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# Impact of Selected Infrared Wavelengths on Inactivation of Microbes on Rough Rice

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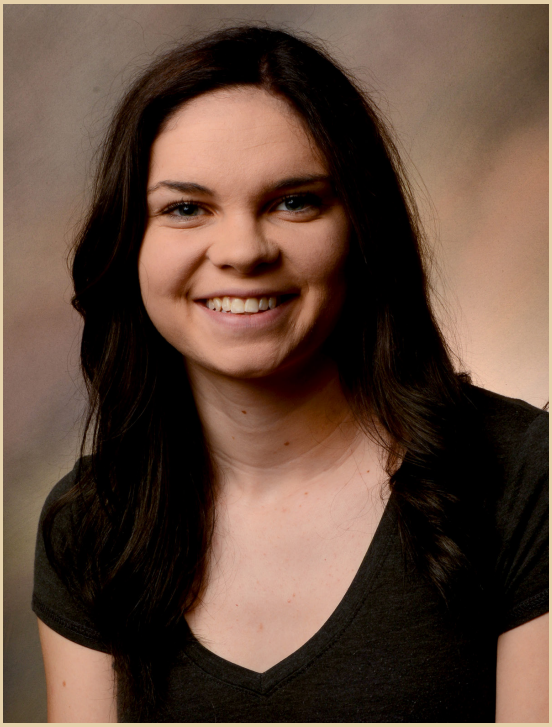
## *Meet the Student-Author*



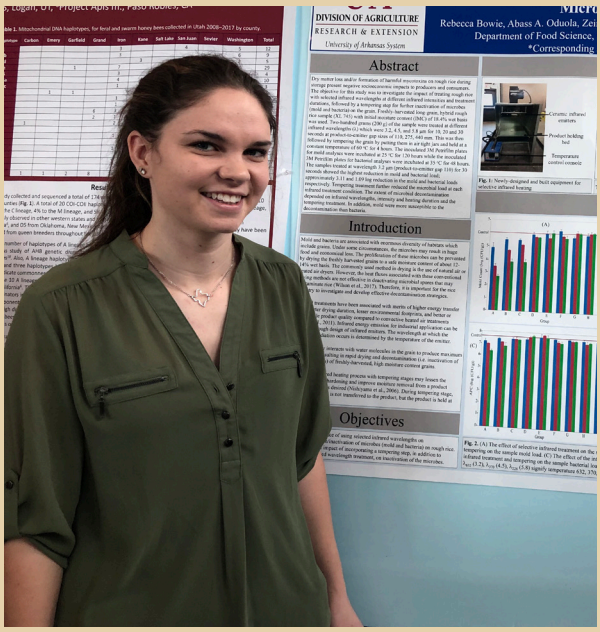
**Rebecca Bowie**

## *Research at a Glance*

- Storage of rough rice in an inappropriate condition may lead to the growth of microbes such as mold and bacteria and may affect the quality and safety of the rough rice.
- Infrared heat treatment and tempering are effective in inactivating the microbes that may contaminate rough rice.
- The inactivation efficiency of infrared heat treatment and tempering is greater than that attainable by current heated air methods used in the industry and is more pronounced for mold than bacteria.

I am from Magnolia, Texas and graduated from Magnolia High School in 2016. I am currently in the Dale Bumpers College of Agricultural, Food and Life Sciences and plan to graduate with a degree in Food Science and a minor in Agriculture Business in May 2020. I interned with Simmons Foods as the Food Safety and Quality Assurance Corporate Intern in the summer of 2019. While interning, I researched the use of Whole Genome Sequencing for the analysis of microbial data within the poultry industry. Also in 2019, I studied abroad for a food sustainability program in Ghent, Belgium. There I was able to learn about successful water and food sustainability methods that they have implemented in the European Union. I have always had a strong desire to study food and enjoy learning more about food production and processes every day.

I am grateful for the help of my Honors mentor, Dr. Griffiths Atungulu, for allowing me to do research. He has helped me every step of the way and graciously shared his knowledge of rice with me. I would also like to thank my committee members, Luke Howard and Sammy Sadaka. Lastly, I would like to thank the following graduate students, Abass Oduola, Shantae Wilson and Zeinab Mohammadi-Shad for their collaboration and guidance throughout the research process.




Rebecca presenting her poster at the 2019 Honors Student Board Poster Contest. She was one of the very few undergraduate presenters.

# Impact of selected infrared wavelengths on inactivation of microbes on rough rice

*Rebecca Bowie*[*], *Abass A. Oduola*[†], *Zeinab Mohammadi Shad*[§], *Shantae A. Wilson*[‡], *and Griffiths G. Atungulu*[¶]

## Abstract

The formation of harmful microbes and their associated mycotoxins on rough rice during storage presents negative socioeconomic impacts for producers and consumers. The objective of this study was to investigate the impact of treating rough rice with selected infrared (IR) wavelengths at different IR intensities and heating durations, followed by a tempering step for further inactivation of microbes (mold and bacteria) on the grain. Freshly harvested long-grain, hybrid, rough rice (RT CLXL745) with initial moisture content (IMC) of 18.4% wet basis (w.b.) was used. Two-hundred grams (200 g) samples of rice were treated at different IR wavelengths (λ), 3.2, 4.5, and 5.8 μm for 10, 20 and 30 seconds (s); at product-to-emitter gaps of 110, 275, and 440 mm. This was then followed by tempering the grain; putting samples in air-tight jars and holding at a constant temperature of 60 °C for 4 hours (h). Inoculated Petrifilm plates for mold and bacterial analyses were incubated at 25 °C for 120 h and 35 °C for 48 h respectively. Samples treated at wavelength 3.2 μm (product-to-emitter gap 110 mm) for 30 s showed the greatest reduction in mold and bacterial load; approximately 3.11 and 1.09 log reduction in the colony forming unit of mold and bacteria, respectively. Microbial analysis was performed on the rice prior to tempering, then all of the rice was tempered and microbial analysis was performed again to analyze the effectiveness of a tempering step. Tempering treatment further reduced the microbial load at each IR treatment condition. Molds showed more susceptibility to the IR decontamination than bacteria. This study provides useful information on the effectiveness of IR heating and tempering on microbial inactivation on rough rice.

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## Introduction

Rice is known to be the primary food source for almost 50% of the total world population, thereby contributing about 20% of the total human dietary energy supply. In order to satisfy import/export demand and supply industries, huge amounts of rice are stored after harvest, often for more than a year (Fleurat-Lessard, 2017). When stored in an inappropriate condition, rice is susceptible to microbial contamination that directly or indirectly affects the quality and safety of the stored rice (Mohammadi Shad and Atungulu, 2019).

The proliferation of microorganisms on rice leads to musty odors, dry matter loss, discoloration, and accumulation of mycotoxin (Christensen and Kaufmann, 1969). This is as a result of the action of the spoilage microorganism interacting with themselves, with the grain, and with the environment of the storage facilities (Atungulu et al., 2018). The moisture content (MC) and temperature of rough rice are the two major parameters that influence microbial growth. Therefore, to prevent the proliferation of microbes, freshly harvested rice must be dried within a short duration to an MC of about 12–14% wet basis (w.b.).

The widely used conventional methods of drying employ the use of natural air or heated air dryers (Atungulu et al., 2019). Unfortunately, these conventional drying methods are ineffective in inactivating microbes and microbial spores that may contaminate the rice kernels (Wilson et al., 2017a). Therefore, it is of high importance to develop alternative methods of drying that can concomitantly dry and disinfect rough rice.

Infrared (IR) heating has been linked with the merits of higher energy transfer rate, shorter duration of drying, mild environmental footprints, and better or comparable product quality compared to convectively heated air treatments (Wang et al., 2011). In addition, IR heating has the potential to simultaneously dry and disinfect rough rice. For industrial applications, IR energy emission can be realized through design of IR emitters. The temperature of the emitter is used to determine the wavelength at which the maximum radiation occurs. Infrared can be classified into near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR) with wavelength ranges 0.75–1.4 μm, 1.4–8 μm, and 8–1000 μm, respectively (Krishnamurthy et al., 2008).

Generally, radiation penetration depth associated with IR heating is rather shallow. Therefore, IR treatment supplies high heat flux on the surface of the treated product. In the case of grain treatment, the IR heat dissipated on the surface of the grain may lead to case-hardening, surface discoloration or even burning before maximum moisture removal is achieved (Wilson et al., 2017b). Incorporating tempering steps may help to alleviate these challenges. The tempering process allows moisture redistribution throughout the grain and eliminates moisture gradients generated during previous IR heating cycles; hence, it makes the next IR heating cycle effective in moisture removal (Li et al., 1998; Nishiyama et al., 2006). During the tempering stage, there is no transfer of IR energy to the grain, but the grain is allowed to rest at a constant temperature. Therefore, IR heating followed by a tempering step may have a higher potential to simultaneously dry and disinfect rough rice than just the application of IR heating.

This study aimed to investigate (i) the influence of using selected IR wavelengths on decontamination/inactivation of microbes (mold and bacteria) on rough rice and, (ii) the impact of incorporating a tempering step, in addition to selected IR wavelength treatment, on inactivation of the microbes.

## Materials and Methods

The rice used was long-grain, hybrid, rough rice (RT CLXL745) obtained in 2016 from Poinsett Rice Inc., Waldenburg, Arkansas. Freshly harvested rough rice with initial MC of 18.4% w.b. was immediately cleaned using dockage equipment (MCi Kicker Dockage Tester, Mid-Continent Industries Inc., Newton, Kan.). The cleaned rice was put in tubs, sealed, and stored in a laboratory cold room set at 4 °C for 24 months. Twenty-four hours prior to conducting experiments, the rice was retrieved from the cold room and allowed to equilibrate with a room temperature of about 26 °C. The MC of the samples was determined by using an AM 5200 Grain Moisture Tester (PERTEN Instruments, Hägersten, Sweden) calibrated with a convective oven method.

A newly built, laboratory-scale IR system (Tempco Electric Heater Corporation) was used. The system consists of three ceramic emitters in one panel, heating chamber, product holding bed, and a temperature control console as shown in Fig. 1. The emitter has a metamorphic yellow (cold) to orange (hot) color. The equipment is made of low profile 20-gauge aluminized steel housing. The standard stocked voltage includes 220–240 V with watt density range from 17.1 kW/m$^2$–54.3 kW/m$^2$; the temperature generated can be as high as 740 °C. This equipment produces IR radiation wavelengths of 3 to 6 μm.

The temperature console is used to vary the IR radiation wavelength generated. For instance, wavelengths of 5.8 μm, 4.5 μm, and 3.2 μm are produced at temperatures of 226 °C, 370 °C, and 632 °C, respectively. The wavelength was calculated using Wien's Displacement Law (Eq. 1).

$$\lambda_{max} = \frac{b}{T} \qquad \text{Eq. 1}$$

Where $\lambda_{max}$ is peak wavelength (μm), *b* is constant of proportionality (2900 μm·K) and *T* is the absolute temperature in Kelvin.

A flat rectangular pan was covered with sterile aluminum foil and 200 g of rice samples were weighed into the pan and spread out to form a thin layer. Then, the thin-layered rice samples were put in the IR equipment and treated at selected wavelengths of 3.2 μm, 4.5 μm and 5.8 μm at three different product-to-emitter gaps (corresponding to different intensities) for different heating durations. The different intensities corresponding to each treatment combination is shown in Table 1. Three replications were done at each treatment combination level. After IR treatment, the samples were allowed to cool down to about 26 °C before they were carefully poured into sterile bags for microbial analysis. A control rice sample received no treatment.

Following the IR treatments described above, all samples were placed inside cleaned 16 oz. jars and covered tightly. The jars were then put in an incubator (Thelco Model 4, Precision Scientific Instruments, Inc., Chicago, Ill.) set at 60 °C for 4 h. After the incubation period, the jars were brought out and allowed to cool down to room temperature. The samples were carefully poured into sterile bags for microbial analysis. A control rice sample received no IR and tempering treatment.

Standard procedures for isolation, plating, and counting were employed (AOAC, 2002) to determine rice total microbial load. Phosphate-buffered dilution water (0.5 M, pH = 7.2) was prepared and autoclaved at 121 °C for sterilization (AOAC, 2002).

Microbial analysis was performed twice, once before tempering and then once after tempering. A 10-g sample of rice was weighed and placed into a sterile stomacher bag. Then, 90 mL of sterile phosphate-buffered dilution water was added to the stomacher bag and masticated. A lab masticator (Silver Panoramic, iUL, S.A., Barcelona, Spain) was used to dislodge the microorganism. The masticator was set at 240 s and 0.7 stroke/s. This process ensured that the rough rice samples were pulverized into powder for total microbial load analysis when mixed with dilution water. Serial dilutions were carried out by mixing 1 mL of the original mixture in the stomacher bag (first dilution–$10^{-1}$) with 9 mL of sterilized phosphate-buffered dilution water in a test tube (second dilution–$10^{-2}$) and so on until the sixth dilution ($10^{-6}$) was made.

The 3M Petrifilm Mold Count Plates and 3M Petrifilm Aerobic Count Plates (3M Microbiology Product, Minneapolis, Minn.) were used in enumerating mold and bacteria count, respectively. The plates were placed flatly in the biosafety cabinet. The top film of the plate was carefully lifted

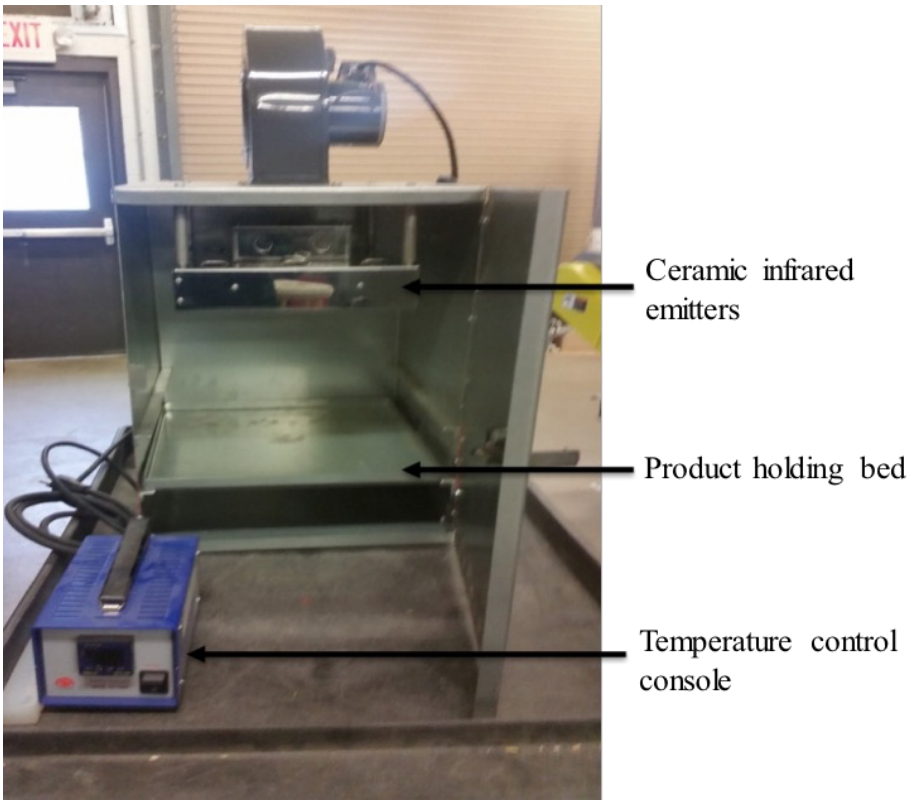


**Fig. 1.** Newly designed and built equipment for selective infrared heating.

and a P1000 micropipette (Finnpipette F2, Thermo Fisher Scientific, Inc., Vantaa, Finland), placed perpendicularly to the plates, was used to transfer 1 mL each of the sample solutions onto the center of the two 3M Petrifilm Plates (i.e., mold and aerobic plates). The top film was then gently lowered. The center of a plastic spreader was placed on the plates to align with the center of the plates. Light manual pressure was then applied on the plastic spreader to ensure even distribution of the inoculum on the Petrifilm plate. The gel was allowed to solidify for one minute. The inoculated Petrifilm plates with clear sides up were stacked to a maximum of 20 units and incubated.

The Petrifilm mold count plates and aerobic count plates were placed in an incubator (Thelco Model 4, Precision Scientific Instruments, Inc., Chicago, Ill.) at 25 °C for 120 h and 35 °C for 48 h, respectively, before counting. After the incubation periods, the colony forming units (CFU) on each plate were counted. Mold colonies on the plates appeared blue, black, yellow, or green, while bacteria colonies on the plates appeared red with a regular shape. The colony forming unit per gram (CFU/g) for each sample was obtained using Eq. 2:

$$T_{cfu} = \frac{P_{cfu}}{D_r} \qquad \text{Eq. 2}$$

Where $T_{cfu}$ is total colony forming units per gram of rice (CFU/g), $P_{cfu}$ is colony forming units counted on plate per gram of rough rice (CFU/g) and $D_r$ is dilution rate ($10^{-1}$ to $10^{-6}$ times).

A statistical software JMP version 14.0.0 (SAS Institute, Inc., Cary, N.C.) was used to carry out analysis of variance (ANOVA) using fit model (full factorial analysis) and Tukey's honestly significant difference (HSD) test to determine significant differences within and among samples. All tests were considered to be significant when $P < 0.05$.

## Results and Discussion

The initial mold load for the control samples was 5.74 log CFU/g. The effect of IR intensity and heating duration on the mold load of the samples is shown in Fig. 2. From the two-factor factorial analysis carried out, there was an IR intensity and heating duration interaction effect on the mean mold load of the samples. Only the highest three intensities (15.71, 10.08, and 7.27 kW/m$^2$), all belonging to wavelength 3.2 µm, reduced the mold load of the rice samples. Greatest mold reduction was observed at the highest intensity (15.71 kW/m$^2$) and longest heating duration (30 s) which brought about 3.11 log CFU/g reduction in the mold load. Other intensities belonging to wavelengths of 4.5 µm and 5.8 µm showed no reduction in the mean mold load of the samples regardless of the heating duration. Similar results with the current study were reported by Wilson et al. (2017b) where the IR heating of corn resulted in about 2.88 log reduction in the mold load. Also, Bingol et al. (2011) reported a 5-log reduction in the mold load of almond when treated with IR.

The effect of the IR treatment followed by the tempering step is shown in Fig. 3. The tempering step resulted in further reduction of the mold count after every IR treatment combination. All IR treatment combinations followed by the tempering step had significant effects on reducing the mean mold load of the samples. Compared to the IR treatment without tempering at the highest intensity of 15.71 kW/m$^2$ and longest heating duration 30 s, tempering further reduced the mold load by an additional 1.40 log CFU/g to bring the mold load reduction to 4.03 log CFU/g. In addition, for all the IR treatments that showed no significant effect, incorporating a tempering step led to a significant reduction in the mold load when compared to the control samples. For instance, the initial mold load of 5.74 log CFU/g was reduced to 5.53 log CFU/g after IR treatment at 0.73 kW/m$^2$ intensity for 30 s. However, incorporating a tempering step at the same IR intensity (0.73 kW/m$^2$) and

**Table 1. Experiment design of different combinations of infrared (IR) processing parameters.**

| Infrared heating duration (s) | Peak wavelength $\lambda_{temp}$ °C (µm) | Product to emitter gap size (mm) | Intensity (kW/m$^2$) |
|---|---|---|---|
| 10 | $\lambda_{226}$ (5.8) | 110 | 1.55 |
| | | 275 | 1.10 |
| | | 440 | 0.73 |
| 20 | $\lambda_{370}$ (4.5) | 110 | 4.13 |
| | | 275 | 2.87 |
| | | 440 | 1.86 |
| 30 | $\lambda_{632}$ (3.2) | 110 | 15.71 |
| | | 275 | 10.08 |
| | | 440 | 7.27 |

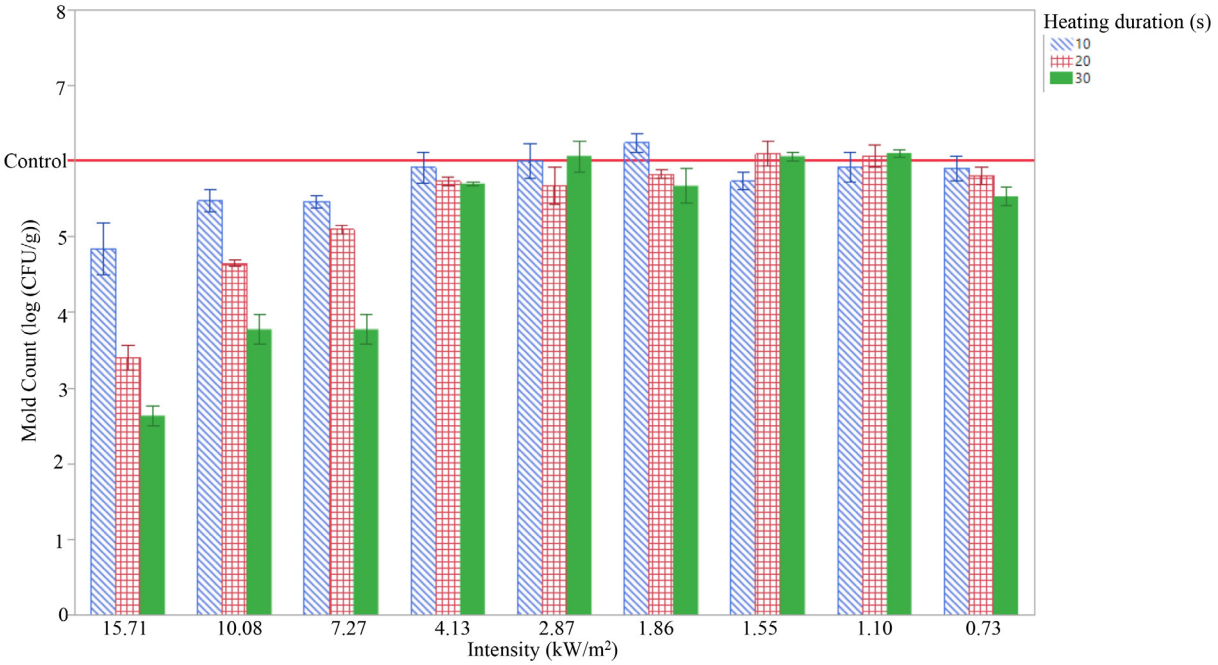


**Fig. 2.** The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s on the mold count on the rough rice samples; CFU signifies colony forming unit.

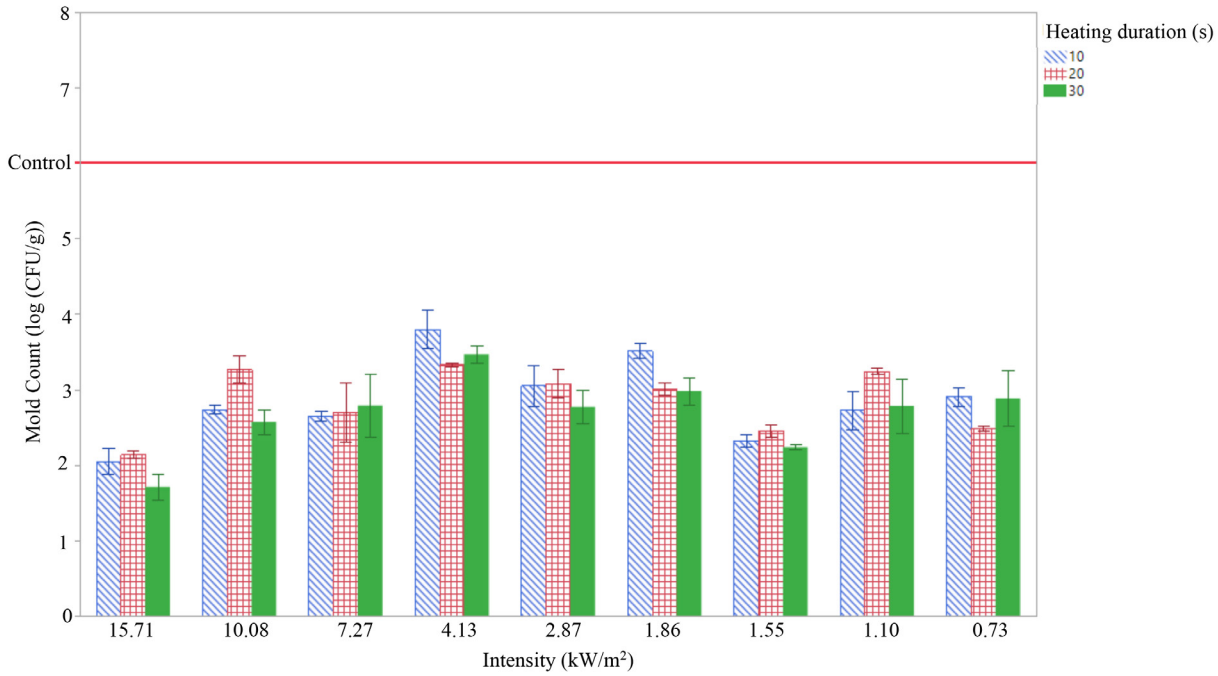


**Fig. 3.** The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s followed by tempering at 60 °C for 4 hours on the mold count on the rough rice samples; CFU signifies colony forming unit.

heating duration (30 s) statistically reduced the initial mold load to 2.88 log CFU/g. In agreement with the current result, Wilson et al. (2017a) reported that IR treatment of corn followed by tempering at 50 °C for 4 h resulted in 3.8–4.5 log mold reduction.

The effect of IR treatment on the aerobic plate count (APC) of the samples is shown in Fig. 4. The control samples had an initial APC of 7.44 log CFU/g. The IR treatment showed low efficiency in deactivating bacteria on the samples when compared to its effect on mold decontamination. Like the result of mold load, the highest intensity (15.71 kW/m$^2$) at the longest heating duration (30 s) showed the maximum reduction; it brought the APC of the sample to 6.35 log CFU/g, i.e., a reduction of 1.09 log CFU/g. Other IR treatment combinations showed less reduction in the APC of the samples. Statistical analysis showed that the intensities of 15.71 kW/m$^2$ and 10.08 kW/m$^2$ had effects on the mean APC of the treated samples. The low reduction in APC by IR heating could be as a result of the presence of heat-resistant bacterial spores in the rough rice samples. A similar result was found by Staack et al. (2008), where they reported that IR heating resulted in a maximum reduction of 1 log CFU/g. In addition, Bingol et al. (2011) reported a very low reduction (0.62 ± 0.18) in the bacterial load when almond was treated using IR treatment. Mackey and Derric (1986) reported that the heat resistance of bacteria increased when bacteria were heated to elevated temperatures for a relatively short time.

Figure 5 shows the effect of the tempering step incorporated into the IR heating treatment on the APC of the samples. Tempering caused a further reduction in the APC when compared to the samples treated without tempering. For instance, tempering the samples that were treated at an intensity of 15.71 kW/m$^2$ for 30 s brought about 3.50 log CFU/g reduction in the APC. Likewise, tempering the samples that were treated at an intensity of 0.73 kW/m$^2$ for 10 s brought about 1.52 log CFU/g reduction in the APC. Incorporating the tempering step made all the intensities that initially had no effects produce significant reductions in the mean bacterial load.

## Conclusions

The IR treatment was effective in inactivating microbes (mold and bacteria) on rough rice. However, incorporating a tempering step led to further microbial inactivation. This treatment combination was more effective on mold inactivation than bacteria. Therefore, a longer heating duration may be required to further reduce the bacteria load on rough rice. For further study, these findings should be extended to inactivating aflatoxin- producing mold—*Aspergillus flavus*—to prevent the production and accumulation of aflatoxin on rice. In addition, the implication of the studied treatments on milled rice yields and quality characteristics should be evaluated.


## Acknowledgements

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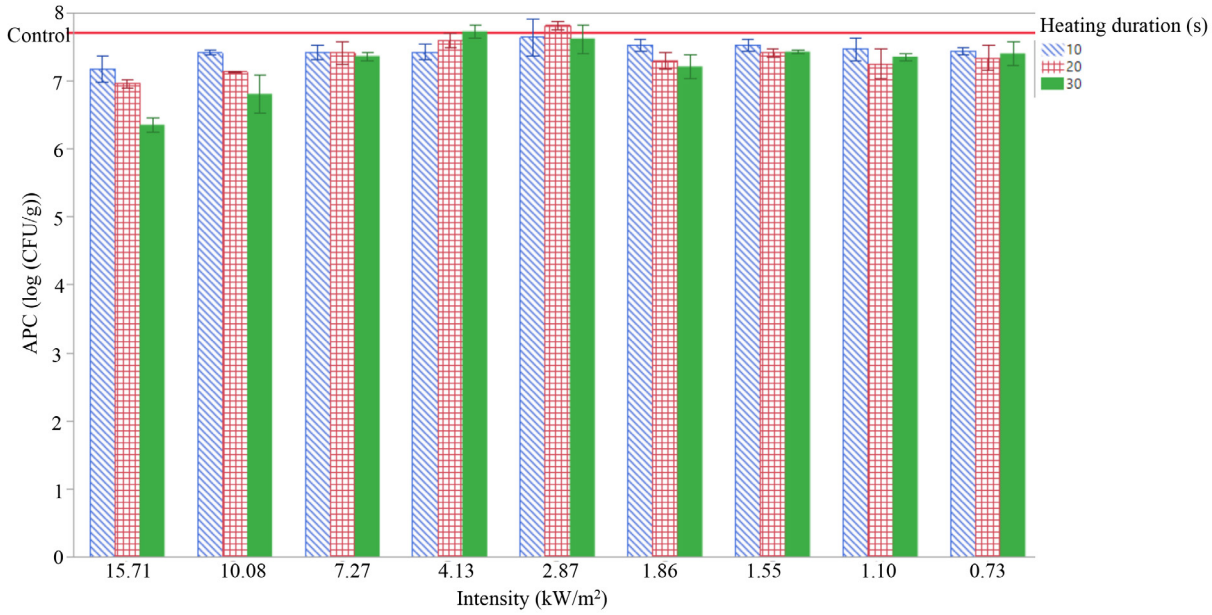


**Fig. 4.** The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s on the aerobic plate count (APC) on the rough rice samples; CFU signifies colony forming unit.

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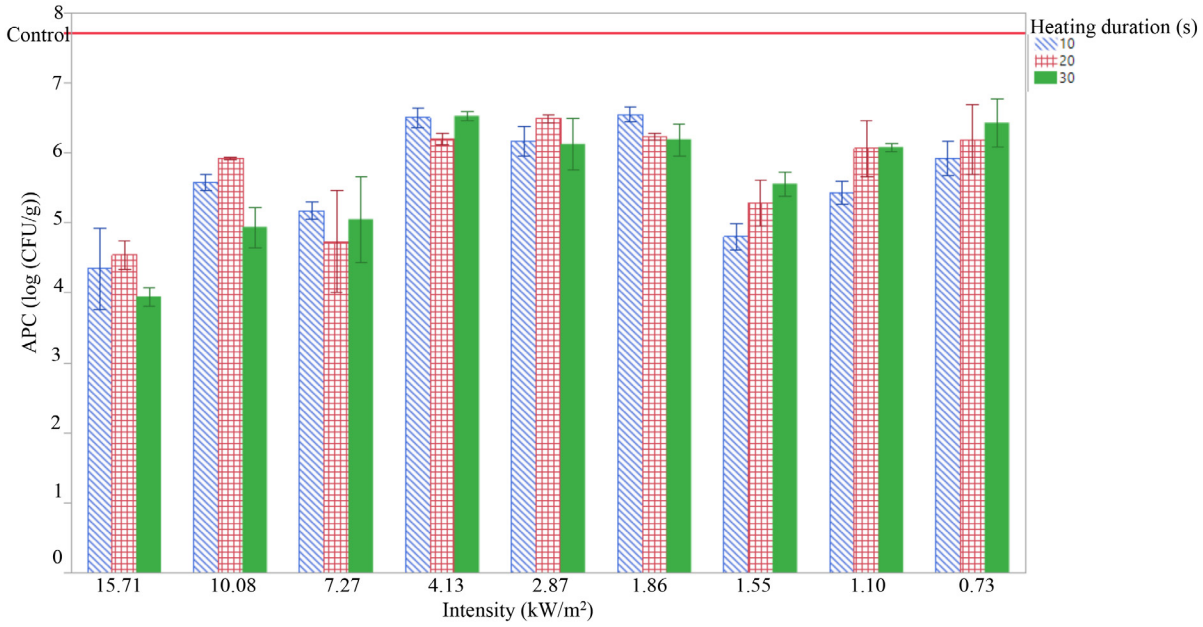


**Fig. 5.** The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s followed by tempering at 60 °C for 4 hours on the aerobic plate count (APC) on the rough rice samples; CFU signifies colony forming unit.