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Abstract

Formation of harmful microbes and their associated mycotoxins on rough rice during storage present negative socioeconomic impacts to producers and consumers. The objective for 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 (XL 745) with initial moisture content (IMC) of 18.4% wet basis (w.b.) was used. Two-hundred grams (200 g) of the samples were treated at different IR wavelengths (λ) which were 3.2, 4.5, and 5.8 μm for 10, 20 and 30 seconds (s) at product-to-emitter gaps of 110, 275, 440 mm. This was then followed by tempering the grain; putting them in air-tight jars and held at a constant temperature of 60 ^{o}C for 4 hours (h). The inoculated Petrifilm plates for mold and bacterial analyses were incubated at 25^{o}C for 120 h and 35^{o}C for 48 h respectively. . The samples treated at wavelength 3.2 μm (product-to-emitter gap 110 mm) for 30 s showed the highest reduction in mold and bacterial load; approximately 3.11 and 1.09 log reduction in the mold and bacterial loads, respectively. Tempering treatment further reduced the microbial load at each IR treatment condition. Molds showed more susceptibility to the IR decontamination than bacteria population. This study provides useful information on the effectiveness of IR heating and tempering on microbial inactivation on rough rice.

Keywords: Wavelength, intensity, infrared, rough rice, microbes.

1.0 Introduction and Literature Review

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 and export demand and supply industries, huge amount of rice is stored after harvest, often longer 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, for example, rice discoloration, and safety in terms of microbial contamination 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).

Mycotoxins are secondary metabolites, usually toxic substances that are produced by fungi that contaminate/infect crops. Unlike bacterial toxins, fungal toxins (mycotoxins) are not proteins and therefore not detectable by human immune system and have the potential to cause immune system suppression, mutations, cancer and teratogenic effects (Zheng et al., 2018). These mycotoxins may disrupt cell structures and their processes such as protein, DNA and RNA synthesis (Zheng et al., 2018). The main concern is that these mycotoxins are heat stable and therefore not destroyed during common drying processes (Christensen and Kaufmann 1969). Out of all the mycotoxins produced by molds, aflatoxin is one of the most potent. Aflatoxin is carcinogenic and mainly produced by *Aspergillus* species (Creppy, 2002). Aflatoxin has been found to contaminate grains including rice, corn, and wheat. Aflatoxin contamination of grains leads to huge economic losses and health threats (Amaike and Keller, 2011). Low doses (chronic

exposure) consumption of aflatoxin by humans may results in cancer, immunosuppression, and growth impairment (Raduly, 2020). High doses (acute exposure) consumption of aflatoxin by animals and humans may lead to poisoning, which may result to death. Animals that feed on contaminated feeds can act as transmitting agents, as meat, milk, or eggs can pass aflatoxin to other species in the food chain. It is difficult to remove aflatoxin from rice after it has be produced by molds. Aflatoxins are heat-stable in a 150^{o}C – 200 ^{o}C temperature range (Herzallah et al., 2008).

The moisture content (MC) and temperature of rough rice are two major parameters that influence microbial growth. Therefore, to prevent the proliferation of microbes, freshly harvested rice must be dried within short duration to a 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 including in-bin, high-temperature crossflow, and rotary drying systems (Atungulu et al., 2019). Unfortunately, these conventional drying methods are not capable of rapidly drying the rice before molds proliferation. Also, the conventional methods are ineffective in inactivating microbes and microbial spores that may have contaminated the rice kernels in the field, during harvest, handling, and storage (Park et al., 2005; Wilson et al., 2017a). Therefore, it is of high importance to develop alternative method of drying that can concomitantly dry and disinfect rough rice.

The 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 convective heated air treatments (Wang et al., 2011). In addition, IR heating has the potential to simultaneously dry and disinfect rough rice. For industrial application, 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. IR can be classified into near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR) with ranges 0.75 – 1.4 μm, 1.4 – 8 μm, and 8 – 1000 μm respectively (Krishnamurthy et al., 2008). The amount of radiant energy emitted from a heat source (E, W/m2) is proportional to the surface temperature T (K) and the emissivity ε of the material. The use of IR wavelengths, in theory, may disrupt the structural integrity of the toxin producing fungi. This disruption may result in eliminating the more heat stable microbes.

The Planck's Law states that the spectral distribution and radiant intensity are a function of wavelength and emitter temperature. As the temperature increases, the peak output of the source shifts to the left of the electromagnetic spectrum with a greater percentage of the output energy in the near IR range (Pan and Atungulu, 2019). Or as the temperature of the emitting object increases, the spectral distribution is shifted towards the shorter wavelengths, and the total energy at each wavelength increases. Emissivity is defined as the ratio of the energy flux emitted by the real body to the flux emitted by a blackbody at the same temperature, where a blackbody is an ideal body which absorbs incident radiation and also emits the maximum radiant energy. In addition, from Wein's Law, the peak wavelength is inversely proportional to the emitting body's temperature. The phenomenon associated with Planck's Law produces the Wien Displacement Curve which is an important feature in equiptment selection. Food materials absorb MIR and FIR energy most efficiently through stretching modes of vibrations, which leads to the radiative heating process (Pan and Atungulu, 2019). It remains a challenge in the food industry to efficiently use selective heating for targeting water without heating the molecular components within the food material (Pan and Atungulu, 2019). Generally, radiation penetration depth associated with IR heating is rather shallow. Therefore, IR treatment supplies high heat flux on the surface of treated product.

In 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., 2017). Incorporating tempering steps may help to alleviate these challenges. The tempering process allows moisture redistribution throughout the grain and eliminate moisture gradient 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 higher potential to simultaneously dry and disinfect rough rice than just application of IR heating.

The aims of this study were 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.

2.0 Materials and Methods

2.1 Samples

The sample used was long-grain, hybrid, rough rice (XL 745) obtained from Poinsett Rice Inc., Waldenburg, Arkansas. Freshly harvested rough rice with IMC of 18.4% w.b. were immediately cleaned using dockage equipment (MCi Kicker Dockage Tester, Mid-Continent Industries Inc., Newton, Kan.). The cleaned samples were put in tubs, sealed, and stored in a laboratory cold room set at 4^{o}C. Twenty-four hours prior to conducting experiments, the samples were retrieved from the cold room and allowed to equilibrate with room temperature of about 26^{o}C. The MC of

the samples were determined by using an AM 5200 Grain Moisture Tester (PERTEN Instruments, Hägersten, Sweden) calibrated with convective oven method.

2.2 Infrared Instrument

A newly-built, laboratory scale IR system (Tempco Electric Heater Corporation) was used. The system consists of three ceramic emitter heaters in one panel, heating chamber, product holding bed, and a temperature control console as shown in figure 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 11 W/in^2 – 35 W/in^2 (17.1 kW/m^2 – 54.3 kW/m^2); the temperature generated can be as high as 740^{o}C (1364^{o}F). 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 temperature of 226^{o}C (439^{o}F), 370^{o}C (689^{o}F), and 632^{o}C (1170^{o}F), respectively.

The wavelength was calculated using Wien's Displacement Law (equation 1).

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

Where λ_{max} is peak wavelength (µm), b is constant of proportionality (2900 µm.K) and T is absolute temperature in Kelvin.

2.3 Infrared heat treatment

A flat rectangular pan was covered with sterile aluminum foil and 200 g of rice samples were weighed onto the pan and spread out to form a single layer. Following this, the thin-layered rice samples were put in the IR and treated at selected wavelengths of 3.2 µm, 4.5 µm and 5.8 µm at

three different product-to-emitter gaps. The product-to-emitter gaps correspond 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^{o}C before they were carefully poured into sterile bags for further analysis. A control rice sample received no treatment.

2.4 Tempering

Immediately after IR treatments described above, 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, IL) set at 60^{o}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 further analysis. A control rice sample received no treatment. This tempering treatment allowed for the rice kernel to re-equilibrate the internal water distribution. Holding the kernel at this temperature for four hours put the rice in a warm environment where the enthalpy within the kernel increased therefore making water want to evaporate from the surface of the kernel. The warm temperature and moisture level in the incubator environment did not allow for that water to leave the kernel because the environmental moisture content was saturated. This acted as a suffocation of the micrboes on the kernel. Microbes did not have room to develop and grow because the kernel distributed the water evenly across its surface leaving no room for the microbial growth, therefore they die.

2.5 Microbial Analysis

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^{o}C for sterilization (AOAC, 2002).

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 ensures 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 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, MN) 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 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 in order 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, IL) at 25ºC for 120 h and 35ºC 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 in color 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 following equation(2):

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

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).

2.6 Statistical Analyses

A statistical software (JMP version 14.0.0, SAS Institute) was used to carry out Analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test to determine significant differences within and among samples. All tests were considered to be significant when $p < 0.05$.

3.0 Results and Discussion

3.1 Mold Count

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 figure 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 kW/m^2, 10.08 kW/m^2, and 7.27 kW/m^2), all belonging to wavelength 3.2 µm, had significant effects in

reducing the mold load of the rice samples. Highest mold reduction was observed at the highest intensity (15.71 kW/m^2) and highest 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 significant 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., (2017) where the IR heating of corn resulted in about 2.88 log reduction in mold load. Also, Bingol et al., (2011) reported a 5-log reduction in mold load of almond when treated with IR. The full factor factorial analysis shows that intensity, heating duration and intensity*heating duration interaction all had significant effect on the sample mean mold load ($P<0.05$).

The effect of the IR treatment followed by the tempering step is shown in figure 3. 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 highest heating duration 30 s, tempering further reduced the mold load by 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 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 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., (2017) reported that IR treatment of corn followed by tempering at 60^{o}C for 4 h resulted in 3.8 – 4.5 log mold reduction. Statistical analysis shows that intensity had a significant effect on the sample mean load

250 (P<0.05). On the other hand, heating duration and intensity*heating duration interaction effects
251 do not have significant effect on the sample mean load (P<0.05).

252 **3.2 Aerobic Plate Count (APC)**

253 The effect of IR treatment on the APC of the samples is shown in figure 4. The control samples
254 had initial APC of 7.44 log CFU/g. The IR treatment showed low efficiency in deactivating
255 bacteria on the samples when compared to its effect on mold decontamination. Like the result of
256 mold load, the highest intensity (15.71 kW/m^2) at the highest heating duration (30 s) showed the
257 maximum reduction; it brought the APC of the sample to 6.35 log CFU/g i.e. reduction of 1.09
258 log CFU/g. Other IR treatment combinations showed less reduction in the APC of the samples.
259 Statistical analysis showed that the intensities of 15.71 kW/m^2 and 10.08 kW/m^2 had significant
260 effects on the mean APC of the treated samples. The low reduction in APC by IR heating could
261 be as a result of presence of heat resistant bacterial spores in the rough rice samples. A similar
262 result was found by Staack et al., (2008), where they reported that IR heating resulted in a
263 maximum reduction of 1 log CFU/g. In addition, Bingol et al., (2011) reported a very low
264 reduction (0.62 ± 0.18) in the bacterial load when almond was treated using IR treatment.
265 Mackey and Derric (1986) reported that the heat resistance of bacteria increased when bacteria
266 are heated to elevated temperatures for a relatively short period of time. Full factor factorial
267 analysis showed that the effect intensity, heating duration, and intensity*heating duration
268 interaction effect all had significant effect on mean APC of the sample (P<0.05)

269 Figure 5 shows the effect of the tempering step incorporated into the IR heating treatment on the
270 APC of the samples. Tempering caused a further reduction in the APC when compared to the
271 samples treated without tempering. For instance, tempering the samples that were treated at
272 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 was treated at intensity 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 significant effects to produce significant reductions in the mean bacterial load. Statistical analysis showed that heating duration did not have significant effects on the mean APC of the samples but tempering and intensities showed significant effects ($p<0.05$).

Many microbial important cellular components including cell wall, DNA, RNA, ribosome, and proteins are destroyed by IR heat thereby leading to microbial inactivation (Nguyen, Corry, & Miles, 2006; Krishnamurthy et al., 2008). The most affected microbial cellular components affected by IR heat is protein; cellular proteins of both mold and bacteria are easily denatured by IR heat. Infrared heat denatures microbial cellular components in the order of protein—RNA—cell wall—DNA (Krishnamurthy et al., 2008; Hamanaka et al., 2000).

4.0 Conclusion

Rice is one of the largest agricultural commodities in the world. This fact makes food safety and quality assurance of rice an uptmost importance. The well-being of the worlds rice crop has a ripple effect on many other markets and has the ability to directly devastate entire communities, cultures and farmers. The development of a technology with the capability to simulatniously dry and decontaminate rice that will be stored for long periods of time is vital. This study demonstrated that the selective IR treatment was effective in inactivating microbes (mold and bacteria) on rough rice. However, incorporating a tempering step led to the further microbial inactivation. Thus, compared with only IR treatment, combining IR heating and tempering was more effective in inactivating both mold and bacteria on rough rice. The treatment combinations used in this study were more effective on mold inactivation than bacteria. Therefore, a longer heating duration may be required to further reduce the bacteria load on rough rice. This study did

a broad testing for fungi and bacteria, it did not test to identify the specific fungi and bacteria that were present on the rice. For further study purposes, the findings in this work should be extended to inactivating aflatoxin producing mold – *Aspergillus flavus* – in order to prevent the production and accumulation of aflatoxin on rice. In addition, the implication of the studied treatments on the milled rice yields and quality characteristics should be evaluated.

Since rice industries and farmers are in need of a safe, cost effective as well as environmentally and consumer friendly drying process, the results gotten from studying the impact of the treatment combinations used in this study on rice quality parameters will provide supplement information needed to scale up this novel technology (selected IR equipment).

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Tables and figures

Table 1: Experiment design of different combination of infrared (IR) parameters

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

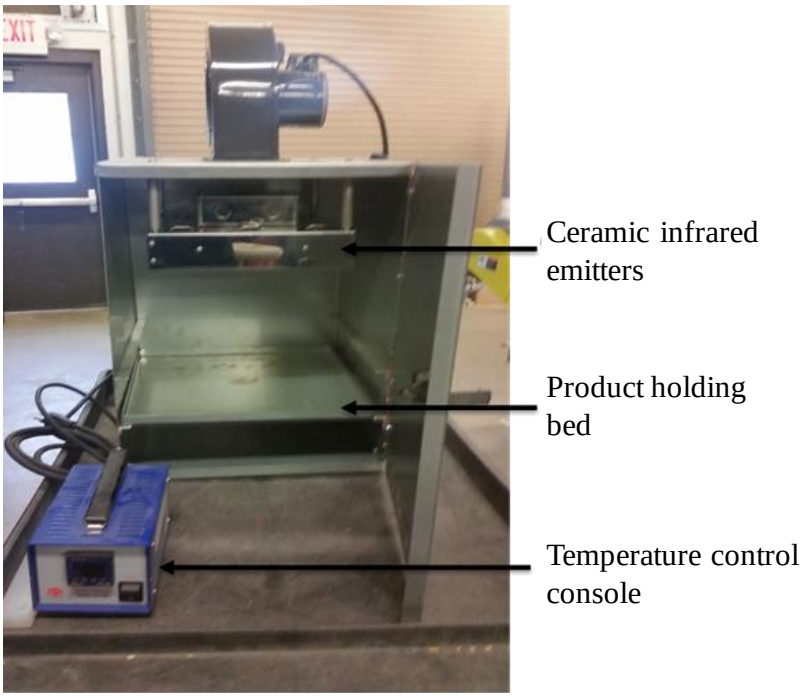

Figure 1: A newly-designed and built equipment for selective infrared heating

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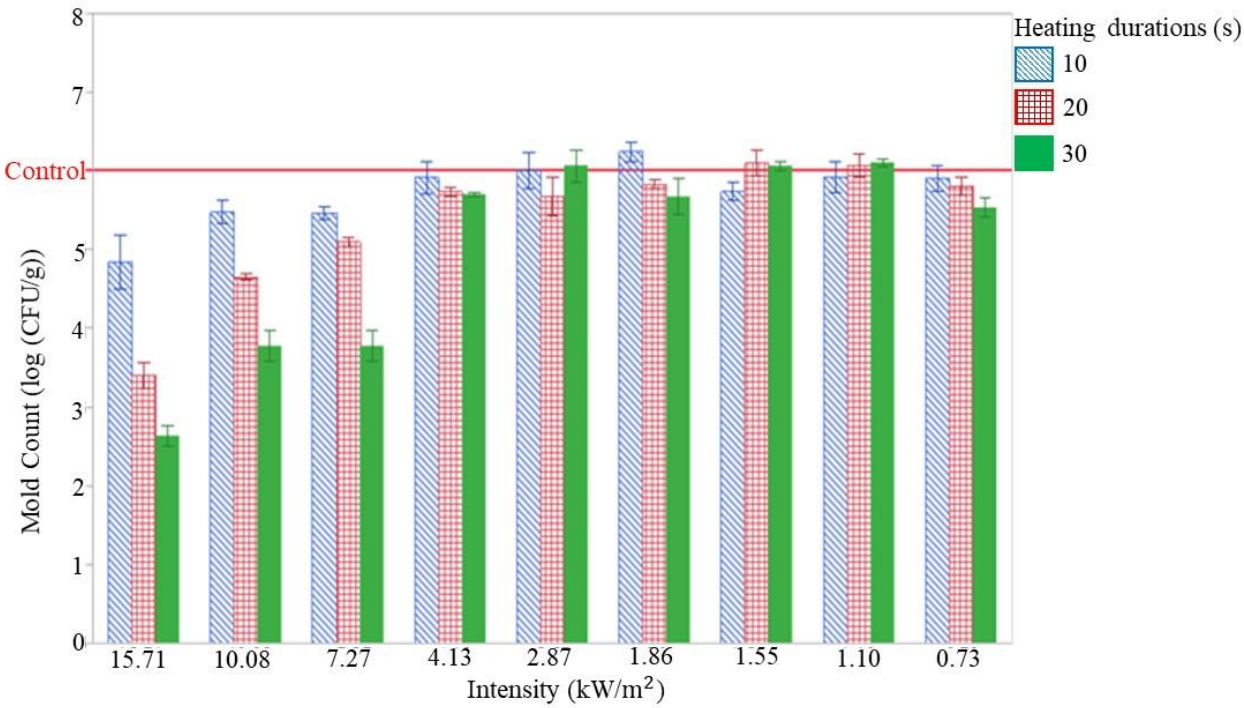


384

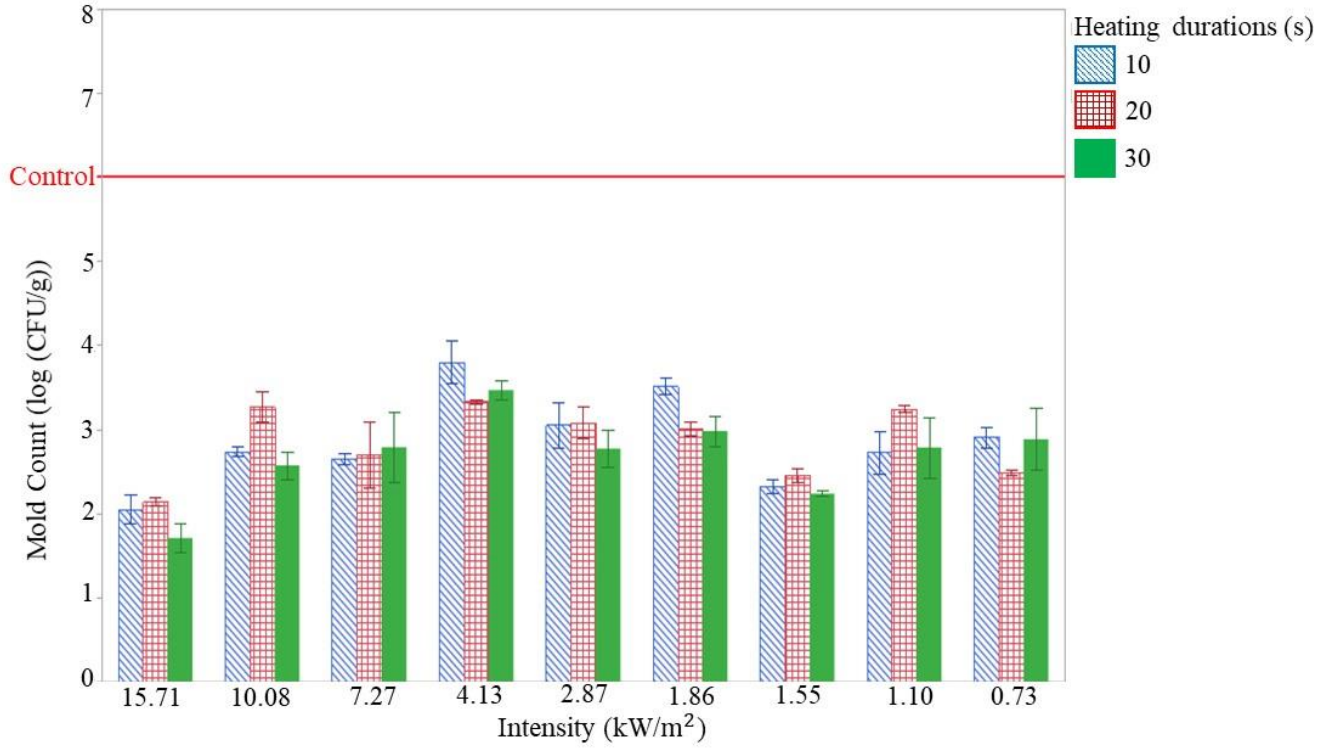
385 Figure 2: The effect of infrared heat treatment at different intensities for different heating

386 durations of 10, 20, and 30 s on the mold count on the rough rice samples; CFU signifies colony

387 forming unit.

388

389



390

391 Figure 3: The effect of infrared heat treatment at different intensities for different heating

392 durations of 10, 20, and 30 s followed by tempering at 60°C for 4 hours on the mold count on the

393 rough rice samples; CFU signifies colony forming unit.

394

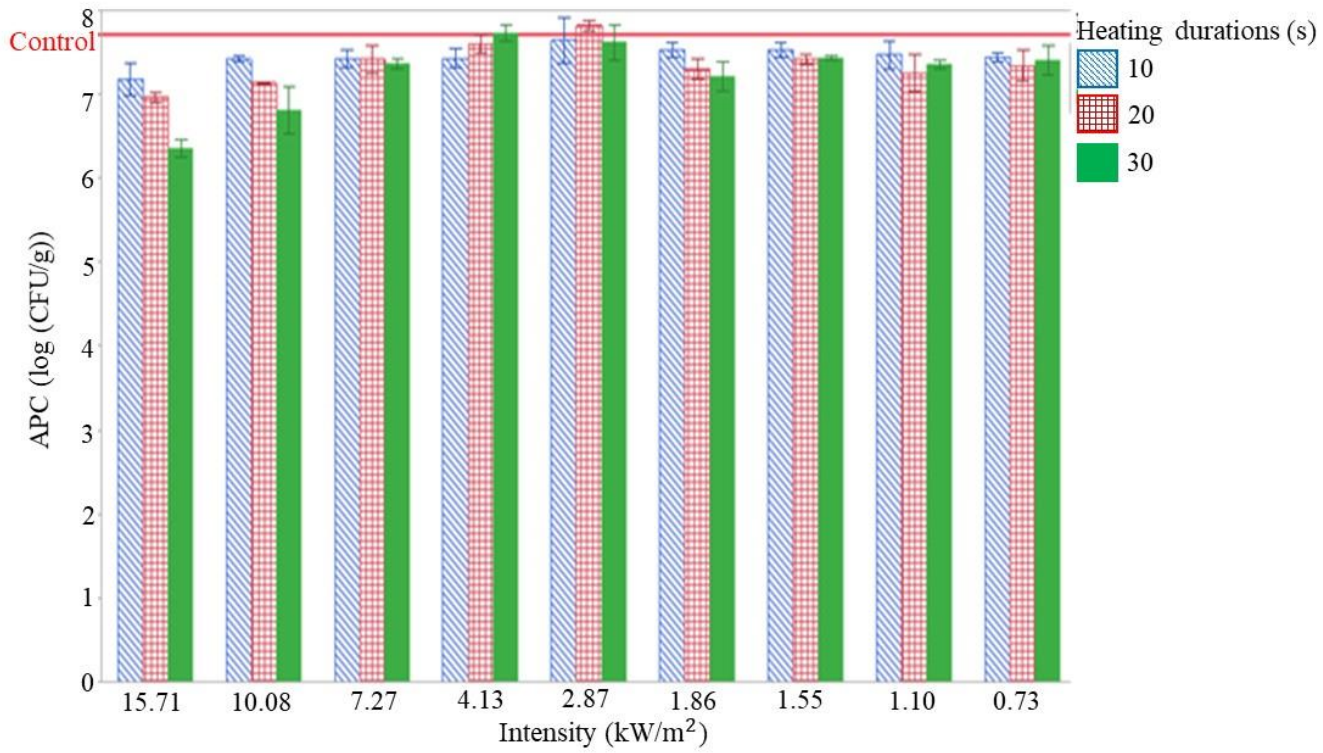


Figure 4: The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s on the aerobic plate count on the rough rice samples; APC signifies aerobic plate count; CFU signifies colony forming unit.

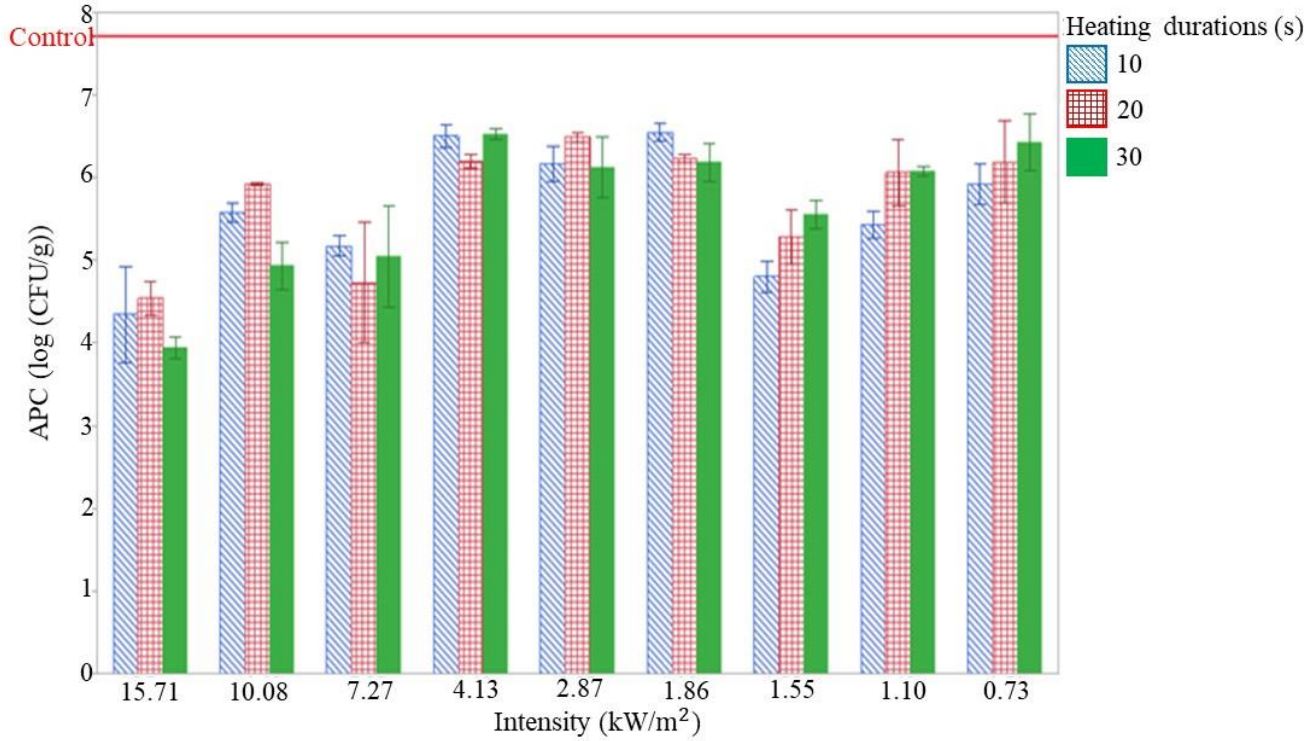


Figure 5: The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s followed by tempering at 60°C for 4 hours on the aerobic plate count on the rough rice samples; APC signifies aerobic plate count; CFU signifies colony forming unit.