Journal of Stored Products Research 69 (2016) 265–271

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

# Hot air-assisted radio frequency heating effects on wheat and corn seeds: Quality change and fungi inhibition

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ARTICLE INFO

*Article history:*
Received 23 September 2016
Accepted 24 September 2016
Available online 13 October 2016

*Keywords:*
Hot air-assisted radio frequency (RF)
Fungi
Seed vigor
Germination rate
Enzyme activity

ABSTRACT

Hot air-assisted radio frequency (RF) heating has been proposed and studied as an alternative disinfestation method for grain seeds. In this study, hot air-assisted RF heating effects on fungi inhibition and its influence on physiological (seed vigor, germination rate) and biochemical (color, enzyme activity) qualities of wheat and corn seeds were further investigated. Results showed that high intensity hot air-assisted RF treatment (70 °C, 10 min) reduced seed vigor and germination rate for both wheat and corn seeds, but relatively mild intensity treatment (65 °C, 10 min) showed no significant influence on color and germination rate, and even enhanced enzyme activity (Superoxide dismutase-SOD, catalase-CAT and peroxidase-POD). Moreover, hot air-assisted RF treatment (65 °C, 10 min) reduced *Aspergillus flavus* by 2 and 3 log-scale (CUF/g) in wheat and corn seeds with the moisture content of 12.0%, and 3 and 4 log-scale for wheat and corn seeds when moisture content increased to 15.0%, respectively. These results indicated higher moisture content ($a_w$) of grain seeds would benefit fungi inhibition for hot air-assisted RF treatment, and with proper control of treatment conditions, hot air-assisted RF heating has the potential to inhibit fungi and maintain physiological and biochemical quality of grain seeds.



## 1. Introduction

Wheat and corn are two of the most important and principal food crops in the world. Long-term storage of grain seeds always accompanies with insects invade, which would result in significant quality and economic loss. Many methods have been studied to control the insects inside the grain seeds, including conventional thermal treatment, chemical fumigation, UV and irradiation (Azin et al., 2006). Conventional thermal treatment can kill insects by properly controlling temperature and time, but energy efficiency is low and it could also affect the quality of the food crops (Feng et al., 2004). Chemical fumigation has advantages of low cost, easy operation and huge production, but negative impact to environment and human health, and many of the chemical fumigants have been banned in lots of countries (Wang and Tang, 2001). Irradiation is an effective method, such as X-ray and γ-ray, but its application is limited by radiation source and consumers' acceptance (Huang et al., 2010). Radio frequency (RF) heating is a novel thermal treatment method at a frequency range from 3 kHz to 300 MHz. It generates heat inside the food products by friction of molecules dominated by ionic conduction and dipole rotation, thus heating dielectric materials rapidly and volumetrically. Hot air-assisted RF heating has been applied in controlling insects in legumes (lentils and peas) without obvious quality degradation of the product (Jiao et al., 2012). Shrestha and Baik (2013) studied the feasibility of hot air-assisted RF heating for controlling insects in wheat, and Shrestha et al. (2013) also investigated hot air-assisted RF heating effects on insects (*Cryptolestes ferrungineus* S.) in wheat at different moisture contents. More recently, Zhou and Wang (2016a, b) developed industrial-scale RF treatment protocol to control *Sitophilus oryzae* in rough, brown, and milled rice with heating uniformity and insects mortality study. All those studies indicated that hot air-assisted RF heating is an effective disinfestation method for low moisture grain seeds.

Except insects invade, another problem associated with grain seeds storage is the putrefactive spoilage caused by fungi, especially in some areas with high relative humidity. Some of the fungi can produce mycotoxin which would not only cause serious quality loss, but also food safety concern when grains are used as semi-prepared food (Lukšienė et al., 2007). Several novel methods including low pressure cold plasma (Selcuk et al., 2008; Suhem et al., 2013) and gamma radiation (Maity et al., 2011) have been

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http://dx.doi.org/10.1016/j.jspr.2016.09.005
0022-474X/

studied to inhibit mold such as *Aspergillus flavus,* a common and typical fungi in most grain seeds. Hot air-assisted RF treatment has also been evaluated to extend the shelf life of pre-packaged bread loaf by inhibition of *P. citinum* spores (Liu et al., 2011). In addition, hot air-assisted RF heating has been studied as a pasteurization method to control pathogens in low-moisture food products, such as almonds (Gao et al., 2011). All those studies indicated the great potential of using hot air-assisted RF treatment to control microorganisms and extend the storage time of low moisture agricultural products.

Conventional methods used for inactivating molds on seeds are not always effective or ecologically and environmentally friendly. A highly successful grain seeds decontamination treatment must be able to inhibit fungi and preserve product quality at the same time. Very few studies had systematically investigated the grain seeds quality changes under different hot air-assisted RF treatments, especially for physiological properties. Wu and Feng (2011) reported that microwave heat strikes could enhance germination rate and vigor of soybean seeds by suppressing electrolyte leakage and increasing soluble sugar content and peroxide enzyme activity, whether or not RF treatments have similar effect on grain seeds obviously needs solid evidence.

Therefore, the objectives of this study were (1) to investigate the influence of hot air-assisted RF treatments on the physiological and biochemical properties of wheat and corn seeds, (2) to further evaluate the effects of hot air-assisted RF treatments on fungi (*Aspergillus flavus*) inhibition in wheat and corn seeds.

## 2. Materials and methods

### *2.1. Hot air-assisted RF heating system*

A 12 kW, 27 MHz pilot scale RF unit ($310 \times 100 \times 165$ cm$^3$) with a built-in hot air heating system and an imbedded conveyor belt system (GJD-6A-27-JY, HuashiJiyuan Co. Ltd., Hebei, China) was used in this study. The RF system had two parallel electrodes, top electrode (75 L × 55 W cm$^2$) position can be adjusted to obtain different RF heating rate, and the gap between the two parallel electrodes was fixed at 10 cm in this study to obtain better heating rate and uniformity based on our preliminary experimental results. The RF system has the similar configuration with that described by Wang et al. (2010) and more information related the RF system can be found elsewhere (Jiao et al., 2015). Fiber optic sensor (ThermAgile-RD Optsensor, Xi'an Heqiguangdian Co. Ltd., Shanxi, China) with 6 channels was used to monitor the real-time temperature changes during RF heating process.

### *2.2. Hot air-assisted RF treatment of seeds and seeds quality evaluation*

#### *2.2.1. Hot air-assisted RF treatments*

Wheat and corn seeds were purchased from a local seed station in Anhui Province, China. Moisture content of the seeds was determined using the oven drying method. Briefly, 2–3 g of ground seeds were placed in an aluminum dish and dried in an oven (GZX-9240 MBE, Shanghai Boxunshiye Co. Ltd., Shanghai, China) at 130 °C for 1 h (AOAC, 2002). The initial moisture content was 11.4 ± 0.2% (w. b.) for wheat seeds, and 12.7 ± 0.1% for corn seeds. Grain seeds (900 g) were placed into polypropylene (PP) plastic container in cuboid shape (16.0 L × 10.5 W × 6.8 H cm$^3$) with small holes on the side and bottom for hot air-assisted RF treatment, and the container with grain seeds was placed on the bottom electrode to obtain better RF heating uniformity based on our previous study (Jiao et al., 2015). To obtain conservative seeds quality changes results, fiber optic sensor was placed at the cold spot inside the container to record the temperature changes during hot air-assisted RF heating. The cold spot position was at the center of top layer (Jiao et al., 2015), and in practice the sensor was placed at about 1 cm below the top layer for easy operation. Based on literature review and our preliminary experiments, hot air-assisted RF treatment with the temperature higher than 70 °C would significantly reduce the grain seeds germination rate, therefore, hot air-assisted RF treatments at 60–70 °C and 5–30 min holding time were selected to study the associated biochemical and physiological quality changes. The built-in hot air system with different temperatures was used to hold the samples temperature constant after the samples were heated to the different target temperatures by RF energy. After holding process completed, samples were immediately cooled down to room temperature by spreading out the samples into thin layer and using forced ambient air to reduce the influence on samples quality. After that, the samples were collected for further quality evaluation. Grain seeds without hot air-assisted RF treatment were used as control.

#### *2.2.2. Electrical conductivity of seed leachates*

Electrical conductivity test has been widely accepted and used for evaluating seed vigor of many species due to its easy and fast measurement process, including wheat seeds (Hasan et al., 2013) and corn seeds (Li et al., 2007), therefore, in this study, the electrical conductivity of wheat and corn seeds soaking solution was determined first before and after hot air-assisted RF treatment to indicate seeds vigor changes. The test of electrical conductivity was carried out based on the method described by Resende et al. (2012). Three sub-samples of 50 grains were weighed for each grain type of seeds. The samples were placed in beakers contained DI water (water: seed = 5:1), covered with parafilm, and maintained at room temperature for 24 h. Subsequently, electrical conductivity of seed soaking solution was measured using a DDS-307A conductivity meter (INESA Scientific Instrument Co., Ltd, Shanghai, China), and the results were expressed as μS cm$^{-1}$ g$^{-1}$. Triplicate tests were performed for each sample.

#### *2.2.3. Enzyme activity determination*

Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were selected as typical enzymes for wheat and corn seeds, and their activities were measured to indicate and better understand seeds vigor. The control and treated grain seeds were grounded into powders. A 1.0 g of powder was extracted by homogenizing with 10 mL of pre-cold 50 mmol L$^{-1}$ PBS (pH = 7.8) in ice bath. The homogenate was centrifuged at 5000 rpm at 4 °C for 20 min and the supernatant was used as crude enzyme extract for quantification of enzyme activity. Three replications were carried out for each sample.

The activity of SOD was measured using an S311 SOD assay kit-WST (Dojindo Molecular Technologies Inc., Kumamoto, Japan) according to its protocol. One unit of the SOD activity was defined as the amount corresponding to 50% inhibition and expressed as U g$^{-1}$ sample. CAT activity was measured according to the method of Lu et al. (2014) with some modifications. The reaction mixture consisted of 1.5 mL of 50 mmol L$^{-1}$ PBS (pH = 7.8), 0.2 mL of crude enzyme extract, 1.0 mL of DI water, and 0.3 mL of 100 mmol L$^{-1}$ $H_2O_2$ as the substrate. The absorbance at 240 nm was measured for 4 min once every 30 s. One unit of the CAT activity was defined as the decrease in absorbance of 0.1 per min and expressed as U g$^{-1}$ sample. POD activity was quantified according to guaiacol color-developing method described by Wu et al. (2014). The reaction mixture included 0.2 mL of crude enzyme extract, 1 mL of 30 mM $H_2O_2$, and 2 mL of 5 mmol L$^{-1}$ guaiacol solution, and the final volume was brought up to 4 mL with PBS (pH = 7.8). The absorbance at 470 nm was measured for 3 min once every 30 s. One unit