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Process protocols based on radio frequency energy to control field and storage pests in in-shell walnuts

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Abstract

A practical process protocol was developed to control insect pests in in-shell walnuts using a 27 MHz pilot scale radio frequency (RF) system. Fifth-instars, that had been determined to be the most heat resistant life stage for navel orangeworm (*Amyelois transitella* [Walker]) using a heating block system, were selected as the targeted insect in the protocol development. RF heating to 55 °C and holding in hot air for at least 5 min resulted in 100% mortality of the fifth-instar navel orangeworm. Rancidity, sensory qualities and shell characteristics were not affected by the treatments. The process slightly reduced the moisture content of the walnut kernels, which could prove an additional benefit by providing even nut moisture content and reducing the growth of microorganisms. If this method can be economically integrated into the handling process, it should have excellent potential as a disinfestation method for in-shell walnuts.

Keywords: Codling moth; Heat; Indianmeal moth; Navel orangeworm; Nut; Phytosanitary quarantine; Quality

1. Introduction

Infestation by insect pests is a major problem encountered during the production, storage and marketing of walnuts (*Juglans regia* L.). The three most economically significant of these pests are codling moth (*Cydia pomonella* [L.]), navel orangeworm (*Amyelois transitella* [Walker]), and Indianmeal moth (*Plodia interpunctella* [Hübner]). Larvae of codling moth and navel orangeworm are field pests and may be present in harvested walnuts. Codling moth is targeted by quarantine regulations in Japan and South Korea, and navel orangeworm is of phytosanitary concern in Australian and European markets. Indianmeal moth is a common pest of stored walnuts and is the insect most often responsible for consumer returns and complaints.

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Methyl bromide (MeBr) fumigation is the usual treatment applied to walnuts to meet quarantine and phytosanitary requirements before shipment to domestic and international markets. Under the Montreal Protocol of the United Nations, MeBr will be banned by 2005 from use for purposes other than preshipment or quarantine treatments (USEPA, 2001). Greater regulation and restriction of MeBr use will likely increase the cost of the fumigant, as well as reduce its availability (Mitcham, 2001). There is, therefore, interest in developing an alternative, non-chemical process protocol to control insect pests in walnuts while retaining acceptable product quality.

Conventional hot air is currently used for initial and final drying of in-shell walnuts after bleaching and washing. The final drying process typically requires 4–6 h in 52 °C air. The slow heating is due to the interior air pockets in in-shell walnuts (Tang et al., 2000; Wang et al., 2001b). Radio frequency (RF) treatment has the potential for use as an alternative thermal treatment for quarantine (Wang et al., 2001a) as well as a fast drying method (Jones and Rowley, 1996). This is because RF energy interacts directly with dielectric materials to provide fast heating (Nelson, 1996). Nelson and Payne (1982) used a laboratory RF unit to control pecan weevil in pecans, but the treatments resulted in a reduction in pecan seed germination. Recently, Wang et al. (2001a) developed a RF treatment to control third- and fourth-instar codling moths in in-shell walnuts. This treatment increased walnut core temperature to 53 °C in 3 min. A 5 min holding time at this temperature resulted in 100% kill of insects without causing quality degradation based on peroxide values (PV) and fatty acids (FA). Thus, it would be desirable if a similar treatment could be used to control other postharvest insect pests, such as Indianmeal moth and navel orangeworm.

In developing a disinfestation protocol for walnuts, it was important to determine which of the targeted insects is the most heat resistant, as well as the most heat resistant life stage for each species. To accomplish this, information was required on the minimum time–temperature combinations that result in 100% mortality for each insect over a relatively large range of temperatures. Several researchers have reported mortality of codling moth instars subjected to hot water bath treatments (Yokoyama et al., 1991; Neven, 1994; Neven and Rehfield, 1995). The thermal death kinetics for fifth-instars of Indianmeal moth, codling moth and navel orangeworm have been separately reported (Johnson et al., 2002; Wang et al., 2002a,b). The thermal death time (TDT) curve for each of these insects is summarized in Fig. 1. The results suggest that navel orangeworm is the most heat resistant insect at the fifth-instar life stage. Yokoyama et al. (1991) reported that the fifth-instar larva was the most heat tolerant developmental stage of codling moth, but additional research is needed to determine the most heat tolerant life stage of the navel orangeworm.

To be effective, phytosanitation procedures must also retain product quality. Quality factors for walnuts include crackability, kernel color, moisture content and flavor. Walnuts contain high concentrations of polyunsaturated fatty acids. This makes them susceptible to the development of oxidative and hydrolytic rancidity, especially at high temperatures. The two main parameters indicating walnut oxidative rancidity are peroxide values (PV, meq/kg) and fatty acids (FA, % oleic). According to the industry standard (Diamond Walnut Company, Stockton, CA), good quality walnuts should have a PV < 1.0

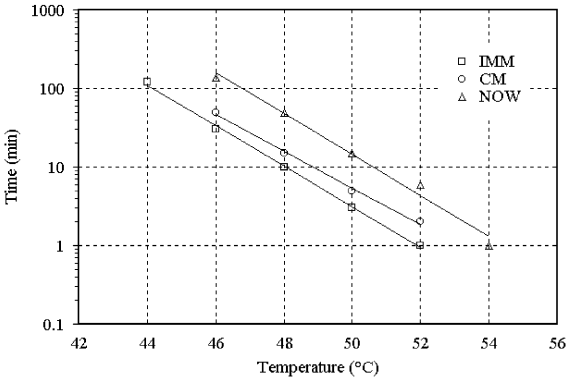

Fig. 1. Experimentally determined minimum time–temperature combinations for complete kill of 600 fifth-instar Indianmeal moths (IMM), codling moths (CM) and navel orange worms (NOW) after heating at 18 °C/min in a heating block system (after Johnson et al., 2002; Wang et al., 2002a,b).

meq/kg and a FA < 0.6%. Buranasompob et al. (2001) reported that heating shelled walnut kernels to 60 °C and hold them for up to 10 min did not increase rancidity compared to untreated walnuts. However, the effect of RF treatments on rancidity needs to be tested.

Objectives of this research were: (1) to determine the most tolerant life stage for the navel orangeworm; (2) to develop a practical process protocol to control the targeted insects using RF energy with hot air; and (3) to study the impact of these treatment protocols on walnut quality.

2. Materials and methods

2.1. Effect of temperature on insect mortality

We used a heating block system (Wang et al., 2002b) developed at Washington State University (WSU), Pullman, WA, to determine the most heat resistant life stage for navel orangeworms among three different life-stages. The life stages most commonly found on dehydrated walnuts are larvae and pupae. Navel orangeworm eggs normally hatch before reaching the processing plant. For this reason the life stages selected for comparison were third-instar larvae, fifth-instar larvae and pupae. The heating block system directly heated the insects at a heating rate of 18 °C/min, which was similar to the heating rate during RF treatments. Heating block temperatures were controlled by the visual software WorkBench PC 2.0 (Strawberry Tree, Sunnyvale, CA) via a solid state relay with a mean error of less than 0.2 °C from the set temperature. Detailed information on this heating block system can be found in Wang et al. (2002b).

Navel orangeworms were reared at the USDA Horticultural Crops Research Laboratory (HCRL), Fresno, CA, packed in an insulated shipping carton, and shipped via overnight delivery to WSU. Tested insects were treated at three time–temperature combinations just below the TDT curve (Fig. 1), these being 48 °C + 25 min holding, 50 °C + 10 min holding, and 52 °C + 4 min holding. Control insects were placed in the unheated block chamber for 25 min. For all the insect mortality tests, 200 insects were treated each run, and each time–temperature combination was repeated three times. At the end of each treatment, the insects were held at 23 °C, 60% RH with a 14:10 (L:D) h photo-period for 1 day before examination.

Insects were considered dead if no movement was observed. Moribund and surviving larvae were observed for an additional 5 days. Mortality of pupae was evaluated by the level of adult emergence after about 12 days at 26 °C. Mortality was calculated as the percentage of dead insects relative to total treated insects for each treatment. Mean values and standard deviations were calculated from three replications for each time–temperature combination. Treatment mortality was corrected for control mortality using the Abbott (1925) formula. To normalize the data, an arcsine transformation was used before analysis. Time–temperature treatments were compared using the SAS analysis of variance test (ANOVA) procedure (SAS Institute, 1989). Where ANOVA showed significant differences ($P \leq 0.05$), means were separated using least significant difference (LSD) t-test (SAS Institute, 1989).

2.2. RF treatment system and procedure

RF energy was used as the main source of heating in all treatment protocols. To reduce temperature drop during the holding period and to improve the surface heating of in-shell walnuts, heated air was also used in combination with RF energy (Fig. 2). The RF power was supplied by a 6 kW, 27 MHz pilot-scale RF system (COMBI 6-S, Strayfield International Limited, Wokingham, UK). The RF system consisted of a transformer, rectifier, oscillator, an inductance-capacitance pair commonly referred to as the 'tank circuit', and the work circuit. The gap between the electrode plates was adjusted so that the samples were treated with 0.8 kW RF energy. A tray drier (UOP8, Armfield Limited, UK) was used to provide forced hot air, and a fan and electric power settings maintained the airflow speed (1 m/s) and air temperature (55 °C) in the RF cavity. Two preliminary tests were conducted

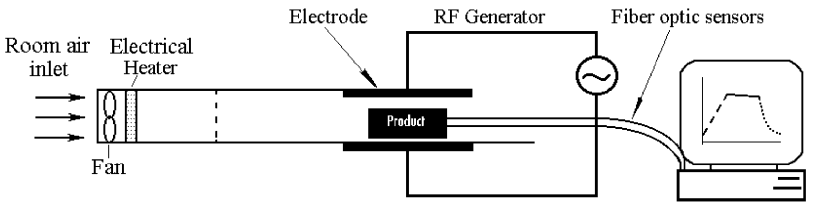

Fig. 2. Schematic view of the combined RF and hot air treatments of in-shell walnuts for insect control.

to determine the power and ramp time by using 60 walnuts from the same batch of samples used for infestation and quality tests. The kernel temperature of a walnut in the center of the samples was measured using a fiber-optic sensor (Nortech Fiberonic, Quebec, Canada) inserted through pre-drilled holes in the shell. These sensors provided 0.5 °C accuracy in the test temperature range between 20 and 60 °C.

Walnuts were infested at HCRL in Fresno, CA, with the most heat resistant life stage of navel orangeworm. An insect larva was placed in each walnut through a 4 mm pre-drilled hole in the shell. Each hole was sealed with a small amount of clay to prevent the insects escaping from the walnuts. An insulated box containing 240 infested walnuts was shipped by overnight delivery to WSU for RF treatments. Sixty infested in-shell walnuts were treated in each run. The walnuts were placed in two layers occupying approximately a square of 28×18 cm from the electrode plates of 70×50 cm. The power was set to 0.8 kW, which is equivalent to about 1 kW/kg.

Three different process protocols were selected based on the TDT curve for the most heat resistant insect and the known thermal effects on walnut quality (Buranasompob et al., 2001). The final temperature (55 °C) and 5 min holding time were selected and that provided room to achieve a complete control of the insects in walnuts based on about 3 °C variation in temperature within the treatment area. In-shell walnuts were heated by RF energy alone to 55 °C mean kernel temperature in protocols A and B, and heated by RF energy together with 55 °C hot air in protocol C. The walnuts were then held in heated air of 55 °C for 5 (protocol A) and 10 min (protocols B and C) (Table 1). Unheated walnuts were used as a control for each protocol. Each test was repeated three times.

Following RF treatments, the walnuts were cooled with forced air (1 m/s) at 22 °C for 20 min before transfer to cold storage at 4 °C and stored for 1 day to reduce probable adverse effects of the elevated temperatures on walnut quality. Infested walnuts were then placed at 23 °C, 60% RH with a 14:10 (L:D) h photo-period for 1 day before the shells were opened for examination. Insect mortality was recorded as previously described: moribund and living insects being kept under observation for another 5 days. The mortality was calculated based on three replicates.

Walnuts used for quality assessments were shipped overnight from the University of California, Davis, CA (UCD) to WSU and handled under the same conditions as the infested samples. The treated walnuts were stored for 1 day at 4 °C before being transported to the Diamond Walnut Company, CA, for chemical analysis or returned to UCD for quality evaluation.

2.3. Walnut quality analyses

Accelerated shelf life tests were conducted in which in-shell walnuts were stored in an incubator

Table 1
Treatment conditions for the infested walnut study and quality analysis

Treatment protocols	Heating to 55 °C	Holding with heated air at 55 °C
A (RF + 5 min)	RF heating	5 min
B (RF + 10 min)	RF heating	10 min
C (RF with hot air + 10 min)	RF with heated air at 55 °C	10 min

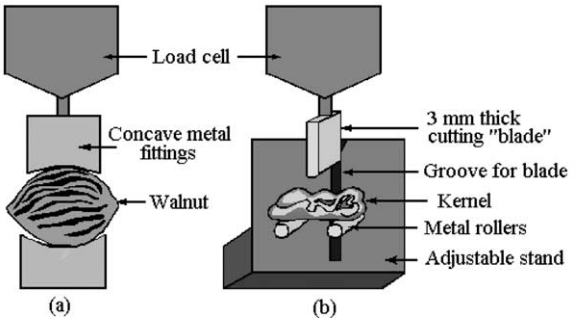

Fig. 3. Setup of Instron compression tester used to measure the force required to fracture walnut shell (a) and kernel (b).

at 35 °C and 30% relative humidity (RH) for 10 and 20 days. These conditions simulated approximately 1 and 2 year storage periods at 4 °C, respectively, based on a Q_{10} value of 3.4 at 35 °C, which is defined as the increase of shelf-life, as a ratio, when storage temperature is increased by 10 °C (Taoukis et al., 1997). Oxidative rancidity involves a reaction with oxygen, and increases as the temperature rises and storage time increases. To test for both oxidative and hydrolytic rancidity in the treated walnuts, the peroxide values (PV) and fatty acid (FA) content of the kernels 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 were described in Wang et al. (2001a). Mean values and standard deviations were calculated for both values for each time–temperature treatment. Within each storage regime, time–temperature treatments were compared using the SAS ANOVA procedure (SAS Institute, 1989).

Quality analysis of treated in-shell walnuts was also carried out based on their physical properties. The force required to crack the shells and kernels of three replicate groups of 15 walnuts was measured using the Instron tension/compression test system (load frame model 1122, Instron, UK) (Fig. 3a). Each nut was placed onto a 50 mm wide concave metal base. A similar metal fitting was attached to a 2.5 kg load cell. The fittings were machined so as to approximate the curvature of the walnut shells, thereby distributing the force over as much of the total nut surface as possible. Each walnut shell was compressed 3 mm at a rate of 20 mm/min, this being sufficient to crack the shell. The Instron software (Merlin, Version 4.31) recorded the force applied every 0.3 s. From these data, the distance traveled and force at which the first crack occurred could be summarized.

The shells were then removed, and a trimmed 0.25 kernel from each nut was placed flattest side down on a pair of 3 mm diameter metal rollers spaced 9 mm apart (Fig. 3b). A 3 mm thick cutting 'blade' with a rounded leading edge was attached to a 50 g load cell and positioned centrally above each kernel. As the blade traveled 5 mm down between the rollers, the force and distance were recorded, as previously.

Weights of the shells and kernels of five nuts from each replicate group were recorded. The nuts were then dried at 70 °C until no further weight loss occurred in order to calculate percentage moisture content for each.

A separate group of walnuts was shelled by hand and used for sensory analysis. Trained panelists were presented with portion cups containing walnut meats from three replicate groups from each treatment in a random order, along with a blind control sample. A labeled sample of the control kernels was also provided. So as to neutralize possible color differences, panelists were asked to wear green tinted glasses and tests were conducted under red lighting. The panelists scored the extent to which flavor and texture of the kernels differed from the labeled control kernels on a scale of 0—no difference from control, to 5—extremely different from control. They were also asked to comment on the flavor, texture and overall appeal of each sample as it compared to the control.

Mean values and standard deviations were calculated from three replicates for each RF treatment and 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 Duncans Multiple Range Test (SAS Institute, 1989).

3. Results and analyses

3.1. Heat resistant life-stage

Comparison of the three different life-stages of navel orangeworm showed that the mortality in unheated controls was 3.5 ± 1.9, 1.3 ± 0.6 and $8.1 \pm 1.0\%$ for third-instars, fifth-instars and pupae, respectively. Although this low control mortality suggested that the effects of shipping and handling were negligible, statistical analysis showed that differences in control mortality for the three life stages were significant ($P < 0.05$). Therefore, treatment mortality was corrected for control mortality before further analysis. Table 2 shows corrected mean values and standard deviations of mortality for navel orangeworm for each life stage and time–temperature combination. Third-instars were significantly less heat tolerant than the other life stages at all temperatures ($P < 0.05$). Although mortalities for fifth-instars were consistently less than those for pupae, the difference was significant only at 52 °C ($P < 0.05$). As the RF treatment protocols were conducted at 55 °C, fifth-instars were used in our study to infest in-shell walnuts for treatment confirmation studies.

3.2. Protocol confirmation studies with infested walnuts

Fig. 4 shows a typical time–temperature history for an in-shell walnut kernel subjected to RF treatment with 10 min hot air holding followed by cooling using forced room air. The kernel temperature increased linearly from 22 to 55 °C within 5 min, as shown in our previous study (Wang et al., 2001a). Over the holding period, the kernel temperature decreased by about 2 °C. The forced room air at 1 m/s cooled the walnut kernel temperature from 53 to about 27 °C during 20 min.

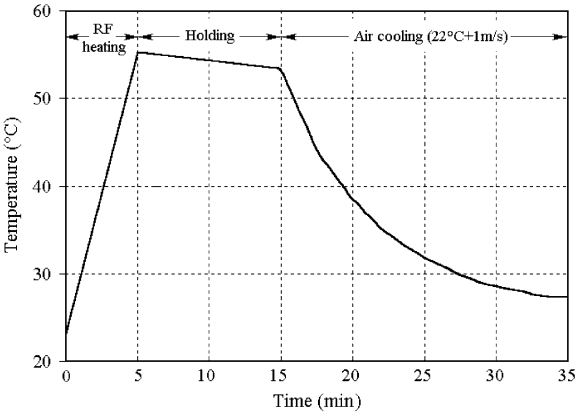

Fig. 4. Typical time–temperature history for in-shell walnut kernels when subjected to RF heating plus 10 min holding with forced hot air (55 °C; 1 m/s) and 20 min cooling with forced room air (22 °C; 1 m/s).

Table 2
Mortality (Mean ± S.D., %) of navel orangeworms at three life stages after heating at 18 °C/min (3 replicates) in a heating block system

Temperature +holding time	Third-instars	Fifth-instars	Pupae
48 °C+25 min	87.4 ± 3.0a*	26.5 ± 1.5b	27.9 ± 2.1b
50 °C+10 min	97.6 ± 0.6a	38.0 ± 8.4b	59.3 ± 6.3b
52 °C+4 min	99.8 ± 0.2a	73.5 ± 8.0b	98.2 ± 0.8a

* Different letters within row indicate that means are significantly different ($P < 0.05$).

Treatment results showed that insect mortality for the control was 0% because all 180 larvae were found alive. Shipment and handling did not cause any mortality in the samples. All of the RF treatments including protocol A (RF + 5 min), protocol B (RF + 10 min) and protocol C (RF with hot air + 10 min), resulted in 100% mortality in the tested samples.

3.3. Walnut quality

Table 3 shows the mean values and standard deviations of the PV and FA values of the control and the three treated walnut kernel samples. The PV and FA values for all treatments increased with storage time. The final PV and FA values during accelerated storage of up to 20 days remained lower than the industry standard values (PV < 1.0 meq/kg and FA < 0.6%) for good walnut quality. Hydrolytic rancidity increased slightly after storage, as indicated by increased FA values from 0 to 20 days at 35 °C. There was no statistically significant difference, however, between untreated controls and RF treated walnuts.

None of the RF treated walnuts differed from the control in terms of the force required to crack the shell or the brittleness of the kernels (Table 4). Similarly, the percentage moisture content of the walnut shells was unaffected by RF treatments, whereas all of the treatments significantly reduced the moisture content of the kernels relative to the control ($P = 0.05$).

Despite the reduced moisture content, the sensory panelists did not detect any differences between the control and the treated walnut kernels ($P = 0.05$). The tasters recorded a range of comments about the walnut samples; however, these did not show any consistent pattern. These results suggest that the RF treatments tested are not likely to affect consumer acceptance of the treated walnuts.

4. Discussion

This study was conducted with only 60 walnuts in a small area treated at a time in a pilot scale unit. Over the holding period with hot air, the kernel temperature decreased by about 2 °C as compared with an approximate 5 °C decrease when hot air was not used (Wang et al., 2001a). The fact that the process protocols achieved 100% mortality suggests that the final kernel temperatures among the walnuts were relatively uniform. The minimum insect temperature was at least higher than 52 °C based on the TDT curve for fifth-instar navel orangeworm in Fig. 1. Because navel orangeworm is more heat resistant than codling moth and Indianmeal moth (Johnson et al., 2002; Wang et al., 2002a,b), we expect that the same treatments will be effective in controlling these latter two insects.

Further research is needed to determine if the treatment protocol could be scaled up to a commercial process. This would include an analysis of the additional cost on a per unit mass basis to evaluate the economic feasibility of the RF process. In this case, thousands of tons of in-shell walnuts would need to be treated within a relatively short harvest season from August to October (Johnson et al., 1992). A major concern in scaling up the pilot-scale treatments to commercial application is the heating uniformity in walnuts in a much larger RF machine than used this study. For commercial systems, walnuts would likely be treated on conveyer belts moving between RF electrodes. Special attention needs to be paid in designing RF applicators to apply a uniform electromagnetic field through the mass of walnuts during the RF treatments.

The chemical quality evaluations confirmed earlier results in which 3 min RF heating to 53 ± 3.6 °C with a 5 min hold at that temperature did not increase rancidity (Wang et al., 2001a). In fact, the PV values of RF treated walnuts after 10 and 20 day storage periods were lower than those of untreated controls. This might be due to possible inactivation of the lipoxygenase enzymes by short heat treatments (Buranasompob et al., 2001).

In summary, this study did not find any negative effects of RF treatments designed to control navel orangeworm on the quality attributes of walnuts. During commercial operations, in-shell walnuts are often bleached in water solution and then dried by heated air. The drying effect on the walnuts during RF treatments is, therefore, more likely to be beneficial for industry to reduce overall operation time and cost. The treatment re-

Table 3
Chemical characteristics of in-shell walnuts treated by radio frequency energy (mean ± S.D. from 3 replicates)

Storage time at 35 °C (days)	Peroxide value* (meq/kg)				Fatty acid (%)			
	Control	RF + 5min	RF + 10min	RF with hot air + 10min	Control	RF + 5min	RF + 10min	RF with hot air + 10 min
0	0.01 ± 0.01	0.05 ± 0.05	0.12 ± 0.08	0.02 ± 0.01	0.10 ± 0.01	0.08 ± 0.02	0.10 ± 0.02	0.10 ± 0.02
10	0.28 ± 0.11	0.12 ± 0.01	0.12 ± 0.08	0.18 ± 0.12	0.15 ± 0.01	0.16 ± 0.04	0.14 ± 0.02	0.15 ± 0.01
20	0.64 ± 0.16	0.37 ± 0.16	0.36 ± 0.20	0.61 ± 0.03	0.21 ± 0.01	0.22 ± 0.06	0.15 ± 0.03	0.17 ± 0.03

* Accepted PV and FA values for good quality are less than 1.0 meq/kg and 0.6%, respectively.

Table 4
Physical characteristics of in-shell walnuts treated by radio frequency energy (Mean ± S.D. from three replicates)

Treatments	Crackability (N/mm)		Moisture content (%)		Difference to control (0–5)*
	Kernel	Shell	Kernel	Shell	
Control	8.5 ± 5.8a**	593 ± 38a	2.6 ± 0.3a	7.8 ± 0.7a	1.88 ± 1.2a
RF + 5 min	6.0 ± 3.4a	559 ± 50a	2.2 ± 0.2b	7.1 ± 0.5a	1.78 ± 1.3a
RF + 10 min	8.3 ± 6.4a	544 ± 41a	2.4 ± 0.2b	7.7 ± 0.6a	1.65 ± 1.2a
RF with hot air + 10 min	10.3 ± 6.8a	588 ± 40a	2.3 ± 0.2b	7.4 ± 0.5a	1.73 ± 1.3a

* Sensory analysis: 0 = no difference from control, 5 = extremely different from control.
** Different letters within column indicate that means are significantly different ($P < 0.05$).

quires only a few minutes exposure, does not involve chemical applications, and seems likely to be acceptable to consumers. If this technology can be economically integrated into a continuous packing line system, it would appear to have potential as a disinfestation method for in-shell walnuts.

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