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Rice bran real-time stabilisation technology with flowing microwave radiation: its impact on rancidity and some bioactive compounds

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RESEARCH ARTICLE

Abstract

A pilot-scale real-time flowing microwave drum (PRFM) heating system was designed and adopted to stabilise rice bran (RB) for retarding rancidity. Meanwhile, the influence of PRFM radiation on fat-soluble bioactive compounds of RB were evaluated. Results revealed that PRFM processing could effectively inactivate lipase (LA) and lipoxygenase (LOX) of RB in minutes. The RB sample irradiated at 4 kW for 10 min with PRFM acquired the optimal stabilisation effect, and its residual LA activity was 7.94% and LOX was completely destroyed. The free fatty acids content and peroxide value of RB sample treated at optimal condition only increased by 1.06% and 5.32 meq O_2/kg rice bran oil at 35 °C for 60 days, respectively. No significant decrease in fatty acid, tocopherols and γ-oryzanol content were noticed in bran sample treated at optimal condition. Therefore, PRFM radiation would be used as a practical and real-time technology for stabilising RB on-line.

Keywords: lipase, microwave, real-time, rancidity, rice bran

1. Introduction

Rice bran (RB), a natural by-product of rice milling, consists of germ, seed coat and part of broken endosperm, and comprises about 10% of total rice grain (Pourali *et al.*, 2009). RB is a considerable source of essential fatty acids, protein, dietary fibre, B vitamins and minerals (Mian Kamran *et al.*, 2014; Saunders, 1985). Rice bran oil (RBO) is recognised as a kind of healthy oil due to its considerable amounts of unsaturated fatty acids and bioactive compounds, such as γ-oryzanol, tocopherols (Henderson *et al.*, 2012; Lerma-García *et al.*, 2009; Sohail *et al.*, 2017).

However, RB is usually utilised as animal fodder in developing countries (Mian Kamran *et al.*, 2014), for it is highly susceptible to rancidity due to the endogenous lipase (LA) and lipoxygenase (LOX) (Shastry and Raghavendra Rao, 1975; Shastry and Rao, 1971). After rice milling, triglycerols is enzymatically hydrolysed to glycerol and free fatty acids (FFA) in a short time (Takano, 1993). It is reported that 5-7% of FFA can release from fresh bran per day (Saunders, 1985). Furthermore, the unsaturated FFA are also better substrate for LOX and autoxidation (Gardner, 1995). As a result, RB undergoes further oxidative decomposition with formation of off-flavour, and it makes RB unsuitable for human consumption (Loypimai *et al.*, 2009).

Since lipids of RB can become rancid during the transportation of bran to oil factory. Therefore, a stabilisation procedure to inactivate LA and LOX with retaining a maximum level of nutrients is indispensable after producing immediately. Various inactivation techniques for RB have been explored including chemical methods (Gopinger *et al.*, 2015), hot air heating (Fernando and Hewavitharana, 1990), extrusion (Randall *et al.*, 1985; Sharma *et al.*, 2004), infrared heating (Yılmaz, 2016) and microwave heating (Vetrimani *et al.*, 1992). Steaming heating makes bran humid and lumpy (Malekian *et al.*, 2000). Large capital investment, high operating and

equipment maintenance cost make extrusion uneconomical for RB stabilisation (Malekian *et al.*, 2000).

Microwave heating is highly popular in food processing due to the advantages of low energy-consumption, rapid heating rate, low maintenance cost and few detrimental effect on nutritional quality (Chandrasekaran *et al.*, 2013; Thanonkaew *et al.*, 2012). Published literatures of RB stabilisation with domestic microwave oven are promising (Ramezanzadeh *et al.*, 1999b; Rizk *et al.*, 1995). However, flowing radiation and scaling up are main obstacles for industrial application of microwave-based stabilisation technology, and there is no literature specifically reporting real-time flowing radiation for RB stabilisation to our best knowledge.

Therefore, a pilot-scale real-time flowing microwave drum (PRFM) heating system was designed with the purpose of developing an on-line stabilisation technology for RB. The main objectives of this study were: (1) to investigate the optimised processing parameters of PRFM stabilisation on RB; and (2) to determine the effect of the PRFM heating on quality of RBO, such as γ-oryzanol, tocopherols and fatty acid content.

2. Material and methods

Samples and reagents

Fresh RB sample was supplied by a local rice factory (Hengshun Rice Industry Co., Ltd, Zhenjiang, Jiangsu, China). Oleic acid (99% pure), tocopherols (α, β, γ and δ), γ-oryzanol and F.A.M.E. Mix (C4-C24) standards were all purchased from Sigma-Aldrich (Shanghai, China), linoleic acid (99% pure) was purchased from Fluka (Ronkonkoma, NY, USA), olive oil was purchased from Mueloliva (Priego de Cordoba, Spain). All other reagents were analytical grade.

The PRFM stabilisation system

The PRFM system (model NJ-6-1) is a self-designed, multifunctional and flowing microwave drum heater, and it was assembled by local microwave company (Nanjing Jiequan Microwave Development Co., Ltd, Jiangsu, China). As shown in Figure 1, the microwave cavity contains 6 magnetrons (6×1000 W, 2,450 MHz) with a maximum power of 6 kW. A rotatable teflon drum inside the chamber can bear a feeding rate of 0-120 kg RB/h, and the feeding rate can be adjustable with a vibration motor. Three teflon rods are installed in the drum with the purpose of stirring RB particles for attaining uniform heating. The device is controlled based on PLC and touched-screen control system with automatic and manual mode. The real-time temperature of stabilised RB can be monitored with an infrared thermometer fixed near outlet. A centrifugal fan is adopted as dehumidifier on top of the outlet with the ventilation rate of 20, 40, 60 and 80 N m^3/h.

Rice bran stabilisation

The feeding rate of RB was set at about 20 kg/h. The stabilisation parameters that resulted in undesirable changes in the sensory quality (mainly burnt flavour) of RB were discarded first. And considering the power consumption, high microwave power (6 kW) and long duration time were not adopted. The optimised processing parameters (microwave power, duration time and ventilation rate) for inactivating the LA and LOX of RB were explored with single factor tests as follows: (1) duration time 7 min, ventilation rate 60 N m^3/h and microwave power 1, 2, 3, 4 and 5 kW; (2) microwave power 3 kW, ventilation rate 60 N m^3/h and duration time 4, 7, 10, 13 and 16 min; and (3) microwave power 3 kW, duration time 4 min and ventilation rate 20, 40, 60 and 80 N m^3/h. The significant factors were then subjected to a full-factor test for further optimisation. And the oil quality of raw and stabilised RB samples were analysed.

Water activity and water content

Water activity (Aw) and water content (WC) of raw and PRFM-treated RB were measured with a dew point hygrometer (AquaLab Series 3TE; Decagon Devices, Pullman, WA, USA) and a halogen moisture analyser (model HB43-S; Mettler Toledo International Inc., Greifensee, Switzerland).

Determination of lipase and lipoxygenase activity

LA and LOX activity of RB was determined according to the method of Xu *et al.* (2012) and Xu *et al.* (2013). For LA activity, briefly, 1.5 ml of purified olive oil was added to 2 g of defatted RB sample (sifted with 30 mesh sieve) and incubated at 40 °C for 8 h. Liberated FFA during incubation was quantified at 715 nm with this method. LA activity was expressed as units/gram (U/g), where 1 U was defined as micro-equivalents of oleic acid liberated per hour.

For LOX activity, briefly, 5 g of RB powder (sifted with 30 mesh sieve) was extracted with 50 ml cold acetic buffer (0.1 mol/l, pH 4.5) by stirring for 30 min at 4 °C and centrifuged at 10,000 rpm for 10 min. The supernatant was used for analysing the activity of LOX with a DU-800 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA) at the intervals of 15 s for 5 min. Substrate solution and the reaction system were prepared with linoleic acid and Tween-20 in phosphate buffer solution as the method of Xu *et al.* (2012). One unit of LOX activity was defined as an increase in absorbance of per minute at 234 nm per mg of protein under assay conditions.

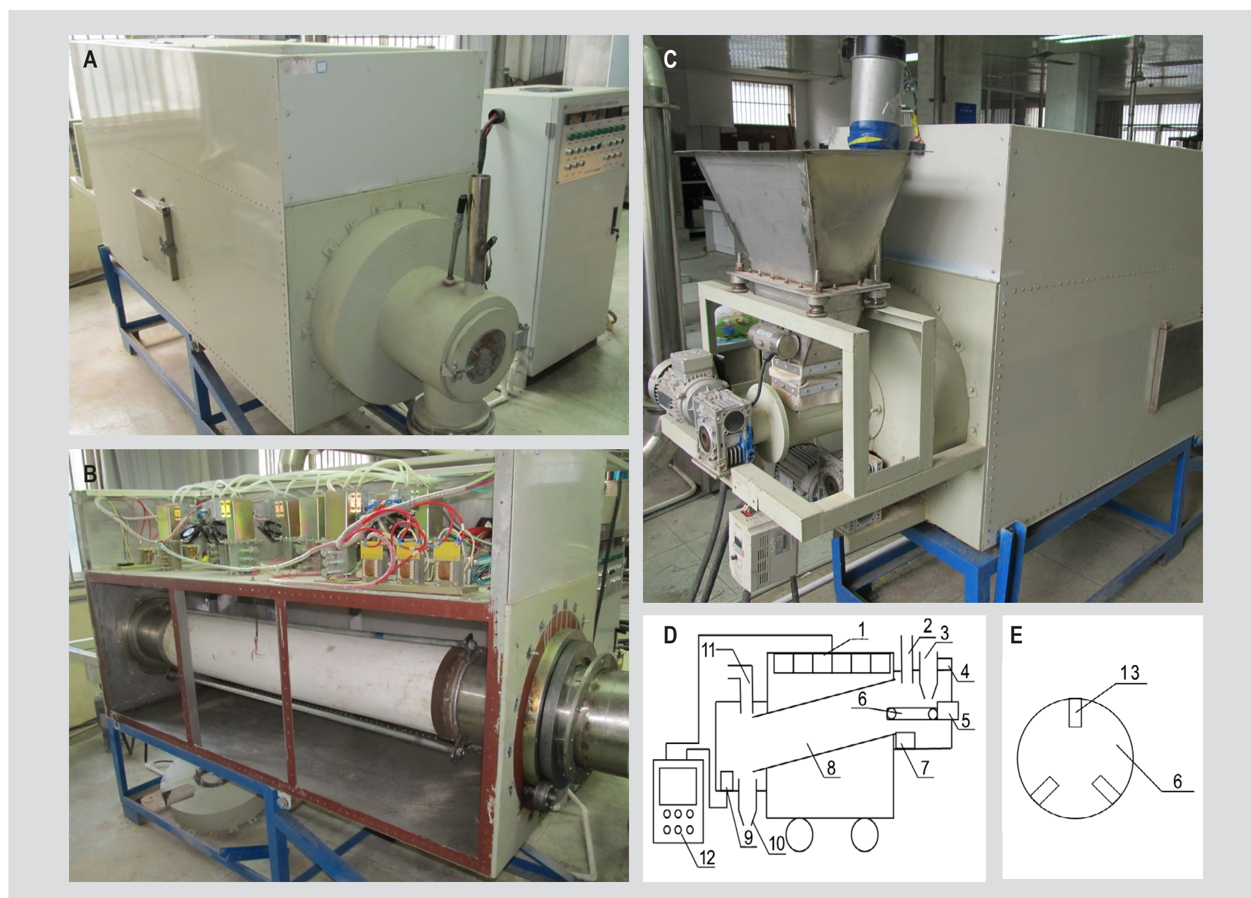

Figure 1. Schematic diagram of the pilot-scale real-time flowing microwave drum (PRFM) stabilisation system: (A) outlet module and control terminal; (B) microwave generator module and drum; (C) feedstock module; (D) structural section; and (D) drum section (1 = magnetrons; 2 = inlet air fan; 3 = feedstock inlet; 4 = vibration motor; 5 = feed motor; 6 = screw feeder; 7 = rotating motor; 8 = rotatable drum; 9 = temperature detector; 10 = outlet; 11 = outlet air fan; 12 = control terminal; 13 = stirring rod).

Accelerated storage experiments

Raw and PRFM-treated RB samples (100 g) were sealed in polyethylene zip-top bags and stored at 35 °C at the relative humidity of 75% in dark for 60 days. The storage stability of RB samples was estimated with FFA content (% of oleic acid) and peroxide value (PV) during storage according to the AOCS official method Ca 5a-40 and Cd 8-53 at the time interval of every 15 days, respectively (AOCS, 2003, 2009).

Rice bran oil preparation

Briefly, 30 g of RB samples (sieved with 30 mesh) was extracted with 300 ml n-hexane (containing 100 mg butylated hydroxytoluene) in conical flask with stopper (500 ml), then the flask was purged with nitrogen for 30 seconds and vibrated at 30 °C for 2 h and filtered through a Buchner funnel quickly, the extraction procedures was repeated for three times. The solvent was removed at 40 °C for 30 min with a rotary evaporator. The residual RBO was nitrogen-filled packaged and stored at -40 °C for further analysis.

Determination of tocopherols and γ-oryzanol content

Tocopherols (α, β, γ and δ) content of RBO was evaluated according to the method of Shao-Hua and Lean-Teik (2011) with some modifications. Briefly, 1.0 g of RBO samples was dissolved in 10 ml hexane, vortexed (30 sec), centrifuged (3,220×*g* for 10 min), filtered (0.45 μm) and injected (20 μl) into the Shimadzu LC-20A HPLC system (Shimadzu Corp., Kyoto, Japan) with a Zorbax RX-SIL column (5 μm i.d., 250×4 mm; Agilent Technologies, Santa Clara, CA, USA) and a Shimadzu RF-10AXL fluorescence detector with excitation and emission wavelength of 290 and 330 nm. The mobile phase was n-hexane/isopropanol (98.5:1.5, v/v) and the flow rate was 1.0 ml/min. Individual tocopherols quantified as mg/kg RBO with external standards.

γ-oryzanol content of RB was quantified according to the method of Shao-Hua and Lean-Teik (2011) with some modifications. Briefly, 200 mg of RBO samples was dissolved in 10 ml hexane, vortexed (30 sec), centrifuged (3,220×*g* for 10 min), filtered (0.45 μm) and injected (20 μl) into the Shimadzu LC-20A HPLC system equipped with a

photodiode array and a silica gel column (Zorbax RX-SIL, 5 μm i.d., 250×4 mm). The mobile phase was n-hexane/isopropanol (98.5:1.5, v/v) and the flow rate was 0.9 ml/min. The detection was performed at 325 nm. γ-oryzanol was quantified as g/kg RBO with external standards.

Fatty acid composition and oxidative stability

The fatty acid composition and oxidative stability of RBO according to the method of Li *et al.* (2016).

Statistical analysis

All the determinations were made in three replicates, and results were expressed as means ± standard deviations. Variance analysis was performed with SPSS statistical software at $P<0.05$ (version 17.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

Effect of PRFM parameters on lipase activity of rice bran

PRFM power

The final temperatures and residual LA activity of RB samples during PRFM treatment with different power are described in Table 1A. The final temperatures of RB samples significantly raised with microwave power ($P<0.05$). When microwave power reached 5 kW, the final temperature of RB reached about 92.4±3.62 °C. There is negative correlation between LA activity and bran temperature. The residual LA activity of RB samples significantly decreased with microwave power increasing ($P<0.05$), and it was linearly correlated to microwave power ($y = -1.37x + 13.3$, $R^2=0.983$) during PRFM processing. The minimal LA activity of RB was about 6.01±0.31 U/g (at 5 kW), with losing 54.33% of its original activity. The LA inactivation may be ascribed to the 'thermal effect' by transformation of microwave energy into thermal energy in short time (Xu *et al.*, 2016). The microwave power was set in the range of 3-5 kW for further optimising technological parameters.

PRFM time

The final temperatures and residual LA activity of RB samples during PRFM treatment with different duration time are described in Table 1B. With subsequently prolonging duration time, the final temperature of processed RB samples significantly increased ($P<0.05$). The maximum bran temperature (82.8±4.1 °C) was observed in samples treated for 16 min. There was a linearly negative correlation between residual LA activity and duration time ($y = -0.36x + 13.15$, $R^2=0.991$) during PRFM processing. The bran samples exposed to PRFM radiation at 3 kW for 16 min attained the least LA activity (7.58±0.37 U/g), with losing 40.61% of its original activity. However, the LA activity of RB samples displayed a slight reduction when the processing time was beyond 13 min, and longer radiation time with microwave could make bran turn brown. Consequently, the duration time of PRFM was set in the range of 7-13 min for further optimising technological parameters.

Table 1. Effect of (A) microwave power, (B) duration time, and (C) ventilation rate, on lipase (LA) activity of rice bran.[1]

		Final temperature (°C)	LA activity (U/g)
A	Microwave power (kW)[2]	Final temperature (°C)	LA activity (U/g)
	0	room temperature	13.16±0.66a
	1	39.3±1.9d	12.16±0.31ab
	2	50.5±2.5cd	11.26±0.51bc
	3	65.7±3.3bc	10.33±0.26bc
	4	76.4±3.8ab	8.81±0.42cd
	5	92.4±4.6a	6.01±0.15d
B	Duration time (min)[3]	Final temperature (°C)	LA activity (U/g)
	0	room temperature	12.79±0.31a
	4	52.6±2.6b	12.38±0.24a
	7	64.9±3.1ab	11.39±0.19ab
	10	72.3±1.6a	9.75±0.17bc
	13	76.5±3.8a	8.19±0.21c
	16	82.8±4.4a	7.58±0.20c
C	Ventilation rate (N m^3/h)[4]	Final temperature (°C)	LA activity (U/g)
	0	73.2±2.2a	9.61±0.33a
	20	70.2±1.5a	10.53±0.20a
	40	67.5±2.4a	10.94±0.26a
	60	65.7±2.3a	10.7±0.14a
	80	62.6±1.1a	10.41±0.06a

[1] Means with different letters in the same column were significantly different at the level $P<0.05$.
[2] Duration time 7 min; ventilation rate 60 N m^3/h.
[3] Microwave power 3 kW; ventilation rate 60 N m^3/h.
[4] Microwave power 3 kW; duration time 4 min.

Ventilation rate

The final temperatures and residual LA activity of RB samples during PRFM treatments with different ventilation rate are shown in Table 1C. The highest temperature (73.2±2.7 °C) and minimal LA activity (9.61±0.28 U/g) were observed in bran treated with no ventilation, the reason may be ascribed to the additional steam heating effect inside the PRFM chamber by hot vapour escaped

from RB. But high moisture content can lead to lumpy and humid bran, and it will be harmful for preservation. With improving the ventilation rate, the hot vapour inside the drum was rapidly drew out, and the final temperature and WC of bran gradually declined. It seems that the ventilation rate has no significant influence upon residual LA activity of RB (P>0.05). Given that low moisture content contributes to food preservation. 80 N m^3/h was chosen as the optimal ventilation rate.

Lipase and lipoxygenase activity

The final temperature, Aw, WC, LA and LOX activity of RB samples during PRFM treatments with different technological parameters are shown in Table 2. The PRFM power and duration time had significant influences on final temperature, Aw and WC of RB (P<0.05). According to Oliveira and Franca (2002), heating efficiency of microwave is quite dependent on radiation frequency and power absorption. Final bran temperatures significantly increased with microwave power and duration time (P<0.05). The maximum bran temperature was about 112.5±5.6 °C at 5 kW for 13 min. The evaporation rate of moisture significantly increased with power level, the lowest WC of RB was 1.37±0.07% at 5 kW for 13 min, followed by 2.48±0.12% (4 kW, 13 min) and 3.22±0.16% (5 kW, 10 min). Similar results were observed for Aw of RB. A significant reduction of LA activity was observed in PRFM-treated bran samples (P<0.05). The residual LA activity of RB samples treated with PRFM at 5 kW for 13 min was only 1.25±0.06 U/kg, followed by 3.08±0.15 U/kg (4 kW, 13 min) and 3.69±0.18 U/kg (5 kW, 10 min), whereas the LA activity of raw samples was 13.21±0.63 U/kg. LOX of bran samples was completely destroyed with PRFM radiation for 7 min.

Storage stability

Free fatty acids content change

FFA content of raw and PRFM stabilised RB samples during storage are displayed in Figure 2A. The FFA content of raw RB increased rapidly from 4.29 to 49.36% at 35 °C during 60 days storage. In contrast, PRFM treatment significantly retarded the development of FFA in RB (P<0.05). Similar inhibitory action on FFA content of RB with domestic microwave oven heating was observed by Ramezanzadeh *et al.* (1999a). The influences of both PRFM power and duration time on FFA content of RB was statistically significant (P<0.05). This may due to the fact that PRFM radiation can effectively inactivate the LA activity of RB according to Table 2 (P<0.05). According to Tao *et al.* (1993), RB with more than 5% FFA is unprofitable for edible oil extraction. The FFA content of RB samples treated at 3 kW for 13 min attained approximately 9.51% at the end of 60 days. The increased FFA content of bran samples treated at 4 and 5 kW for 10 min kept below 3% at 35 °C for 60 days. The results confirmed that PRFM radiation is an effective and rapid stabilisation technology for RB. However, it is also easy to produce brown bran during PRFM radiation at 4 and 5 kW for more than 10 min. Therefore, the suitable PRFM parameters for effectively preventing RB from hydrolytic rancidity were 4 kW for 10 min (FFA increased by 1.06% at 35 °C for 60 days), 5 kW for 7 min (1.79%) and 4 kW for 7 min (2.91%).

Peroxide value change

PV reflects the oxidative rancidity extent of oil. The PV of raw and PRFM-treated RB samples during storage are displayed in Figure 2B. The PV of raw bran remarkably

Table 2. The final temperature, water content, water activity, residual lipase (LA) and lipoxygenase (LOX) activity of raw and PRFM-treated rice bran samples.[1]

Microwave power (kW)	Duration time (min)	Final temperature (°C)	Water content (%)	Water activity	LA activity (U/g)	LOX activity (U/mg)
Raw	–	17.9±1.2a	12.23±0.51a	0.56±0.13a	13.21±0.63a	0.15±0.02a
3	7	66.3±3.3e	8.92±0.36b	0.44±0.09ab	10.95±0.45b	0
	10	70.9±2.6de	7.48±0.32bc	0.36±0.11ab	9.92±0.39b	0
	13	74.8±2.7cde	4.81±0.24de	0.18±0.07ab	9.45±0.25b	0
4	7	77.4±3.9cde	6.21±0.31cd	0.25±0.08ab	9.61±0.48b	0
	10	90.6±4.5bcd	4.02±0.20efg	0.14±0.06b	4.96±0.31c	0
	13	95.3±3.7abc	2.48±0.12gh	0.07±0.03b	3.08±0.15de	0
5	7	92.4±4.6abc	4.73±0.23def	0.17±0.05ab	6.32±0.31c	0
	10	98.7±3.9ab	3.22±0.16fg	0.10±0.01b	3.69±0.18d	0
	13	112.5±5.6f	1.37±0.07h	0.03±0.02b	1.25±0.06e	0

[1] Data are expressed as mean ± standard deviation (n=3). Means with different letters in the same column were significantly different at the level P<0.05.

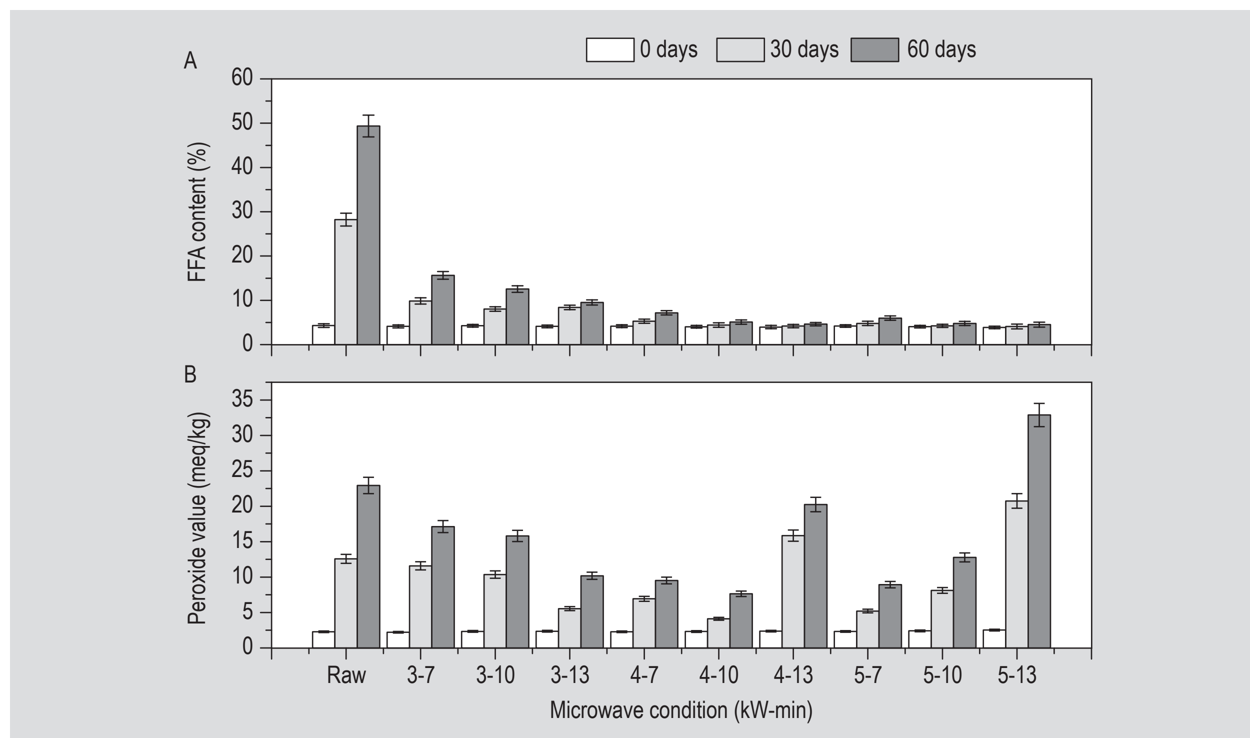

Figure 2. The changes in (A) free fatty acid (FFA) content and (B) peroxide value of rice bran during storage (microwave power: 3-5 kW, microwave time: 7-13 min, ventilation rate: 80 N m^3/h).

increased from 2.28 to 22.93 meqO_2/kg RBO at 35 °C during storage (*P*<0.05). This may be ascribed to the synergistic effects of enzymatic and non-enzymatic oxidation in raw bran. The development of peroxides in most bran samples were significantly retarded by PRFM processing (*P*<0.05), and this may be ascribed to the elimination of enzymatic oxidation since the LOX activity of the PRFM-treated samples was completely destroyed. However, PV of the RB samples treated at 4 and 5 kW for 13 min increased to unacceptable high level during storage (20.24 and 32.88 meqO_2/kg RBO), and it may be related to the low Aw (0.07±0.03 and 0.03±0.02) of samples treated at this condition. According to Barden and Decker (2016), monolayer of water in specific food products is necessary to cover the surface of the lipid, preventing it from direct exposure to air. So the reason of tremendous PV increase in samples treated at 4 and 5 kW for 13 min may be ascribed to the disrupting of monolayer water in RB with excessive PRFM radiation, and it makes the RBO directly expose to oxygen. Thus over-dehydration (Aw<0.07) with PRFM radiation made the dried RB samples more sensitive to oxidation. The minimal PV was observed in RB treated at 4 kW for 10 min (7.64 meqO_2/kg RBO), followed by sample treated at 5 kW for 7 min (8.93 meqO_2/kg RBO). Therefore, the suitable PRFM parameters for preventing RB from oxidative rancidity were 4 kW for 10 min, followed by 5 kW for 7 min.

Hydrolytic and oxidative rate

The rate constants of hydrolytic reaction (K_{FFA}) and oxidative reaction (K_{PV}) of lipids in raw and PRFM-treated bran during storage as a function of Aw are shown in Figure 3A and 3B, respectively. The K_{FFA} and K_{PV} were calculated with the zero-order reaction rate equations. The K_{FFA} was Aw-dependent and significantly decreased with residual Aw of RB (*P*<0.05). The K_{FFA} of the RB samples with Aw of 0.03-0.14 (treated at 4 and 5 kW beyond 10 min) were in the range of 0.009-0.014 g/kg/day. This may be related to the fact that water can act as both reactant and solvent in lipids hydrolytic reaction. Low Aw results in low molecular mobility of both substrate and LA. Duckworth and Smith (1963) declared that solute movement was not detectable below monolayer value.

However, the K_{PV} curve reveals a different tendency. Initially the K_{PV} of RB gradually decreased along with Aw. Once the Aw of RB was lower than 0.14, the K_{PV} significantly increased with further radiation with PRFM (*P*<0.05). According to Labuza and Dugan (1971), water can play both protective and pro-oxidative roles in lipids oxidation reaction. It is reported that lipid in dehydrated foodstuffs is more stable to oxidation at the Aw of about 0.33 (Labuza and Dugan, 1971). According to Romani *et al.* (2016), lipid oxidation rate of biscuits was slower at Aw

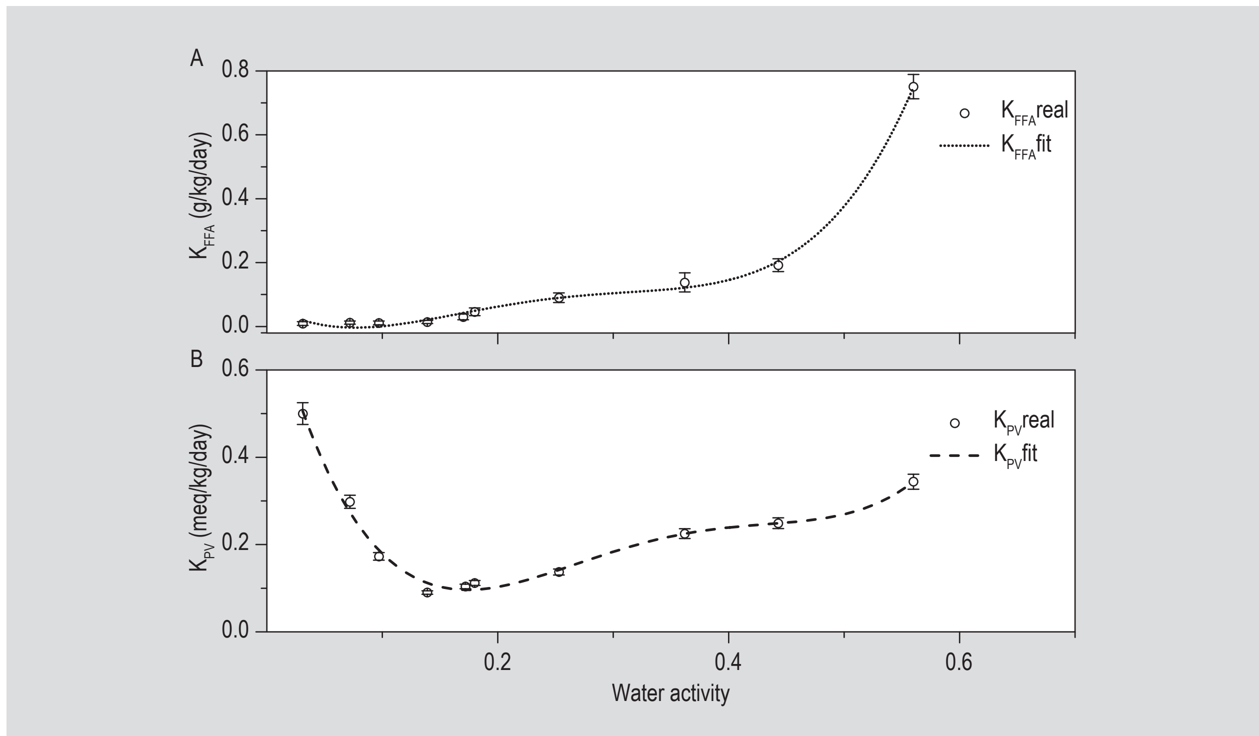

Figure 3. The (A) hydrolytic reaction (K_{FFA}) and (B) oxidative reaction (K_{PV}) of rice bran with different residual water activity during storage.

of 0.35. A minimal oxidative rate of oatmeal was observed at Aw of 0.23 by Jensen and Risbo (2007). The monolayer of water is thought to be essential to cover the surface of lipids in foodstuff, preventing it from directly exposing to oxygen (Barden and Decker, 2016). In this research, the optimum storage Aw of RB for inhibiting both hydrolytic and oxidative rancidity is near 0.14 (WC = 4.02±0.20%).

Oil quality

Fatty acid composition

The main fatty acid of RB samples are shown in Table 3. It is reported that oleic and linoleic acid contents of RB samples were in the range of 35.9-49.2% and 27.3-41.0% respectively (Goffman *et al.*, 2003). Approximately

Table 3. Main fatty acid content of rice bran oil.[1,2]

Microwave power (kW)	Processing time (min)	Fatty acid composition (%)					
		Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	γ-linolenic acid	∑UFA
Raw	–	17.51±0.19[a]	1.51±0.04[a]	38.30±0.44[a]	39.11±0.35[a]	2.69±0.19[a]	80.10±0.46[a]
3	7	17.45±0.02[a]	1.48±0.05[a]	38.32±0.25[a]	39.08±0.14[a]	2.67±0.08[a]	80.07±0.16[a]
	10	17.49±0.18[a]	1.47±0.03[a]	38.29±0.21[a]	39.05±0.22[a]	2.64±0.11[a]	19.98±0.19[a]
	13	17.58±0.11[a]	1.45±0.05[a]	38.26±0.12[a]	39.06±0.13[a]	2.65±0.11[a]	79.96±0.12[a]
4	7	17.57±0.23[a]	1.51±0.01[a]	38.33±0.11[a]	39.03±0.15[a]	2.71±0.23[a]	80.07±0.21[a]
	10	17.43±0.07[a]	1.47±0.04[a]	38.11±0.20[a]	39.14±0.26[a]	2.63±0.04[a]	79.91±0.17[a]
	13	17.62±0.01[a]	1.45±0.07[a]	38.23±0.09[a]	39.03±0.23[a]	2.65±0.01[a]	79.91±0.13[a]
5	7	17.62±0.33[a]	1.49±0.00[a]	38.28±0.18[a]	39.06±0.21[a]	2.66±0.03[a]	80.01±0.18[a]
	10	17.41±0.21[a]	1.44±0.06[a]	38.12±0.07[a]	39.02±0.21[a]	2.62±0.12[a]	79.76±0.15[a]
	13	17.24±0.10[a]	1.43±0.04[a]	38.24±0.16[a]	38.92±0.39[a]	2.57±0.08[a]	79.73±0.23[a]

[1] Data are expressed as mean ± standard deviation (n=3). Means with different letters in the same column were significantly different at the level P<0.05.
2 UFA = unsaturated fatty acids.

80.10±0.46% unsaturated fatty acids were determined in oil extracted from raw bran, including 38.30±0.44% oleic acid, 39.11±0.35% linoleic acid and 2.69±0.19% γ-linolenic acid. The PRFM radiation has insignificant effect on the fatty acids content of RB samples (*P*>0.05). Similar conclusion was proposed by Ramezanzadeh *et al.* (2000), they found no significant difference in fatty acid content between raw and microwave treated bran sample (800 w, 3 min). According to Yilmaz *et al.* (2014), no significant change was observed in fatty acid content between raw and IR-stabilised RB samples.

Tocopherols content

Four tocopherol homologs (α-, β-, γ-, and δ-tocopherol) were identified and quantified in this research. α-tocopherol was the predominant component (1,026.18 mg/kg), followed by γ-tocopherol (190.76 mg/kg), β-tocopherol (156.58 mg/kg) and δ-tocopherol (75.03 mg/kg). The concentration of α-tocopherol gradually decreased with increasing radiation time and microwave power according to Table 4. However, the individual and total tocopherol contents were insignificantly affected by the PRFM heating at 3 and 4 kW (*P*>0.05). Even after heated at 4 kW for 10 min, more than 91.83% of tocopherols still remained. A significant reduction of tocopherols content was observed in bran samples treated at 5 kW for more than 10 min (*P*<0.05), and this may be ascribed to the high temperature generated at this condition. Yang *et al.* (2013) and Wroniak *et al.* (2016) found that concentration of α-tocopherol in rapeseed gradually decreased with microwave heating. Ko *et al.* (2003) pointed out that longer duration time with microwave oven heating led to a significant degradation of tocopherols in RB. RB stabilised with extrusion at 120-140 °C for 6 min lost 16.0-23.8% α-tocopherol (Shin *et al.*, 1997). Yilmaz *et al.* (2014) showed a significant loss of tocopherol (up to 50%) in RB samples stabilised with short wave infrared heating. Pradeep *et al.* (2014) observed that bran treated with parboiling resulted in a significant reduction (58-94%) in total tocopherols. It is obvious that the tocopherols of RB are relatively stable during PRFM radiation at 3 and 4 kW.

γ-oryzanol content

γ-oryzanol is one of the strongest antioxidants in RB. According to Table 4, γ-oryzanol content of raw RB was 17.53±1.25 g/kg. The γ-oryzanol insignificantly changed in PRFM-treated bran samples (*P*>0.05), even after heated at 5 kW for 13 min, only 2.40% of its γ-oryzanol degraded. Shin *et al.* (1997) reported that RB stabilised with extrusion at 120-140 °C for 6 min lost 7.4-10.8% of γ-oryzanol. Thanonkaew *et al.* (2012) and Kim *et al.* (2014) showed that γ-oryzanol content of RB stabilised with microwave oven was relatively higher than that of raw bran (*P*<0.05). This indicates that PRFM radiation is an ideal technology for RB stabilisation continuously with retaining a maximum level of these natural nutraceuticals.

Oxidative stability

The Rancimat method was adopted to evaluate the antioxidant activity of oil extracted from raw and PRFM-treated RB samples. As shown in Figure 4, the induction time of PRFM-treated RB samples decreased with processing time at 3 and 4 kW (*P*>0.05). Significant reduction of induction time was observed in bran sample treated at 5 kW for more than 10 min, and similar finding was observed in our previous research on wheat germ with flameless catalytic infrared (Li *et al.*, 2016). Yang *et al.* (2011) proved that the Rancimat induction time was positively correlated with the concentrations of the anti-

Table 4. Tocopherols and γ-oryzanol content of rice bran oil (RBO).[1]

Microwave power (kW)	Processing time (min)	Tocopherols (mg/kg RBO)				Total tocopherol (mg/kg RBO)	γ-oryzanol (g/kg RBO)
		α	β	γ	δ		
Raw	–	1,026.31±14.99^{a}	156.58±12.98^{a}	190.76±10.06^{a}	75.03±11.47^{a}	1,448.47±21.26^{a}	17.53±1.25^{a}
3	7	1,022.92±11.12^{a}	155.62±8.05^{ab}	189.14±6.15^{a}	74.28±1.76^{a}	1,437.52±17.09^{ab}	17.65±0.37^{a}
	10	1,017.13±9.62^{a}	153.11±6.69^{ab}	187.03±4.59^{a}	73.59±2.91^{a}	1,431.51±10.44^{ab}	17.81±0.66^{a}
	13	1,010.29±7.64^{ab}	151.47±9.50^{ab}	183.21±3.62^{ab}	72.11±2.70^{a}	1,421.22±16.31^{ab}	17.68±0.55^{a}
4	7	1,003.53±10.79^{ab}	153.14±10.05^{ab}	186.57±16.27^{ab}	73.36±9.31^{a}	1,416.61±28.22^{ab}	18.04±1.43^{a}
	10	942.34±16.03^{bc}	142.27±13.49^{ab}	173.33±11.32^{ab}	68.17±8.35^{a}	1,316.12±14.79^{cd}	17.79±0.73^{a}
	13	916.63±11.69^{cd}	136.71±9.96^{ab}	161.14±1.15^{ab}	65.03±3.72^{a}	1,289.61±16.93^{de}	17.32±0.26^{a}
5	7	976.68±15.73^{abc}	148.94±11.13^{ab}	181.45±9.97^{ab}	70.7±10.37^{a}	1,377.78±13.88^{bc}	17.95±0.93^{a}
	10	906.45±12.54^{cd}	134.67±7.86^{ab}	166.01±8.76^{ab}	67.04±2.85^{a}	1,257.43±25.06^{e}	17.43±0.43^{a}
	13	847.20±15.09^{d}	129.44±5.26^{b}	157.94±4.26^{b}	62.83±4.33^{a}	1,179.16±17.62^{f}	17.11±0.32^{a}

[1] Data are expressed as mean ± standard deviation (n=3). Means with different letters in the same column were significantly different at the level *P*<0.05.

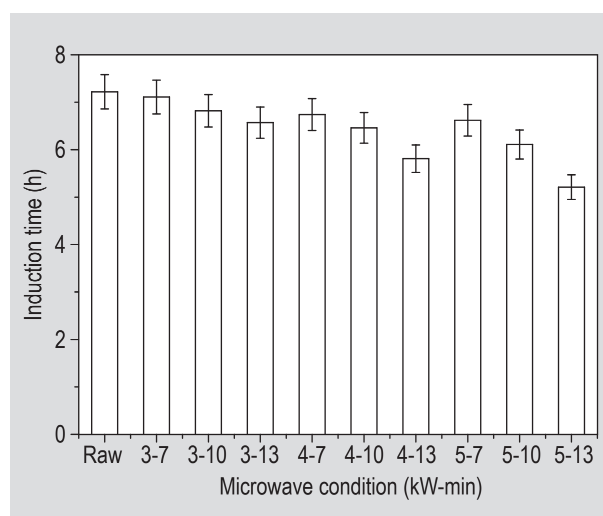

Figure 4. The oxidative stability of rice bran oil (x-axis values reflect microwave condition, the number before the hyphen is microwave power: 3-5 kW, the number after the hyphen is processing time: 7-13 min).

oxidative constituents in oil, such as tocopherols, phenolic acid. So the shorter induction time of treated RBO may be related to degradation of tocopherols during PRFM radiation.

4. Conclusions

In conclusion, the results of this study revealed that the PRFM stabilisation system can effectively inactive LA and completely destroy LOX of RB in minutes. Both of the PRFM power and duration time have significant influence on LA and LOX activity of RB. The optimum technological parameter of RB stabilisation with PRFM radiation was 4 kW for 10 min at the ventilation rate of 80 N m^3/h. Under this condition, the residual LA activity was 7.94% and LOX activity was completely destroyed. The FFA content and PV of this RB sample only increased by 1.06% and 5.32 meq O_2/kg RBO at 35 °C for 60 days, respectively. The optimal storage Aw for RB is about 0.14. No significant decrease in fatty acid, tocopherols and γ-oryzanol content of bran treated at optimum condition. These results manifest that the PRFM radiation is a commercially feasible technology for RB stabilisation on line.

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