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Short communication

Validation of radio frequency treatments as alternative non-chemical methods for disinfesting chestnuts

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ABSTRACT

Chemical fumigation has been widely used to control insects in postharvest chestnuts but is inherent dangers when using fumigants. The purpose of this study was to validate application of radio frequency (RF) treatments for disinfesting chestnuts as an alternative to chemical fumigation. A practical process protocol was developed to control insect pests in chestnuts using a 27.12 MHz free-running oscillator RF system. Fifth-instar yellow peach moth, *Conogethes punctiferalis*, more heat tolerant than chestnut weevil, *Curculio elephas*, under three temperature and time combinations using a heating block system, was selected as the targeted insect to validate the RF treatment protocol. Mortality of fifth-instar *C. punctiferalis* increased with increasing holding time at 55 °C using RF heating and reached 100% while holding in hot air for at least 5 min. Furthermore, there was no significant quality difference in color, fat, firmness, moisture content, protein, and soluble sugar content of chestnuts observed between RF treatments and controls. RF treatment methods hold potential to scale up for industrial applications of disinfesting chestnuts.

1. Introduction

Chestnuts have higher moisture and starch contents, but lower fat and protein contents than most other nuts (Chenlo et al., 2009; Suárez et al., 2012; Vasconcelos et al., 2010). Thus, they provide a good resource for two key insects, chestnut weevil (*Curculio elephas*) and yellow peach moth (*Conogethes punctiferalis*). It is estimated that annual losses of chestnuts due to pests are about 20% of the total production during storage in China, resulting in high economic losses (Gao et al., 2011). Therefore, it is necessary to develop an effective and efficient postharvest method for disinfesting chestnuts and reducing damage to their product quality during storage (Tang et al., 2007).

Methyl bromide fumigation has been widely used to disinfest chestnuts but in accordance with the Montreal Protocol, production and application of methyl bromide will be banned in developing countries by 2015 (UNEP, 1992). Therefore, there is an increasing interest in developing a nonchemical alternative method to control postharvest insects in chestnuts. Radio frequency (RF) interacts directly with the entire volume of agricultural products, provides fast heating (Marra et al., 2009; Zhao et al., 2000), and has been mainly proposed as physical methods for disinfesting agricultural commodities, such as almonds (Gao et al., 2010), apples (Wang et al., 2006), cherries (Ikediala et al., 2002), oranges (Birla et al., 2005), pecans (Nelson and Payne, 1982), rice (Lagunas-Solar et al., 2007; Mirhoseini et al., 2009; Zhou et al., 2015), and walnuts (Wang et al., 2002b, 2007a,b). A RF treatment protocol has been developed for disinfesting chestnuts after improving RF heating uniformity using hot air surface heating, moving, and mixing (Hou et al., 2014). To be scaled up for commercial applications, it is essential to determine the treatment efficacy using infested chestnuts with the target insects and evaluate the effects of RF treatment protocols on product quality.

In validating the developed RF treatment protocol for disinfesting chestnuts, it is necessary to determine the most heat resistant insect species and life stage for the targeted insects. A heating block system has been widely used to determine the thermal death kinetics and thermotolerance of many insects, such as codling moth (Ikediala et al., 2000; Wang et al., 2002a), naval

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orangeworm (Wang et al., 2005), Indianmeal moth (Johnson et al., 2003), red flour beetle (Johnson et al., 2004), and rice weevil (Yan et al., 2014). The thermal resistance results of life stages show that the latest-instar is the most heat tolerant life stage for several insects (Johnson et al., 2003, 2004; Wang et al., 2005). Hou et al. (2015) report that the fifth-instar is the most heat tolerant stage of *C. punctiferalis* and the minimum exposure time between 44 and 50 °C to achieve 100% mortality. For developing effective RF treatments, it is needed to determine the more heat resistant insect between fifth-instar *C. elephas* and *C. punctiferalis* using the same heating block system.

Many RF treatment protocols have been developed by including hot air surface heating, moving, mixing, holding, and cooling (Gao et al., 2010; Wang et al., 2014a, 2014b). High temperature and short holding time have been commonly used to develop the effective RF treatments (Tang et al., 2000). Based on the existed non-uniform heating in RF treatments, the final insect mortality could be different from that obtained by the model heating block system. Therefore, it is desirable to validate the efficacy of infested chestnuts at the target temperature of 55 °C with different holding times.

The objectives of this study were: (1) to determine the more heat tolerant pest between fifth-instar *C. punctiferalis* and *C. elephas* using the heating block system, (2) to validate the practical RF treatment protocol using the infested chestnuts with different holding times at 55 °C hot air, and (3) to evaluate the effect of the RF treatments on chestnut quality.

2. Materials and methods

2.1. Samples

Chinese chestnuts (*Castanea mollissima*) were obtained in September, 2014 from a local wholesale market in Yangling, Shaanxi, China. The moisture content and individual weight of tested chestnuts were 47.94 ± 1.93% on wet basis (w.b.) and 6.97 ± 2.35 g, respectively. The chestnuts were stored with mesh bags in a refrigerator (BD/BC-297KMQ, Midea Refrigeration Division, Hefei, China) at 3 ± 1 °C. They were taken out from the refrigerator and kept at ambient room temperature (20 ± 1 °C) for 12 h before experiments.

2.2. Determining the more heat tolerant pest

A heating block system (Fig. 1) was used to determine the more heat tolerant pest between *C. elephas* and *C. punctiferalis* with the fifth-instars. Heating rate, set-point temperature, and holding time of the heating block system were controlled by customized Visual Basic software and PID controllers (i/32 temperature & process controller, Omega Engineering Inc., Stamford, CT) via a solid state relay. Detailed information about the heating block system can be found elsewhere (Wang et al., 2002a). Three temperature and time combinations: 46 °C + 8 min, 48 °C + 4 min, and 50 °C + 2 min, were selected for this comparison test to achieve 60–90% mortality. The heating rate of 5 °C/min was selected to simulate the heating rate in chestnuts during RF treatments (Hou et al., 2014).

Both *C. punctiferalis* and *C. elephas* larvae were collected from the infested chestnuts and reared at the Northwest A&F University, Yangling, China. Fifty actively moving insect larvae were placed in the insect chamber of the heating block system (HBS) and closed for each test under the given three time-temperature combinations. Control tests were conducted in the unheated block chamber for 10 min. Each treatment was repeated thrice. At the end of each exposure, the insects were removed immediately and held in a glass rearing containers (6 cm diameter × 9 cm height) covered by a fine mesh cloth for air exchange, and maintained in a rearing room at 25 ± 1 °C, 70–80% RH, and a photoperiod of 16:8 (L:D) h with artificial light for 2 days before evaluation. Insects were considered dead if no movement was observed or the body color was dark. Mortality was calculated as the percentage of dead insects relative to total treated ones for each treatment.

2.3. Procedure of RF treatments

Chestnuts were infested with the fifth-instar life stage of *C. punctiferalis*, which was the more heat resistant insect than *C. elephas*. An insect was placed through a pre-drilled hole in kernel of each infested chestnut, which was sealed with insulating tape to prevent escaping of the insects. Before each treatment, the 25 infested chestnuts were randomly mixed into the un-infested chestnuts (2.5 kg) in an insulating container (26 cm × 18 cm × 8 cm, HF-932, Zhejiang Howfun Company, Taizhou, China). This represented an artificial infestation level of 7%, well within 4–8 % of the natural infestation rate in chestnuts (Debouzie et al., 1996; Wells and Payne, 1980). The container made of polypropylene with perforated side and bottom walls was heated each time to provide oxygen for insects, and allow hot or room air to pass through the samples for heating or cooling.

Since the minimum exposure time to achieve 100% mortality of the most heat tolerant stage of *C. punctiferalis* was 3 min at 50 °C (Hou et al., 2015), the final temperature (55 °C) and four different holding times (0, 1, 3, and 5 min) were selected according to the heating non-uniformity of chestnuts using RF energy (Hou et al., 2014). The infested chestnuts mixed with other un-infested samples were heated to the target temperature by a 6 kW, 27.12 MHz free-running oscillator RF system (SO6B, Strayfield International, Wokingham, U.K.) with a hot air system supplied by a 6 kW electric heater. The detailed description of the RF system could be found in Hou et al. (2014) and Zhou et al. (2015). The gap between the top and bottom electrodes was fixed at 12 cm based on the appropriate heating rate of chestnuts achieved by RF energy (Hou et al., 2014). The RF treatment protocol consisted of RF heating to 55 °C with forced hot air, moving conveyor at 9.2 m/h, twice mixing, and holding at 55 °C hot air for 4 different times, followed by forced room air cooling in single-layer samples as determined

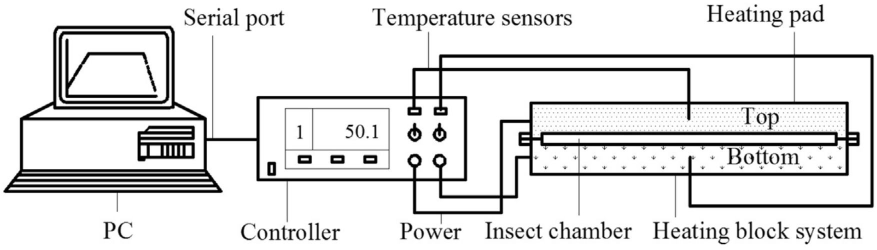

Fig. 1. Schematic view of the heating block system (Yin et al., 2006).

by Hou et al. (2014). Unheated chestnuts were used as a control for each treatment.

After RF treatments, the infested chestnuts were separated from non-infested ones and broken manually. The insects were moved into glass rearing containers, and maintained under the same conditions described above in the rearing room for 2 days before evaluation. Insect mortality was counted as previously described: the percentage of dead larvae relative to total treated ones for each treatment.

2.4. Chestnut quality analyses

Before and after RF treatments, the quality of chestnuts taken from each treatment was evaluated immediately. Color, fat, firmness, protein, moisture content, and soluble sugar contents were selected as major parameters to evaluate chestnut kernel quality. Moisture content of the kernel was determined by the vacuum oven drying method following the National Standard of China (GB5009.3–2010, 2010) issued by China State Bureau of Standards. Specifically, the chestnuts were sliced into chips with thickness of 5 mm. About 5 g chestnuts were placed in aluminum dishes, and then dried in a vacuum oven (DZX-6020B, Nanrong Laboratory Equipment Co., Ltd, Shanghai, China) at 105 °C and 101.3 kPa until constant weight of chestnuts was attained. The samples with aluminum dishes were placed in desiccators with $CaSO_4$ until the temperature of samples reached room one before weighing.

The fat content was determined by Soxhlet extraction method following the National Standard of China, GB/T5009.6–2003 (2003), measured by extracting a known weight of samples with petroleum ether. The protein was detected using the Kjeldahl method following GB5009.5–2010 (2010). The percentages of nitrogen were transformed into protein content by multiplying a conversion factor of 5.3 suggested elsewhere (Korel and Balaban, 2006; Mccarthy and Meredith, 1988). The soluble sugar content of the samples was determined by the Anthrone colorimetry method. Detailed information on evaluating the soluble sugar content of the chestnuts can be found elsewhere (Hou et al., 2014).

Firmness of chestnuts was determined by a texture analyzer (TA-XT.PLUS/50, Stable Micro System Ltd., Survey, England), and recorded as the maximum compression force (kg) required to push a probe of 2 mm diameter to a depth of 10 mm at 2 mm/s test speed. Readings were averaged for 6 replicates from each treatment. The force–time graphs were recorded and analyzed using the Exponent software (version 6.0.7.0, Stable Micro Systems Ltd., Survey, England).

Chestnut kernel color was measured with a computer vision system (CVS). The CVS and measurement procedures of color image were described by Hou et al. (2014). Color images of 10 chestnut kernels from each treatment were obtained by the camera, stored in the computer, and analyzed by Adobe Photoshop CS (Adobe Systems Inc., USA). The color values obtained from Photoshop (L, a, b) were converted to CIE LAB (L*, a* and b*) values using the following formulas (Briones and Aguilera, 2005):

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

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

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

2.5. Statistical analysis

All measurements were conducted in triplicate. Mean values and standard deviations (SD) of all measuring values were obtained from the replicates for each RF treatment. All statistical analyses were performed at a 5% significance level on replication data using the Microsoft Excel variance procedure (Microsoft Office Excel, 2007).

3. Results and discussion

3.1. Heat tolerance comparison between *C. elephas* and *C. punctiferalis*

The mortality in unheated controls both for *C. elephas* and *C. punctiferalis* larvae was 0%, suggesting that the effects of handling procedure could be negligible. Table 1 shows the mortality of *C. punctiferalis* and *C. elephas* at three treatment conditions with the heating rate of 5 °C/min in the HBS. Overall, the fifth-instar *C. punctiferalis* showed a greater thermal tolerance than *C. elephas* larvae, since the average mortality of fifth-instar *C. punctiferalis* was lower than that of *C. elephas* under the three given conditions, but with significant difference ($P < 0.05$) only at two temperature-time combinations (50 °C + 2 min and 46 °C + 8 min). Therefore, the *C. punctiferalis* larvae were chosen as the target pest for validation studies.

3.2. Protocol validation studies with infested chestnuts

Treatment results also showed that insect mortality for the control was 0% because all *C. punctiferalis* larvae were found alive in unheated samples, suggesting that handling procedure did not cause any insect mortality in the infested chestnuts. The mortality of *C. punctiferalis* increased from 74.90% to 100% when the hot air holding time increased from 0 min to 5 min after RF heating to 55 °C. In general, increasing holding time at given temperatures resulted in increased insect mortality. The mortality curve for fifth-instar *C. punctiferalis* was described by the linear regression equation $M = 5.14\ t + 75.05$ with the coefficient of determination $R^2 = 0.992$, where t is exposure time (min) and M is mortality (%) of *C. punctiferalis*. But the minimum time-temperature of 3 min at 50 °C to achieve 100% mortality using HBS (Hou et al., 2015) did not result in complete control of fifth-instar *C. punctiferalis* in RF treatments, which was probably caused by non-uniform RF heating. A similar phenomenon was observed in earlier studies with codling moth larvae in microwave treated cherries (Ikediala et al., 1999) and RF treated walnuts (Wang et al., 2001), and with navel orangeworm larvae in RF treated in-shell walnuts (Wang et al., 2007b).

3.3. Chestnut quality

Table 2 lists the moisture contents in the chestnut kernels and

Table 1
Mortality (mean ± SD, %) of fifth-instar *Conogethes punctiferalis* and *Curculio elephas* larvae at three treatment conditions.

Species	Temperature + holding time		
	50 °C + 2 min	48 °C + 4 min	46 °C + 8 min
Curculio elephas	95.65 ± 4.55a[a]	65.22 ± 6.92a	86.96 ± 7.17a
Conogethes punctiferalis	85.84 ± 4.44b	59.43 ± 10.02a	62.63 ± 1.59b

[a] Means followed by different lowercase letters are significantly different at P = 0.05 between two pests.

Table 2
Quality characteristics (mean ± SD) of chestnut kernels before and after radio frequency (RF) treatments.

Quality parameters	Moisture (% w.b)		Protein (%)	Fat (%)	Soluble sugar (%)	Firmness (kg)	Color		
	Shell	Kernel					*L*[a]	*a*[a]	*b*[a]
Control	25.63 ± 0.23a	47.11 ± 1.89a	4.41 ± 0.36a	6.54 ± 0.47a	13.52 ± 2.50a	2.09 ± 0.29a	83.07 ± 0.97a	−1.41 ± 1.19a	57.41 ± 2.29a
RF	13.98 ± 0.14b	46.09 ± 8.09a	4.67 ± 0.83a	6.02 ± 0.28a	10.51 ± 3.40a	2.23 ± 0.54a	79.53 ± 2.39a	2.04 ± 2.03a	60.86 ± 1.62a

[a] Same letters in column indicate that means are insignificantly different at $P = 0.05$ between control and RF treated samples.

shells before and after RF treatments. The average moisture content of the chestnut kernel was higher than that of the shells. The RF treatment reduced moisture contents of chestnut kernels and shells, but the significant difference ($P < 0.05$) was only observed in chestnut shells. The moisture content of chestnut shells was reduced from 25.63% w.b. to 13.98% w.b. after RF treatments. But the moisture content of chestnut kernels was slightly reduced from 47.11% w.b. to about 46.09% w.b. after RF treatments. Less moisture losses in RF treated kernels were probably caused by the short heating time and preventing moisture diffusion through the special texture of the shell (Hou et al., 2014).

Table 2 also summarizes the major quality attributes of control and RF treated chestnuts before and after RF treatments. Under the thermal treatment at 55 °C for 5 min, chestnuts quality was not significantly affected ($P > 0.05$), and remained similar to the control samples, even though the average fat, protein, soluble sugar, firmness, L^*, a^*, b^*, and other quality parameters were slightly changed. Furthermore, there were no significant differences in these quality parameters between RF treatments and untreated controls after 8 days at 35 °C, simulating one year of storage at 4 °C (Hou et al., 2014).

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