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Verification of radio frequency pasteurization treatment for controlling *Aspergillus parasiticus* on corn grains

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ABSTRACT

Radio frequency (RF) has been proposed and tested to achieve a required anti-fungal efficacy on various food samples due to its advantage of deeper penetration depth and better heating uniformity. The purpose of this study was to validate applications of RF treatments for controlling *Aspergillus parasiticus* in corn while maintaining product quality. A pilot-scale, 27.12 MHz, 6 kW RF heating system together with hot air heating was used to rapidly pasteurize 3.0 kg corn samples. Results showed that the pasteurizing effect of RF heating on *Aspergillus parasiticus* increased with increasing heating temperature and holding time, and RF heating at 70 °C holding in hot air for at least 12 min resulted in 5–6 log reduction of *Aspergillus parasiticus* in corn samples with the moisture content of 15.0% w.b. Furthermore, thermal resistance of *Aspergillus parasiticus* decreased with increasing moisture content (MC) of corn samples. Quality (MC, water activity – a_w, protein, starch, ash, fat, fatty acid, color, electrical conductivity and germination rate) of RF treated corn met the required quality standard used in cereal industry. Therefore, RF treatments can provide an effective and rapid heating method to control *Aspergillus parasiticus* and maintain acceptable corn quality.

1. Introduction

Corn (*Zea mays* L.) is the primary food crop of China both in terms of planting area and total yield production (Wang et al., 2014). It often loses nutritional and economic value due to mold invasion during storage each year (FAO, 2007). *Aspergillus parasiticus* is commonly associated with stored corn grain, and it can produce aflatoxins, which are very toxic for animal and human health (Ellis et al., 1991; Tsai and Yu, 1999). Aflatoxins are difficult to remove because of their stable molecular structures. Therefore, it is of considerable importance to eliminate the *Aspergillus parasiticus* and similar molds before aflatoxins can be produced, ensuring corn safety and quality.

Several methods have been studied to eliminate *Aspergillus parasiticus* spores, such as ethylene (Gunterus et al., 2007), atmospheric pressure fluidized bed plasma reactor (Dasan et al., 2016), enzyme-linked immunosorption (Tsai and Yu, 1999), low pressure cold plasma (Basaran et al., 2008), low-dose microwave radiation (Fang et al., 2011), and irradiation (Kanapitsas et al., 2015). Although these methods can reduce surface fungal contamination, they often require either sophisticated equipment and expensive chemicals or drastic treatment conditions and may cause unacceptable detrimental effects to edible materials. Even more importantly, they may present health hazards and some methods may be impractical, costly, not completely effective, or time-consuming for the treatment of large quantities (Dasan et al., 2016; Gao et al., 2011; Huang et al., 2010; Jiao et al., 2016; Li et al., 2017; Zhou and Wang, 2016b). Considering the above mentioned disadvantages and problems, a new and rapid alternative decontamination method for the specific elimination of *Aspergillus parasiticus* in corn grains is urgently needed.

Radio frequency (RF) heating holds potential for pathogen control in agricultural commodities due to its rapid and volumetric heating with little quality loss (Hou et al., 2016; Marra et al., 2009). Many studies have explored the possibility of using RF energy to pasteurize produce and indicate that RF heating may achieve > 4 log reductions of target pathogens in agricultural commodities (Gao et al., 2011; Jeong and Kang, 2014; Jiao et al., 2016; Kim et al., 2012; Li et al., 2017; Schlisselberg et al., 2013). There are, however, few studies on determining the effective RF process parameters during heating and holding to validate the inactivation rate of targeted pathogens in corn grain. Since a RF treatment protocol has been developed for pasteurizing after improving RF heating uniformity in corn using hot air surface heating, moving, stirring and holding (Zheng et al., 2016), it is critical to evaluate the efficacy of pasteurization and quality changes of corn samples when using combination of RF treatment with hot air.

Inactivation kinetic data of target molds in corn are essential to the development of effective RF treatments. The Weibull model is a more accurate model than the first-order model and has been successfully used to describe thermal inactivation of microorganisms in various food (Buzrul and Alpas, 2007; Jin et al., 2011; van Boekel, 2002). Thermal resistance of *Aspergillus parasiticus* in the Weibull model has shown that after heating to 50, 55, 60, 65, 70 and 75 °C, about 21.4, 18.3, 17.5, 14.7, 14.0 and 13.9 min are needed, respectively, for holding to achieve 6-log reductions of spores (Jin et al., 2011). These

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ranges of final temperatures and holding times should be practical for RF control of the *Aspergillus parasiticus* in corn without negative effects on corn quality.

The objectives of this research were: (1) to determine the thermal resistance of *Aspergillus parasiticus* spores at 55, 60, 65 and 70 °C for the hold time of 14 min in RF heated corn samples with the moisture content of 15.0% w.b., (2) to evaluate the effects of RF treatment protocol on *Aspergillus parasiticus* inhibition with different holding times at 70 °C in corn samples with the MC of 15.0% w.b., (3) to determine effects of MC of 9.1%, 12.0%, 15.0%, and 17.9% w.b. in corn samples on reduction of *Aspergillus parasiticus* after RF treatments at 70 °C for 14 min, and (4) to evaluate the quality parameters (MC, a_w, protein, starch, ash, fat, color, fatty acid, electrical conductivity, and germination rate) of corns after RF treatments and during an accelerated storage.

2. Materials and methods

2.1. Materials

The strain of *Aspergillus parasiticus* (CICC 41000) used throughout this study was purchased from China Center of Industrial Culture Collection, Beijing, China. The spores were suspended in sterile 0.9% NaCl solution at a concentration of 10^7 CFU/g, and stored at a low temperature (4 °C) until use. Corn grain was obtained from a local famer's market in Yangling, Shaanxi, China. The average initial MC of corn samples was 9.1 ± 0.16% wet basis (w.b.). To explore the thermal resistance of *Aspergillus parasiticus* in RF heated corn with different moisture levels, other samples with MC of 12.0%, 15.0%, and 17.9% w.b. were prepared for the experiment. The initial samples were conditioned by direct addition of predetermined amount of distilled water to obtain the targeted MC (Wang et al., 2015; Zheng et al., 2016). Then all corn samples were sealed into polyethylene bags and stored at 4 ± 1 °C until testing. Before each test, corn samples were placed in an incubator (BSC-150, Boxun Industry & Commerce Co., Ltd., Shanghai, China) for 12 h at 25 ± 0.5 °C to equilibrate.

2.2. Hot air-assisted RF heating system

The heating process was conducted in a 6 kW, 27.12 MHz pilot-scale free running oscillator RF system (SO6B, Strayfield International, Wokingham, U.K.) together with a hot air system supplied by a 6 kW electric heater. The size of the parallel perforated electrode plates was 40 cm × 83 cm, and the top electrode was moved to change RF power coupled to the samples. The details of the RF and hot air systems can be found in Wang et al. (2010). The air distribution box under the bottom electrode provided a forced hot air flow into the RF cavity to achieve surface heating and maintain the sample temperature when the RF power was turned off.

Corn samples (125 g) were spread into a thin layer in a polypropylene container and exposed to ultraviolet light for 30 min before inoculation. Each preprocessed corn sample (25 g) was placed into a polyethylene bag (80 × 120 mm) and 1 ml of the inoculum was added. The bag was closed and then mixed thoroughly for 10 min. Then the inoculated corn was placed in a bio-safety hood (22 ± 2 °C) for 12 h for moisture equilibration. The inoculated corn samples were wrapped in paper (150 × 210 mm) and placed at five representative locations (1–5) of the polypropylene container (Fig. 1). One paper bag placed at room temperature served as an untreated control.

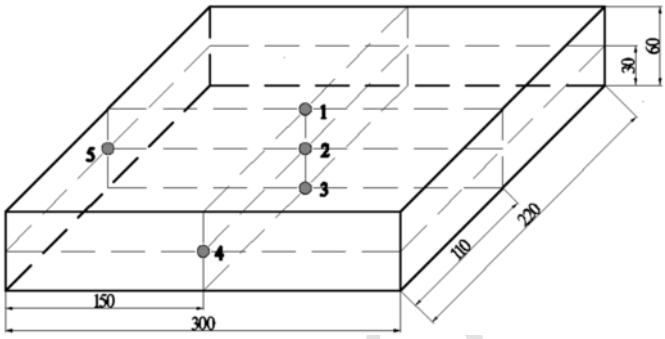

Fig. 1. Location of temperature measurement points in the corn samples during holding in the block shaped container (all dimensions are in mm).

2.3. Inoculation m

~~2.3.1.~~ RF treatment

~~2.3.1.1.~~ Effects of sample temperature on thermal resistance of *Aspergillus parasiticus*

According to the inactivation results of *Aspergillus parasiticus* spores in moldy maize by microwave processing (Jin et al., 2011), 55 °C, 60 °C, 65 °C, and 70 °C were selected as target temperatures for RF pasteurization. Corn samples (3.0 kg) with MC of 15.0% w.b. were filled into the polypropylene container, including 125 g inoculated corn samples. The polypropylene container was placed in the middle of two electrodes in the RF system, and the electrode gap was fixed at 11.0 cm based on the appropriated heating rate of corn achieved by RF energy. Based on previous experiments on determining RF heating uniformity in almonds (Gao et al., 2011; Li et al., 2017), chestnuts (Hou et al., 2014), corn (Jiao et al., 2016; Zheng et al., 2016), rice (Zhou et al., 2015), and wheat (Jiao et al., 2016), the cold spot was always located at the geometric center of the rectangular container. The RF system was turned off until the cold spot located at the position 2 (Fig. 1) of corn samples achieved the target temperatures, and hot air was circulated within the RF cavity to hold the target temperatures for 14 min. After RF treatments, inoculated samples were immediately placed in sealed plastic bags and put into ice water (≈ 4 °C, for at least 2 min) for cooling, ready for enumeration (Li et al., 2017). Each test was repeated three times.

~~2.3.1.2.~~ Effect of holding time on thermal resistance of *Aspergillus parasiticus*

Jin et al. (2011) proposed the minimum exposure time to achieve the desired target inactivation level of *Aspergillus parasiticus* was 14 min at 70 °C using a microwave heating system. By considering the heating non-uniformity of corn samples, the holding times of 4, 8, 12 and 16 min were chosen after RF heating to 70 °C. Corn samples (3.0 kg) with MC of 15.0% w.b. were heated from 25 °C to 70 °C, then the RF system was turned off and hot air was circulated within the RF cavity to hold the target temperature for 4 different times. After RF treatments, the corn samples were moved out and then placed in sealed plastic bags, and finally put into ice water (≈ 4 °C, for at least 2 min) for cooling until further analysis was performed. This process was repeated thrice.

2.3.1.3. Effect of MC of corn on thermal resistance of Aspergillus parasiticus

Water activity levels can affect thermal resistance of microorganisms, and the RF treatment protocols have been developed after improving RF heating uniformity in corn samples (Zheng et al., 2016). Therefore, effects on RF pasteurization of corn samples with MC of 9.1%, 12.0%, 15.0%, and 17.9% w.b. were determined. The whole treatment protocol developed for 3.0 kg corn samples (6 cm in depth) with 125 g inoculated corn samples consisted of RF heating under the suitable electrode gap as determined by Zheng et al. (2016) with hot air surface heating at 70 °C. Then the RF system was turned off and the corn samples were held at 70 °C hot air for 14 min. The inoculated samples were taken out for cooling after hot air-assisted RF treatments.

2.4. Enumeration method

After cooling, each RF treated corn sample (25 g) was immediately poured into a dilution bottle with 225 ml of sterile physiological saline (0.9% NaCl) to achieve a 10-fold dilution. Serial dilutions were performed and 1 ml of each one was mixed with plated onto PDA agar and incubated at 28 ± 2 °C for 4 d to obtain counts of *Aspergillus parasiticus*. The plate counts were expressed as CFU/g.

2.5. Corn quality analyses after conducting RF treatment protocol

Evaluating the physicochemical properties of agricultural products after disinfesting or pasteurizing process is important for evaluating the effectiveness of the RF treatment protocol. Before and after RF treatments, the quality of control and RF treated corn samples with 15.0% w.b. were evaluated immediately and after accelerated shelf life storage. In this study, MC, a_w, starch content, protein content, fat content, ash content, color, fatty acid content, electrical conductivity, and germination rate were chosen for quality analysis after pasteurizing using RF energy.

Corn samples were stored at 35 ± 0.5 °C with 68 ± 0.5% relative humidity (RH) for 17 d to simulate commercial storage at 10 °C for 1 year. The accelerated storage time at 35 °C was calculated based on a Q_{10} value of 3.41 for nutrition loss (Taoukis et al., 1997) and the relatively low temperature storage time (1 year) (Wang and Zhang, 2011). Similar accelerated tests were successfully used in various RF treated products (Gao et al., 2010; Hou et al., 2014; Jiao et al., 2012; Li et al., 2017; Ling et al., 2015; Wang et al., 2002; Wang et al., 2006; Zhang et al., 2016; Zheng et al., 2016; Zhou et al., 2015; Zhou and Wang, 2016a, 2016b), indicating that the accelerated test was reliable to simulate the real-time storage experiments. Control and RF treated samples (600 g) were packed individually in 5 bags and stored in the incubator and taken out from each treatment for quality analysis after 0, 4, 8, 12 and 17 d of storage. Each test was replicated three times.

The MC of corn samples was determined by using oven drying following the AOAC standard (AOAC, 2000a). Each aluminum dish containing about 5 g corn was placed in a vacuum oven (DZX-6020B, Nanrong Laboratory Equipment Co., Ltd., Shanghai, China) at 105 °C, and 103.1 kPa until a constant weight of samples was attained. Then the weight of corn samples was recorded at room temperature after cooling in a desiccator with $CaSO_4$. The MC was calculated by the weight loss between initial and final corn samples. Water activity of corn samples was measured by an Aqua Lab water activity meter (Model 4TE, Decagon Devices, Inc., Pullman, WA, USA) under ambient temperature (25 °C), and the values were read directly from the instrument panel.

The method of Kjeldahl following the AOAC standard (AOAC, 2005) was used for determining the nitrogen content, and the protein was calculated by the nitrogen content multiplying the conversion factor of 6.25 (Haros et al., 2003; Malumba et al., 2009). Starch content was detected by using the enzymatic hydrolysis method described by AOAC (2000b). Total ash content was determined by weighing the residual ash obtained by combustion in a Muffle furnace at 550 °C for 4 h. Fat content in corn was measured by the Soxhlet extraction method following the AOAC method (AOAC, 2006). The fatty acid content is frequently considered as a quality index during production storage, and was determined by the manual titration method following the National Standard of China (GB/T 20570-2015, 2015). All those quality values were estimated based on dry weight basis of the tested samples.

Color values of control and RF treated samples at 15.0% w.b. were measured by a computer vision system (CVS). A detail description of the CVS unit can be found in Hou et al. (2014) and Zhou et al. (2015). Based on the measurement procedures described by Zheng et al. (2016), corn flour (20 g) was placed in a Petri dish (8 cm diameter) for color images. Adobe Photoshop CS3 (Adobe Systems Inc., USA) was used to analyze the color values: lightness (L), redness-greenness (+ or − a) and yellowness-blueness (+ or − b), which were obtained from Photoshop and could be converted to CIE LAB (L*, a* and b*) values (Briones and Aguilera, 2005). The L* value changes from 0 to 100 represented dark to light. A higher positive a* and b* values indicated more redness and yellowness, respectively (Sandhu et al., 2007).

The electrical conductivity value was chosen to assess seed vigor of corn samples because of its easy and fast measurement process (Fessel et al., 2006; Viloria and Méndez-Natera, 2011). The methodology proposed by Cordovatellez and Burris (2002) for electrical conductivity determination was used in intact and non-deformed corn seeds of 150 samples. Three replications of 50 corn seeds were weighed and rinsed twice with distilled water, then washed with ultrapure water. Each prewashed corn sample was placed on filter paper to remove excess water, then soaked in a 100 ml stoppered conical flask containing 50 ml ultrapure water at 25 °C for 24 h. Electrical conductivity values both for control and RF treated samples were detected using a conductivity meter (DDSJ-308A, INESA Scientific Instrument Co., Ltd., Shanghai, China), and results were expressed in $\mu S\ cm^{-1}\ g^{-1}$.

The germination rate was measured as an important quality parameter of corn seeds according to the method of Govender et al. (2008) with slight modification. Due to the quantity limitation of the experiments, fifty-four corn seeds were randomly chosen from each sample. Three replicates of eighteen seeds were placed equidistantly on the sterilized Petri dishes (d = 86 mm) containing two sheets of moist germination paper. Other two sheets of moist germination papers were placed on the corn samples, and 5 mL of water was sprinkled over the paper daily to maintain the saturated environment for corn seeds (Jiao et al., 2016). Percentage germination rate was determined after incubated at 25 °C for 5–7 d. Three replications were carried out for corn seed germination tests, and results were presented as the percentage of seedlings that were germinated by the end of the test period.

2.6. Statistical analysis

Results were reported as mean values and standard deviations calculated from the three replicates for each treatment. Differences were estimated by the analysis of variance (ANOVA) followed by Tukey's test and considered significantly at $P \leq 0.05$. All statistical analyses

were performed using the statistical software SPSS 16.0 version (SPSS Inc., Chicago, IL, USA).

3. Result and discussion

3.1. Hot air-assisted RF heating temperature-time evolution

Fig. 2 shows the typical temperature-time evolution at the geometric center of polypropylene container for hot air-assisted RF heating of corn samples with electrode gap of 11.0 cm. The center temperature of corn samples achieved the target values by RF energy and then maintained a stable value by hot air. During holding, the temperature fluctuated in a narrow range around 0.5 °C. About 4.6 min, 5.5 min, 6.6 min and 7.5 min were needed to heat 3.0 kg corn samples with MC of 15.0% w.b. from 25 °C to 55 °C, 60 °C, 65 °C and 70 °C, respectively. The RF heating rates were relatively close to avoid the non-uniformity in corn samples and heat resistance of microorganisms being affected by heating rates (Huertas et al., 2016).

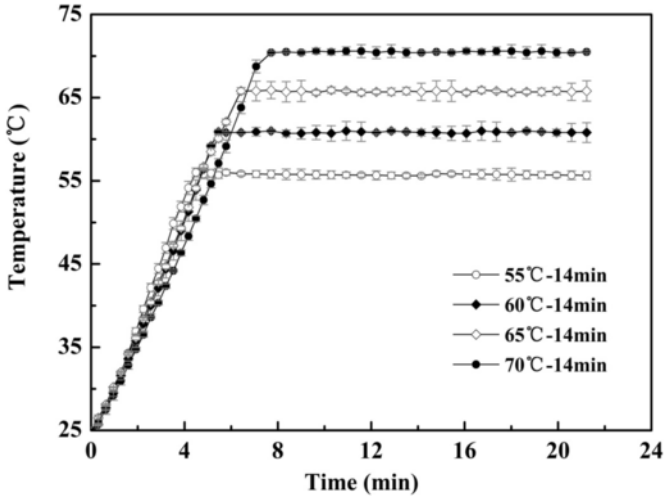

Fig. 2. Temperature–time evolution of hot air-assisted RF heating for corn samples in the center of polypropylene container (electrode gap = 11.0 cm).

3.2. Effect of temperature on thermal resistance of Aspergillus parasiticus

Table 1 shows the reduction of *Aspergillus parasiticus* under four different temperatures with holding time of 14 min. The thermal resistance of *Aspergillus parasiticus* was reduced with the rising temperature, with about 2-, 3-, 5-, and 6-log reductions at 55 °C, 60 °C, 65 °C, and 70 °C, respectively. The reductions of *Aspergillus parasiticus* in five locations were different at same heating temperatures, which may be a result of non-uniformity of RF treatments. The reduction *Aspergillus parasiticus* at positions 4 and 5 were more than that at other positions, which might be caused by RF overheating at the edge and corner of the container (Zheng et al., 2016). Similar phenomena were observed in RF treated chestnuts (Hou et al., 2015) and rough, brown, and milled rice (Zhou and Wang, 2016b).

3.3. Effect of holding time on thermal resistance of Aspergillus parasiticus

Table 2 shows the reductions of *Aspergillus parasiticus* under different holding times at 70 °C. The reduction data of *Aspergillus parasiticus* as affected by locations were similar to those in Table 1. After RF heating to 70 °C, viability of *Aspergillus parasiticus* spores decreased with increasing holding time, and 5–6 log reductions were achieved within 12–16 min. The pasteurizing effect of RF heating on *Aspergillus parasiticus* was higher during the early treatment period (4–8 min) than the later treatment period (12–16 min). Similar results were observed in low pressure cold plasma treated nut surfaces reported by Basaran et al. (2008). This was probably caused by the remaining spore cells adapting to certain stress conditions (van Boekel, 2002).

Table 1
Effect of hot air-assisted RF treatments with 4 temperatures and holding for 14 min on *Aspergillus parasiticus* spores (CFU/g) of corn samples with moisture content of 15.0% w.b. at 5 locations of the container.

Treatment	Locations	Temperature (°C)			
		55	60	65	70
Control		$(3.0 \pm 0.5) \times 10^7$	$(2.0 \pm 0.8) \times 10^7$	$(2.2 \pm 0.2) \times 10^7$	$(4.6 \pm 0.6) \times 10^7$
Hot air + RF	1	$(2.6 \pm 0.9) \times 10^5$	$(7.4 \pm 0.1) \times 10^4$	$(7.2 \pm 0.6) \times 10^2$	$(1.8 \pm 0.7) \times 10^1$
	2	$(3.2 \pm 0.8) \times 10^5$	$(8.1 \pm 0.9) \times 10^4$	$(8.9 \pm 0.5) \times 10^2$	$(2.4 \pm 0.6) \times 10^1$
	3	$(2.3 \pm 0.3) \times 10^5$	$(1.6 \pm 0.7) \times 10^4$	$(6.8 \pm 0.7) \times 10^2$	$(1.1 \pm 0.4) \times 10^1$
	4	$(1.9 \pm 0.5) \times 10^5$	$(6.8 \pm 0.5) \times 10^3$	$(4.7 \pm 0.4) \times 10^1$	–
	5	$(1.7 \pm 0.6) \times 10^5$	$(5.7 \pm 0.3) \times 10^3$	$(5.9 \pm 0.8) \times 10^1$	–

Table 2
Effect of hot air-assisted RF treatments with 70 °C for four holding times on *Aspergillus parasiticus* spores (CFU/g) of corn samples with moisture content of 15.0% w.b. at 5 locations of the container.

Treatment	Locations	Holding times (min)			
		4	8	12	16
Control		$(2.0 \pm 0.8) \times 10^7$	$(3.8 \pm 0.4) \times 10^7$	$(5.3 \pm 0.\ 6) \times 10^7$	$(4.5 \pm 0.9) \times 10^7$
Hot air + RF	1	$(7.2 \pm 0.2) \times 10^5$	$(4.5 \pm 0.7) \times 10^3$	$(3.4 \pm 0.5) \times 10^2$	$(2.0 \pm 0.2) \times 10^1$
	2	$(9.8 \pm 0.7) \times 10^5$	$(6.4 \pm 0.6) \times 10^3$	$(4.8 \pm 0.6) \times 10^2$	$(2.3 \pm 0.6) \times 10^1$
	3	$(4.4 \pm 0.8) \times 10^5$	$(4.3 \pm 0.3) \times 10^3$	$(2.3 \pm 0.4) \times 10^2$	$(1.9 \pm 0.6) \times 10^1$
	4	$(1.7 \pm 0.4) \times 10^5$	$(2.0 \pm 0.5) \times 10^2$	$(2.1 \pm 0.9) \times 10^1$	–
	5	$(1.1 \pm 0.9) \times 10^5$	$(1.9 \pm 0.3) \times 10^2$	$(1.3 \pm 0.6) \times 10^1$	–

3.4. Effect of RF treatment on Aspergillus parasiticus inhibition in corn samples with different MC

The reductions of *Aspergillus parasiticus* after RF treatment in corn samples with different MC are shown in Table 3. The thermal resistance of *Aspergillus parasiticus* decreased with increasing MC. Specifically, 1–2, 3–4, and 6–7 log reductions of *Aspergillus parasiticus* in corn samples were obtained with MC of 9.1%, 12.0% and 15.0%/17.9% w.b., respectively. The results indicated that RF heating can achieve the required inhibition level by changing MC in food samples. Similar effects were also found in in-shell almonds (Li et al., 2017) and wheat (Jiao et al., 2016) after RF treatments.

Although low moisture content or water activity (a_w) is a key factor in controlling microbial *growth*, it actually presents a significant impediment to microbial inactivation. Many studies have demonstrated that the thermal resistance of microorganisms increases with decreasing water activity in several food samples (Villa-Rojas et al., 2013). Since elevated temperatures cause protein unfolding and denaturation, ribosomal damage, and enzyme inactivation, resulting in inactivation of microorganisms, the principal cause of thermal inactivation of bacterial cells under high moisture conditions is irreversible destabilization of ribosomes. Microorganisms may have lower molecular flexibility of protein structures at lower sample moisture content. The enhanced heat resistance of bacteria in low-moisture environments is probably because desiccation of bacterial cells sharply reduces molecular mobility and helps stabilize ribosomal units against irreversible damage due to thermal energy (Syamaladevi et al., 2016).

3.5. Corn sample quality

The changes of MC and a_w of corn samples before and after RF treatment during accelerated storage are shown in Fig. 3. The MC and a_w decreased slightly with storage time and presented a significant difference ($P < 0.05$) between control and RF treated samples. As shown in Fig. 3a, the samples lost about 0.7–1.0% w.b. of MC both for control and RF treated samples after storage life. A similar phenomenon was found in sweet corn after 12 d storage at 1 °C and an additional 2 d of marketing simulation at 20 °C (Aharoni et al., 1996). The a_w fluctuated in a narrow range both for control and RF treated samples after storage time (Fig. 3b). Similar results were observed by Zia-Ur-Rehman (2006), who reported that during storage at 10 °C for 180 d, the MC of corn showed lowering trend with increasing storage time.

Fig. 4a shows that the mean protein values both for control and RF treated corn samples during storage time. The RF treatment resulted in an approximate 0.22% increase in protein as determined from nitrogen content, but there was no significant difference ($P > 0.05$) between control and RF treated samples. Similar phenomena were found in RF treated milled rice (Zhou et al., 2015; Zhou and Wang, 2016a, 2016b), and chestnuts (Hou et al., 2015). The protein content decreased with the increase of storage time and there were no significant differences ($P > 0.05$) among all corn samples. The reduction of protein content was probably caused by the reduction of free amino nitrogen content (Onigbinde and Akinyele, 1988), and similar results were also obtained by Palaciosfonseca et al. (2009) in corn flour.

Fig. 4b shows the starch content of RF treated samples and slight reductions with respect to untreated samples. No significant differences ($P > 0.05$) were observed among treatment and storage time. Fig. 4c shows that the ash content decreased with increasing storage time both for control and RF treated corn samples. However, there were no significant differences ($P > 0.05$) between control and RF treated samples. Similar results were reported by Zhou et al. (2015) and Zhou and Wang (2016a), Zhou and Wang, (2016b), indicating that RF treatment had no influence on starch and ash contents of the grain samples during storage time.

Fig. 4d shows the changes of fat values both in control and RF treated corn samples during storage. The fat content of RF treated corn samples was slightly higher than that of the untreated samples, but no significant differences ($P > 0.05$) were observed in two samples. A similar phenomenon was found by Zhou et al. (2015), who reported that after RF heating at 50 °C with holding 5 min, there were no significant differences in fat content of milled rice ($P > 0.05$). The fat content lost about 0.36% both for control and RF treated corn samples and had a significant difference ($P < 0.05$) after storage life. This trend was probably caused by lipase and oxidation (Zhou et al., 2002). The changes of fat content in control and RF treated corn samples during storage were similar to those of corn during storage for 8 weeks at 25 °C reported by Reed et al. (2007), and those of corn flour collected monthly over a 6-month duration in a local supermarket reported by Palaciosfonseca et al. (2009).

It is of great importance to analyze the fatty acid content of corn samples after RF treatment and storage. The fatty acid content of corn samples before and after RF treatment during storage is reported in Fig. 5. With increasing storage time, the fatty acid content in control samples increased at a faster speed than that in RF treated corn samples. Specifically, the mean fatty acid values were similar ($P > 0.05$) both for control and RF treated samples during 0–4 d storage periods. However, a significant difference ($P < 0.05$) was observed in 8, 12 and 17 d storage time between control and RF treated samples. This reduction in RF treated corn samples might be due to possible inactivation of the lipoxygenase enzymes by heat treatments (Buransompob et al., 2003). For both RF treated samples and control, the fatty acid content after the accelerated storage life was still lower than the standard values (78 KOH/100 g) in corn samples following the National Standard of China (GB/T 20570-2015, 2015), indicating that the RF treated corn could be stored for 10 °C for 1 year with acceptable product quality.

Table 3
Inhibition of *Aspergillus parasiticus* spores (CFU/g) after hot air –assisted RF treatments (70 °C, 14 min) in corn samples with four moisture contents at 5 locations of the container.

Treatment	Locations	Moisture content (w.b.)			
		9.1%	12.0%	15.0%	17.9%
Control		$(1.6 \pm 0.4) \times 10^7$	$(3.0 \pm 0.8) \times 10^7$	$(4.6 \pm 0.6) \times 10^7$	$(2.3 \pm 0.6) \times 10^7$
Hot air + RF	1	$(2.1 \pm 0.3) \times 10^6$	$(3.2 \pm 0.6) \times 10^4$	$(1.8 \pm 0.7) \times 10^1$	–
	2	$(3.3 \pm 0.7) \times 10^6$	$(3.8 \pm 0.5) \times 10^4$	$(2.4 \pm 0.6) \times 10^1$	–
	3	$(1.4 \pm 0.8) \times 10^6$	$(1.8 \pm 0.6) \times 10^4$	$(1.1 \pm 0.4) \times 10^1$	–
	4	$(1.9 \pm 0.5) \times 10^5$	$(2.1 \pm 0.4) \times 10^3$	–	–
	5	$(2.9 \pm 0.8) \times 10^5$	$(2.9 \pm 0.8) \times 10^3$	–	–

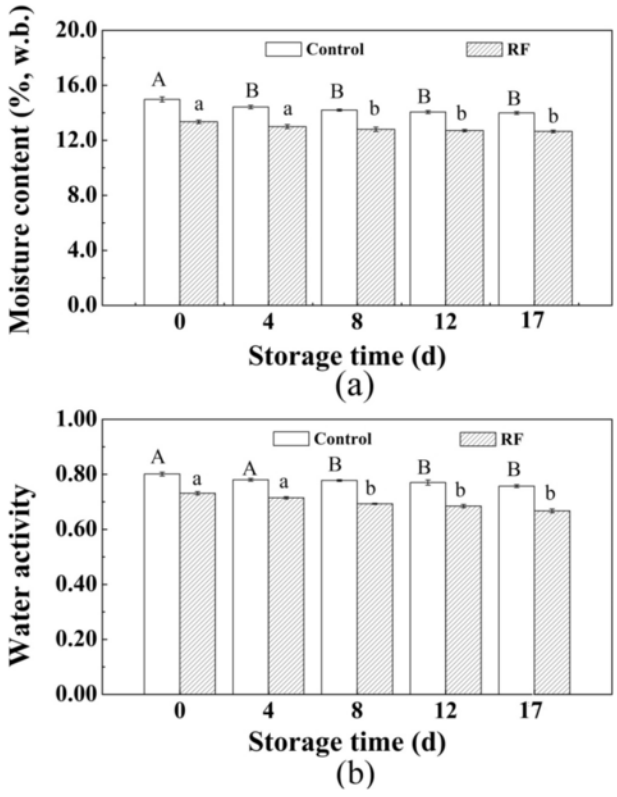

Fig. 3. Changes in moisture contents (a) and water activity (b) of RF treated and untreated corn samples during storage at 35 °C, 68% RH. Different lower and upper case letters indicate significantly different at $P = 0.05$ among storage times.

Table 4 shows that the color values of corn samples at 15.0% w.b. before and after RF treatment. For treated corn samples, a* and b* values increased while L* values decreased. However, no significant differences ($P > 0.05$) were observed in L*, a* and b* values of corn samples between the control and RF treatment. Similar results were found in cooked corn by Odjo et al. (2012). The L* value of RF treated samples slightly increased with increasing storage time, whereas a* and b* values decreased. This trend probably resulted from the decrease of protein content. Similar results were found by Jamin and Flores (1998) and Sandhu et al. (2007), who reported that a* and b* values were positively correlated with protein content whereas the L* values were negatively correlated with protein content.

The electrical conductivity values of corn samples at 15.0% w.b. before and after RF treatment are summarized in Table 5. The electrical conductivity of RF treated corn samples was higher than that in the control, but a significant difference ($P < 0.05$) was only observed at storage time of 0 d. The similar result was reported by Jiao et al. (2016), who found the electrical conductivity of corn seed leachates significantly ($P < 0.05$) increased after RF heating at 70 °C with > [illegible] in holding. The electrical conductivity increased with increas-

ing storage time both for control and RF treated samples. There was a significant difference ($P < 0.05$) observed in control samples between initial and final storage times. A similar phenomenon was observed in low lipoxygenase maize varieties (Li et al., 2007) stored in silo bags (Costa et al., 2010) and airtight bags (Santos et al., 2012). Higher electrical conductivity of seed leachates indicates that lower seed vigor was probably caused by lipid peroxidation, enzyme inactivation, and disruption of cellar membranes (McDonald, 1999; Walters, 1998). During the same storage time, the electrical conductivity values of RF treated samples increased at a similar rate with respect to control samples, indicating that RF treatment did not affect the aging speed of corn seed at storage life.

The germination rates of control and RF treated corn samples were 94.4% and 74.1%, respectively. Obviously, the germination rate of the RF treated corn sample sharply declined and had the significant difference ($P < 0.05$) to control samples (Table 5). This may be because higher treatment temperatures would inactive some of enzymes inside the seed and ultimately damage the germination and seedling vigor (Farooq et al., 2005; Jiao et al., 2016). With increasing of storage time, the germination rate of control samples reduced from 94.4% to 85.2%, and that for RF treated samples declined from 74.1% to 68.5%. There were no significant differences ($P > 0.05$) observed in the storage period both for control and RF treated samples. Similar results were found by Costa et al. (2010), who reported that after storing at 35 °C for 30 d, germination rate of corn sample with MC of 14.5% slightly declined but retained a value of 85%.

4. Conclusion

The combined final temperature and holding time were used to control *Aspergillus parasiticus* in corn using hot air assisted RF treatments. The higher temperatures and longer holding time resulted in less residual spores in corn samples. Holding time of 12 min achieved at least 5-log reductions in 15% MC (w.b.) corns after RF heating at 70 °C. The effect of RF treatment on controlling *Aspergillus parasiticus* was enhanced by increasing the MC of corn samples, resulting in 7-log reductions in corn samples with MC of 17.9% w.b. after RF heating at 70 °C with holding time 14 min. After the RF pasteurizing process, the physiological characteristics and quality of corn (15.0% MC, w.b.) were maintained according to the market standards. Future research is needed to develop an industrial-scale RF treatment to control *Aspergillus parasiticus* in stored corn.

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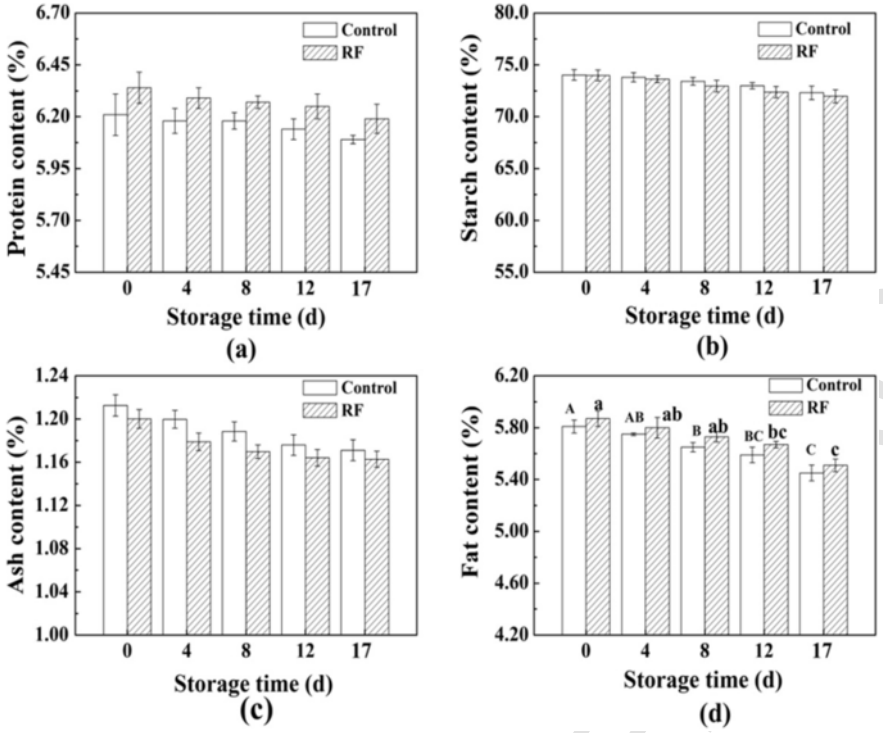

Fig. 4. Changes (% on dry basis) in protein content (a), starch content (b), ash content (c), and fat content (d) of RF treated and untreated corn samples during storage at 35 °C, 68% RH. Different lower and upper case letters indicate significantly different at $P = 0.05$ among storage times.

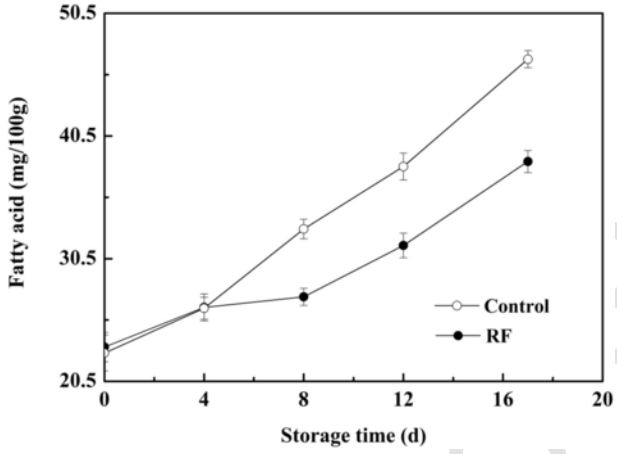

Fig. 5. Changes in fatty acid content of RF treated and untreated corn samples during storage at 35 °C, 68% RH.

Table 4
Changes in color values of RF treated and untreated corn samples with 15.0% MC (w.b.) during the accelerated storage.

Color parameters	Storage time (days)[b]					
	Treatment	0	4	8	12	17
L*[c]	Control	72.9 ± 3.8[a]	73.4 ± 4.2	73.6 ± 4.9	74.1 ± 5.2	74.7 ± 4.0
	RF	72.5 ± 4.1	73.2 ± 3.9	73.3 ± 5.1	73.9 ± 5.1	74.2 ± 4.6
a*[c]	Control	(− 1.0 ± 1.6)	(− 1.3 ± 2.5)	(− 1.7 ± 2.1)	(− 2.8 ± 1.7)	(− 3.8 ± 1.9)
	RF	(− 0.7 ± 1.8)	(− 1.0 ± 2.1)	(− 1.4 ± 2.3)	(− 2.5 ± 1.8)	(− 3.1 ± 2.0)
b*[c]	Control	36.6 ± 5.3	36.3 ± 5.5	36.1 ± 4.4	35.6 ± 4.1	35.2 ± 4.2
	RF	37.1 ± 5.8	36.9 ± 5.6	36.5 ± 4.9	36.0 ± 4.1	35.4 ± 4.3

[a] No significant difference was observed both for treatments and storage times ($P > 0.05$).

[b] 17 days at 35 °C, 68% RH to simulate 1-year storage at 10 °C.

[c] L* (lightness); a* (redness-greenness); b* (yellowness-blueness).

Table 5
Changes in seed vigor and germination rate of RF treated and untreated corn samples with 15.0% MC (w.b.) during the accelerated storage.

Parameters	Storage time (days)[#]					
	Treatment	0	4	8	12	17
Electrical conductivity (μS cm^{-1} g^{-1})	Control	7.6 ± 0.7aAa[&]	8.4 ± 0.5aAB	8.6 ± 0.4aAB	8.9 ± 0.7aAB	9.0 ± 0.4aB
	RF	9.1 ± 0.7bA	9.2 ± 0.4aA	9.4 ± 0.5aA	9.8 ± 0.5aA	10.2 ± 0.7aA
Germination rate (%)	Control	94.4 ± 5.6aA	90.7 ± 8.5aA	88.9 ± 5.6aA	87.0 ± 8.5aA	85.2 ± 3.2aA
	RF	74.1 ± 8.5bA	72.2 ± 5.6bA	70.4 ± 3.2bA	70.1 ± 8.4bA	68.5 ± 3.2bA

[&] Different lower and upper case letters indicate that means are significantly different at $P = 0.05$ among treatments and storage time, respectively.

[#] 17 days at 35 °C, 68% RH to simulate 1-year storage at 10 °C.

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