



Quality and mold control of enriched white bread by combined radio frequency and hot air treatment

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ABSTRACT

This study explored the application of radio frequency (RF) energy in conjunction with conventional hot air treatment to provide uniform heating for control of mold in pre-packaged bread loaf. A 6 kW, 27.12 MHz RF system was used to develop treatment protocols. The treatment parameters were selected based on minimum time–temperature conditions that were required for 4-log reduction of *Penicillium citrinum* spores while yielding acceptable bread quality. During combined RF and hot air treatments, the core and periphery of the bread loaf were heated together with almost the same heating rate. The maximum temperature difference within one bread slice was less than 5 °C. The moisture contents and water activities of RF treated samples first increased and then decreased compared to those of untreated samples, while firmness increased during the storage for both heat treated and untreated samples, yet the overall differences in sample qualities between RF treated bread samples and control were not significant. Because of better heating uniformity, much lower mean product temperature and shorter holding time were used for control of *P. citrinum* spores with combined RF and hot air treatment as compared to conventional heating alone. Heating bread to 58 °C or higher resulted in 4-log reduction of *P. citrinum* spores isolated from moldy bread. The storage life at room temperature (23 °C) was extended by 28 ± 2 days for the treated white bread.

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1. Introduction

Bread is prone to rapid microbial spoilage, particularly mold growth, due to post-baking contamination during cooling, slicing and wrapping, which greatly limits its shelf life. Thus methods of mold control are of great importance to the bakery industry (Smith et al., 2004). Radio frequency (RF) heating has been studied as a means to control mold growth and extend shelf life of finished bakery products (Cathcart et al., 1947; Bartholomew et al., 1948; Zhao et al., 1999; Piyasena et al., 2003; Tang et al., 2005). Effects of RF treatments on bread quality are also considered in those studies. Cathcart et al. (1947) reported that heating wrapped white bread to 60 °C by means of RF energy could control mold growth within 10-day storage without causing reduction of thiamin. Bartholomew et al. (1948) found that the storage life of inoculated Boston brown bread was extended by 12 days after heated by RF to 66 °C. The RF treatment resulted in 4-log reduction of *Penicillium* and *Aspergillus* spores. Yet according to Cathcart et al. (1947) and Bartholomew et al. (1948), severe condensation appeared from the surface of bread loaves after RF treatment, which resulted from temperature difference between bread and the surrounding air; air

has a relative loss factor of zero and cannot be heated by RF energy. Thus, in this study, we used surface hot air to maintain a relatively high surface temperature of bread loaves while using RF for volumetric heating. In order to design an effective combined RF and hot air treatment, lethal thermal conditions for bread molds, together with mold growth and quality changes of treated bread during storage, must be studied.

Bread spoilage mainly results from mold contamination, especially *Penicillium* and *Aspergillus* (Smith et al., 2004; Guynot et al., 2005; Pateras, 2007). Lethal thermal condition for *Penicillium* and *Aspergillus* has been reported to be 68–70 °C for 20 min with conventional heating method (Olsen, 1965), while brining bread to 57 and 60 °C by RF heating resulted in no mold development within 4 and 10-day storage period, respectively, and their untreated control grew molds on the 4th storage day as a contrast (Cathcart et al., 1947). Similar findings were reported by Bartholomew et al. (1948) that mold control can be achieved at lower temperatures with shorter holding time when using RF heating compared with conventional heating. But all the above researches were conducted only using either RF heating or conventional surface heating methods, data about lethal effect of combined RF and hot air treatment on bread mold control is unavailable.

There is also a lack of information in the literature on mold growth and bread quality changes during storage after combined

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RF and hot air treatments. The objectives of this study were to: (1) determine the lethal thermal condition for mold isolated from moldy bread using combined RF and hot air treatments. We selected target sample temperatures of 53, 58, 63 and 68 °C and determined minimum conditions to achieve 4-log reduction of bread mold spores; and (2) study mold growth and quality changes in the treated bread during storage. The quality indexes included moisture content, water activity and bread firmness.

2. Materials and methods

2.1. Sample preparation

Sliced enriched white breads (Oven Joy White Enriched Bread made by Lucerne Foods, Inc., Pleasanton, CA, USA) were purchased from a local grocery store in Pullman, WA, USA. The ingredients of the bread are shown in Table 1, among which ash content (AOAC, 2000a) and porosity (Rahman, 1995, 2005) were measured in the laboratory and the others were based on the labels.

2.1.1. Mold spore suspension

Two white bread loaves were stored at room temperature for the incubation of molds. Individual organisms in pure culture were then isolated from moldy breads using streak plate method and inoculated onto the surface of potato dextrose agar medium, which were incubated afterward for the growth of mold colony at 25 °C in an electric constant temperature incubator (Precision Economy Incubator, Jouan, Inc., Winchester, VA, USA) for 5 days. Mold spores were gathered and made into spore suspension with a concentration level of 10^7 CFU/mL for future use.

2.1.2. Inoculated bread samples

Bread columns with a diameter of 20 mm and thickness of 12.5 mm (1 slice) in Petri dishes were exposed to ultraviolet lights for 30 min, and then inoculated with 1×10^6 CFU/g of pure mold strain isolated from moldy bread. The bread columns inoculated with mold spores were then placed under a sterile hood for the moisture content to equilibrate for 12 min and kept in sterile Low-Density Polyethylene (LDPE) Ziploc bags for future use.

Bread loaves treated with ultraviolet lamp were inoculated with 3.2×10^6 CFU/g of pure mold strain isolated from moldy bread. Mold suspensions were evenly squirted to the cold spot of bread slices predetermined by preliminary tests. The bread slices were

then placed under a sterile hood for moisture equilibration for 12 min and put back into original packages for future use.

2.2. Combined RF and hot air treatment

A 6 kW, 27.12 MHz RF system (COMBI 6-S, Strayfield International, Wokingham, U.K.) was used in this research, with an area of 750 mm × 550 mm for the top RF plate electrode. The gap between the top electrode and bottom electrode was adjusted between 130 and 240 mm (Fig. 1) to regulate coupling of RF energy to the bread samples for obtaining desired heating rate in breads. A conveyor belt with changeable moving direction and speed was equipped to assure different moving styles and residence time of food products in RF field. An auxiliary hot air system was installed to increase air temperature in RF field for maintaining product surface temperature (Fig. 1). The ambient air entered from the inlet to a 5.6 kW electric heater, heated to target temperature and blown upwards by a fan through air distribution box and air holes on the bottom electrode.

To provide stable hot air condition, 1 h was allowed for the hot air to circulate in the empty RF cavity prior to the combined RF and hot air treatment. After the stable target hot air temperature was obtained, bread samples were placed into the RF cavity and the RF system was immediately started. The hot air temperature resulting in target treatment temperature in the RF cavity was determined as the target hot air temperature for the holding period to maintain appropriate bread surface temperature. Since the RF cavity was not airtight, heat transfer occurred between hot air in the RF cavity and the surrounding air of room temperature. Therefore, target hot air temperatures higher than the target treatment temperatures were used to maintain the desired holding temperatures in the RF cavity.

The electrical heater was immediately turned off after the cold region of bread samples were heated to target temperatures. Ambient air was then blown through air distribution box and air holes on the bottom electrode to cool down the bread temperature to room temperature for future use.

2.2.1. Selecting treatment conditions

Inoculated bread samples prepackaged in Ziploc bags were placed in-between the two plate electrodes of the RF system described above and heated by combined RF and hot air system till the highest temperature in breads reached 53, 58, 63, or 68 °C.

Table 1

Proximate analysis of the white bread samples (mean of three replicates).

Product	Protein (% wet basis)	Ash (% wet basis)	Fat (% wet basis)	Carbohydrate (% wet basis)	Moisture content (% wet basis)	Porosity
White bread	6.90	2.89 ± 0.02	1.72	51.72	37.10 ± 0.17	0.80 ± 0.00

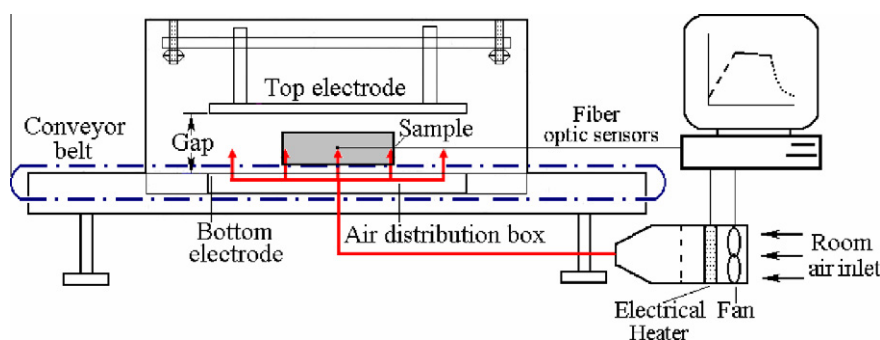


Fig. 1. Schematic diagram of combined RF and hot air treatment system (Wang et al., 2010).

After that, the bread samples were held in hot air till the temperature of cold spot got to a target treatment temperature listed above. Different target hot air temperatures were also tried to select appropriate temperature for control of surface molds on breads. Duplicate independent experiments were made for each target temperature.

Combined RF and hot air treated and untreated inoculated bread samples were aseptically collected and placed into sterile centrifugal tubes. A total 3 mL of 0.1% peptone water was added in one centrifugal tube to dilute the sample, which was then homogenized by vortex oscillating. Serial dilutions were performed and 0.1 mL of three dilutions of each sample was plated onto potato dextrose agar. The Petri dishes were then stored at 25 °C in the electric constant temperature incubator for 3–5 days for the count of mold.

Time–temperature combinations were selected based on minimum conditions required for 4-log reduction of *Penicillium citrinum* spores inoculated into the bread columns, in order to obtain best retention of bread quality. The lowest target sample temperature and the used hot air temperature which resulted in 4-log reduction of the mold number were determined as the mold lethal thermal condition for mold control when using combined RF and hot air treatment.

2.2.2. Treatment validation

The efficacy of selected treatment condition was validated by separate experiments in which inoculated and packaged bread loaves were treated and stored at room temperature (23 °C) to see if mold indeed grow over an extended period of time. The extended time period was dependent on the treatment temperature and holding time. Higher treatment temperature and longer holding time resulted in longer extended storage time. If untreated inoculated bread loaves developed molds, while combined RF and hot air treated inoculated bread loaves developed no mold within an extended storage period, then the mold lethal thermal condition determined above is effective for commercial mold control of bread loaves. According to Cathcart et al. (1947), heating white bread to 60 °C by RF treatment extended bread storage life by 6 days. Considering the introduction of hot air treatment resulted in better heating uniformity in RF treated bread loaf, if the target treatment temperature and holding time were comparative to those in the study of Cathcart et al. (1947), the extended storage period in this research should be longer than 6 days.

The lethal thermal treatment condition was also applied to uninoculated bread loaves to study the quality changes of the treated breads during storage at 23 °C. If combined RF and hot air treated uninoculated bread loaves maintained good quality as compared to untreated bread loaves, then the mold lethal temper-

ature and the used hot air temperature determined above are applicable to combined RF and hot air treatment of bread loaves.

For RF heating experiments, pre-packaged bread loaves in LDPE bags were placed in-between the RF electrodes and heated by combined RF and hot air treatment till the highest temperature in breads reached a desired target temperature. After that, the breads were held in hot air till the temperature of cold spot reached the target treatment temperature. Breads were then cooled by ambient air and stored at room temperature (23 °C) together with untreated inoculated and uninoculated bread loaves, respectively. The inoculated bread loaves were stored for mold growth. The uninoculated bread loaves were stored to check mold growth and for quality analyses.

2.3. Temperature distribution in bread

Bread loaves were located in-between two plate electrodes with bread slices perpendicular to the electrodes along the moving direction of conveyor belt. On going through the RF field with the running conveyor belt, each slice of the whole loaf passed the same route in RF field, for this reason, bread slices in one loaf had the same heating pattern. Therefore, temperature distribution of each slice was representative of the whole loaf. Preliminary experiment showed that the cold spot lied in the center of each bread slice.

Three sample temperatures above, below and right at the center (as a, c and b in Fig. 2) of one bread slice were measured during combined RF and hot air treatment using pre-calibrated FISO fiber optic sensors (UMI, FISO Technologies Inc., Saint-Foy, Quebec, Canada) with an accuracy of ± 1 °C. Surface temperatures of bread slices were immediately recorded by infrared thermal camera with an accuracy of ± 2 °C (Thermal CAM™ SC-3000, FLIR Systems, Inc., North Billerica, MA, USA) slice by slice right after the bread loaf was heated to lethal temperature. Two replicates were made for each experiment.

2.4. Moisture content

Bread moisture content on wet basis was calculated from the difference in weight after vacuum-drying at 98–100 °C for about 5 h (AOAC, 2000b). Three replicates were conducted for each measurement.

2.5. Water activity

Bread water activity at room temperature was measured using an AquaLab Series 3 water activity meter (Decagon Devices Inc., Pullman, WA, USA). Three replicates were conducted for each measurement.

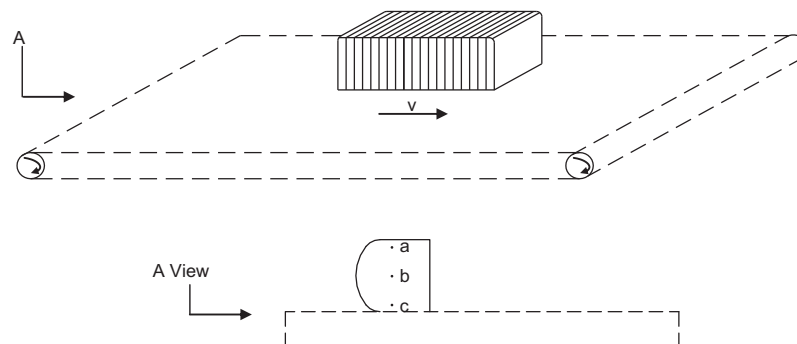


Fig. 2. The placement of bread loaf within the RF field.

2.6. Firmness

Bread firmness was measured using a TA-XT2i texture analyzer (Stable Micro Systems, London, UK). Measurements were made on “Measure Force in Compression” mode, pre-test speed, test speed and post-test speed were set as 1.0, 1.7 and 10.0 mm/s, respectively, compression ratio was 40%. A 36 mm-diameter aluminum probe was used and the thickness of bread sample was 25 mm (two slices). The pressure related to a compression ratio of 25% was defined as bread firmness (AACC, 1988).

2.7. Statistical analyses

The experimental design was completed in a randomized mode. Data were analyzed using SYSTAT 6.0.1 (SYSTAT Software Inc., San Jose, CA, USA). All the statistically significant comparisons were made at significant level of $\alpha = 0.05$.

3. Results and discussion

3.1. Mold type

The mold isolated from moldy bread was inoculated onto the surface of potato dextrose agar and incubated at 25 °C. Mold colony appeared after 3–4 days' incubation and grew up to 20–25 mm in diameter in 10–14 days. The mold colony was flat, filamentous and velvety in texture with obvious radial grooves in the surface (Fig. 3). The colony was initially white and became gray green in time with a white edge. The plate reverse was orange.

Mycelia and conidia fixed on a microscopic slide was placed under 400 \times microscope, septate hyaline hyphae (1.5–5 μ m in diameter), branched conidiophores, metulae, phialides, and conidia were observed. They formed 3–4 cyclical brush-like branched

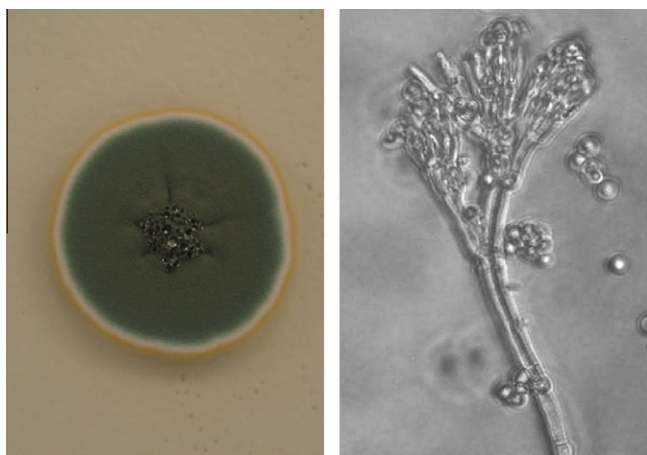


Fig. 3. Colony and microscopic morphologies of the mold.

conidial chain. The conidia (2.5–5 μ m in diameter) were round and unicellular at the tips of the phialides (Fig. 3).

According to the colony and microscopic morphologies, the mold was identified as *P. citrinum* (Robinson et al., 2000; Standardization Administration of China, 2003). *P. citrinum* is the major producer of mycotoxin-citrinin which is known to be nephrotoxic and carcinogenic to humans and to a wide variety of animals (Semple et al., 1989; Mazur et al., 2006). Nowadays food safety problems caused by citrinin have been a growing concern among governments and the public. It is desirable to control the growth of *P. citrinum* in breads.

3.2. Mold lethal condition

According to Table 2, bread target temperature higher than 58 °C resulted in 4-log reduction of *P. citrinum* spores. But when hot air temperature was lower than 59 °C, accordingly surface temperature of bread columns lower than 58 °C, the desired lethal effect could not be achieved, which further validated the necessity of the introduction of auxiliary hot air treatment to keep the required surface temperature of bread loaves. Therefore, target temperature of 58 °C and hot air temperature that kept bread surface temperature of 58 °C were determined as the mold lethal condition in this research.

3.3. Mold growth of inoculated bread loaves during storage

White hyphae appeared from two untreated bread loaves on the 7th and 8th storage day, respectively, and gray green mold spot formed on the 10th day. No hyphae was found from two RF treated bread loaves until they were stored for 41 and 43 days at 23 °C, respectively, as shown in Table 3.

The results demonstrated that the target temperature at 58 °C or higher was effective for commercial mold control of bread loaves. In order to maintain a surface temperature of 58 °C for bread loaves, hot air temperature of 63 °C was introduced for the RF system. This condition was determined based on the minimum time–temperature conditions required for 4-log reduction of *P. citrinum* spores in order to obtain best retention of bread quality. In practical applications, the storage life of bread can be extended even more with increased target temperature on the precondition of good bread quality.

3.4. Temperature distribution within bread loaf after combined RF and hot air treatment

When bread loaf was located in-between the two plate electrodes which had a gap of 202 mm, conveyor belt running at a speed of 1 cm/s, hot air temperature of 63 °C applied, temperatures at three different locations (shown in Fig. 2) as recorded by fiber optic sensors in one bread slice increased almost linearly with treatment time during combined RF and hot air treatment (Fig. 4). After the RF system was turned off, the bread temperatures still increased to the mold lethal temperature under the effect of

Table 2
Thermal lethal effect of *P. citrinum* spores after different treatments.

Treatment	Treatment conditions			Average colony count over two replicates after treatment (CFU/g)
	Target treatment temperature (°C)	Target hot air temperature (°C)	Heating time (min)	
Control	23	23	0	10 ⁶
Combined RF and hot air treatment	53	54	1.6	10 ⁵
	58	54	1.9	10 ⁴
	58	59	1.8	<10 ²
	63	64	2.2	<10 ²
	68	69	2.6	<10 ²

Table 3
Effect of combined RF and hot air treatment on the extension of inoculated bread storage lives.

Treatment	Target treatment temperature (°C)		Target hot air temperature (°C)	Heating time (min)	Mold growth (Days after)				
					7	8	10	41	43
Control	Rep 1	23	23	0	-	*	**		
	Rep 2	23			*	*	**		
Combined RF and hot air treatment	Rep 1	58	63	4.6	-	-	-	*	
	Rep 2	58			-	-	-	-	*

-, No mold growth.
* Slight mold growth.
** Pronounced mold growth.

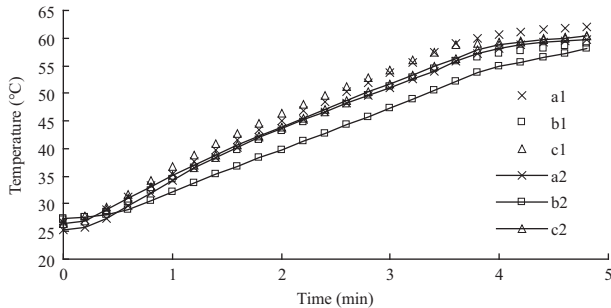


Fig. 4. Temperature–time histories of three positions for one bread slice during combined RF and hot air treatment with two replicates at the target temperature of 58 °C.

hot air and heat transfer within bread samples. The cold spot (b1 or b2) lied in the core of the bread slice.

During RF treatment, the core and periphery of bread loaves were heated together with similar rate. Thus, temperature was fairly evenly distributed in breads after RF heat treatment. While during hot air heating, surface of bread loaves were heated first, accordingly the temperature of bread surface was highest and thermal energy transferred from surface to the core of bread loaves. Therefore, after combined RF and hot air treatment, periphery of bread loaves were heated more compared to the core, that is, the cold spot lied in the core of bread loaves after combined RF and hot air treatment. The heating pattern was good for mold control of bread loaves, for molds appeared mainly from surface of bread loaves.

Since RF treatment played the major role with the help of hot air holding the surface temperature (Liu et al., 2009), bread loaves were heated fairly uniformly by combined RF and hot air treatment. Surface temperatures of bread slices were recorded right after combined RF and hot air treatment as shown in Fig. 5 as an example. The temperature was fairly evenly distributed within each slice, with maximum differences in each slice less than 5 °C.

3.5. Quality changes in bread crumb during storage

Combined RF and hot air treated and untreated bread loaves were kept at room temperature (23 °C) for mold growth and quality analyses. Mold was observed from the surface of untreated bread loaves after 5 weeks' storage. The samples after combined RF and hot air treatment took four extra weeks to show visible mold growth. During storage, combined RF and hot air treated bread loaves maintained as good quality as untreated bread loaves at the same storage period.

3.5.1. Moisture content

Moisture was redistributed from crumb to crust in bread, and accordingly moisture content in bread crumb decreased over time.

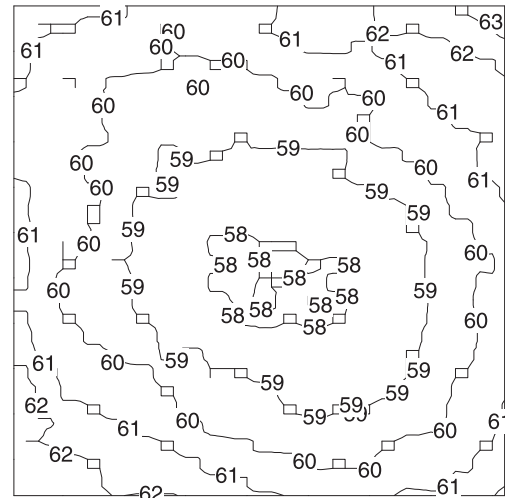


Fig. 5. Surface temperature distributions of one bread slice after combined RF and hot air treatment (°C) at the target temperature of 58 °C.

During combined RF and hot air treatment, certain amount of moisture evaporated inside bread samples migrated to the bread surface and escaped through the wrapper, which resulted in totally 4% moisture loss. Moisture content of combined RF and hot air treated bread crumbs rose slightly after 1 week's storage because of the moisture equilibration between bread crumb and crust within the bread loaf, then decreased over time till the sixth week and finally stabilized till the end of storage life (Fig. 6). Moisture content of untreated bread crumbs decreased over time within the whole storage life due to staling. Moisture migration from bread crumb to the crust during combined RF and hot air treatment was caused by generation of internal vapor pressure during the RF heating. The consequent moisture loss in the bread crumb and increased moisture in the crust led to a more even distribution of moisture in the treated bread samples. Before and after combined

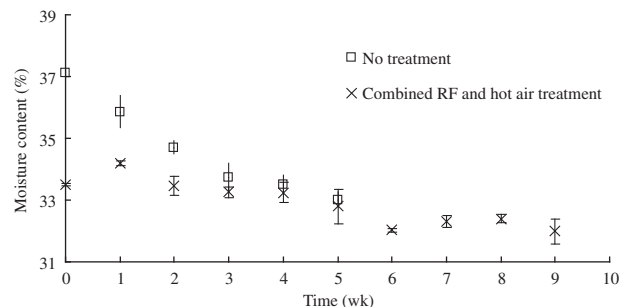


Fig. 6. Bread moisture contents during the storage (two replicates for each of two independent experiments).

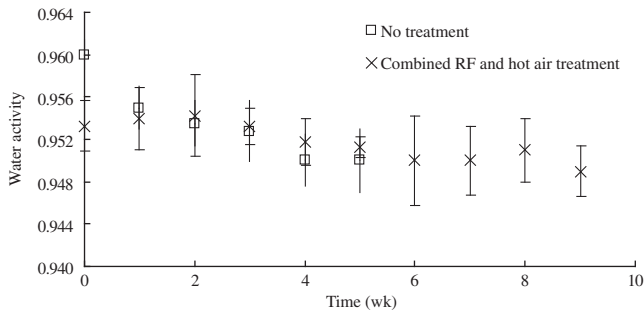


Fig. 7. Bread water activities during the storage (two replicates for each of two independent experiments).

RF and hot air treatment, as well as within the storage life, bread moisture content was higher than 31%, that is, met the standard of fresh bread moisture content (Li et al., 2000; Song et al., 2005).

3.5.2. Water activity

Before and after combined RF and hot air treatments, bread loaves had high water activities which lay in the area of 0.950–0.960 suitable for most bacteria and molds to grow. Combined RF and hot air treated bread crumbs had an initial water activity of 0.953, which increased a little bit after 1 week storage due to the reabsorption of surface moisture which was driven from bread crumb to the crust and the wrapper during combined RF and hot air treatment (Cathcart et al., 1947), then decreased till the sixth week and finally stabilized as shown in Fig. 7. The untreated bread crumbs had a highest water activity of 0.960, which decreased rapidly to the same level as combined RF and hot air treated ones after 1 week's storage and decreased gradually afterwards.

Combined RF and hot air treatment had little effect on bread water activity during storage. Since most molds grow normally at water activities higher than 0.8 (Smith et al., 2004), the mechanism of combined RF and hot air treatment was not to inhibit the growth of the targeted microorganisms by decreasing water activity, but to kill them by thermal effect.

3.5.3. Firmness

Combined RF and hot air treatment slightly reduced bread firmness, possibly resulting from the gelatinization of some aged starch into α -starch (Cathcart et al., 1947; Li et al., 2000). During storage, firmness of combined RF and hot air treated bread samples increased with storage time till the sixth week, and finally stabilized (Fig. 8). Crumb firmness of combined RF and hot air treated bread loaves increased over time at a higher rate compared with that of untreated bread loaves. This is consistent with findings of Persaud et al. (1990) that storage modulus, accordingly the firmness of

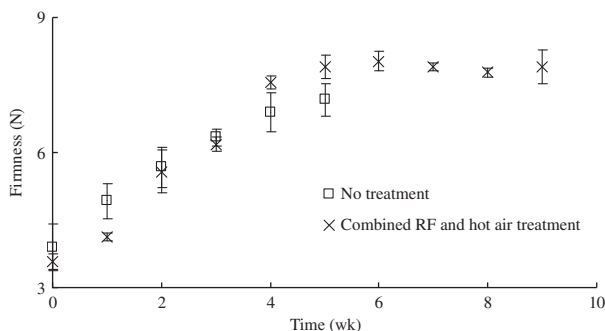


Fig. 8. Bread firmnesses during the storage (two replicates for each of two independent experiments).



(a) Combined RF and hot air treatment **(b)** No treatment

Fig. 9. Appearances of bread samples with/without combined RF and hot air treatment after storage.

microwave and conventionally reheated bread crumbs increased at a greater rate than that of their unheated control. The same result was reported by Song et al. (2002) that reheating increased staling rate of bread. This was attributed to the smaller specific volume of combined RF and hot air treated bread loaves (Liu et al., 2009) compared with that of untreated bread loaves since staling rate and extent increased linearly as bread loaf volume decreased (Pomeranz and Shellenberger, 1971). The overall difference quantitatively between the firmness of the RF treated bread samples and control was yet not significant ($p > 0.05$). In all cases, the bread firmness was less than 9 N, which was acceptable to consumers (Fan, 2007).

3.6. Mold growth after storage

Bread had a storage life of 66 ± 1 and 38 ± 1 days for combined RF and hot air treated (for 4.6 ± 0.2 min to 58°C) and untreated samples, respectively. This demonstrated that combined RF and hot air treatment provided good potential in extending bread storage life. The appearances of combined RF and hot air treated and untreated bread samples after 60-day storage are shown in Fig. 9, which clearly showed the effectiveness of the mold control using the combined treatment method developed in this study.

4. Conclusions

Much lower mean product temperature and shorter holding time were used for required control of *P. citrinum* spores with combined RF and hot air treatment as compared to conventional heating. Heating bread to 58°C or higher resulted in 4-log reduction of *P. citrinum* spore isolated from moldy bread.

Combined RF and hot air treatment demonstrated good potential in extending bread shelf life. The storage life at room temperature (23°C) was extended by 28 ± 2 days for white bread after combined RF and hot air treatment.

During combined RF and hot air treatment, the core and periphery of the bread loaf were heated together with almost the same

heating rate. The maximum temperature difference within one bread slice was less than 5 °C.

During storage, the RF treated samples' moisture contents and water activities first increased and then decreased compared to those of untreated samples which decreased over time, while firmness increased during the storage for both heat treated and untreated samples. The overall differences in sample qualities, in terms of moisture content, water activity and firmness between RF treated bread samples and control were not significant during the storage.

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