



Thermal death kinetics of adult *Sitophilus oryzae* and effects of heating rate on thermotolerance



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ABSTRACT

Information on thermal death kinetics of targeted stored insects under different heating conditions is essential for developing postharvest disinfestation treatment protocols. Using a heating block system, the thermal death kinetics of adult rice weevil, *Sitophilus oryzae* (L.), were determined at temperatures from 44 to 50 °C at 2 °C intervals and a heating rate of 5 °C/min. The effects of heating rates (0.1, 0.5, 1, 5 and 10 °C/min) on mortality were also examined. The results showed that thermal death curves of *S. oryzae* followed a 0-order kinetic reaction model. The required holding times for achieving 100% mortality were 130, 50, 12, and 4 min at 44, 46, 48, and 50 °C, respectively. The activation energy for killing *S. oryzae* was 505 kJ/mol and the *z* value obtained from the thermal-death-time curve was 3.9 °C. Insect mortality after a 20 min exposure to 46 °C at low heating rates (0.1 or 0.5 °C/min) was significantly lower than that at high heating rates (1–10 °C/min). The information provided by thermal death kinetics for *S. oryzae* is useful in developing effective postharvest thermal treatment protocols.

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1. Introduction

Infestations of grain by various stored product pests may occur at any time from harvest to consumption (Lee et al., 2001). It is estimated that the overall damage caused by such pests is about 10–40% of the annual worldwide production of stored grains (Mishra et al., 2013), with annual losses of about 27% of the total rice yield (Alfonso-Rubi et al., 2003). Among these pests, the rice weevil, *Sitophilus oryzae* (L.), is one of the most destructive and widespread in stored grains and legumes, causing reduction in weight, quality, commercial value, and seed germination, and increased susceptibility to fungal infestation (Jian et al., 2012). The adult female *S. oryzae* (L.) commonly bores a shallow cavity in the kernel in which to deposit eggs, sealing the egg cavity with a gluey secretion (Feng et al., 2004). Although pest management practices often target the most visible adult stage, postharvest treatments are often required to prevent additional product damage during

storage and avoid re-infestation of products before shipment to domestic and international markets.

Traditional chemical fumigations using methyl bromide and phosphine are common to control stored grain pests. Despite its effectiveness, global use of methyl bromide is being phased out due to its listing as an ozone depleter by the Montreal Protocol (USEPA, 2001). In China, populations surveyed over 8 years from 55 locations showed resistance to phosphine increased 10.7% for *S. oryzae* and 71.2% for lesser grain borer (*Rhyzopertha dominica*) (Yan et al., 2004). Therefore, it is urgent to develop an alternative non-chemical method to completely control *S. oryzae* in grain.

As an efficient and safe method, heat treatments have been widely studied to control insect pests in agricultural products (Shellie and Mangan, 1994; Jin, 2011; Purohit et al., 2013). Thermal mortality data of targeted insects have been obtained by directly exposing insects in a water bath (Thomas and Mangan, 1997; Wang et al., 2009), heating insects in glass or metal tubes (Thomas and Shellie, 2000; Lurie et al., 2004) or heating infested fruits in water baths (Hansen et al., 2004). Due to large variations in insect mortality data resulting from these traditional methods, a heating block system (HBS) was developed and used successfully to obtain reliable thermal death kinetic data for several insect pests of fruits and nuts (Wang et al., 2002a,b; Johnson et al., 2004; Armstrong et al., 2009). When compared with the two water immersion

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methods, the HBS yields lower mortality data with less variation, resulting in more conservative treatment recommendations (Wang et al., 2009). Results obtained by the HBS were confirmed for radio frequency heat treatment of walnuts infested with fifth-instar codling moth, *Cydia pomonella* (Wang et al., 2001a) or fifth-instar navel orangeworm, *Amyelois transitella* (Mitcham et al., 2004), and with hot water treatments for cherries infested with third-instar *C. pomonella* (Feng et al., 2004; Hansen et al., 2004).

Thermal death kinetic models derived from thermal mortality data may be useful in predicting the efficacy of different treatment conditions and designing new treatment protocols. A 0.5th-order kinetic model was successfully applied to data from *C. pomonella* (Wang et al., 2002a), Indian meal moth, *Plodia interpunctella* (Johnson et al., 2003), *A. transitella* (Wang et al., 2002b), Mediterranean fruit fly, *Ceratitis capitata* (Gazit et al., 2004; Armstrong et al., 2009), red flour beetle, *Tribolium castaneum* (Johnson et al., 2004), and Mexican fruit fly, *Anastrepha ludens* (Hallman et al., 2005). To develop practical heat treatments for *S. oryzae*, it is desirable to develop a similar model based on HBS data.

Heating rate may have an important effect on the mortality of treated insects (Evans, 1986). Neven (1998) reported that *C. pomonella* larvae may experience thermal conditioning and acclimation at relatively slow heating rates (0.13–0.2 °C/min). Thomas and Shellie (2000) reported that the exposure times needed to achieve 99% mortality of *A. ludens* at 44 °C are 62 and 42 min when heated at 0.175 and 1.4 °C/min, respectively, suggesting that slower heating rates require longer exposures to the target temperature to achieve the same mortality. For conventional heating, heating rates at the center of the product mass may range between 0.05 and 2 °C/min, depending on heating method, product size, and final temperature (Wang et al., 2001b). Target insects may have adequate time to adapt to the heat and increase their thermotolerance (Waddell et al., 2000; Garczynski et al., 2011). As a result, conventional heat treatments typically require long treatment times to achieve adequate mortality levels. Wang et al. (2002a,b) confirmed that heating rates between 5 and 15 °C/min, which correspond to that used in microwave and radio frequency heating, did not increase thermotolerance of fifth-instar *C. pomonella* and *A. transitella*. Therefore, understanding the effect of different heating rates on the mortality of *S. oryzae* would be useful in the development of a treatment protocol.

The HBS system is more difficult to use with internal stages such as *S. oryzae* larvae and pupae. Because removal of internal stages from the seeds causes high mortality, they must be treated within the seed. However, insulation by the seed slows the heating rate and makes it difficult to quantify. The treatment response of internal stages is normally measured by adult emergence, which complicates direct comparisons between stages. The heating rates for treating external, mobile adult stages are easier to quantify, and evaluation is immediate and consistent. For these reasons, we selected the adult stage for this initial study.

Our objectives were to 1) determine the thermal mortality of adult *S. oryzae* at 4 selected temperatures as a function of holding time using the heating block system, 2) develop the thermal death kinetic model of the adult *S. oryzae*, 3) predict the holding time needed to achieve the required mortality at given populations, and 4) explore the effects of heating rates on the thermal mortality of the adult *S. oryzae*.

2. Materials and methods

2.1. Heating block system (HBS)

The HBS was composed of top and bottom aluminum blocks (254 × 254 × 18 mm) which fit together to form the insect

treatment chamber (214 × 214 × 3 mm), heating pads, and a data acquisition/control unit (Fig. 1). Calibrated type-T thermocouple sensors were used to monitor the temperatures of the top and bottom blocks. Heating rates (0.1–15 °C/min) and the set-point temperature were controlled by Visual Basic software via a solid-state relay. Two PID controllers (I32, Omega Engineering, Inc., Stamford, CT) regulated the two block surface temperatures separately. The high thermal capacitance of the blocks provided smooth temperature profiles over the heating and holding periods with temperature deviations from the set point (≤ 60 °C) of less than 0.3 °C (Wang et al., 2002b). More detailed descriptions of the HBS can be found in Gazit et al. (2004) and Johnson et al. (2003).

2.2. Test insects

Adult *S. oryzae* were obtained from the College of Plant Protection, Northwest A&F University, Yangling, China. The adults were kept in a glass jar containing 10 g of wheat grains covered with nylon-mesh-screen (Tilley et al., 2007; Zhao et al., 2007) and were reared under ambient conditions of 27 ± 2 °C, 65% relative humidity and a photoperiod of 14:10 (L:D) h with artificial light.

2.3. Treatment procedures

Based on the thermal-death-time curves for *P. interpunctella* (Johnson et al., 2003) and *T. castaneum* (Johnson et al., 2004), four or five exposure times (1–130 min) at 44, 46, 48 and 50 °C and a heating rate of 5 °C/min were selected to provide a wide range of mortality levels including 100% for adult *S. oryzae*. To determine the effect of heating rate on *S. oryzae* mortality, heating rates of 0.1, 0.5, 1, 5 and 10 °C/min were used. Based on previous test results, a treatment temperature of 46 °C with an exposure of 20 min was selected to provide mortality levels of below 100%. Heating rates ≤ 1 °C/min simulate conventional hot air and hot water heat treatments and fast heating rates ≥ 5 °C/min simulate rapid heating methods using microwave and RF energies.

Fifty actively moving adults were randomly selected for each temperature–time combination test. Because of their small size and speed, *S. oryzae* adults were treated in the heat treatment chamber in a nylon-mesh bag. Since the insect chamber in the HBS was only 3 mm in height, the heat transfer effect of the bag on insect mortality was negligible. For all treatments, the HBS began at a pretreatment temperature of 20 °C. Control insects were placed in the unheated HBS for the longest exposure time at each temperature. Immediately upon completion of the exposure time, the block was opened and the bag was removed from the block. Treated adults were gently brushed into a glass jar containing wheat. The tested *S. oryzae* adults were held for 6 days after treatment for observation. The pests were considered dead if no movement was observed. All trials were repeated three times.

Mean values and standard deviations were calculated from three replicates for each treatment. The mean values for insect mortality under different heating rates were separated at $P = 0.05$ level using least significant difference (LSD) *t*-test.

2.4. Insect thermal kinetic modeling

The thermal death kinetic response of heat treated insects can be described by a temperature–time model, using mean survival ratios as a function of exposure time for each temperature. The model has been previously used for third-instar *C. capitata* (Gazit et al., 2004; Hallman et al., 2005) and fifth-instar *C. pomonella* (Wang et al., 2002b). The general equation for the kinetic model is as follows:

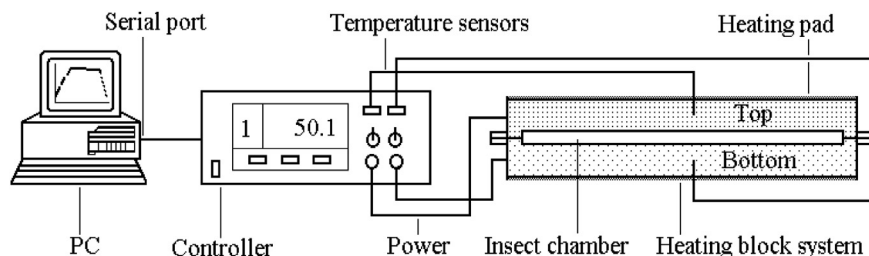


Fig. 1. Heating block system for insect mortality studies (Yin et al., 2006).

$$\frac{d(N/N_0)}{dt} = -k(N/N_0)^{1-n}, \quad (1)$$

The integration form above can be converted to different reaction orders as follows by taking the logarithm of both sides of equation (1):

$$\begin{aligned} \ln(N/N_0) &= -kt + c \quad (n = 1), \\ (N/N_0)^{1-n} &= -kt + c \quad (n \neq 1). \end{aligned} \quad (2)$$

N and N_0 are the surviving and initial numbers of *S. oryzae*, respectively, k is the thermal death rate constant, t the exposure time (min), and n is the kinetic order of the reaction. Results using the 0-, 0.5th-, 1st-, 1.5th- and 2nd-reaction order were calculated. The best-fitted reaction order was determined by comparing the average coefficients of determination (R^2) over all four treatment temperatures. Upon selection of the reaction order, the constants of k and c were obtained from the regression equation, and the model was used to estimate the time needed to kill 95, 99, 99.33 and 99.99% of the test insects (LT_{95} , LT_{99} , $LT_{99.33}$ and $LT_{99.99}$, respectively). The thermal-death-time (TDT) curve for *S. oryzae* adults was then developed by plotting the minimum exposure time required at each temperature to achieve 100% kill of test adults on a semi-log scale, and the z value (the temperature difference by which the mortality rate is altered by a factor of 10) was obtained from the TDT curve (Wang et al., 2002b).

2.5. Activation energy

The thermal death activation energy (E_a , J/mol) is the minimum energy needed to achieve the death of pests, and reflects the sensitivity of the insect response to temperature changes. E_a of *S. oryzae* adults was calculated by two independent methods. For the first method, E_a was calculated by the equation below:

$$E_a = \frac{2.303RT_{\min}T_{\max}}{z}, \quad (3)$$

where R is the universal gas constant (8.314 J/mol K), T_{\min} and T_{\max} are the minimum and maximum absolute temperatures (K) of the test range, respectively, and z is the negative inverse of the slope of the TDT curve ($^{\circ}\text{C}$) (Armstrong et al., 2009). Another method for calculating activation energy was through the slope of an Arrhenius plot of $\log k$ versus the reciprocal of the absolute temperature ($1/T$) as follows (Hallman et al., 2005):

$$\log k = \log k_0 - \frac{E_a}{2.303RT}, \quad (4)$$

where k_0 is the reference thermal death rate constant (min^{-1}).

3. Results and discussion

3.1. Thermal death kinetics of adult *S. oryzae*

The average survival rate for unheated controls at the four temperatures was $94 \pm 1.4\%$, showing that little mortality was caused by handling. Consequently, treatment mortality in subsequent temperature–time combination tests was not corrected for control mortality. Table 1 shows coefficients of determination (R^2) for different reaction orders at four treatment temperatures to kill the *S. oryzae*. Because the 0th-order had the largest average coefficient of determination over the four temperatures, it was selected for further model development. This contrasts with earlier studies on *A. ludens* (Hallman et al., 2005), *T. castaneum* (Johnson et al., 2004), and *C. pomonella* (Wang et al., 2004), in which the 0.5th-order was most applicable.

The thermal mortality curves of adult *S. oryzae* with the best-fit 0th-order model are shown in Fig. 2. The slopes of the thermal mortality curves decreased several-fold when the treatment temperature increased from 44 to 50 $^{\circ}\text{C}$. The minimum holding time for 100% mortality of 150 insects at 44, 46, 48, and 50 $^{\circ}\text{C}$ was about 130, 50, 12, and 4 min, respectively.

Table 2 shows the model constants fitted by the 0th-order for thermal mortality of adult *S. oryzae*. As expected, the k value increased with increasing temperature, suggesting that higher temperatures require shorter exposures to achieve the same insect mortality. Except for 46 $^{\circ}\text{C}$, the c value was within the margin of error for the ideal of 1 at time zero. Table 3 shows the minimum time for 100% mortality of 150 insects and predicted lethal time (LT) to obtain 95%, 99%, 99.33% and 99.99% mortality. The predicted LTs increased with increasing insect mortality levels. The observed minimum time for 100% mortality was close to the estimated $LT_{99.33}$ but with slight over estimations at 46 and 48 $^{\circ}\text{C}$ and small under estimations at 44 and 50 $^{\circ}\text{C}$ at 99%, 99.33% and 99.99% mortality levels.

These results suggest that a 4 min exposure at 50 $^{\circ}\text{C}$, easily obtained using radio frequency energy (Wang et al., 2010), should be effective in disinfecting grain of adult *S. oryzae*. However, earlier studies suggest that the adult *S. oryzae* is not the most heat tolerant

Table 1
Coefficients of determination (R^2) from kinetic order (n) models for thermal mortality of adult *Sitophilus oryzae* at four temperatures.

Temperature	N_0	$n = 0$	$n = 0.5$	$n = 1$	$n = 1.5$	$n = 2$
44 $^{\circ}\text{C}$	150	0.998	0.944	0.734	0.612	0.592
46 $^{\circ}\text{C}$	150	0.990	0.945	0.844	0.749	0.696
48 $^{\circ}\text{C}$	150	0.969	0.839	0.554	0.411	0.388
50 $^{\circ}\text{C}$	150	0.964	0.969	0.801	0.572	0.510
Average	150	0.980	0.924	0.734	0.586	0.547

N_0 = the initial number of insects tested.

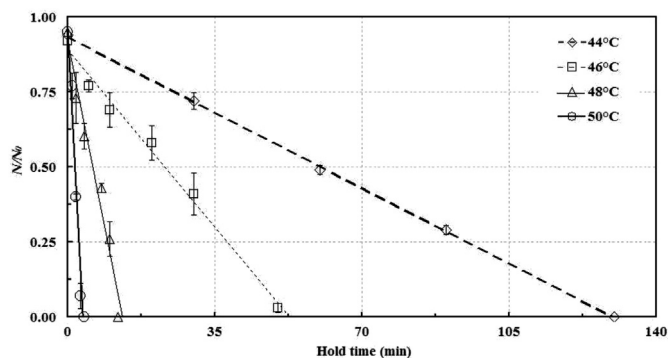


Fig. 2. Thermal mortality curves of adult *Sitophilus oryzae* at different temperatures and exposures. N and N_0 are the surviving and initial numbers of *Sitophilus oryzae*, respectively.

Table 2

Thermal death constants of 0th-order reaction model for adult *Sitophilus oryzae* at four temperatures.

Temperature	$(N/N_0)^{1-0} = -kt + c$	
	k	c
44 °C	0.0072	0.9353
46 °C	0.0169	0.8899
48 °C	0.0707	0.9197
50 °C	0.2598	0.9578

N and N_0 = surviving and initial numbers of *Sitophilus oryzae*, respectively, k = thermal death rate constant, t = the exposure time (min).

stage. Becket et al. (1998) looked at the relative tolerance of *S. oryzae* life stages using a system that rapidly heated infested grain, however, the method used did not take into account differences in heating rate experienced by different stages due to insulation by the grain. Based on LT_{99s} , adults were consistently the least tolerant life stage, but as treatment temperature increased the differences between stages decreased. This is similar to the results found for *T. castaneum* where significant differences between the stages were noted at lower treatment temperatures, but no significant differences occurred at the highest treatment temperature (Johnson et al., 2004). Consequently, there may be little difference in the heat tolerance of difference *S. oryzae* stages at the relatively high treatment temperature of 50 °C, although additional studies are needed to confirm this.

3.2. Thermal-death-time curve and activation energy of *S. oryzae*

The thermal-death-time (TDT) curve for adult *S. oryzae* is shown in Fig. 3. The curve for *S. oryzae* was described by the linear regression equation $\log t = 13.608 - 0.2602T$ ($R^2 = 0.996$) where t = time and T = temperature. The z value obtained from the negative inverse of the slope of the thermal-death-time curve was

Table 3

Comparison of lethal times (min) obtained by experiments and 0th-order kinetic models for adult *Sitophilus oryzae* at four temperatures.

Temperature	N_0	Minimum time for 100% mortality of 150 insects	Predicted treatment time (min) (95% CI)			
			LT_{95}	LT_{99}	$LT_{99.33}$	$LT_{99.99}$
44 °C	150	130	122.96 (120.0–125.5)	128.51 (125.4–131.2)	128.98 (125.9–131.7)	129.89 (126.7–132.6)
46 °C	150	50	49.70 (39.5–63.5)	52.07 (41.3–66.6)	52.26 (41.4–66.9)	52.65 (41.7–67.4)
48 °C	150	12	12.30 (10.4–16.0)	12.87 (10.8–16.8)	12.91 (10.8–16.8)	13.01 (10.9–17.0)
50 °C	150	4	3.49 (2.7–4.2)	3.65 (2.8–4.3)	3.67 (2.8–4.4)	3.69 (2.9–4.4)

N_0 = the initial number of insects tested.

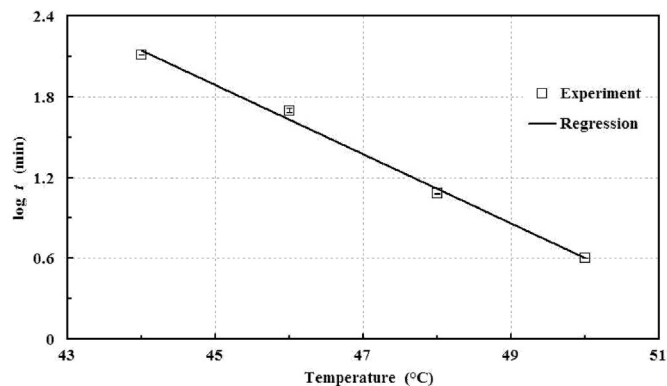


Fig. 3. Thermal mortality curve for adult *Sitophilus oryzae* at a heating rate of 5 °C/min. The regression equation was $\log t = 13.608 - 0.2602T$ with the coefficient of determination $R^2 = 0.996$, where t is exposure time and T is treatment temperature.

3.9 °C, resulting in a thermal death activation energy of 505.0 kJ/mol (Eq. (3)).

Figure 4 shows the Arrhenius plot for temperature effects on thermal death rates of adult *S. oryzae*. From the slope of the regression equation, the activation energy calculated by Eq. (4) was 523.0 kJ/mol, which was slightly greater than that obtained by Eq. (3). This activation energy was smaller than that for *T. castaneum* (814.1 kJ/mol) (Johnson et al., 2004) and *A. ludens* (560.7 kJ/mol) (Hallman et al., 2005), but larger than for *C. pomonella* (473.0 kJ/mol) (Wang et al., 2002a), *P. interpunctella* (506.3 kJ/mol) (Johnson et al., 2003), and *A. transitella* (519.0 kJ/mol) (Wang et al., 2002b). This indicates that adult *S. oryzae* is more sensitive to temperature change than the moth species, but less sensitive than either *A. ludens* or *T. castaneum*.

3.3. Effect of heating rate on thermal mortality

Figure 5 shows the mortality changes of adult *S. oryzae* at 46 °C for 20 min as influenced by heating rates. The average mortality was similar at heating rates of 1 °C/min and above ($P > 0.05$), which was in agreement with the findings in Wang et al. (2005). However, mortality at heating rates below 1 °C/min was significantly reduced from those at the higher heating rates ($P < 0.05$), and was in agreement with observations reported by Neven (1998) and Thomas and Shellie (2000). This enhanced thermal tolerance may be caused by heat acclimation of test insects during lengthy exposures to non-lethal temperatures. Our results suggest that the rapid temperature rise characteristic of heating with microwave or radio frequency would avoid acclimation and be a distinct advantage for the use of this technology.

4. Conclusions

Our study provided a model describing the response of *S. oryzae* adults to high temperatures. Such a model and the activation

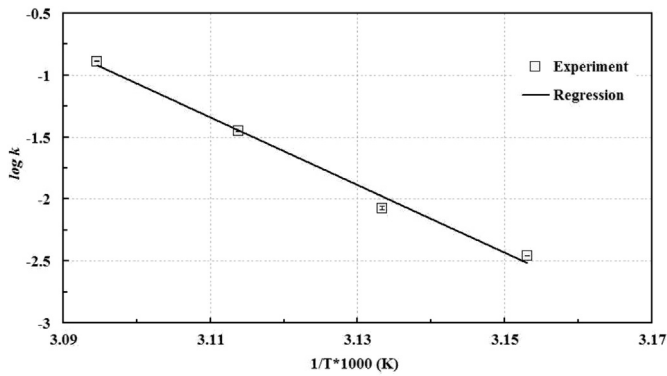


Fig. 4. Arrhenius plot for temperature effects on thermal death rates of *Sitophilus oryzae*. The regression equation was $\log k = 83.564 - 27.301 \times 1/T^*1000$ with the coefficient of determination $R^2 = 0.990$, where k is the thermal death rate constant and T is the treatment temperature (Kelvin).

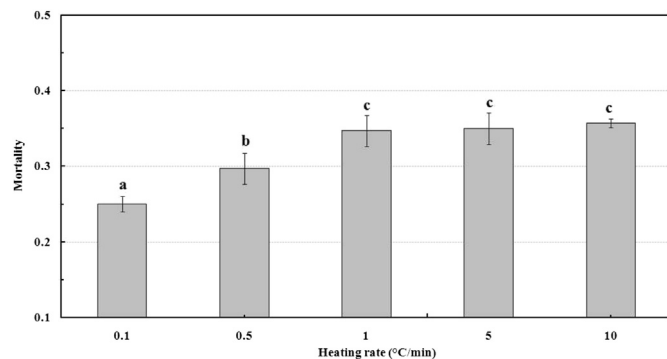


Fig. 5. The mortality curve of *Sitophilus oryzae* at different heating rates. Different letters indicate that means are significantly different ($P < 0.05$).

energy derived from it were useful in comparing the relative heat tolerance of *S. oryzae* adults with other species, and developing heat treatment protocols. Although care must be taken when applying any such protocol to those stages feeding within the seed, the high temperatures and rapid heating available with microwave or radio frequency heat treatments should also provide control of these stages, and avoid acclimation often found in conventional heat treatments.

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