



Effects of hot air-assisted radio frequency heating on enzyme inactivation, lipid stability and product quality of rice bran

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ABSTRACT

Rice bran (RB) is a valuable by-product of rice milling but highly susceptible to lipid rancidity induced by inherent lipase (LA) and lipoxygenase (LOX) activities. Therefore a stabilization step is required to reduce activities of these enzymes to ensure RB quality. Effects of hot air-assisted radio frequency (HAARF) heating on enzyme inactivation, lipid stability and product quality of RB were investigated. Influence of water activity (a_w) in stabilized RB on lipid rancidity during storage was also evaluated. Results showed that average residual LA and LOX activities in RB treated by RF heating at electrode gap of 10cm–100 °C with holding in hot air for 15min decreased to 19.2% and 5.5% of their original values. Free fatty acid content and peroxide value of RB oil extracted samples treated under these conditions remained below acceptable limits even following 60d storage at 35 °C. The lowest reaction rate for RB rancidity during storage occurred at a_w value of 0.241. No significant adverse quality changes in RB were observed immediately following HAARF heating, and subsequent storage stability of RB was also enhanced by HAARF heating. HAARF heating can potentially provide rapid and effective methods for RB stabilization without adverse impact on product quality.

1. Introduction

Rice bran (RB) is a by-product of rice milling and consists of the outer layer and germ of the grain and accounts for 6–8 g/100 g w.b of total grain weight (Sharif, Butt, Anjum, & Khan, 2014). In 2014, global paddy rice production was 741 million tons resulting in production of more than 50 million tons of RB (FAOSTAT, 2016). RB has traditionally been used as a cost-efficient ingredient for animal feed or as raw material for oil extraction. More recently, many studies have shown that stabilized RB can be used directly as an ingredient in the preparation of meat and bakery products, but also can be used as a source from which high quality protein isolates and phytochemicals can be extracted (Kim, Chung, & Lim, 2014a; Sharif et al., 2014; Thanonkaew, Wongyai, McClements, & Decker, 2012). However, its short shelf life due to hydrolytic and oxidative rancidity of lipids induced by lipase (LA) and lipoxygenase (LOX) activities has limited a more widespread usage of RB in food processing.

Various methods have been employed to reduce LA and LOX activities and stabilize RB. Conventional thermal treatments in the presence or absence of steam are commonly employed. However, large volume samples generally require long exposure times using this method, in turn leading to adverse head induced damage to RB quality. Novel thermal processing techniques, such as infrared (IR), microwave (MW), radio frequency (RF) and ohmic (OH) heating, have been considered as alternatives to conventional heating with the potential for stabilization of RB (Lakkakula, Lima, & Walker, 2004; Ramezanzadeh, Rao, Prinyawiwatkul, Marshall, & Windhauser, 2000; Yilmaz, Tuncel, & Kocabiyik, 2014). Although IR, MW and OH heating had the added benefit of increasing the extracted oil yields, studies on using RF heating for RB stabilization are needed to overcome their disadvantages.

RF heating is used to heat dielectric materials with electromagnetic waves at frequencies between 1 and 300MHz. As RF generates heat within the commodity by molecular friction, it can rapidly raise temperature in a uniform, volumetric fashion and significantly reduce

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heating time compared to conventional heating methods. Unlike OH, RF heating does not require direct contact with product. RF heating also has greater penetration depths compared with IR or MW heating while also having simpler and more uniform field patterns compared to MW heating (Awuah, Ramaswamy, & Tang, 2015). RF heating is commonly used for rapidly elevating products to target temperatures (i.e. preheating), which can then be maintained for appropriate holding times in conventional hot air environments. In the past five years, hot air-assisted RF (HAARF) heating has been studied in food and agriculture products for various purposes, such as pasteurization and roasting (Boreddy, Birla, Froning, Thippareddi, & Subbiah, 2014; Jiao, Zhu, Deng, & Zhao, 2016). Available results suggest that HAARF heating could be used to replace or supplement conventional heating in food processing which would suggest it also has great potential in the stabilization of RB.

While enzyme activity is a key factor in the stabilization of RB, other factors such as moisture content (MC) or water activity (a_w) are also key parameters in the storage stability of foodstuffs. For dried food products, there is a critical MC (or a_w) value below which quality loss is minimised and ensures a products stability throughout its shelf life (Bell, 2007). In fat containing foods, there is a positive correlation between product MC and rate of hydrolysis reactions due to reactant and solvent effects of water (Kim, Kim, & Lee, 2014b). Moreover, the rate of oxidation reactions could be accelerated at both at low and high a_w due to protective and pro-oxidative effects of water (Labuza & Dugan, 1971). Although substantial research has focused on optimizing thermal process treatment parameters for prevention of lipid rancidity in RB, studies related to the influence of product MC (or a_w) of stabilized RB on developing lipid rancidity during storage and shelf life are limited.

Based on issues identified above, objectives of present study were: (1) to explore the efficacy of HAARF heating as a method for the inactivation of LA and LOX in RB, (2) to determine effects of HAARF heating on lipid stability of RB by monitoring lipid hydrolysis and oxidation, (3) to establish the relationship between lipid rancidity of RB and its a_w (or MC) during storage following HAARF heating, and (4) to evaluate influences of HAARF heating on the overall product quality of RB.

2. Materials and methods

2.1. Materials

Freshly milled RB obtained from the paddy variety 'Shanyou63' (indica type) was directly collected from a local milling plant and placed in polyethylene (PE) bags. After sieving the bran with a British standard sieve no. 20 (750 μ m aperture) to remove broken grains, sand and other foreign materials, the bran was stabilized by HAARF heating (all being performed within 3h of milling). The sieved bran had 11.16 ± 0.08 g/100 g water, 13.24 ± 0.25 g/100 g protein, 22.01 ± 0.93 g/100 g fat, and 11.67 ± 0.06 g/100 g ash of sample (fresh weight basis) (AOAC, 2005). Chemicals used in this research were purchased from either Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) or Sigma-Aldrich (St. Louis, MO, USA).

2.2. Hot air-assisted radio frequency heating procedure

HAARF heating was performed using a 6kW, 27.12MHz pilot-scale free running oscillator RF system (SO6B, Strayfield International, Wokingham, U.K.) combined with a hot air oven (DGG-9203A, Shanghai Sumsung Laboratory Instrument Co., Ltd, Shanghai, China). The RF system allowed for adjustments of the parallel electrode gap between 9 and 19cm, with adjustments made using the movable top

electrode (40 cm \times 83 cm) to regulate the RF power. According to thermal inactivation studies reported by Brunswiler, Heine, Kappeler, Conde-Petit, and Nyström (2013), thermal treatments with involving product temperatures in excess of 80 °C would begin to inactivate LA in RB at MCs of about 10g/100g w.b. Thus, RF heating the product to 80, 90 or 100 °C and holding at these target temperatures in hot air oven for three different holding times at each temperature were selected to study efficacy of HAARF stabilization.

During HAARF treatment, each batch of 200g RB sample (starting temperature \sim 25 °C) was placed in a cylindrical container (inner: 12 cm diameter and 5.5 cm deep) made of polypropylene (PP) (Fig. 1). The RB was spread uniformly in the container, and then tapped 3–4 times on tables to remove air voids within the material. Overall this resulted in a sample thickness of 5 cm. The cylindrical container filled with RB was placed on the center of the bottom electrode, and the electrode gap was maintained at 10cm in this study to obtain a faster heating rate without arcing. Four fiber-optic temperature sensors (HQ-FTS-D120, Heqi Technologies Inc., Xian, China) connected to a data logger used to record sample temperatures during RF heating. Fiber optics were inserted through holes on the lid of the PP container and fixed at a depth of 2.5cm along radial dimensions of 0 (geometric center of the sample), 1.8, 3.6, and 5.4 cm (Fig. 1).

Our previous study showed that the geometry center (0cm) of the container filled with RB was the cold spot during RF heating and the temperature difference between the geometry center and the outer-

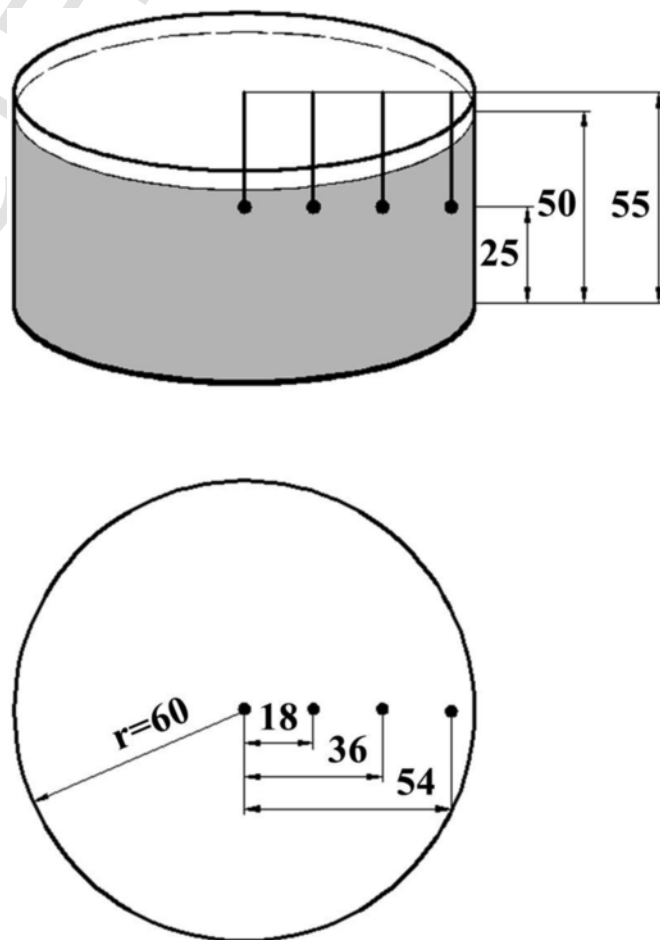


Fig. 1. Dimensions of the cylindrical polypropylene container used to contain rice bran samples during heat treatments and locations of four fiber optic sensors (all dimensions are in cm).

most measured radial distance (i.e. 5.4cm-container radius 6cm) was about 12°C when heated to 100°C. At this point, the RF system was turned off until the cold spot (0cm) achieved the 80, 90 or 100°C, then RB samples were immediately (in 10s) removed from the RF cavity and transferred to a hot air oven to maintain the target temperatures for a given period. After holding, samples were immediately removed from hot air oven and cooled to room temperature (~25°C) using ambient natural air in laboratory by spreading the samples in a thin layer (~5mm) for 15min, following which the samples were stored in zip-lock PE bags until required for analysis. In addition, the aforementioned cylindrical container filled with RB (as described above) was heated in hot air, with the temperature-time profile at the geometry center of RB in hot air of 80 and 100°C recorded using the fiber-optic temperature sensor system. Under this condition the heat transfer was more effective from the top surface due to certain insulation effects of PP material. Therefore, results were only used to compare heating rates between RF and hot air treatments. RB samples without HAARF or conventional treatment (i.e. raw) were used as a control.

2.3. Enzyme activity determination

LA and LOX activity of RB samples was determined following the method reported by Laokuldilok and Rattanathanan (2014) and Ramezanzadeh et al. (2000), respectively. The results were expressed as percentage of enzyme activity in raw samples as below:

$$\begin{aligned} & \text{Relative enzyme activity (\%)} \\ &= \frac{\text{residual enzyme activity}}{\text{enzyme activity of raw rice bran}} \times 100 \end{aligned} \quad (1)$$

2.4. Moisture content and water activity determination

MC and a_w of RB samples were respectively measured using a moisture analyzer (Model HE53, Mettler Toledo International Inc., Switzerland) and a water activity meter (Model 4TE, Decagon Devices, Inc., Pullman, WA, USA).

2.5. Storage experiment

2.5.1. Storage conditions

Control and HAARF treated RB samples were all packed in zip-lock PE bags (300g each) and stored in the dark at 35°C and 70±2% relative humidity (RH) for 60d in an incubator (HWS-150, Shanghai Sumsung Laboratory Instrument Co., Ltd, Shanghai, China) to simulate about six months storage under ambient conditions (25°C, RH 65–80%). This storage time was calculated based on Q_{10} value of 3.4 for lipid rancidity (Taoukis, Labuza, & Saguy, 1997). Q_{10} was commonly used for quantifying the rate of quality changes (k) of foodstuffs as influenced by temperature (T , °C) during storage and is calculated as follows:

$$Q_{10}^{(T_2-T_1)/10} = \frac{\theta_s(T_1)}{\theta_s(T_2)} \quad (2)$$

where T_1 and T_2 are normal and accelerated storage temperatures (°C), respectively, and θ_s stands for the storage time (d).

2.5.2. Oil extraction and chemical analysis

RB oil (RBO) was extracted by a laboratory scale Soxhlet extractor using *n*-hexane. The storage stability of RB was estimated by determining the free fatty acid (FFA) content and peroxide values (PV)

of RBO at 15d intervals according to standard methods of AOCS (1997). FFA content and PV were important indicators for evaluating the extent of hydrolytic and oxidative rancidity in lipids during storage, according to Codex Alimentarius Commission (2001) in which non-refined plant oils with more than 5g/100g FFA and 10meq/kg were generally considered to be unsuitable for human consumption. Before and after storage, a_w , MC, activity of LA and LOX in control and HAARF treated samples were also measured.

2.6. Quality analysis

Fatty acid (FA) composition of RBO was determined using the method reported by Ling, Yang, Li, and Wang (2016) and the results were expressed as g of FA per 100g of total FAs. Tocopherol content of RBO was determined using the method reported by Ling, Hou, et al. (2016) and Ling, Yang, et al. (2016). Individual tocopherol content was expressed as mg/kg RBO. γ -oryzanol content in RBO was analyzed using the method described by Thanonkaew et al. (2012) and expressed as g/100g RBO.

2.7. Statistical analysis

Data were expressed as mean±standard deviations of triplicate measurements. Significant differences ($p < 0.05$) within means were analyzed using variance and Tukey's honestly significant difference (HSD) test in the statistical software SPSS 16.0 version (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Temperature-time profiles of rice bran during radio frequency heating

Fig. 2A shows the typical temperature-time profile of RB during RF heating using a 10cm electrode gap. About 2.5–3min was required to heat 200g RB from 25 to 80 and 100°C. However, with hot air heating the corresponding heating time was 180min (Fig. 2B). The temperature difference between the center and the edge of RB sample increased gradually with increasing target temperature, and the maximum difference was about 12°C when the temperature was attained to 100°C. Similar phenomenon was also reported by Boreddy et al. (2014) that the temperature difference between center and edge of egg white powder filled in a cylindrical container could reach 19°C after RF heating to 90°C. It is generally considered that the non-uniform RF heating was mainly due to the uneven distribution of electromagnetic field between the center and the edge of the sample container (Awuah et al., 2015). The non-uniform RF heating existed for a relatively short time (2.5–3min) when combined with the longer holding times (5–45min) for enzyme inactivation. Previous studies also reported that an extended hot air holding process during HAARF disinfestations not only could be helpful for insect control but also improved the heating uniformity (Ling, Hou, Li, & Wang, 2016).

3.2. Hot air-assisted radio frequency heating influence on moisture content and water activity in rice bran

MC is an important factor influencing storage stability of RB. Table 1 shows that the initial MC and a_w of control were 11.16g/100g w.b. and 0.671, respectively. The MC and a_w of HAARF treated samples decreased significantly ($p < 0.05$) with increasing treating temperature and holding time, and reached their minimum values of 3.07g/100g w.b. and 0.141 in samples treated at 90°C for 45min, respectively. Both MC and a_w changed after 60d of storage, among which the MC of control decreased significantly ($p < 0.05$), while by

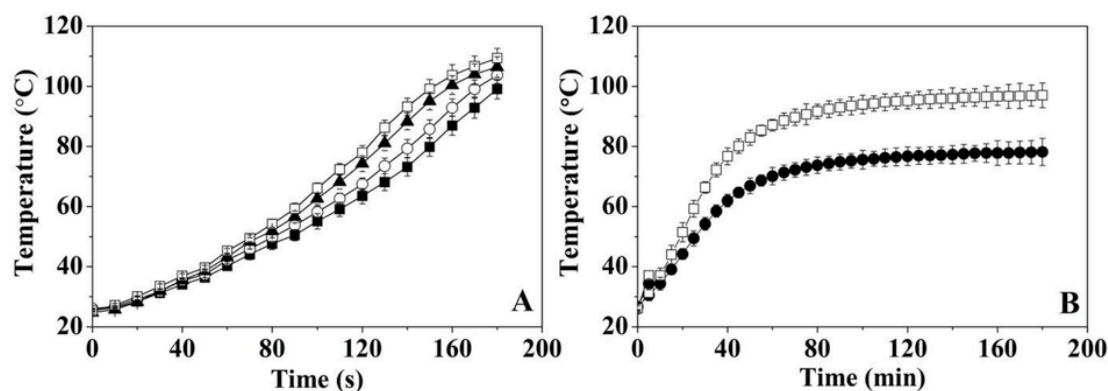


Fig. 2. Temperature-time histories of rice bran in cylindrical container when subject to (A) radio frequency heating (■0mm ○18mm ▲36mm □54mm) and (B) hot air heating (●80°C □100°C).

Table 1

Moisture content and water activity (25°C) of rice bran samples subjected to different hot air-assisted radio frequency (HAARF) heating conditions before and after storage.

HAARF treatment	Moisture content (g/100g, w.b.)		Water activity	
	Storage time (d)			
	0	60	0	60
Control	11.16 ± 0.08 ^{aA}	9.22 ± 0.16 ^{aB}	0.671 ± 0.021 ^{aA}	0.601 ± 0.007 ^{aB}
80°C/15min	7.21 ± 0.14 ^{bB}	8.65 ± 0.15 ^{bA}	0.476 ± 0.013 ^{bB}	0.581 ± 0.011 ^{bA}
80°C/30min	5.17 ± 0.10 ^{eB}	7.55 ± 0.10 ^{eA}	0.321 ± 0.005 ^{eB}	0.519 ± 0.010 ^{dA}
80°C/45min	3.31 ± 0.13 ^{hB}	6.14 ± 0.09 ^{hA}	0.161 ± 0.011 ^{hB}	0.412 ± 0.010 ^{gA}
90°C/10min	6.61 ± 0.12 ^{cB}	8.26 ± 0.18 ^{cA}	0.443 ± 0.007 ^{cB}	0.556 ± 0.008 ^{cA}
90°C/20min	4.79 ± 0.11 ^{fB}	7.06 ± 0.14 ^{fA}	0.286 ± 0.006 ^{fB}	0.482 ± 0.011 ^{eA}
90°C/30min	3.07 ± 0.15 ^{hB}	6.03 ± 0.12 ^{hA}	0.141 ± 0.009 ^{hB}	0.404 ± 0.008 ^{gA}
100°C/5min	6.29 ± 0.03 ^{dB}	8.05 ± 0.09 ^{dA}	0.426 ± 0.005 ^{cB}	0.551 ± 0.009 ^{cA}
100°C/10min	5.33 ± 0.06 ^{eB}	7.89 ± 0.10 ^{dA}	0.341 ± 0.010 ^{dB}	0.539 ± 0.006 ^{cA}
100°C/15min	4.29 ± 0.14 ^{gB}	6.69 ± 0.09 ^{gA}	0.241 ± 0.008 ^{gB}	0.452 ± 0.008 ^{fA}

*Mean values are not significantly different ($p > 0.05$) for the same capital letters within a row among the storage time, and for the same lower case letters within a column among the treatment.

contrast, all the HAARF treated samples absorbed the water from environment. This could be due to the moisture permeability of the zip-lock bags, and so the RB samples with different initial MC absorbed or desorbed water until a new moisture equilibrium was reached.

3.3. Hot air-assisted radio frequency heating inactivation of lipase and lipoxigenase

During HAARF heating, the average residual LA and LOX activity in RB decreased significantly ($p < 0.05$) with increasing treatment temperature and holding time as shown in Fig. 3. After treating at 80°C for 45min, 90°C for 30min, and 100°C for 15min, the average residual LA activity decreased to 18.3%, 16.0% and 19.2%, respectively. However, the corresponding values for LOX decreased to 4.1%, 4.4%, and 5.5%. The lower residual enzyme activities of LOX suggest that LA displays greater thermal resistance than LOX, which was also observed by Orthoefer (2004).

After 60d of storage, the average residual LA and LOX activity of the control increased significantly to 406% and 221%, respectively ($p < 0.05$). Oliveira, Bassinello, Lobo, and Rinaldi (2012) reported that microorganisms could grow and produce endogenous LA and LOX in stored RB. Thus, the rapid increase in enzyme activity in control might be due to the increase in microflora that produce endogenous enzyme. On the contrary, the corresponding values in HAARF treated samples were lower than those in control, which were between 19.5 to 53.6% and 7.8–53.1% for LA and LOX, respectively. Moreover,

the residual enzyme activity in all HAARF treated samples increased after 60d of storage. Among which the average residual LA activity in RB samples treated with HAARF heating at 80°C for 45min, 90°C for 20 and 30min, and 100°C for 10 and 15min increased significantly ($p < 0.05$) from 18.3 to 22.4%, 29.3–33.9%, 16.0–19.5%, 33.2–40.5%, and 19.2–24.5%, respectively. These increases might be due to the water absorption in RB during storage and were in agreement with the increases of a_w presented in Table 1. Based on previous studies of Labuza and Dugan (1971), the enzyme activity in foodstuff had significantly positive correlation on its MC (a_w). Similarly, Xu et al. (2013) also observed that the LA activity in MW stabilized wheat germ began to increase again after absorbing water during storage. This study further proves that it is difficult to irreversibly inactivate LA and LOX in RB with low MC conditions and the endogenous enzymes activity in RB stabilized by dry heating can be partly inhibited only if the bran moisture increased to atmospheric equilibrium during storage.

3.4. Hot air-assisted radio frequency heating influence on lipid rancidity in rice bran

As shown in Table 2, the initial FFA content between control and HAARF treated samples was not significant difference ($p > 0.05$), regardless of the time temperature combination applied. However, as expected, the FFA content of control increased sharply beyond the acceptable limit within the first 15d of storage and reached 50.67g/

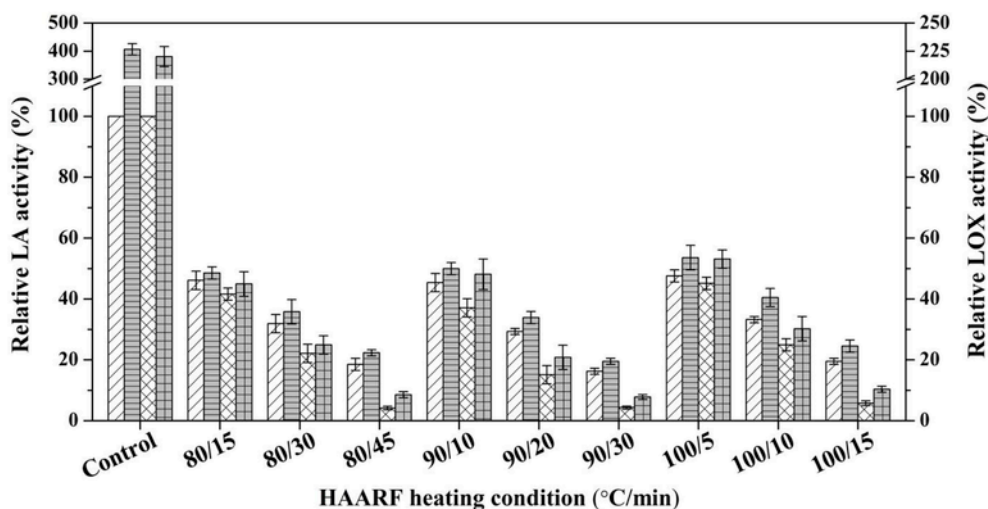


Fig. 3. Lipase (LA) (▨) 0 day (▩) 60 day) and lipoxygenase (LOX) (▧) 0 day (▨) 60 day) activity in rice bran samples subjected to different hot air-assisted radio frequency (HAARF) heating conditions before and after storage.

Table 2

Changes of free fatty acid content (oleic g/100g) in rice bran samples subjected to different hot air-assisted radio frequency (HAARF) heating conditions during storage.

HAARF treatment	Storage time (d)				
	0	15	30	45	60
Control	2.15 ± 0.15 ^a	21.64 ± 0.52 ^a	32.78 ± 0.66 ^a	40.61 ± 0.91 ^a	50.67 ± 0.88 ^a
80 °C/15 min	2.34 ± 0.13 ^a	10.87 ± 0.27 ^b	16.85 ± 0.34 ^b	21.16 ± 0.93 ^b	29.11 ± 0.14 ^b
80 °C/30 min	2.17 ± 0.11 ^a	4.23 ± 0.14 ^c	6.85 ± 0.25 ^f	9.96 ± 0.24 ^e	12.93 ± 0.39 ^f
80 °C/45 min	2.15 ± 0.09 ^a	2.95 ± 0.16 ^f	3.36 ± 0.13 ^g	3.97 ± 0.08 ^g	4.48 ± 0.12 ⁱ
90 °C/10 min	2.01 ± 0.04 ^a	10.15 ± 0.37 ^b	15.96 ± 0.27 ^c	20.65 ± 0.52 ^b	26.74 ± 0.49 ^c
90 °C/20 min	2.24 ± 0.10 ^a	4.34 ± 0.17 ^e	7.06 ± 0.39 ^f	9.02 ± 0.22 ^f	10.69 ± 0.31 ^g
90 °C/30 min	2.21 ± 0.15 ^a	2.57 ± 0.22 ^f	3.01 ± 0.12 ^h	3.46 ± 0.17 ^h	4.01 ± 0.06 ⁱ
100 °C/5 min	2.08 ± 0.14 ^a	9.21 ± 0.19 ^c	13.65 ± 0.41 ^d	17.95 ± 0.22 ^c	24.64 ± 0.65 ^d
100 °C/10 min	2.11 ± 0.09 ^a	6.21 ± 0.37 ^d	9.15 ± 0.25 ^e	13.04 ± 0.61 ^d	15.86 ± 0.34 ^e
100 °C/15 min	1.98 ± 0.19 ^a	2.75 ± 0.09 ^f	3.22 ± 0.13 ^g	3.96 ± 0.16 ^g	4.85 ± 0.09 ^h

*Mean values are not significantly different ($p > 0.05$) for the same lower case letters within a column among the treatment.

100 g at the end of storage. However, the FFA accumulations of HAARF treated samples were all significantly ($p < 0.05$) lower than those of the control at each storage interval. The FFA contents of samples treated at 80 °C for 45 min, 90 °C for 30 min, and 100 °C for 15 min were lower than the recommended upper limit (FFA < 5 g/100 g) within 60 d of storage, and the lowest accumulation of FFA was obtained in samples treated at 90 °C for 30 min.

Table 3

Changes of peroxide value (meq/kg RBO) in rice bran samples subjected to different hot air-assisted radio frequency (HAARF) heating conditions during storage.

HAARF treatment	Storage time (d)				
	0	15	30	45	60
Control	0.98 ± 0.02 ^a	11.25 ± 0.32 ^a	23.99 ± 0.76 ^a	32.71 ± 0.78 ^c	38.21 ± 0.82 ^c
80 °C/15 min	1.02 ± 0.03 ^a	6.11 ± 0.41 ^c	9.07 ± 0.57 ^d	13.26 ± 0.41 ^d	19.14 ± 0.29 ^d
80 °C/30 min	0.95 ± 0.03 ^a	2.39 ± 0.15 ^e	5.81 ± 0.17 ^f	7.14 ± 0.33 ^h	8.42 ± 0.17 ^h
80 °C/45 min	0.98 ± 0.02 ^a	13.25 ± 0.53 ^b	25.99 ± 0.63 ^c	34.71 ± 0.29 ^b	40.21 ± 0.42 ^b
90 °C/10 min	1.06 ± 0.11 ^a	5.03 ± 0.25 ^d	8.85 ± 0.41 ^d	12.14 ± 0.26 ^e	16.95 ± 0.38 ^e
90 °C/20 min	1.07 ± 0.14 ^a	2.41 ± 0.18 ^e	5.38 ± 0.22 ^f	7.14 ± 0.27 ^h	8.05 ± 0.17 ^h
90 °C/30 min	1.04 ± 0.05 ^a	13.14 ± 0.31 ^b	29.56 ± 0.54 ^b	37.15 ± 0.86 ^a	45.11 ± 0.97 ^a
100 °C/5 min	1.08 ± 0.04 ^a	5.15 ± 0.11 ^d	8.45 ± 0.38 ^d	11.02 ± 0.47 ^f	15.64 ± 0.39 ^f
100 °C/10 min	0.96 ± 0.08 ^a	2.65 ± 0.27 ^e	6.85 ± 0.33 ^e	7.96 ± 0.36 ^g	9.85 ± 0.14 ^g
100 °C/15 min	0.99 ± 0.06 ^a	1.88 ± 0.10 ^f	3.64 ± 0.28 ^g	5.47 ± 0.31 ⁱ	7.56 ± 0.22 ⁱ

*Mean values are not significantly different ($p > 0.05$) for the same lower case letters within a column among the treatment.

PV of these two samples increased rapidly from 0.98 to 1.04 meq/kg to 40.21 and 45.11 meq/kg, respectively, and were significantly ($p < 0.05$) higher than that of other HAARF treated samples at the end of storage. According to Codex Alimentarius Commission (2001), RB samples treated by HAARF heating at 80 °C for 30 min, 90 °C for 20 min, and 100 °C for 10 and 15 min can achieve a shelf life of more than 60 d at 35 °C (PV < 10 meq/kg). Based on the FFA and PV results observed in the present study, HAARF heating to 100 °C followed by 15 min holding time at this temperature was the optimal stabilization condition for retarding both hydrolysis and oxidation reactions in RB samples. Under these conditions, the RB can be stored for a period of 60 d at 35 °C and remain under the threshold limits of FFA < 5 g/100 g and PV < 10 meq/kg.

3.5. Relationship between water activity (or moisture content) and lipid rancidity in rice bran

To better understand the relationships between a_w (MC) and lipid rancidity of stabilized RB during storage, a zero order kinetic model for lipid rancidity in low moisture foodstuffs as suggested by Labuza (1982) was used to fit the FFA content and PV versus storage time. The relationship between reaction rate constants k_{FFA} or k_{PV} and a_w of stabilized RB during storage all followed the polynomial relationship (Fig. 4), and the fitted models for hydrolytic and oxidative rancidity were $k_{\text{FFA}} = -6.154a_w^3 + 8.434a_w^2 - 2.02a_w + 0.161$ ($R^2 = 0.995$) and $k_{\text{PV}} = 121.1a_w^4 - 218.3a_w^3 + 144.7a_w^2 - 40.75a_w + 4.229$ ($R^2 = 0.979$), respectively. For hydrolytic rancidity, the k_{FFA} decreased significantly with decreasing a_w , and reached the lowest value of 0.029 g/100 g/day at a_w of 0.141 (Fig. 4A). This might be because water molecules are both reactant and solvent in lipid hydrolytic rancidity reactions, thus reducing MC could effectively inhibit the hydrolysis of lipid (Kim et al., 2014b). However, for oxidative rancidity, the k_{PV} decreased with decreasing a_w and reached the lowest value of 0.111 at a_w of 0.241, then rapidly increased to 0.666 and 0.747 with a further decrease in a_w to 0.161 and 0.141, respectively (Fig. 4B). This was agreement with the rapid oxidation rate in samples treated at 80 °C for 45 min and 90 °C for 30 min presented in Table 3. Bell (2007) reported that the oxidation rate could increase as a_w of foodstuff decreased lower than its a_w corresponding to monolayer of water due to direct contact of oxygen molecules and lipids. Thus, the significant increase of k_{PV} at a_w below 0.241 might be because the water molecules exert a protective layer on the surface of lipids which then disappeared due to over drying. Labuza and Dugan (1971) reported that the lipids in most dried foods are most stable for oxidation at a_w of about 0.33. Similarly, Jensen and Risbo (2007) found that

oatmeal muesli, peanuts and pork scratchings had minimal oxidation rate at a_w from 0.23 to 0.43. Domínguez, Azuara, Vernon-Carter, and Beristain (2007) also reported that the minimal oxidation rate in the macadamia nut occurred when maintained at a_w of 0.436 during storage. It can be seen that different food products have different optimal a_w for retarding rancidity. In our study, the lowest rate both for hydrolytic and oxidative rancidity of RB occurred at a_w value of 0.241 (MC = 4.29 g/100 g w.b.). Therefore, to achieve a successful dry heating stabilization, the heating process should not be conducted to obtain a_w of RB lower than 0.241 to avoid over-drying leading to auto-oxidation during storage in RB.

3.6. Hot air-assisted radio frequency heating influence on product quality of rice bran

3.6.1. Fatty acid composition of rice bran

Because RB sample treated by HAARF heating at 100 °C for 15 min have the longest shelf life, only this sample was chosen for quality evaluation. It was observed that HAARF treatment at 100 °C for 15 min had no significant influence on the FA composition of RB ($p > 0.05$) (Table 4). Similar results were also observed in RB stabilized by MW heating (Ramezanzadeh et al., 2000). After 60 d of storage, the FA composition and content in HAARF treated sample did not change significantly ($p > 0.05$), while the content of linoleic acid and linolenic acid in control showed a significant decrease ($p < 0.05$). The possible explanation for the decrease of FAs contents during storage could be due to the abundant FFAs hydrolyzed by LA in control. FFAs, such as linoleic and linolenic acid, are an excellent substrate for LOX and auto-oxidation leading to the generation of primary oxidation products.

3.6.2. Tocopherol and γ -oryzanol contents of rice bran

Table 5 shows the total tocopherols were 48.06 mg/kg in control and α -tocopherol was the predominant component, followed by $\beta + \gamma$ -tocopherol, while no δ -tocopherol was detected. After HAARF treatment, α -tocopherol and total tocopherol contents increased from 30.12 to 48.06 mg/kg to 34.64 and 55.05 mg/kg, respectively. Kim et al. (2014a) reported that the most of the tocopherol was bound to proteins or phospholipids and thermal process could break these linkages. After 60 d of storage, the total tocopherol content of control and RF treated sample decreased from 48.06 to 30.01 and 55.05 to 45.74 mg/kg, decreasing by 38 and 17%, respectively. Similar decreases were also observed in α and $\beta + \gamma$ -tocopherol. This implied that the tocopherols in raw RB were less stable than the HAARF treated RB during storage. Chun, Lee, and Eitenmiller (2005) reported that tocopherol had a significant antioxidant effects during lipid

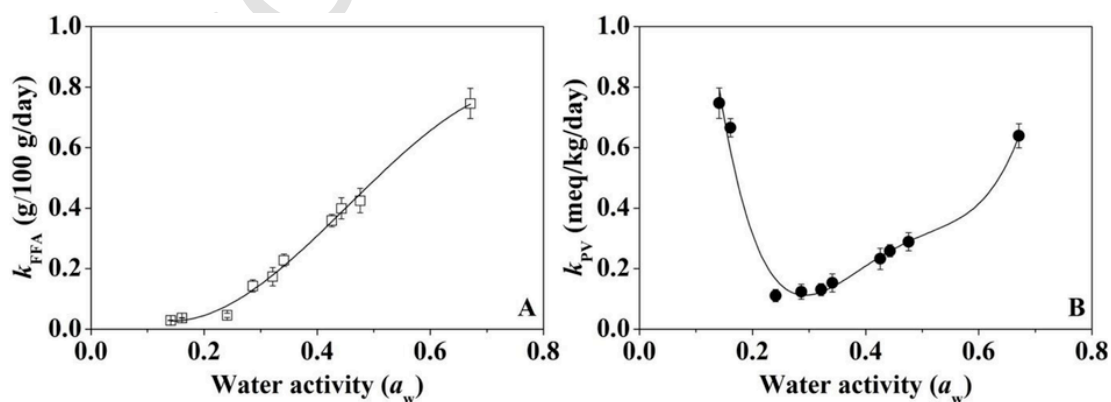


Fig. 4. Relationship between lipid rancidity rate constant (k) and water activity (a_w) of rice bran samples during storage with (A) hydrolytic reaction (\square Hydrolytic rancidity rate — Predicated value) and (B) oxidative reaction (\bullet Oxidative rancidity rate —Predicated value).

Table 4

Changes in fatty acid compositions of rice bran oil (RBO) extracted from control and hot air-assisted radio frequency (HAARF) treated rice bran samples before and after storage.

Fatty acid (relative g/ 100 g RBO)	Storage time (days)	Treatment	
		Control	HAARF treated at 100 °C + 15 min
Palmitic (C16:0)	0	16.72 ± 0.52 ^{Aa}	16.59 ± 0.31 ^{Aa}
	60	16.39 ± 0.68 ^{Aa}	16.17 ± 0.49 ^{Ba}
Stearic acid (C18:0)	0	1.64 ± 0.11 ^{Aa}	1.61 ± 0.09 ^{Aa}
	60	1.72 ± 0.22 ^{Aa}	1.45 ± 0.26 ^{Aa}
Oleic acid (C18:1)	0	39.15 ± 0.55 ^{Aa}	39.09 ± 0.41 ^{Aa}
	60	40.15 ± 0.48 ^{Aa}	39.12 ± 0.37 ^{Aa}
Linoleic acid (C18:2)	0	39.79 ± 0.69 ^{Aa}	39.54 ± 0.47 ^{Aa}
	60	37.14 ± 0.67 ^{Bb}	39.16 ± 0.51 ^{Aa}
Linolenic acid (C18:3)	0	1.02 ± 0.08 ^{Aa}	1.09 ± 0.11 ^{Aa}
	60	0.88 ± 0.05 ^{Bb}	1.18 ± 0.14 ^{Aa}

*Mean values are not significantly different ($p > 0.05$) for the same capital letters within a row among the treatment, and for the same lower case letters within a column among the storage time.

oxidation in stored peanuts, and its losses were highly correlated with PV increase.

As show in Table 5, control sample contained 2.97 g/100 g oil of γ -oryzanol. Statistically, the effect of HAARF heating and storage on

Table 5

Changes in tocopherol and γ -oryzanol contents of rice bran oil (RBO) extracted from control and hot air-assisted radio frequency (HAARF) treated rice bran samples before and after storage.

Sample	Storage time (days)	Individual tocopherols (mg/kg RBO)		Total tocopherols (mg/kg RBO)	γ -oryzanol (g/100 g RBO)
		α	$\beta + \gamma$		
Control	0	30.12 ± 0.58 ^b	17.89 ± 0.69 ^b	48.06 ± 0.72 ^b	2.97 ± 0.25 ^a
	60	18.14 ± 0.52 ^c	11.82 ± 0.37 ^d	30.01 ± 0.35 ^d	2.91 ± 0.12 ^a
HAARF treated at 100 °C + 15 min	0	34.64 ± 0.54 ^a	20.37 ± 0.42 ^a	55.05 ± 0.28 ^a	2.85 ± 0.16 ^a
	60	29.56 ± 0.33 ^b	16.18 ± 0.28 ^c	45.74 ± 0.51 ^c	2.89 ± 0.07 ^a

*Mean values are not significantly different ($p > 0.05$) for the same lower case letters within a column among the treatment and storage time.

the γ -oryzanol content of RBO was not significant ($p > 0.05$). Similarly, Rodchuajeen, Niamnuay, Charunuch, Soponronnarit, and Devahastin (2016) also reported that the storage time did not significantly affect the γ -oryzanol content of RB stabilization by different moving-bed drying methods. Overall, γ -oryzanol in RB was stable during HAARF stabilization and storage.

4. Conclusion

The combined target temperature and holding time were used to inactivation of LA and LOX activities in RB using HAARF treatments. The higher temperatures and longer holding time resulted in less residual enzyme activities in RB samples. Under the conditions selected in this study, the RB sample treated by the HAARF heating to 100 °C with 15min holding can be stored and remain below acceptable thresholds for a period of 60 d at 35 °C without adverse effect on product quality. Moreover, the optimal a_w (or MC) for stabilized RB with the lowest lipid rancidity rate occurred at a_w of 0.241. This study indicates that HAARF dry heating can be used as an effective method for inactivating endogenous enzymes to extend the shelf life of RB. Future studies to evaluate the effects of industrial-scale HAARF treatments on stabilization of RB with different MC will be valuable.

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References

- AOAC, 2005. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, DC.
- AOCS, 1997. Official methods and recommended practices of the American oil chemists' society, 5th ed. USA AOCs Press, Champaign, IL.
- Awuah, G.B., Ramaswamy, H.S., Tang, J., 2015. Radio frequency heating in food processing: Principles and applications. CRC Press.
- Bell, L.N., 2007. Moisture effects on food's chemical stability. In: Barbosa-Cánovas, G.V. (Ed.), Water activity in foods: Fundamentals and applications. Black-well Publishing Ltd, Oxford, pp. 173–198.
- Boreddy, S.R., Birla, S.L., Froning, G., Thippareddi, H., Subbiah, J., 2014. Effect of radio frequency assisted thermal processing on quality and functional properties of egg white powder. *Transactions of the ASABE* 57 (6), 1761–1770.
- Brunschwiler, C., Heine, D., Kappeler, S., Conde-Petit, B., Nyström, L., 2013. Direct measurement of rice bran lipase activity for inactivation kinetics and storage stability prediction. *Journal of Cereal Science* 58, 272–277.
- Chun, J., Lee, J., Eitenmiller, R.R., 2005. Vitamin E and oxidative stability during storage of raw and dry roasted peanuts packaged under air and vacuum. *Journal of Food Science* 70, 292–297.
- Codex Alimentarius, 2001. Fats, oils and related products, 2nd ed. FAO/WHO Food Standards Programme, Rome, Italy.
- Domínguez, I.L., Azuara, E., Vernon-Carter, E.J., Beristain, C.I., 2007. Thermodynamic analysis of the effect of water activity on the stability of macadamia nut. *Journal of Food Engineering* 81, 566–571.
- FAOSTAT, 2016. Food and agriculture organization, Available at: <http://www.fao.org/faostat/en/#data/QC/visualize>.
- Jensen, P.N., Risbo, J., 2007. Oxidative stability of snack and cereal products in relation to moisture sorption. *Food Chemistry* 103, 717–724.
- Jiao, S., Zhu, D., Deng, Y., Zhao, Y., 2016. Effects of hot air-assisted radio frequency heating on quality and shelf-life of roasted peanuts. *Food and Bioprocess Technology* 9, 308–319.
- Kim, S.M., Chung, H.J., Lim, S.T., 2014a. Effect of various heat treatments on rancidity and some bioactive compounds of rice bran. *Journal of Cereal Science* 60, 243–248.
- Kim, J., Kim, M.J., Lee, J., 2014b. Role of moisture on the lipid oxidation determined by D₂O in a linoleic acid model system. *Food Chemistry* 146, 134–140.
- Labuza, T.P., 1982. Shelf-life dating of foods. Food and Nutrition Press, Inc, Westport, Connecticut, 44–53.
- Labuza, T.P., Dugan, L.R., 1971. Kinetics of lipid oxidation in foods. *Critical Reviews in Food Science and Nutrition* 2, 355–405.
- Lakkakula, N.R., Lima, M., Walker, T., 2004. Rice bran stabilization and rice bran oil extraction using ohmic heating. *Bioresource Technology* 92, 157–161.
- Laokuldilok, T., Rattanathanan, Y., 2014. Protease treatment for the stabilization of rice bran: Effects on lipase activity, antioxidants, and lipid stability. *Cereal Chemistry* 91, 560–565.
- Ling, B., Hou, L., Li, R., Wang, S., 2016. Storage stability of pistachios as influenced by radio frequency treatments for postharvest disinfestations. *Innovative Food Science & Emerging Technologies* 33, 357–364.
- Ling, B., Yang, X., Li, R., Wang, S., 2016. Physicochemical properties, volatile compounds, and oxidative stability of cold pressed kernel oils from raw and roasted pistachio (*Pistacia vera* L. Var Kerman). *European Journal of Lipid Science and Technology* 118, 1368–1379.
- Oliveira, M.G.D.C., Bassinello, P.Z., Lobo, V.L.D.S., Rinaldi, M.M., 2012. Stability and microbiological quality of rice bran subjected to different heat treatments. *Ciência E Tecnologia De Alimentos* 32, 725–733.
- Orthofer, F.T., 2004. Chapter 19: Rice bran and oil. In: Champagne, E.T. (Ed.), *Rice: Chemistry and technology*, 3rd ed. American Association of Cereal Chemists, St. Paul, Minnesota.
- Ramezanzadeh, F.M., Rao, R.M., Prinyawiwatkul, W., Marshall, W.E., Windhauser, M., 2000. Effects of microwave heat, packaging, and storage temperature on fatty acid and proximate compositions in rice bran. *Journal of Agricultural and Food Chemistry* 48, 464–467.
- Rodchuajeen, K., Niamnuy, C., Charunuch, C., Soponronnarit, S., Devahastin, S., 2016. Stabilization of rice bran via different moving-bed drying methods. *Drying Technology* 34, 1854–1867.
- Sharif, M.K., Butt, M.S., Anjum, F.M., Khan, S.H., 2014. Rice bran: A novel functional ingredient. *Critical Reviews in Food Science and Nutrition* 54, 807–816.
- Taoukis, P.S., Labuza, T.P., Saguy, I.S., 1997. Handbook of food engineering practice. In: Valentas, K.J. (Ed.), *Kinetics of food deterioration and shelf-life prediction*. CRC Press, New York: Boca Raton, p. 374.
- Thanonkaew, A., Wongyai, S., McClements, D.J., Decker, E.A., 2012. Effect of stabilization of rice bran by domestic heating on mechanical extraction yield, quality, and antioxidant properties of cold-pressed rice bran oil (*Oryza sativa* L.). *LWT-Food Science and Technology* 48, 231–236.
- Xu, B., Zhou, S., Miao, W., Gao, C., Cai, M., Dong, Y., 2013. Study on the stabilization effect of continuous microwave on wheat germ. *Journal of Food Engineering* 117, 1–7.
- Yılmaz, N., Tuncel, N.B., Kocabiyik, H., 2014. Infrared stabilization of rice bran and its effects on γ -oryzanol content, tocopherols and fatty acid composition. *Journal of the Science of Food and Agriculture* 94, 1568–1576.