the final kernel temperature was located above and to the right of the TDT curve, and was as good as earlier, small-scale studies with infested walnuts (Wang et al., 2001, 2002c). Specifically, 100% mortality was achieved as long as the mean and the standard deviation of the final walnut kernel temperatures remained above the TDT curve. In the two treatment runs using an electrode gap of 285 mm, only 98% mortality was obtained because final walnut kernel temperatures (52.1 ± 2.8 and 54.9 ± 4.5 °C) dropped below the TDT curve boundary (Fig. 3). Generally, a larger kernel temperature variation requires an increase in mean target temperatures to achieve complete kill.

3.2. *Walnut moisture content*

Table 2 shows the moisture contents of the kernel, shell and whole walnuts before and after RF treatments. The average moisture content was highest in the shell, followed by whole nuts and then the kernel. The moisture content in RF treated walnuts was significantly less than that of untreated control walnuts ($P < 0.05$). In particular, the moisture content in the shell was reduced by 1% (from 7.2% in controls to 6.3% in treated samples). In contrast, moisture content dropped only 0.2–0.6% in kernels and whole nuts, respectively, during RF treatments. The final moisture content difference after RF treatment was less than 0.2% between the unwashed and air-dried in-shell walnuts. The drying effect RF treatments have on air-dried walnuts may be beneficial, allowing the industry to reduce overall static air drying time and associated costs after washing. However, if drying is excessive, this could result in loss of saleable weight.

3.3. *Walnut quality*

Table 3 summarizes the results of quality evaluations during accelerated storage. Only samples from treatments that resulted in 100% kill were evaluated. Mean PVs increased with storage time for both control and RF treated walnuts, but FA values did not show a similarly clear trend during storage. There was no statistically significant difference in PVs and FA values between control and RF treated samples except for the PVs of air-dried walnuts stored at 35 °C for 10 days ($P < 0.05$). For both unwashed and air-dried nuts, the final PVs and FA values during accelerated storage for up to 20 days remained within the acceptable range (PV < 1.0 meq/kg and FA < 0.6%) used by industry for good walnut quality. Our quality evaluations confirmed earlier results in which RF heating to 53 °C for 5 min (Wang et al., 2001), 55 °C for 10 min (Wang et al., 2002a,b,c), and 75 °C for 5 min (Mitcham et al., 2004) did not increase walnut rancidity after 10- and 20-day accelerated storage.

On the Hunter scale, *L*-values decrease as product color darkens. In our study, kernel color was slightly darker after RF treatments and after storage (Table 3). However, there was no statistically significant difference between untreated controls and RF treated walnuts for different storage times ($P > 0.05$). According to walnut industry standards, acceptable *L*-values for product color are >40. Consequently, the final color of treated walnuts, even after accelerated storage, would still be acceptable to the walnut industry.

4. Conclusions

By completely controlling navel orangeworm within in-shell walnuts without adversely affecting product quality, our study successfully demonstrated the efficacy of RF treatments as an alternative to methyl bromide fumigation for postharvest insect control in in-shell walnuts. We have previously demonstrated that fifth-instar navel orangeworm larvae are the most heat tolerant species and life stage of the important postharvest walnut pests in California, therefore the RF treatment developed in this study would also control codling moth, Indianmeal moth and red flour beetle in in-shell walnuts. In addition to providing insect control for incoming, unwashed walnuts, RF treatments may be useful in reducing drying time and energy costs for air-dried walnuts.

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