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EFFECT OF HIGH-POWER SHORT-TIME MICROWAVE HEATING ON INACTIVATION OF MICROBES ON ROUGH RICE

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ABSTRACT.
Maintaining the safety of rice has become a priority in the food industry, especially due to rice's gluten-free label and increasing utilization as flour or ingredient for formulating various products. The objective of this study was to investigate the effectiveness of using high-power, short-time treatment of rough rice with microwaves propagated at 915 MHz frequency to inactive microbes on rice without compromising its quality attributes. Freshly-harvested, high moisture content (MC) rough rice at 21% (wet basis) and 20 mm bed thickness was exposed to microwave at powers of 16, 18, and 20 kW, for various durations (1, 2, and 3 minutes). Standard procedures were used to determine microbial load on treated and untreated samples. Serial dilutions of both treated and control samples were carried out and transferred to 3M petrifilms for the total aerobic count (TAC) and total fungal count (TFC) enumeration. The 3M petrifilms were incubated at 30°C for 2 days and 25°C for 5 days for TAC and TFC, respectively. The comparison of the enumeration showed that samples treated with the highest microwave power (20 kW) over the longest exposure durations (3 minutes) had the highest reductions in TAC and TFC - up to 1.21 cfu/g and 5.01 cfu/g log reductions in TAC and TFC, respectively. A separate study reports the impact of these treatments on quality characteristics of rice. Meanwhile, the results from this study suggest that there is potential for using high microwave powers with exposure duration of only 3 min to achieve significant inactivation of microorganism on rough rice.

Keywords.

High- power Short-time treatment, Microwave treatment, Microbial inactivation, Rough rice

Introduction

There has been an increased demand for diversity in the food market driven by consumers thriving for a healthy lifestyle, which has impelled the development of new rice products. Gluten free rice flour has escalated a new range of interest in the food industry for its nutritional properties. Formulations designed using the rice flour combined with other ingredients show potential benefits to individuals on gluten-restricted diets (Wesley et al., 2021). Even though rice is regarded as a low moisture grain, it still is prone to contamination which results in its spoilage. Low moisture foods are presumed to be safe from microbial invasion owing that they have low water activity of less than 0.7. (Blessington et al.,2013; Rifna et al., 2019) Also, the presence of these natural contaminants may render the grains unsafe for consumption, because they can produce a range of potentially toxic substances such as aflatoxins that are of most concern on health. The current understanding of the main abiotic factors found to deteriorate rice quality during storage are temperature and moisture (Atungulu et al., 2016). Toxigenic fungi, *Aspergillus flavus* have been identified as the most common observed microbe capable of colonizing the surface of rice grain (Moreau et al., 2011). Rice is one of the most consumed staple foods in the world. Typically, rice is preferably consumed in white rice form as a source of starch, especially in Asian countries (Lai et al., 2015). When rice is harvested at initial moisture contents (MC) of 23-24%, it is immediately dried to about 13-14% MC (w.b), this facilitates maintenance of the optimal quality during long-term storage (Atungulu et al., 2016). Moreover, the crop is harvested seasonally, it is therefore imperative that the post-harvest processes such as drying, storage, and processing are critical to ensure its safety and availability in the future (Coradi et al., 2022).

Nowadays, the advancement of techniques used to exterminate microbes have endorsed safety of food products. Both chemical and thermal technologies have shown to be effective. However, use of chemical treatments like ozone, chlorine, ethylene oxide that require fumigation have been banned in European Union and the United States because of environmental and health concerns (Shirkole et al., 2021). Whereas non-thermal technologies like pulsed light, high-pressure processing, cold plasma require high treatment intensities to achieve substantial microbial load reduction (Deng et al., 2019). On the other hand, conventional thermal treatments use high heat to denature microbial DNA but at the expense of affecting nutritional and physicochemical properties of products such as loss of desirable bioactive and antioxidant compounds (Rifna et al., 2019). In search of other alternative technologies that could decontaminate and preserve nutritional and sensory properties led to introduction of new technologies such as microwave heating, infrared heating, ionizing radiation and ultra violet rays.

Microwave heating as a drying technique of rough rice has been on the surge especially after (Atungulu et al., 2016) reported that the technology could achieve one pass drying at specific energies of around 600 kJ/kg followed by tempering resulted

in a significant reduction of MC to a final MC of 14% to 16%. Microwave is a type of electromagnetic radiation with a wavelength that falls between infrared and ultra-short waves. The mechanism of action of microwave, which is traced to the heating impact of radiation that causes damage to microbial cell wall, genomic DNA damage, and agglomeration of the cytoplasmic proteins leading to gradual death of the microorganism (Skowron et al., 2022). Microwave has been severally used as an effective treatment for microbial decontamination in food products (Eliasson, Isaksson, Lövenklev, & Ahrne, 2015; Jeevitha, Sowbhagya, & Hebbar,2016). With no doubt, it is a promising technology, however numerous researches reported that the treatment is dependent on attributes like microwave power, temperatures and treatment time.

Previous studies that dealt with use of microwave as a drying and decontamination method employed frequency of 2450 MHz (Kaasova, Kadlec, Bubnik Hubackova, & Prihoda, 2002; Liu, Jiaqiang, Ma, & Xie 2016; Vadivambal & Jayas 2007) and reported that there are limitations of the frequency to penetrate thick grain bed (Kumar, 2015). Conversely, microwave set to operate at 915 MHz has a higher large-scale decontamination potential due to its penetration ability that is three times greater than 2450 MHz (Smith et al., 2018).

The specific objectives designed for this study was to investigate the effect of high-power microwave heating at 915 MHz on rough rice harvested at initial MC of 21% (w.b) to inactivate aerobic bacteria and fungi at different treatment durations and powers. The data obtained will be useful to optimize drying conditions and determine optimal powers settings and durations for treatment to improve the efficiency of the technology for microbial reduction.

Materials and methods

Rice Samples

Freshly harvested long grain rice samples, variety RT 7321 was used in this study. The initial MC of the rough rice was 21% (wet basis). The obtained samples were cleaned using a dockage equipment (MCi Kicker Dockage Tester, Mid-Continent industries Inc, Newton, KS, USA). The equipment used appropriate sieves to sort out weed, chaff, grain and any other foreign material other than the rice grain. The cleaned rice was placed in tubs and stored in laboratory cold room set at 4°C. Prior to the beginning of the experiments, all the samples were obtained from the cold room and allowed to equilibrate with room conditions 25°C until period of use. The MCs (wet basis) of the samples were determined using an AM 5200 Grain Moisture Tester (PERTEN Instruments, Kurva Sweden).

Microwave equipment and treatments

An industrial MW system (AMTek, Applied Microwaves Technology Inc.., Cedar Rapids IW, USA) was used in this study.

The system consists of a transmitter, a waveguide and a MW heating zone (oven), which was set to operate at a frequency of 915 MHz. The system transmitter, a high-powered vacuum tube which operates as a self -excited oscillator. It is used to convert high voltage electric energy to MW radiation. The waveguide consists of a rectangular metal tube through which electromagnetic field propagates lengthwise. It is used to couple MW power from the magnetron into the laboratory oven, which then provides uniform temperatures throughout while in use. The laboratory oven is the internal cavity of the MW. The implication of MW heat intensity and heating duration on the microbial load reduction for the microbial load reduction for rice bed thickness at 20mm were studied. A sample of 3 kg rough rice was weighed and placed into MW safe trays for the treatment. The outside of the trays were made with polypropylene with Teflon coated fiberglass mesh at the bottom of the samples. The trays containing the rice sample were then placed in the oven on the belt and treated at various power levels and duration. The specific energy (kJ/kg- rough rice) was determined based on the MW power (kW), the treatment duration (min) and loading mass (kg) of the treated sample.

After the treatment, the rice samples were separated by layer and then transferred immediately to jars and sealed airtight. A HOBO sensor (Onset Computer Corporation, Bourne, MA, USA) was placed in the jars to determine the changes in temperature and relative humidity inside the jars. The jars were placed in an environmental chamber (Platinous chamber, ESPEC North America, Inc. Hudsonville, MI, USA) set at a temperature of 25°C and relative humidity of 65%. The samples were allowed to cool naturally to 25°C. After cooling, the MC was determined using AM 5200 Grain Moisture Tester (PERTEN Instruments) and then 10 g of the treated sample was taken out for microbial analysis. Control samples were not treated with MW, but gently dried to a MC of 12.5% in an EMC chamber (Platinous chamber, ESPEC North America, Inc) set at a temperature of 25°C and relative humidity of 65%.

Table 1. Experimental design

Factor	Levels	Number of experiments
Power level, kW	16 kW, 18 kW, 20 kW	× 3
Duration (min)	1 min, 2 min, 3 min	× 3
Replication	1, 2, 3	× 3

Microbial Enumeration

The microbial enumeration was carried out using standard procedures to determine total microbial load. Phosphate buffered-dilution water (0.5M, Ph= 7.2) was prepared, then autoclaved at 121°C for sterilization. A 10 g sample of the rough 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) set at 240 s and 0.7 stroke/s was used to dislodge the microorganism by ensuring that the rough rice samples were pulverized into powder for microbial 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 fungal 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 safely placed in the biosafety cabinet. The top film of the plate was carefully lifted and a P1000 micropipette (Finnpippete F2, Thermo Fisher Scientific, Inc, Vantaa, Finland), placed perpendicularly to the plates, was used to transfer 1 mL of the sample solutions onto the center of the two 3M Petrifilm plates (i.e., fungi 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 Petri plate. The gel was allowed to solidify for 1 min. The inoculated Petrifilm plates with clear sides up were stacked to maximum of 20 units and incubated. The Petrifilm fungal count plates and aerobic count plates were placed in an incubator (Thelco Model 4, Precision Scientific Instrument, Inc.) at 25°C for 120 hrs and 35°C for 48hrs respectively, before counting. After the incubation periods, the colony forming units (CFU) on each plate were counted. Mold colonies on the plate appeared blue, black, yellow or green in color while bacteria colonies on the plates appeared red with a regular shape.

The colony forming units per gram of rice (CFU/g) for each sample was obtained using the equation

$$T_{cfu} = \frac{P_{cfu}}{D_r}$$

where

T_{cfu} = total colony forming units per gram of rice (CFU/g)

P_{cfu} = colony forming units counted on plate per gram of rough rice

D_r = dilution rate 10^{-1} to 10^{-6} times

Results and discussions

Effect of increasing Microwave power on microbial loads

The microbial analysis of aerobic bacteria colonies on petrifilm appeared red in color whereas the yeast colonies appeared blue-green with defined edges. The mold color varied from brown, beige, orange and blue green with diffusive edges. The initial population mean of aerobic bacteria and fungi in control samples were 6.67 CFU/g and 7.19 CFU/g with standard deviations of 0.34 and 0.2 respectively. Atungulu, Zhong, Thote, Okeyo, and Couch (2015) conducted a study in 2013 and 2014 to investigate microbial growth kinetics on freshly harvested rough rice, different cultivars, from different locations in

USA and Arkansas and reported average levels of Aerobic bacteria and fungi plate count to be 8.19 and 7.75 log CFU/g respectively. Statistical analysis performed showed that the effects of the different treatments on the microbial load to be statistically significant ($p < .001$) **Tables 2 and 3** . Increasing the microwave power (16 kW, 18 kW and 20kW) on the rice sample resulted in significant reduction of both aerobic bacterial and fungal load as depicted on **Table 4**. The least aerobic bacterial and fungal reduction were seen at power levels of 16 kW and had least square means of 0.55 log CFU/g and 2.63 log CFU/g reduction and standard errors of 0.059and 0.054, respectively. The low reduction of aerobic bacteria could be attributed to heat resistance of predominant bacteria and bacterial spores to degradation. Bacterial contaminants formed biofilms around tofu coagulates which significantly increased their heat tolerance to microwave treatment (Zhao et al., 2021). Through another work performed by Smith and Atungulu (2018) with a 915 MHz frequency at power level of (5, 10 and 15 kW) discovered that rice bed thickness was a crucial parameter in the response of aerobic bacteria while power was the most significant for the fungal response. In their study, for different rice bed thicknesses (5, 10, and 15 cm) resulted in higher aerobic counts at the top layers than in the bottom layers.

Table 2. Summary table showing effects of microwave power, heating duration on aerobic bacteria reduction response (LOG CFU/g)

Source	Log worth	*p* value
Power (kW)	1.101	0.0001
Duration (min)	2.098	<.0001
Power level, kW*Duration	0.359	0.0636

Table 3. Summary table showing effects of microwave power, heating duration on fungi reduction response (LOG CFU/g)

Source	Log worth	*p* value
Power (kW)	3.909	<.0001
Duration (min)	65.186	<.0001
Power level, kW*Duration	2.428	<.0001

Effect of increasing heating durations on microbial load

The effects of increasing heating duration were found to be insignificant (p=0.0636) when combined with microwave power for aerobic plate count. Fungi, at large had the greatest load reduction than aerobic bacteria on treatment with the highest MW intensities and heating durations. At 20 kW MW power level with a 3 minutes heating resulted in only a 1.2 log CFU/g reduction with a standard error of 0.104 in aerobic bacteria. Conversely, this was not the case for fungal load response, this is to say that there was a significant interaction effect (p< 0.0001) on the two factors which led to least square mean of 5.01 log CFU/g reduction with a 0.094 standard error. Similar outcome was reported by Smith and Atungulu (2017) on microbial response to microwave set at 915 MHz where they discovered heating duration was insignificant with no quadratic effects

Table 4. Effect of increasing microwave power levels and heating durations on aerobic bacteria and fungal reduction response

	Aerobic bacteria log reduction count (Log CFU/g)			Fungal log reduction count (Log CFU/g)		
Heating duration (min)	1	2	3	1	2	3
Microwave Power level						
16	0.21 ± 0.104^{c}	0.75± 0.104^{b}	0.673±0.104^{b}	1.022±0.094^{e}	2.84± 0.094^{d}	4.02±0.094^{c}
18	0.24±0.104^{c}	1.22±0.104^{a}	0.66±0.104^{b}	1.00±0.094^{e}	4.13±0.094^{c}	4.86±0.094^{a}
20	0.70±0.104^{b}	1.18±0.104^{a}	1.21±0.104^{a}	0.99±0.094^{e}	4.52±0.094^{b}	5.01±0.094^{a}

Mean ± SE followed by different letters are significantly different (p < .05)

Table 3 shows the combination effect of increasing microwave power levels and heating duration with least square means log reductions for aerobic and fungal response. It can be inferred that the use of microwave treatment for shorter durations may not guarantee inactivation of aerobic bacteria. Eliasson, L., Isaksson, S., Lövenklev, M., & Ahrné, L. (2015) evaluated microbial load after MW treatment at a power level of 650 W for 20 minutes and reported a 4.8 log reduction which was attributed to the high exposure durations.

Conclusions

The results demonstrate that increasing microwave powers resulted in a significant microbial load reduction. Control rice

samples had a higher microbial population count than samples that were treated with microwave. The selected 915 MHz MW had an impact on both fungal and aerobic bacterial counts. Effects of increasing heating durations resulted to significant reduction (<.0001) in fungal counts. On the contrary, there was no significant reduction in aerobic counts (p= 0.06). The use of microwave treatment can be a promising technology for microbial decontamination in rice. This may help to reduce toxins-related problems associated with the occurrence of these microbes in rice. In addition to providing safety, it also preserves nutritional qualities by eradicating contributing factors that may lead to quality deterioration. More studies on modelling analyses are still needed to establish optimal MW settings for maximum microbial inactivation of aerobic bacterial and fungal species.

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