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Research article

Stabilization of rice bran using ohmic heating or ultra-superheated steam

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Abstract

Rice bran (RB) is stabilized to improve its quality and extend its shelf life. RB stabilization reduces lipases, peroxidase, lipoxygenases and auto-oxidation enzymatic activities to prevent rancidity. This study investigated the effects of ohmic heating (OH) or ultra-superheated steam (USS) treatments on the free fatty acid (FFA) content and lipase activity (LA) compared to a hot-air (HA) oven during 15 d of storage in open containers at room temperature. After 15 d, the FFA level of the untreated RB was 23.55%, while the FFA levels for the RB subjected to HA for 15 and 90 min were 12.40% and 7.82%, respectively. On the other hand, the FFA levels for the RB subjected to OH at 100°C for 15 min and USS at 400°C for 25 s were 3.91% and 4.71%, respectively, and were considered suitable for industrial purposes (FFA< 5%) in edible oil extraction. A low LA (< 0.1 international units per gram of RB (IU/g RB)) was observed in the RB treated using the OH or USS treatments, whereas an LA range of 0.654–1.051 IU/g RB was detected for HA. The OH and USS treatments effectively inactivated LA and inhibited FFA formation in shorter heating times, compared to HA. OH-applied electricity as the heating source had rapid and uniform energy conversion that might be advantageous compared to HA. USS used very high temperatures for short times with high throughput. In conclusion, OH at 100°C for 15 min and USS at 400°C for 25 s were recommended for effective RB stabilization.

Introduction

Rice bran (RB) is a sub-product of rice milling and is an excellent source of nutrients; consequently, RB is gaining substantial importance for use in industrialized processes. RB consists of 8.7–18.9% fat, which have excellent nutritional properties food and health benefits (Liao et al., 2020). Lipase is normally dormant in RB; however, the milling step increases the contact surface between lipase and oil, which could accelerate the oxidation process. As RB is milled, the spherosome membranes are disrupted, and a native lipase hydrolyzes the

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neutral lipids leading to an increase in the free fatty acid (FFA) content by 1–7% per day under favorable conditions (Lacerda et al., 2013; Arora et al., 2016). RB containing over 5% free fatty acid (FFA) content develops a rancid flavor and is considered uneconomical for industrialized purposes due to the loss of oil yield during oil extraction (Patil et al., 2016). Therefore, RB should be treated to inactivate the native lipase enzyme in a process called stabilization. Stabilizing RB improves its quality and extends its shelf-life. Different stabilization methods have been reported such as drying in a hot-air oven or steaming (Thanonkaew et al., 2012), microwave heating (Pongrat and Songsermpong, 2019), hot air-assisted radio frequency heating (Liao et al., 2020), infrared radiation heating (Irakli et al., 2018), extrusion (Rafe and Sadeghian, 2017), ultra-superheated steam bed drying (Rodchuajeen et al., 2016) and ohmic heating (Loypimai et al., 2009).

Ohmic heating (OH) is an innovative method to potentially extend the shelf-life of RB by converting and dissipating electrical current into heat in a very short time. Stabilization of RB using OH is a method that effectively degrades enzymes, preventing odd flavors and rancidity (Lakkakula et al., 2004; Makroo et al., 2020). Loypimai et al. (2015) and Dhingra et al. (2012) investigated the effect of OH on RB and found that the application of an electric field (EF) could retard the formation of FFA during storage. However, available information is limited on the stabilization of RB using OH with different heating times and on post-processing storage in open containers at room temperature. Most studies have focused on the storage of stabilized RB in closed containers under refrigerated conditions at 4°C (Lakkakula et al., 2004; Loypimai et al., 2009). However, storage in an open area at room temperature for several days is practically applied in the rice industry. In addition, most studies involved ohmically heating the RB to the target temperature without any holding time, which is probably not sufficient to lower the moisture content (MC), inactivate microorganisms and stabilize the RB to be kept at room temperature for 15 d.

Ultra-superheated stream (USS) is another innovative food technology used in RB stabilization. USS uses dry steam to inhibit enzymatic activity and prolong the shelf-life of RB. USS is a technology primarily used in drying due to its high energy efficiency and output (Jia et al., 2018). The application of USS to stabilize RB has shown promising results in the inhibition of the formation of FFA and preserving bioactive compounds such as γ-oryzanol and phenolic compounds during extended storage (Satou et al., 2010; Rodchuajeen et al., 2016). However, there is insufficient information supporting the use of very high temperatures (≥ 300°C) for a short time in RB stabilization to extend its shelf-life. The use of a short heating time (in terms of seconds) potentially increases the capacity output of stabilized RB and inhibits FFA formation.

This research aimed to study the effects of OH and USS treatments of RB compared with conventional heating on FFA and lipase activity (LA) during storage for 15 d in open containers at room temperature.

Materials and Methods

Chemicals

Triton X-100, gum Arabic and p-nitrophenol-butyrate (p-NPB) were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). Hexane, toluene and isopropanol were purchased from RCI Labscan (Bangkok, Thailand). All chemicals and reagents were of analytical grades. All other reagents and chemicals were acquired from Ajax Finechem Pty. Ltd. (Auckland, New Zealand) or Merck & Co. (Darmstadt, Germany) unless otherwise stated.

Rice bran preparation

Freshly milled RB (*Oryza sativa*) was purchased from a local supplier located in Nonthaburi province, Thailand. The RB was passed through a 60-mesh sieve (Standard 425 μm; Endecotts Ltd.; London, UK) to remove foreign matter. Then, it was mixed thoroughly to obtain homogenous samples, packed in aluminum zipper foil bags and stored at -20°C until use. The RB samples were analyzed to determine the initial values of FFA, MC and lipase activity (LA). Untreated RB was used as the control in this study.

Moisture content measurement

The MC was determined using the AOAC 925.10 method (Association of Official Analytical Chemists, 2000). RB samples (1 g) were dried in a hot-air oven at 130°C until the measured weights were constant. The data were expressed on a dry weight basis (%). An MC over 10% can promote the residual LA to hydrolyze the oil during storage (Sharma et al., 2014). Therefore, control of the MC after treatment should be considered.

Rice bran oil extraction

The RB oil extraction process followed the method modified from Prabhu et al. (1999). A RB sample (30 g) was weighed, placed in a 250 mL Erlenmeyer flask with 150 mL of n-hexane and stirred for 20 min. The mixture was filtered to remove the solvent using Whatman paper No. 4 (GE Healthcare; Chalfont St Giles, UK) and extracted three times with n-hexane. For the last filtration, 35 mL of fresh n-hexane was added to the sample to wash it. The solvent in the extract was evaporated using a rotary evaporator (Rotavapor R-300; BÜCHI Labortechnik AG; Flawil, Switzerland), collected and stored at -20°C for further analysis.

Free fatty acid determination

The FFA content was determined using the AOCS Cd 3d-63 procedure (Association of Oil Chemists' Society, 1998). The method estimates the acid value of the oil using titration with phenolphthalein as the end-point indicator. The acid value was defined as the number of milligrams of potassium hydroxide required to neutralize the acids in 1 g of fatty material. The acid value was expressed as the FFA content by calculating the percentage of oleic acid according to the Cd 3d-63 procedure.

Lipase extraction

The lipase extraction method was adapted from Prabhu et al. (1999). A sample of 10 g of RB was weighed and placed in a 75 mL Erlenmeyer flask. The 10 g of RB was defatted by stirring for 30 min in 50 mL of n-hexane. Whatman paper No. 4 (GE Healthcare; Chalfont St Giles, UK) was used to filter the mixture and the residue was called defatted RB. The defatted RB was allowed to air-dry for 1 h to evaporate any n-hexane residue.

The defatted RB was stirred in 30 mL of 0.5 mM calcium chloride and 50 mM of potassium phosphate buffer at pH 7.0 at 10°C for 30 min. The mixture was centrifuged at 12,000 revolutions per minute (rpm) for 30 min at 4°C; the supernatants were collected as crude lipase extract.

Lipase activity

The LA was measured against p-NPB, as reported by Loypimai et al. (2009). The substrate consisted of two solutions: A and B. Solution A contained 40 mg of p-NPB dissolved in 12 mL of isopropanol. Solution B contained 0.4 g Triton X-100 and 0.1 g gum Arabic dissolved in 90 mL of 100 mM potassium phosphate buffer at pH 7.0. The substrate solutions were prepared by the drop-wise addition of 0.3 mL of solution A (p-NPB) into 3 mL of solution B under intense vortexing. These mixtures were stable for 1 hr at room temperature. For the p-NPB substrate system, 0.1 mL of crude LA extract was added to 3.3 mL of substrate solution. The reaction rate was measured after incubating these mixtures at 35°C for 5 min at 410 nm wavelength using a spectrophotometer (Genesys G10-S UV-Vis; Thermo Fisher Scientific; Waltham, MA, USA). The same treatment was applied to a sample without any enzyme and this was used as a blank. One unit of LA was defined as the amount of the enzyme needed to liberate 1 μmole of p-NPB per minute under assay conditions. LA was expressed in international units per gram of RB on a dry basis (IU/g RB).

Hot-air oven treatment

The hot-air (HA) oven treatment was carried out in a hot-air oven (FED 53; Binder GmbH; Tuttlingen, Germany) at 100°C. A 0.5 cm layer of RB was placed uniformly on stainless steel shallow pans. The samples were placed in the oven at 100°C with a holding time of 15 min to be the same as the stabilization time of ohmic heating. A heating time of 90 min was selected to investigate the effect of a longer heating time on the FFA and LA.

Ohmic heating treatment

OH stabilization was carried out in a Pyrex T-shaped test cell with two platinized titanium electrodes which was held with a holder, as shown in Fig. 1. The diameter and the cross-sectional area of each electrode were 2.5 cm and 4.91 cm^2, respectively. The distance between the electrodes (L) was fixed at 3 cm for every sample (20 g). The temperature was measured using a type K thermocouple (Sang Chai Meter, model PCR-1, Thailand). The variable voltage (V) at 50 Hz was supplied by a 5 kW isolated transformer (TDGC2-5K; Changcheng Electrical Equipment Group Co., Ltd., Wenzhou, China) with the maximum voltage and current being 250 V and 20 A, respectively. The temperature, voltage and amperage were recorded every 3 s using a digital multimeter (GDM-8261A; GW INSTEK; New Taipei City, Taiwan). The EF (measured in volts per centimeter) was determined as the ratio of voltage to the distance between the electrodes (EF = V / L). The MC was adjusted by adding distilled water to the RB to make a current-transmitting medium, thus increasing the electrical conductivity of the RB to increase the heating rate. The RB and distilled water were mixed until the sample became homogenous.

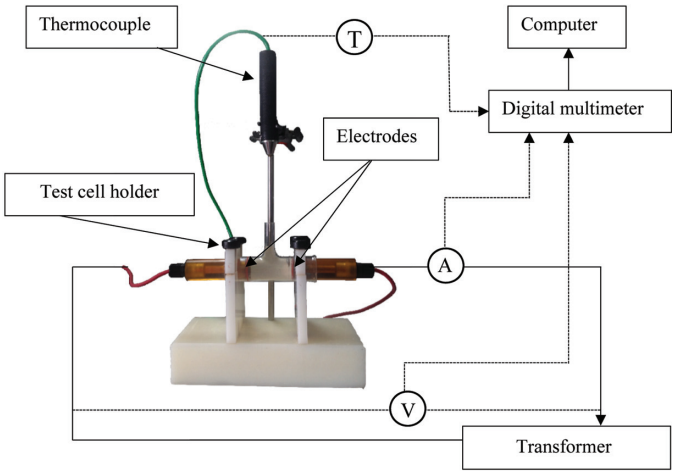

Fig. 1 Ohmic schematic test cell with its components (T = temperature; A = current; V = voltage)

The heating rate from room temperature to 100°C (come-up time) for stabilization of RB was determined using three different MC levels (33.33%, 42.86% and 53.85%) and heating to 100°C using three different voltages (200 V, 220 V and 240 V) to obtain EF values of 66.67 V/cm, 73.33 V/cm and 80.00 V/cm, respectively. Burning was observed at the electrodes when the EF was greater than 80.00 V/cm and the MC was less than 33.33% (data not shown). An air gap which blocked the current flow due to evaporation was observed when the temperature reached 100°C. The sample needed to be tightly compressed by the two electrodes to remove the air. The lowest MC of the RB after treatment, followed by the shortest heating time, was selected for posterior shelf-life studies. For the shelf-life study, the RB was stabilized at 100°C at three different holding times (5 min, 10 min and 15 min).

Ultra-superheated steam treatment

The USS equipment used is schematically represented in Fig. 2. It consisted of a water softener system, a diesel boiler, a radio frequency unit (USS unit; USA Patent No. 8343422. JSP Ltd.; Tokyo, Japan), and a drying chamber. RB samples were stabilized at three temperatures (300°C, 350°C and 400°C) and three different processing times (15 s, 20 s and 25 s). The heating temperature was programmed in the USS equipment; once the set temperature had been reached, the RB sample of 200 g was placed in the sample holder and positioned in the drying chamber. The stabilization time commenced after closing the drying chamber door. The samples were rotated at a constant 60–70 rpm for all temperatures and stabilization times.

Experimental design

Overall, there were 15 treatments as follow: HA for 15 or 90 min; OH at 5, 10 or 15 s; and 300, 350 or 400°C USS each for either 15, 20 or 25 s; and a control (untreated RB).

After stabilization treatment, the samples were subsequently cooled and stored in open containers (open zip-lock bags) at ambient temperature (approximately 33°C). The MC, FFA content and LA were evaluated at 15 d of storage in the shelf-life study by immediately sampling after treatment and every 5 d.

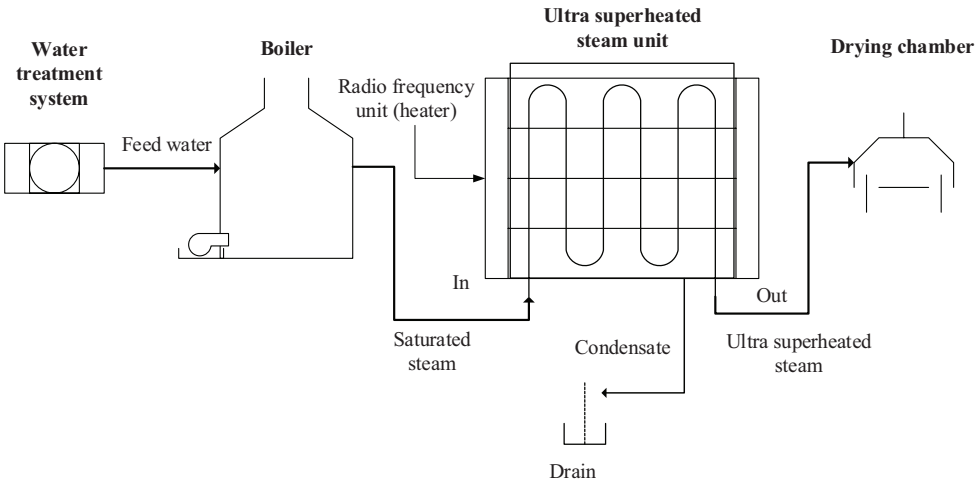

Fig. 2 Schematic diagram of ultra-superheated steam system

The samples were analyzed in duplicate. For each replicate, two samples were assayed, with each sample analyzed in triplicate. More than six data points for each replicate were collected.

Statistical analysis

Statistical analyzes were performed using the SPSS 21 software (IBM Inc.; New York, NY, USA). Data were subjected to analysis of variance and significant differences ($p < 0.05$) between means were tested using Duncan's multiple range test.

Results and Discussion

Moisture content

Table 1 shows the MC of the RB subjected to HA, OH and USS compared to the control for 15 d of storage. The initial MC values of the control sample and the RB subjected to HA and USS were approximately 17.56%. The MC of the control sample decreased from 17.56% to 10.48% by the end of the 15 d of storage. After HA heating, the MC of RB substantially decreased from 17.56% to 3.61–4.17% and then increased to 9.10–9.16% by the end of storage.

For ohmic heating, the post-treated MC of the RB with initial MC values of 33.33%, 42.86% and 53.85% were in the ranges 28.21–31.58%, 35.14–38.89% and 44.93–49.25%, respectively (Table 2). Although the heating rate of RB with 53.85% MC was faster than that with either 42.86% or 33.33%, the MC afterward was the highest. As expected, the increase in the EF raised the heating rate and reduced the come-up time. As a result, the combination of 33.33% MC and 80 V/cm resulted in the fastest come-up time and the lowest MC after treatment and was selected for further stabilization studies. After the treatment, the MC reduced from the initial 33.33% to a range of 21.61–23.36% while an MC of 9–11% is required for commercial industry (Tao et al., 1993). At the end of 15 d, MC values of 9.63–10.4% were observed which was associated with the desorption of moisture to reach an equilibrium state. Although high moisture could promote the growth of fungi, the current results (data not shown) indicated that the OH at 100°C for 15 min could reduce total aerobic bacteria counts (TAC) and total yeast and mold counts (TYMC) from 5.02 log colony forming units (CFU)/g (raw rice bran) and 4.73 log CFU/g (raw rice bran), respectively, to 2.69 log CFU/g and < 1 log CFU/g, respectively, (after OH treatment). Good storage practices in sanitary facilities with sufficient air flow and low humidity might reduce the increase in microbial growth during room temperature storage. Furthermore, a potential solution to reduce the MC of ohmically stabilized RB is to use preconditioned silos or storage rooms with artificial airflow at 35°C for drying RB (Paraginski et al., 2014).

Table 1 Moisture content (MC) of rice bran subjected to hot air (HA), ohmic heating (OH) and ultra-superheated steam (USS) during 15 d of storage

Treatment	MC (%)			
	Day 0	Day 5	Day 10	Day 15
Control	17.56 ± 0.13^{Ac}	16.04 ± 1.87^{Aa}	9.85 ± 0.09^{Bde}	10.48 ± 0.22^{Babc}
HA 100°C, 15 min	3.61 ± 0.33^{Bi}	8.70 ± 0.14^{Ade}	9.23 ± 0.25^{Aef}	9.16 ± 0.71^{Adef}
HA 100°C, 90 min	4.17 ± 0.50^{Ci}	8.30 ± 0.91^{Bde}	7.74 ± 1.05^{Bg}	9.10 ± 0.31^{Adef}
OH 100°C, 5 min	23.36 ± 0.86^{Aa}	16.22 ± 0.44^{Ba}	10.68 ± 0.04^{Ccd}	10.40 ± 1.23^{Cbc}
OH 100°C, 10 min	22.62 ± 0.94^{Aab}	16.16 ± 0.16^{Ba}	10.49 ± 0.18^{Ccd}	9.63 ± 0.12^{Ccde}
OH 100°C, 15 min	21.61 ± 0.51^{Ab}	15.20 ± 0.36^{Ba}	11.82 ± 1.20^{Cab}	10.13 ± 0.09^{Dbcd}
USS 400°C, 15 s	10.19 ± 1.01^{Af}	9.23 ± 1.40^{ABde}	8.98 ± 0.19^{ABef}	8.13 ± 0.41^{Bf}
USS 400°C, 20 s	8.67 ± 0.56^{ABh}	7.92 ± 0.36^{BCe}	7.16 ± 0.46^{Cg}	9.17 ± 0.46^{Adef}
USS 400°C, 25 s	9.44 ± 0.86^{Afg}	9.74 ± 1.08^{Acd}	7.50 ± 0.41^{Bg}	9.07 ± 0.52^{Adef}
USS 350°C, 15 s	11.73 ± 0.21^{Ae}	12.51 ± 0.89^{Ab}	12.24 ± 0.39^{Aa}	9.80 ± 0.74^{Bcd}
USS 350°C, 20 s	10.52 ± 0.67^{Aef}	11.20 ± 0.45^{Abc}	10.83 ± 1.08^{Abcd}	8.23 ± 0.35^{Bf}
USS 350°C, 25 s	10.11 ± 0.78^{Af}	8.29 ± 0.57^{Bde}	8.70 ± 0.58^{Bf}	8.60 ± 0.43^{Bef}
USS 300°C, 15 s	13.00 ± 1.70^{Ad}	11.21 ± 0.83^{Abc}	12.18 ± 0.10^{Aa}	11.44 ± 0.84^{Aa}
USS 300°C, 20 s	10.84 ± 0.62^{Aef}	11.51 ± 1.71^{Ab}	11.10 ± 0.32^{Abc}	11.00 ± 0.22^{Aab}
USS 300°C, 25 s	9.93 ± 0.28^{Afg}	9.37 ± 0.82^{Ade}	10.28 ± 0.20^{Acd}	10.28 ± 0.58^{Abc}

Mean ± SD ($n = 2$) superscripted with different uppercase letters indicate significant ($p < 0.05$) differences within a row and different lowercase superscripts indicate significant ($p < 0.05$) differences within a column.

Table 2 Moisture content (MC) and electric field (EF) under ohmic heating (OH)

Initial MC (%)	EF (V/cm)	Come-up time* (min)	MC after OH (%)
33.33	66.67	29.35±0.37[a]	31.58±0.25[g]
	73.73	18.22±0.07[b]	29.87±0.13[h]
	80.00	10.73±0.83[c]	28.21±0.10[i]
42.86	66.67	3.93±0.09[d]	38.89±0.22[d]
	73.73	3.80±0.07[d]	36.99±0.19[e]
	80.00	2.57±0.15[e]	35.14±0.11[f]
53.85	66.67	1.90±0.13[f]	49.25±0.47[a]
	73.73	1.76±0.15[fg]	47.06±0.30[b]
	80.00	1.22±0.07[g]	44.93±0.28[c]

* = come-up time is the heating time of rice bran from room temperature to 100°C.
Mean ± SD (n = 2) within a column superscripted with different lowercase letters indicate significant ($p < 0.05$) differences.

For USS heating, temperature and time had a noticeable effect on the MC. A high temperature with a long heating time resulted in a low MC and vice versa. The USS treatment at 400°C for 25 and 20 s reduced the MC by approximately 50% (from 17.56 to 8.67–9.43%), while the USS treatment at 300°C for 15 s reduced the MC by 26% (from 17.56 to 13.00%). The USS treatment may have reduced the MC of all the samples due to its unique drying properties. USS could condense the moisture from the surface of the RB with this condensation being evaporated to the medium due to raising the temperature of the evaporation point (Tang, 2010). Diffusion of the moisture to the medium (superheated steam) could also be occurred within the RB structure due to expansion of the cells, which flushes the moisture leading to a highly porous, dried material (Rodchuajeen et al., 2016). Satou et al. (2010) reported similar results for condensed steam at the RB surface being released due to the high temperature in USS, thus decreasing the MC during treatment.

The MC of all treatments in the current study displayed adsorption and desorption so that the samples reached an equilibrium state of relative humidity (Choi et al., 2010). The increase in the MC during storage might be attributed to the hygroscopic property of the RB (Sharma et al., 2014). Wheat bran also had the same phenomenon of moisture equilibrium during storage at room temperature (Sharma et al., 2014).

Free fatty acid content

The FFA content of the RB subjected to HA, OH and USS increased during the 15 d of storage at room temperature (Table 3). The control samples increased gradually from 3.19% to a final value of 23.55%, approximately a seven-fold increase.

The HA treatment at 100°C for 15 min and 90 min increased the FFA content throughout the storage period. After 15 d of storage, the FFA content of RB subjected to HA at 100°C for 15 min treatment was 12.40% higher than the RB subjected to HA at 100°C for 90 min. This difference in FFA confirmed that heating time adversely affected FFA formation in RB. In addition, during storage, the RB absorbed ambient moisture to reach equilibrium. The enzymatic activity might have reactivated after treatment, thus increasing the rate of hydrolysis (Singh and Sogi, 2016). A prolonged HA treatment might diminish the RB oil quality; thus, HA treatment should be applied with caution (Kim et al., 2014).

Compared to the OH treatment at 100°C for 5, 10 and 15 min, the HA heating time was longer yet less effective in RB stabilization; although the MC of the RB sample subjected to HA treatment was lower than the RB sample subjected to OH treatment throughout the 15 d of storage. Gong et al. (2013) suggested that an added-moisture heating treatment such as OH could inactivate lipase enzymes more effectively than a dry-heating process such as HA. The dry-heating method contained insufficient water in the vicinity of the enzyme to bring the thermal transition from the native to the denaturized form. Additionally, the rapid and uniform conversion from electricity to heat in the OH treatment might be another factor in the effective inactivation of lipase. After OH treatment, there were no significant differences in the FFA content among the samples treated at 100°C for 15 min, 10 min or 5 min. However, during the 15 d of storage, the OH treatment at 100°C for 15 min had the lowest FFA (3.91%) compared to the OH treatments for 10 min and 5 min (5.29% and 5.85%, respectively). Heating time, temperature and MC are important key factors inhibiting the formation of FFA; however, an MC of ohmically heated RB higher than 10% could promote hydrolysis and residual LA or support bacterial lipase that hydrolyzes the oil or increases the FFA content during storage (Singh and Sogi, 2016).

Table 3 Free fatty acid (FFA) of rice bran subjected to hot air (HA), ohmic hea3ting (OH) and ultra-superheated steam (USS) during 15 d of storage

Treatment	FFA (%)			
	Day 0	Day 5	Day 10	Day 15
Control	3.19 ± 0.55^{Dc}	12.17 ± 0.88^{Ca}	15.89 ± 0.50^{Ba}	23.55 ± 1.48^{Aa}
HA 100°C, 15 min	3.91 ± 0.41^{Ca}	4.50 ± 0.50^{Cdef}	8.98 ± 0.87^{Bc}	12.40 ± 1.99^{Ac}
HA 100°C, 90 min	3.16 ± 0.20^{Cc}	4.73 ± 0.70^{BCdef}	4.97 ± 1.52^{Bgh}	7.82 ± 1.29^{Ade}
OH 100°C, 5 min	2.62 ± 0.36^{Cd}	4.18 ± 0.66^{Befg}	5.07 ± 0.34^{Bfgh}	5.85 ± 0.39^{Afg}
OH 100°C, 10 min	2.61 ± 0.52^{Cd}	4.22 ± 0.85^{Bdefg}	4.37 ± 0.66^{BAh}	5.29 ± 0.30^{Ag}
OH 100°C, 15 min	2.28 ± 0.30^{Bd}	2.65 ± 0.29^{Bh}	3.10 ± 0.24^{ABi}	3.91 ± 1.18^{Ah}
USS 400°C, 15 s	3.72 ± 0.09^{Dab}	4.85 ± 0.06^{Ccdef}	5.87 ± 0.26^{Bdef}	7.08 ± 0.19^{Adef}
USS 400°C, 20 s	3.33 ± 0.26^{Cbc}	4.11 ± 0.82^{Cefg}	5.27 ± 0.33^{Bfg}	6.61 ± 0.50^{Aef}
USS 400°C, 25 s	3.56 ± 0.28^{Cabc}	3.48 ± 0.06^{BCg}	4.24 ± 0.15^{Bh}	4.71 ± 0.14^{Agh}
USS 350°C, 15 s	3.50 ± 0.26^{Cabc}	9.45 ± 0.26^{Bb}	9.72 ± 0.32^{Bc}	13.32 ± 0.20^{Ac}
USS 350°C, 20 s	3.71 ± 0.12^{Dab}	5.59 ± 0.18^{Cc}	6.39 ± 0.25^{Bd}	7.68 ± 0.18^{Ade}
USS 350°C, 25 s	3.56 ± 0.09^{Dabc}	4.94 ± 0.41^{Ccde}	6.14 ± 0.12^{Bde}	7.51 ± 0.32^{Ade}
USS 300°C, 15 s	3.41 ± 0.11^{Dabc}	9.51 ± 0.44^{Cb}	11.16 ± 0.06^{Bb}	16.09 ± 0.79^{Ab}
USS 300°C, 20 s	3.82 ± 0.07^{Dab}	5.04 ± 0.04^{Ccd}	5.70 ± 0.25^{Bdefg}	7.99 ± 0.14^{Ad}
USS 300°C, 25 s	3.61 ± 0.36^{Cabc}	4.06 ± 0.51^{Cfg}	5.45 ± 0.47^{Befg}	6.85 ± 0.33^{Adef}

Mean ± SD (n = 2) superscripted with different uppercase letters indicate significant ($p < 0.05$) differences within a row and different lowercase superscripts indicate significant ($p < 0.05$) differences within a column.

The FFA value of the RB under the USS treatment at 400°C for 25 s had the lowest FFA value of 4.71% compared to the other USS treatments. However, it was not significantly different from the OH treatment at 100°C for 15 min with both FFA values lower than 5% after 15 d of storage in the shelf-life study. This could have been due to the combined effect of temperature and time, which provided sufficient heat treatment to inhibit LA (Lavanya et al., 2017). On the other hand, the USS samples treated at 300°C for 15 s and at 350°C for 15 s had the highest rates of formation of FFA (both over 13% after 15 d of storage). The FFA for the USS treatments at 300°C for 20 s, at 300°C for 25 s, at 350°C for 20 s, at 350°C for 25 s, at 400°C for 15 s and at 400°C for 20 s ranged from 6.61% to 7.99%. The USS treatments at 400°C for 20 and 15 s and at 300°C for 25 s, 20 s and 15 s resulted in FFA values higher than 5%, indicating that the USS treatments effectively inhibited the LA and retarded the formation of FFA.

The shelf stability of RB was considered in terms of the FFA content and LA, where the FFA activity was the product of the hydrolysis of triglycerides by lipases and LA was the residual lipase activity of the RB after treatment. The control samples had higher values for FFA during storage than the RB samples subjected to the HA, OH or USS treatments. Therefore, a stabilization process is necessary to inhibit the formation of FFA, where such a process would extend the shelf-life and improve the RB quality (Lacerda et al., 2013).

Based on these results, the stabilization treatments should provide adequate MC, heating temperature and time to inhibit any undesirable enzymatic activity during storage. The OH treatment at 100°C for 15 min and the USS treatment at 400°C for 25 s were recommended as effective stabilization methods for rice bran stored for up to 15 d in open containers as the FFA values were less than 5%.

Lipase activity

As expected, the control had the highest enzyme activity compared to all heating treatments during 15 d of storage, in accordance with the FFA results (Table 4). For all HA treatments, LA increased from 0.589–0.666 IU/g RB to 0.948–1.051 IU/g RB after 5 d of storage and decreased to 0.844–0.654 IU/g RB at day 15 of storage. Conversely, the LA in each OH treatment was the least, with an approximately 98% reduction from the untreated sample and slight fluctuation during storage. There was no significant difference in LA between the OH treatment at 100°C for 15 min (0.014 IU/g RB) and the OH treatment at 100°C for 10 min (0.011 IU/g RB) at day 15. These results suggested that the inactivation was sufficient to deactivate LA and retard FFA formation, as discussed earlier. In addition, the increases in the TAC and TYMC from 2.69 log CFU/g and < 1 log CFU/g, respectively, (after OH) to 3.87 log CFU/g and 3.85 log CFU/g, respectively, (data not shown) at the end of the 15 d of storage indicated that FFA formation might be attributed to bacterial lipase. However, the LA of the sample subjected

Table 4 Lipase activity (LA) of rice bran subjected to hot air (HA), ohmic heating (OH) and ultra-superheated steam (USS) during 15 d of storage

Treatment	LA (international units per gram of rice bran on a dry basis)			
	Day 0	Day 5	Day 10	Day 15
Control	1.442 ± 0.025^{Aa}	1.428 ± 0.045^{Aa}	1.333 ± 0.025^{Ba}	1.080 ± 0.014^{Ca}
HA 100°C, 15 min	0.589 ± 0.032^{Cc}	1.051 ± 0.006^{Ab}	0.810 ± 0.012^{Bb}	0.844 ± 0.007^{Bb}
HA 100°C, 90 min	0.666 ± 0.008^{Bb}	0.948 ± 0.046^{Ac}	0.659 ± 0.009^{Bc}	0.654 ± 0.002^{Bc}
OH 100°C, 5 min	0.017 ± 0.000^{Dg}	0.035 ± 0.001^{Bdef}	0.043 ± 0.002^{Afg}	0.031 ± 0.001^{Cf}
OH 100°C, 10 min	0.025 ± 0.000^{Bfg}	0.023 ± 0.001^{Cef}	0.030 ± 0.000^{Ag}	0.011 ± 0.001^{Dg}
OH 100°C, 15 min	0.029 ± 0.000^{Aefg}	0.019 ± 0.001^{Cf}	0.025 ± 0.000^{Bg}	0.014 ± 0.000^{Dg}
USS 400°C, 15 s	0.053 ± 0.001^{Bde}	0.062 ± 0.001^{Ade}	0.064 ± 0.001^{Ade}	0.062 ± 0.002^{Ad}
USS 400°C, 20 s	0.043 ± 0.002^{Ddef}	0.070 ± 0.000^{Ad}	0.060 ± 0.000^{Bef}	0.051 ± 0.001^{Ce}
USS 400°C, 25 s	0.035 ± 0.000^{Aefg}	0.038 ± 0.003^{Adef}	0.031 ± 0.001^{Bg}	0.031 ± 0.001^{Bf}
USS 350°C, 15 s	0.044 ± 0.000^{Adef}	0.048 ± 0.004^{Adef}	0.054 ± 0.013^{Aef}	0.050 ± 0.001^{Ae}
USS 350°C, 20 s	0.054 ± 0.000^{Bde}	0.047 ± 0.001^{Cdef}	0.062 ± 0.004^{Adef}	0.037 ± 0.000^{Df}
USS 350°C, 25 s	0.043 ± 0.000^{Bdef}	0.040 ± 0.001^{Cdef}	0.057 ± 0.000^{Aef}	0.036 ± 0.001^{Df}
USS 300°C, 15 s	0.063 ± 0.003^{Ad}	0.063 ± 0.005^{Ade}	0.066 ± 0.004^{Ade}	0.059 ± 0.002^{Ade}
USS 300°C, 20 s	0.039 ± 0.001^{Ddefg}	0.070 ± 0.003^{Bd}	0.081 ± 0.001^{Ad}	0.063 ± 0.000^{Cd}
USS 300°C, 25 s	0.031 ± 0.001^{Defg}	0.047 ± 0.000^{Cdef}	0.055 ± 0.001^{Bef}	0.061 ± 0.000^{Ad}

Mean ± SD (n = 2) superscripted with different uppercase letters indicate significant ($p < 0.05$) differences within a row and different lowercase superscripts indicate significant ($p < 0.05$) differences within a column.

to OH at 100°C for 5 min was significantly higher than that of the sample subjected to OH at 100°C for10 min and to OH at 100°C for 15 min, but not significantly different from the USS at 400°C for 25 s. The USS samples at 400°C for 25 s, at 350°C for 25 s and at 350°C for 20 s had the lowest levels of LA and were significantly different from the other USS treatments at 15 d of storage.

The LA of the control samples was the highest and was not significantly different during the first 5 d of storage; however, after day 10, the activity significantly declined. This decline was attributed to the relationship of moisture content to equilibrium relative humidity (Reddy and Chakraverty, 2004). The HA treatments at 100°C for 15 min and 90 min produced residual LA values high enough to promote the fast hydrolysis of the oil, increasing the FFA value. The residual LA during storage could be explained by partial inactivation of lipase during oven treatment (Sharma et al., 2004; Kim et al., 2014) and the inefficiency of stabilization using hot-air drying (Gong et al., 2013).

The OH treatment had a significant influence on LA, which agreed with Loypimai et al. (2009), who reported that OH could inhibit the LA and retard FFA formation during 21 d of storage at 4°C. Although OH deactivates enzymes due to its natural thermal effect, evidence of an EF effect also exists. Castro et al. (2004) found that OH could enhance the inactivation rate of lipoxygenases (LOX) and polyphenoloxidase, accounting for reducing the treatment time compared to conventional heating. Samaranayake and Sastry (2016) indicated an EF effect of inactivation of pectin methylesterase in tomatoes occurred at 70°C. The EF might break weak noncovalent bonds between enzyme molecules such as hydrogen bonds, electrostatic forces, van der Waals forces and hydrophobic interaction (Castro et al., 2004). Electrophoretic motion might be another cause; however, this hypothesis should be further investigated because RB has a low MC and is not the same as enzyme molecules in an aqueous environment, as explained by Samaranayake and Sastry (2016).

In the USS treatments, LA diminished as the temperature and time increased. Similar results were reported for studies where USS was applied to brown rice, with LA and LOX being successfully inactivated (Satou et al., 2010). The USS treatment at 400°C for 25 s produced the lowest FFA after 15 d of storage. This result could be explained by a higher degree of thermal inactivation. In contrast, the other USS treatments were ineffective in LA inactivation; therefore, the oil was hydrolyzed during storage. The residual LA in the USS treatments was suspected to have been caused by the incomplete inactivation of the thermostable fraction, which required inactivation equivalent times of 2–4 min above 90°C. Rodchuajeen et al. (2016) stabilized RB using USS bed-drying with temperatures and times of 120–160°C and 1.9–4.4 min, respectively. The FFA content of treated samples increased slightly after 55 d of storage, with values lower than 10%. The USS treatment at 140°C produced the best results, in which

the combination of temperature and time was adequate to inactivate LA completely.

Insufficient heat treatment might partially inactivate indigenous enzymes, resulting in a continuous increase in FFA at a slower rate during storage at room temperature (Kim et al., 2014). The LA decrease during storage was possibly due to the lack of water to diffuse and dissolve the substrate into the active sites of the enzymes and subsequently, modify the proper molecular conformation (Bell, 2008). Furthermore, the fluctuation of LA values during storage was plausibly influenced by the equilibrium MC of substrate availability (Goffman and Bergman, 2003). Kim et al. (2014) found that the stabilization of RB using microwaves resulted in a fluctuation of the lipoxygenase activity during storage at room temperature, due to MC and oxygen availability. Possible reactivation of enzymatic activity could be due to conformational changes within the food. It also could be due to enzyme liberation from chloroplasts due to compartmentalization and mechanical manipulation. Likewise, enzymes located near their substrates tend to increase their activity during storage (Jacobo-Velázquez et al., 2013). Therefore, completely irreversible enzymatic inactivation via HA, OH and USS treatments is mandatory to avoid possible residual activities that might produce high FFA levels during the commercial storage of RB.

The application of USS and OH showed potential in inhibiting the formation of FFA to less than 5% during storage for 15 d at room temperature. The OH treatment at 100°C for 15 min produced effective inactivation of LA. The thermal effect of the uniform EF distribution with sufficient MC and heating time under the OH treatment might be advantageous compared to the HA treatment. The USS treatment at 400°C for 25 s inactivated lipase in a concise time due to the remarkable rapidity of the surface heating, which is one of the features of superheated steam. Further studies are required of OH and USS stabilization techniques at industrial scales.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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