



## Research article

# Effect of superheated steam heating on quality and antioxidant activities of riceberry bran

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## Abstract

Riceberry is known for its health-promoting properties and it has increased in popularity as a pigmented rice. Rapid degradation in terms of enzyme hydrolysis is a major problem in the further utilization of rice bran. Rice bran and pigmented rice bran must be stabilized before use. Superheated steam heating (SSH) could be an effective method to inhibit enzymatic activities as well as rancidity as this method involves high temperature and a short process. This study investigated the SSH conditions influencing the physical, chemical and antioxidant properties of freshly milled riceberry bran (FRB) and 5-month-frozen stored riceberry bran (5M SRB) and the influence of sample handling. Riceberry bran was subjected to SSH at 275–375°C for 5–20 s. Stabilized samples were determined for color, moisture content, peroxide value (PV), free fatty acids (FFA) content, lipase activity and antioxidant activities. All SSH conditions could reduce the moisture content, PV and FFA in riceberry bran. SSH conditions at 275°C ≥ 15 s, 325°C ≥ 10 s and 375°C ≥ 10 s had PVs lower than the maximum acceptable level (15 mg equivalents/kg oil). The total phenolic content of FRB was maintained, while the antioxidant activity of diphenyl-picrylhydrazyl radical scavenging activity increased. SSH significantly reduced lipase activity, compared to the untreated control under both tested conditions of handling. SSH could be potentially used to retard rancidity and to promote antioxidant activities in riceberry bran even with bran stored for a prolonged period (up to 5 mth).

## Introduction

Thailand, one of the world's largest rice producers and exporters, annually produces approximately 35 million tonnes of rice (Office of Agricultural Economics, 2016). Rice bran is a co-product obtained from rice milling and the polishing process and the bran makes up 8–12% depending on the milling degree (Esa et al., 2013). Rice bran is typically used as animal feed due to its high nutrients (Malekian et al., 2000). However, the quality of rice bran can be instantaneously degraded by the presence of endogenous lipase (Malekian et al.,

2000). These limiting factors contribute to the instability of rice bran during storage, rendering it unsuitable for human consumption. The increasing human consumption trend of pigmented rice over the past several years has been due to its attractive and colorful appearance and several health benefits, leading to the leftover pigmented rice bran. Riceberry is a cross-bred strain from the Hom Nin rice variety, which is well known for containing high antioxidant activities, and Jasmine fragrant rice (Sirichokworakit et al., 2015). Rice bran has been reported as a good source of dietary fiber, protein, essential unsaturated fatty acids, minerals, vitamins and phenolic compounds (Malekian et al., 2000;

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Patil et al., 2016). It is a source of gamma-oryzanol, beta-carotene, niacin, thiamin, riboflavin, anthocyanins and total phenolic compounds (Patil et al., 2016). Significant amounts of antioxidants such as phenolic acid, flavonoids, tocopherols, tocotrienols and gamma-oryzanol have been found naturally in rice bran, which could prevent related chronic diseases such as coronary heart diseases and cancer (Gul et al., 2015). Moreover, anthocyanins and proanthocyanidins can be found in pigmented rice bran (Huang and Lai, 2016).

Many stabilizing methods have been applied to improve the utilization and to extend the shelf life of rice bran, such as hot air drying, microwave heating, autoclaving, fluidized bed drying, steaming, chemical stabilization and ohmic heating (Kim et al., 2014; Loypimai et al., 2016). The major limitation of most methods is the extended-period, high-temperature stabilizing process, which can affect the chemical and physical properties of rice bran. In addition, low temperature treatments (such as chilling and freezing) have been typically used to store rice bran (Prabhakar and Venkatesh, 1986). However, the low temperature treatments do not inhibit lipase so that lipase activity still gradually occurs during storage.

Superheated steam technology has been widely used for food applications. A high heat transfer rate and an oxygen-free environment are the major advantages (Ezhil, 2010). Superheated steam is generated by the addition of sensible heat to saturated steam. When water is heated at a specific pressure, it generates saturated steam at its boiling point and by heating saturated steam above boiling point at a certain pressure (for example, 100°C and 1 atmospheric pressure), the saturated steam is converted to superheated steam (Ezhil, 2010). Exhausted steam can be recycled, which saves the cost of energy. Superheated steam heating (SSH) can reduce oxidation in a product because the oxygen from the surroundings of the process is eliminated through air replacement with superheated steam (Tang and Cenkowski, 2000; Ezhil, 2010; Wu et al., 2016). SSH was effective in suppressing lipid oxidation in pork bundles in canned food, which had relatively low peroxide values before and after storage (Huang et al., 2004). Numerous studies have reported on the use of SSH in several food products such as potato (Tang and Cenkowski, 2000), rice (Horrungsiwat et al., 2016), carrot (Hiranvarachat et al., 2008) and avocado (Husen et al., 2014). Tang and Cenkowski (2000) reported that the dehydration time of potatoes using SSH decreased more than with hot air drying when the drying temperature increased. Moreover, the moisture ratio of rice using SSH reduced more rapidly than with microwave-hot air drying (Horrungsiwat et al., 2016). The antioxidant activities of carrot using low pressure superheated steam drying (LPSSD) were higher than those of carrot using hot air drying because LPSSD had lower losses based on the percentage of relative inhibition than with hot air drying (Hiranvarachat et al., 2008). In avocado, superheated steam could promote antioxidant activities and the total phenolic content compared to freeze drying because phenolic compounds were possibly released from the matrix and superheated steam drying at high temperatures (130–170°C) resulted in 735.06–934.61 mg gallic acid equivalents (GAE)/100 g dried sample, which were significantly higher than with fresh avocado at 520.55 mg GAE/100 g dried sample (Husen et al., 2014). Therefore, the current study investigated

the effect of SSH conditions on the physical, chemical and antioxidant properties including the lipase activity of freshly milled riceberry bran and 5-month-frozen stored riceberry bran.

## Materials and Methods

### *Materials and sample preparation*

Two handling procedures of stored riceberry bran were investigated in this study: 1) initial handling, referred to as freshly milled riceberry bran (FRB); and 2) 5-month-frozen stored riceberry bran (5M SRB). The samples were obtained from the Rice Research Center (Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand). FRB was collected after milling and immediately stored in a refrigerator at 8–10°C for 24 hr, while the 5M SRB samples were kept in frozen storage at -18°C for 5 mth. Then, both samples were taken to the laboratory, where the riceberry bran was screened through a 30-mesh sieve to remove broken grains, hull fragments, paddy kernels and foreign material. Riceberry bran samples (250 g) were individually vacuum-packed in an aluminum-foil, laminated pouch and stored at -18°C in the dark to prevent the hydrolysis of fatty acids by lipase activity (Patil et al., 2016). Untreated riceberry bran (without the SSH treatment) was used as a control. A stabilization study using SSH was conducted for two replications and all analyses were assessed within 3 mth.

### *Stabilization using superheated steam heating*

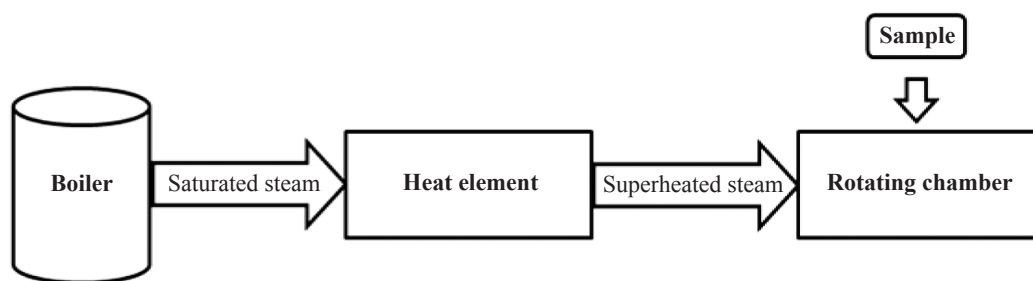
Uniform particle sizes of FRB and 5M SRB were treated using SSH under various processing conditions. The SSH system was courteously provided by JSP Inc., Ltd. (Tokyo, Japan) consisting of a steam generator (boiler), a radio frequency super heater with control panel, conveying pipes, a processing chamber, an exhaustor and a condenser as shown in Fig. 1. Riceberry bran samples were subjected to different processing temperatures (275°C, 325°C and 375°C) from 5 s to 20 s. Each sample of riceberry bran (250 g) was placed in the rotating chamber (60–70 revolutions per minute) for uniform heating. After processing, the samples were cooled and stored in the aluminum-foil pouches and kept at -18°C until analysis.

### *Determination of stabilized riceberry bran quality*

The moisture content was determined using an oven method (AOAC, 2012). A spectrophotometer (model CM-3500d; Minolta; Aichi, Japan) was used to measure the color parameters in the CIE L\*a\*b\* system and total color difference ( $\Delta E^*$ ) was calculated using Equation 1:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where  $\Delta E^*$  is the total color difference,  $\Delta a^*$  is the difference in redness or greenness (+ red, - green) and  $\Delta b^*$  is the difference in yellowness or blueness (+ yellow, - blue).



**Fig. 1** Schematic diagram of batch type superheated steam heating system

Riceberry bran oil was extracted from untreated riceberry bran and SSH-stabilized riceberry bran for determination of the peroxide value (PV) and free fatty acid (FFA) content. Riceberry bran (150 g) was mixed with n-hexane (900 mL) at 1:6 (weight per volume) at room temperature using a stirrer (model ss30; Stuart Scientific; Stone, UK) at 9×g for 2 hr (modified from Kim et al., 2014). The extract was separated using vacuum filtration (model TC-2000vm; Sparmax; Taipei, Taiwan) with no.1 Whatman® filter paper. The residue was further extracted using 120 mL n-hexane before the two extracts were combined. Riceberry bran oil was separated from the supernatant using a rotary evaporator (Rotavapor R-200; Buchi; Flawil, Switzerland) under reduced pressure at 40°C. The residual n-hexane solvent was removed under nitrogen gas flow. Rice bran oil samples were kept at -18°C in the dark. The PV and FFA were determined using the titration method of AOCS (2003). The PV was determined using Equation 2. The FFA was calculated as oleic acid and expressed as the percentage of the total lipids as shown in Equation 3:

$$PV = \frac{[(V_1 - V_0) \times N \times 1000]}{W} \quad (2)$$

where PV is the peroxide value (measured in milligram equivalents per kilogram oil, mg Eqv/kg oil),  $V_1$  is the volume of sodium thiosulphate solution and  $V_0$  is the volume of the blank (both in milliliters), N is the molarity of the sodium thiosulphate solution (M) and W is the weight of the sample (in grams).

$$FFA = \frac{(V \times N \times 28.2)}{W} \quad (3)$$

where FFA is the fatty acid content as a percentage, V is the volume of sodium hydroxide solution (in milliliters), N is the molarity of sodium hydroxide solution (M) and W is the weight of the sample (in grams).

#### Determination of lipase and antioxidant activities

##### Determination of lipase activity

The lipase activity method was modified from Ohenhen and Ikenebomeh (2007), in which 0.2M Tris/malate/NaOH was used as a buffer solution and olive oil was used as a substrate. After incubation in the dark at  $35 \pm 2^\circ\text{C}$ , the free fatty acids liberated over 50 min

were determined using titration with 0.05M NaOH. One unit of lipase activity was defined as the amount of enzyme capable of releasing 1 ml of oleic acid in 1 min.

##### Determination of antioxidant activities

To evaluate the total phenolic content and antioxidant activities, riceberry bran was extracted according to the methodology of Walter et al. (2013). A riceberry bran sample (1 g) was thoroughly mixed with 20 mL of 80% methanol before the centrifuged tube was constantly agitated for 1 hr at room temperature. The sample tube was then centrifuged at 1,008×g for 10 min prior to supernatant separation. The crude extract was kept in amber glass bottles and stored at -18°C until analysis. The total phenolic content of riceberry bran was determined using the modified colorimetric Folin-Ciocalteu method (Wolfe et al., 2003). A volume of 0.5 mL of deionized water and 0.125 mL of a known dilution of the crude extract were added to a test tube. Folin-Ciocalteu reagent (0.125 mL) was subsequently added to the solution and allowed to react for 6 min. After adding 7% sodium carbonate solution (1.25 mL), the mixture was vigorously shaken. Final volume was made up to 3 mL with deionized water. The mixture was left at room temperature for 90 min for color development and the absorbance was read at 760 nm using an ultraviolet-visible spectrophotometer (MRX II; DYNEX Technologies, Inc.; Chantilly, USA). The measurement was compared to a standard curve of gallic acid solutions and expressed as milligrams of gallic acid equivalent (GAE) per 1 g of riceberry bran. Ferric reducing antioxidant power (FRAP) assay was performed according to Tananuwong and Tewaruth (2010). A volume of 10 µL of the crude extract was added to 990 µL of FRAP assay solution (a ratio of acetate buffer:ferric chloride solution:tripyrilidyltriazine solution of 10:1:1 by volume) in a cuvette. The mixture was held for 4 min at room temperature before measuring the absorbance at 593 nm. The corrected absorbance was calculated by subtracting the absorbance of the reagent blank from the absorbance of the sample read after 4 min. The antioxidant activity was calculated as micromoles of Trolox per gram of sample (µmol /g sample) using a Trolox standard curve.

Determination of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay of riceberry bran was conducted. Different dilutions of the crude extracts were prepared. Diluted crude extract (50 µL) was mixed with 950 µL of DPPH solution (0.3 mM) in a cuvette. After vigorously shaking, the mixture was incubated in the dark at room temperature for 30 min. The absorbance was measured at 515 nm, using an ultraviolet-visible spectrophotometer (Tananuwong and

Tewaruth, 2010). DPPH free radical scavenging activity was calculated and expressed as the equivalent amount of the sample in DPPH solution for estimation of the concentration providing 50% inhibition ( $IC_{50}$ ).

#### Statistical analysis

Statistical analysis of experimental data was carried out using analysis of variance using the general linear model procedure with a fixed effects model. Mean comparison was determined using Duncan's multiple range test with the SPSS software (version 12.0; IBM Co., Ltd; Chicago, IL, USA). Significant differences among means were recognized at  $p < 0.05$ .

### Results and Discussion

Numerous technologies have been successfully reported for the purpose of stabilizing rice bran. Most stabilizing techniques involved a wet process, for example, autoclaving, pressure cooking and extrusion (Sharma et al., 2004; Kim et al., 2014). After stabilizing, it was essential to further dry the rice bran to reduce the moisture content. On the other hand, there are other stabilizing techniques such as microwave heating, infrared radiation and ohmic heating (Ramezanzadeh et al., 1999a; Lakkakula et al., 2004; Yilmaz and Tuncel, 2015). These dry-heat processes normally need moisture adjustment at the beginning to prevent over-heating and to obtain acceptable characteristics in the rice bran (Patil et al., 2016). FRB was stored at 8–10°C for a day after milling and 5M SRB was kept frozen for 5 mth after milling. Generally, rapid deterioration of rice bran takes place immediately following the milling as according to Malekian et al. (2000), lipase and oxidase cause the deterioration of crude fat, thus making the bran unsuitable for human consumption. Lipase in the bran hydrolyzes triglycerides to FFAs and glycerols, and the released FFAs can affect rancidity, off-odor, off-flavor, increase the acidity and change the functional properties (Malekian et al., 2000; Patil et al., 2016).

#### Influence of superheated steam heating conditions on riceberry bran quality

A summary of the statistical analysis regarding the contribution of sample handling and storage as well as processing parameters (SSH temperature and time) is presented in Table 1. There was a significant individual effect in this study with total color difference ( $\Delta E^*$ ), moisture content, peroxide value and free fatty acid content at a 5% significant level ( $p < 0.05$ ). However, certain interaction terms on the quality were also significantly affected (Table 1). For example, the  $L^*$ ,  $a^*$  and  $b^*$  of FRB and 5M SRB after SSH treatment significantly decreased compared to the untreated control (Table 2). After SSH exposure over 20 s at 375°C, FRB and 5M SRB were visibly dark in color with a burnt smell (data not shown). The  $\Delta E^*$  values  $\pm$  SD of FRB and 5M SRB were  $2.62 \pm 2.04$  to  $6.92 \pm 0.25$  and  $1.21 \pm 0.42$  to  $3.19 \pm 0.57$ , respectively (Table 2). Slight  $\Delta E^*$  was observed among the treated conditions since the natural color of riceberry bran appeared as dark purple due to the presence of anthocyanin. In black rice

bran, cyanidin 3-glucoside (Cy 3-glc) was found to be the major anthocyanin followed by peonidin 3-glucoside (Pn 3-glc). The total anthocyanin  $\pm$  SD in the pigmented rice bran was reported to be in the range  $0.20 \pm 0.01$  to  $11.27 \pm 0.38$  mg Cy 3-glc Eqv/g dry matter depending on rice varieties (Huang and Lai, 2016). The effect of SSH conditions on moisture content reduction is shown in Table 3. At the same temperature, the moisture content of FRB and 5M SRB decreased with increasing exposure time. Treatment with SSH could rapidly reduce the initial moisture content from about 9% to less than 1%. Fast moisture reduction was observed, which was probably due to the higher heat transfer rates of SSH (Ezhil, 2010). Stabilization using SSH did not require further hot-air drying to remove moisture, unlike with other stabilizing treatments. Lipid oxidation is significantly affected by the moisture content in foods and rapid stabilization could decrease lipid oxidation, thus extending the shelf-life of rice bran (Ramezanzadeh et al., 1999a).

**Table 1** Probability level of each independent parameter and interaction terms on quality of riceberry bran

Parameter	Quality of riceberry bran	p-value
Sample handling & storage	total color difference	0.000
	moisture content	0.006
	peroxide value	0.000
	free fatty acid content	0.000
Temperature	total color difference	0.000
	moisture content	0.000
	peroxide value	0.000
	free fatty acid content	0.000
Time	total color difference	0.000
	moisture content	0.000
	peroxide value	0.000
	free fatty acid content	0.000
Interaction between sample handling & storage and temperature	total color difference	0.000
	moisture content	0.000
	peroxide value	0.000
	free fatty acid content	0.000
Interaction between sample handling & storage and time	total color difference	0.018
	moisture content	0.002
	peroxide value	0.671
	free fatty acid content	0.000
Interaction between temperature and time	total color difference	0.000
	moisture content	0.000
	peroxide value	0.000
	free fatty acid content	0.000
Interaction of sample handling & storage, temperature and time	total color difference	0.026
	moisture content	0.691
	peroxide value	0.017
	free fatty acid content	0.000

**Table 2** Color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) and total color difference ( $\Delta E^*$ ) of: freshly milled riceberry bran (FRB) and 5-month-frozen stored riceberry bran (5M SRB) subjected to superheated steam heating at 275–375°C for 5–20 s

Sample handling & storage†	Temperature (°C)	Time (s)	$L^*$	$a^*$	$b^*$	$\Delta E^*$
FRB	Untreated control		29.64±1.15 <sup>a</sup>	5.43±0.49 <sup>abc</sup>	5.16±1.05 <sup>a</sup>	-
	275	5	27.53±1.07 <sup>b</sup>	5.33±0.36 <sup>abcd</sup>	4.88±0.47 <sup>ab</sup>	2.62±2.04 <sup>efghi</sup>
		10	23.39±1.70 <sup>kl</sup>	4.99±0.28 <sup>bcd</sup>	4.21±0.67 <sup>bcd</sup>	6.75±2.80 <sup>a</sup>
		15	22.95±0.44 <sup>l</sup>	4.80±0.16 <sup>defghi</sup>	3.74±0.30 <sup>fgh</sup>	6.92±0.25 <sup>a</sup>
		20	23.32±1.02 <sup>kl</sup>	4.55±0.42 <sup>fghijkl</sup>	3.73±0.50 <sup>fgh</sup>	6.66±0.21 <sup>a</sup>
	325	5	24.61±1.83 <sup>ijk</sup>	4.96±0.42 <sup>cdefg</sup>	4.27±0.98 <sup>bcd</sup>	5.56±3.01 <sup>ab</sup>
		10	25.34±0.08 <sup>fghij</sup>	4.44±0.33 <sup>fghijklm</sup>	3.53±0.92 <sup>fghi</sup>	5.81±0.17 <sup>ab</sup>
		15	25.39±0.52 <sup>efghij</sup>	4.62±0.15 <sup>fghijk</sup>	4.03±0.28 <sup>cdefg</sup>	4.97±1.44 <sup>bc</sup>
		20	27.26±1.57 <sup>bc</sup>	4.19±0.60 <sup>ijklmn</sup>	3.95±0.35 <sup>defgh</sup>	4.54±1.24 <sup>bcd</sup>
	375	5	26.67±1.89 <sup>bcd</sup>	4.80±0.16 <sup>defghi</sup>	4.79±0.16 <sup>abc</sup>	2.85±1.65 <sup>efgh</sup>
		10	27.23±1.17 <sup>bcd</sup>	4.02±0.21 <sup>lmn</sup>	3.73±0.41 <sup>fgh</sup>	4.40±0.79 <sup>bcd</sup>
		15	28.98±1.03 <sup>a</sup>	3.93±0.40 <sup>mno</sup>	4.67±0.26 <sup>abcd</sup>	2.93±0.25 <sup>efg</sup>
		20	26.86±0.56 <sup>bcd</sup>	4.06±1.03 <sup>klmn</sup>	4.20±1.06 <sup>bcd</sup>	3.59±1.80 <sup>cde</sup>
5M SRB	Untreated control		26.89±0.82 <sup>bcd</sup>	5.53±0.41 <sup>ab</sup>	4.15±0.43 <sup>bcd</sup>	-
	275	5	26.40±1.08 <sup>bcd</sup>	5.65±0.20 <sup>a</sup>	4.60±0.14 <sup>abcde</sup>	1.29±0.35 <sup>hij</sup>
		10	25.14±0.55 <sup>ghij</sup>	5.02±0.70 <sup>bcd</sup>	3.72±0.95 <sup>fgh</sup>	1.59±0.76 <sup>ghi</sup>
		15	24.34±1.83 <sup>kl</sup>	4.49±0.43 <sup>fghijklm</sup>	2.94±0.66 <sup>i</sup>	3.19±0.57 <sup>def</sup>
		20	25.71±1.73 <sup>cdefghij</sup>	4.82±0.37 <sup>defgh</sup>	3.40±0.20 <sup>ghi</sup>	1.75±0.70 <sup>fghi</sup>
	325	5	26.07±1.40 <sup>bcd</sup>	5.24±0.66 <sup>abcde</sup>	3.83±0.88 <sup>efgh</sup>	1.77±0.43 <sup>fghi</sup>
		10	26.01±0.48 <sup>bcd</sup>	4.24±0.31 <sup>hijklmn</sup>	2.83±0.15 <sup>i</sup>	1.94±0.30 <sup>fghi</sup>
		15	24.87±0.47 <sup>hij</sup>	4.70±0.23 <sup>efghij</sup>	3.21±0.42 <sup>hi</sup>	2.10±0.22 <sup>efghi</sup>
		20	25.83±1.46 <sup>cdefghij</sup>	4.40±0.46 <sup>ghijklm</sup>	3.38±0.07 <sup>ghi</sup>	1.92±0.52 <sup>fghi</sup>
	375	5	27.08±0.86 <sup>bcd</sup>	4.86±0.28 <sup>defg</sup>	3.91±0.56 <sup>defgh</sup>	1.21±0.42 <sup>ij</sup>
		10	26.98±0.26 <sup>bcd</sup>	4.47±0.26 <sup>fghijklm</sup>	3.21±0.37 <sup>hi</sup>	1.46±0.32 <sup>ghij</sup>
		15	26.15±0.26 <sup>bcd</sup>	4.23±0.14 <sup>ijklmn</sup>	3.21±0.15 <sup>hi</sup>	1.74±0.40 <sup>fghi</sup>
		20	25.60±1.85 <sup>defghij</sup>	3.77±0.29 <sup>n</sup>	3.93±0.21 <sup>defgh</sup>	2.32±1.01 <sup>efghi</sup>

$L^*$  = lightness;  $a^*$  = redness;  $b^*$  = yellowness.

† <sup>a-n</sup> = different lowercase superscript letters within the same column are significantly different ( $p < 0.05$ ).

Data = mean ± SD of two independent replications ( $n = 6$ ).

Lipase is primarily present in the outer layers of the rice kernel (Patil et al., 2016). During the rice milling process, lipase mainly hydrolyses a triglyceride into glycerol and three molecules of free fatty acids, while other key enzymes are lipoxygenase and peroxidase (Patil et al., 2016). Changes in lipid properties are important in monitoring the quality deterioration of riceberry bran. The untreated control of 5M SRB was more susceptible to degradation regarding oil quality than FRB as shown by higher PV and FFA contents due to the long storage time under frozen conditions (Table 3). The PV is used to determine the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation, which could indirectly indicate the oil freshness (Patil et al., 2016). Previous studies have shown that lipid oxidation occurs in three stages—initiation, propagation and termination (Malekian et al., 2000; Wu et al., 2016). During the first stage, triglycerides are hydrolyzed by lipase. The number of resulting free fatty acids increases. During the second stage, the released unsaturated fatty acids are oxidized to form hydroperoxides (oxidation products) by lipoxygenase or by autooxidation. Finally, the unstable hydroperoxides are decomposed into secondary oxidation products (Wu et al., 2016). Table 3 shows the effect of SSH conditions on the

PV of FRB and 5M SRB. The initial PV values ± SD of FRB and 5M SRB were  $25.7 \pm 1.6$  mg Eqv/kg oil and  $34.0 \pm 0.3$  mg Eqv/kg oil, respectively. Earlier study (Patil et al., 2016) reported a lower PV in the untreated rice bran than measured in the current study, which could be explained by different sample preparation. The riceberry bran samples used for the current study were intentionally stored for a long period (up to 5 mth) to imitate handling of small scale enterprises, while most of the other studies used freshly milled rice bran. The PV of FRB and 5M SRB decreased as the exposure time increased at the same temperature. Heat can inactivate lipoxygenase, thus retarding riceberry bran from lipid oxidation (Malekian et al., 2000). Patil et al. (2016) found that microwave heating and parboiling could maintain and control the rise in the PV of rice bran in the range 2.94–8.95 mg Eqv/kg oil until the end of 3 mth. Both lipase and lipoxygenase are mainly responsible for the formation of hydroperoxides, whose activities are suppressed by efficient heat treatments leading to lowered PV levels in rice bran (Patil et al., 2016). Decomposition of the formed hydroperoxides results in volatile degradation products (Shaker et al., 2013).



**Table 3** Moisture content, peroxide value and free fatty acid content of freshly milled riceberry bran (FRB) and 5-month-frozen stored riceberry bran (5M SRB) subjected to superheated steam heating at 275–375°C for 5–20 s

Sample handling & storage*	Temperature (°C)	Time (s)	Moisture content (%)	Peroxide value (mg equivalent/kg oil)	Free fatty acid content (%)
FRB	Untreated control		9.61±0.48 <sup>a</sup>	25.7±1.6 <sup>bc</sup>	41.8±2.4 <sup>f</sup>
	275	5	8.10±0.06 <sup>b</sup>	23.1±0.0 <sup>c</sup>	31.1±0.2 <sup>i</sup>
		10	5.72±0.06 <sup>e</sup>	19.8±1.5 <sup>de</sup>	20.7±1.4 <sup>k</sup>
		15	4.17±0.23 <sup>ghi</sup>	12.3±2.0 <sup>h</sup>	9.9±0.3 <sup>m</sup>
		20	3.63±0.28 <sup>ij</sup>	7.1±1.4 <sup>jk</sup>	6.0±0.6 <sup>n</sup>
	325	5	7.43±0.23 <sup>c</sup>	24.8±1.6 <sup>bc</sup>	19.9±1.2 <sup>k</sup>
		10	5.91±0.30 <sup>de</sup>	12.8±2.7 <sup>gh</sup>	15.2±1.1 <sup>l</sup>
		15	4.32±0.24 <sup>g</sup>	5.8±0.4 <sup>kl</sup>	9.6±0.9 <sup>m</sup>
		20	2.13±0.12 <sup>l</sup>	2.4±0.1 <sup>m</sup>	6.6±0.1 <sup>n</sup>
	375	5	6.40±0.12 <sup>d</sup>	20.3±2.2 <sup>d</sup>	21.8±1.1 <sup>jk</sup>
		10	4.91±0.93 <sup>f</sup>	9.2±2.7 <sup>ij</sup>	14.3±1.0 <sup>l</sup>
		15	2.84±1.31 <sup>k</sup>	4.1±1.8 <sup>lm</sup>	7.6±1.0 <sup>n</sup>
		20	0.94±0.02 <sup>mn</sup>	2.3±0.1 <sup>m</sup>	5.8±0.2 <sup>n</sup>
5M SRB	Untreated control		9.87±0.11 <sup>a</sup>	34.0±0.3 <sup>a</sup>	62.4±2.3 <sup>a</sup>
	275	5	8.53±0.07 <sup>b</sup>	31.7±2.6 <sup>a</sup>	53.6±0.3 <sup>c</sup>
		10	5.72±0.15 <sup>e</sup>	24.7±1.5 <sup>bc</sup>	55.8±1.1 <sup>b</sup>
		15	4.58±0.26 <sup>fg</sup>	15.3±2.0 <sup>fg</sup>	44.7±1.9 <sup>e</sup>
		20	3.68±0.14 <sup>hij</sup>	11.2±1.6 <sup>hi</sup>	36.2±2.4 <sup>g</sup>
	325	5	7.30±0.26 <sup>c</sup>	26.6±3.3 <sup>b</sup>	54.6±2.3 <sup>bc</sup>
		10	5.03±0.88 <sup>f</sup>	12.7±0.6 <sup>gh</sup>	47.1±3.7 <sup>d</sup>
		15	4.02±1.12 <sup>ghi</sup>	7.9±2.9 <sup>jk</sup>	32.9±1.8 <sup>h</sup>
		20	1.01±0.12 <sup>m</sup>	6.2±1.8 <sup>kl</sup>	23.5±0.2 <sup>j</sup>
	375	5	6.16±0.10 <sup>de</sup>	17.4±2.8 <sup>ef</sup>	48.2±2.1 <sup>d</sup>
		10	4.23±0.06 <sup>gh</sup>	9.5±2.9 <sup>ij</sup>	34.0±1.7 <sup>h</sup>
		15	3.17±0.09 <sup>jk</sup>	3.9±1.6 <sup>lm</sup>	15.8±1.7 <sup>i</sup>
		20	0.45±0.02 <sup>n</sup>	2.4±0.1 <sup>m</sup>	10.9±0.3 <sup>m</sup>

\* a-n = different lowercase superscript letters within the same column are significantly different ( $p < 0.05$ ).

Data = mean ± SD of two independent replications ( $n = 6$ ).

The FFA content was used as an indicator of the degree of hydrolytic rancidity in the riceberry bran. Initial FFA ± SD values of FRB and 5M SRB were 41.8 ± 2.4% and 62.4 ± 2.3%, respectively (Table 3). The FFA content of FRB and 5M SRB at the same temperature decreased with an increase in exposure time (Table 3) because the SSH could inactivate the lipase and delay hydrolytic rancidity (Malekian et al., 2000). Heating might be effective at inactivating the residual enzymes and microorganisms, resulting in retarded FFA formation (Malekian et al., 2000). Another index of lipid oxidation is the thiobarbituric acid (TBA) value that measures malondialdehyde (MDA), a minor component of fatty acids formed on the degradation of polyunsaturated fatty acids during storage (Patil et al., 2016). The moisture content, PV and FFA of stabilized jasmine rice bran by SSH at 300°C for 5 s significantly decreased from untreated rice bran ( $p < 0.05$ ) (Boonmawat and Ratphitagsanti, 2016). Patil et al. (2016) reported that oil could be regarded as high quality when the oil quality indicators were FFA ≤ 3%, PV ≤ 7.63 mg Eqv/kg oil and TBA ≤ 0.071 mg MDA/kg.

According to the Codex Alimentarius Commission (Codex Alimentarius Commission, 1999), the acceptable level of the PV in cold-pressed and virgin oils for human consumption is less than 15 mg Eqv/kg oil. The SSH conditions with the PV below the acceptable level were then selected for the determination of antioxidant

activities and lipase activity. Accordingly, SSH times with the shortest exposure times at each processing temperature were ≥ 15 s at 275°C and ≥ 10 s at 325°C and 375°C.

#### *Selected superheated steam heating conditions on lipase activity*

Rice bran has a short shelf life due to lipase hydrolyzing triglycerides into free fatty acids (Ramezanzadeh et al., 1999b; Malekian et al., 2000). The rate of FFA formation can be very high—up to 5–7% per day and up to 70% in a month (Patil et al., 2016). Hydrolytic rancidity could be minimized by controlling lipase activity (Malekian et al., 2000). Effective stabilizing conditions using SSH were assessed, regardless of initial sample handling. Although high FFA contents were observed in both samples, a rapid SSH treatment could decrease lipase activity, leading to significant reduction of FFAs with higher processing temperatures and longer exposure times (Table 3). The occurrence of free fatty acids can be accelerated by the presence of lipase. Table 4 shows the lipase activity of FRB as influenced by SSH. Similarly, the lipase activity in 5M SRB decreased significantly from untreated 5M SRB. The lipase activity ± SD of untreated FRB and 5M SRB were 1.07 ± 0.08 units (U)/g and 1.06 ± 0.04 U/g, respectively. The high temperature associated with the SSH conditions probably eliminated lipase. Chotimarkorn et al.

(2008) stated that lipase was eliminated following heating at 100°C for 15 min, while lipoxygenase was inactivated after heating at 50–70°C for 10 min (Malekian et al., 2000). Patil et al. (2016) found that the microwave stabilizing technique (4 W/g for 5 min) could maintain low levels of FFA (1.09–1.38%) throughout 4 wk of storage at room temperature. In contrast, untreated rice bran had a substantial increase in FFAs from 1.05% (day 0) to 58.50% (day 28). Rice bran oil quality (FFA, PV and TBA) was stabilized for up to 90 d of storage when the rice bran was microwave treated.

#### *Effect of selected superheated steam heating conditions on antioxidant activities*

The antioxidant activities and total phenolic content of FRB and 5M SRB after SSH were compared with those of untreated riceberry bran (Table 4). By increasing the SSH temperature, the total phenolic compounds slightly decreased in both treatments. The determination of antioxidant activities is reaction-mechanism dependent. The sensitivity and specificity of a single analytical method cannot determine the antioxidant activities of all of the different phenolic compounds in riceberry bran extract. Therefore, a combination of the DPPH and FRAP methods was used in the analysis to provide a more reliable assessment of the antioxidant activities of riceberry bran. DPPH is one of the most stable free radicals and is frequently used in the evaluation of radical scavengers in natural foods (Loypimai et al., 2016). DPPH assay was expressed as  $IC_{50}$ , where a lower  $IC_{50}$  value indicates higher antioxidant efficiency (Loypimai et al., 2016). The FRAP assay has been widely used to directly test the total antioxidant potential of several foods and plant extracts (Loypimai et al., 2016). It determines the reducing potential of the antioxidant reacting with a ferric tripyridyltriazine ( $Fe^{3+}$ -TPTZ) complex to form ferrous tripyridyltriazine ( $Fe^{2+}$ -TPTZ) (Loypimai et al., 2016), which is almost colorless. At 275°C, there was a significant increase in the FRAP value and scavenging ability of DPPH in FRB and 5M SRB. SSH enhanced the release of bound phenolic compounds, which led to an increase in the total phenolic

content and antioxidant activities (Rumruaytum et al., 2014). The extremely high temperatures did not decrease antioxidant activities. In addition, Wang et al. (2012) reported that the scavenging ability of DPPH, the FRAP value and the total phenolic content of sweet potato using superheated steam at 140°C for 40 min increased compared to the control. Likewise, higher antioxidant activities and total phenolic content were obtained in avocado pulp using superheated steam at 130–170°C, compared with freeze drying at 50°C (Husen et al., 2014). Therefore, the temperature of SSH may contribute to less damage of antioxidants and also promote antioxidant activities.

The best treatment combination of SSH temperature and exposure time was selected based on the PV being below the acceptable level and having a minimum FFA and moisture content, where there were no visible changes in the treated samples. Considering a safe level of PVs and a short exposure time, FRB treated using SSH at 275°C for 15 s showed the best mean  $\pm$  SD results of  $12.3 \pm 2.0$  mg Eqv/kg oil. Moreover, SSH elevated the FRAP value and DPPH assay, retained a high total phenolic content and decreased lipase activity. Microwave-stabilized rice bran did not result in any nutritional changes during 3 mth of storage; moreover its oil quality was regarded as high (Patil et al., 2016). Ramezanzadeh et al. (1999a) stated that the FFA content of microwave-stabilized rice bran slightly increased when stored at room temperature (4% FFA) and in a refrigerator (1% FFA), while the FFA content of unstabilized rice bran increased to 19% at room temperature after 4 mth. Lakkakula et al. (2004), reported the FFA content of microwave-stabilized rice bran was in the range 2.80–3.89%, whereas the FFA content of unstabilized rice bran increased from 3.96% to 18.03% after storage for 6 wk. Lipids in the untreated rice bran deteriorated after 6 mth of storage as a result of the alteration in the phospholipid composition, leading to degradation of triacylglycerols to free fatty acids and glycerol through mono- or diacylglycerols (Aibara et al., 1986).

Nowadays, there is increasing consumer awareness regarding healthy and nutritious food, with a special focus of functional food. Natural products obtained from plants receive high attention and are in demand as part of diets aimed at preventing disease (Hu et al.,

**Table 4** Lipase activity and antioxidant activities of freshly milled riceberry bran (FRB) and 5-month-frozen stored riceberry bran (5M SRB) subjected to superheated steam heating (SSH) under selected conditions

Sample handling & storage*	SSH conditions	Lipase activity (units/g)	Total phenolic content (mg GAE/g sample)	FRAP value ( $\mu$ mol trolox/g sample)	DPPH assay $IC_{50}$ (mg/ml)
FRB	Untreated control	1.07 $\pm$ 0.08 <sup>a</sup>	13.78 $\pm$ 0.22 <sup>a</sup>	67.36 $\pm$ 0.29 <sup>b</sup>	8.97 $\pm$ 0.02 <sup>c</sup>
	275°C, 15 s	0.50 $\pm$ 0.09 <sup>c</sup>	13.65 $\pm$ 0.01 <sup>a</sup>	75.37 $\pm$ 0.16 <sup>a</sup>	7.73 $\pm$ 0.06 <sup>b</sup>
	325°C, 10 s	0.52 $\pm$ 0.11 <sup>c</sup>	13.27 $\pm$ 0.01 <sup>b</sup>	63.04 $\pm$ 0.96 <sup>c</sup>	8.28 $\pm$ 0.06 <sup>b</sup>
	375°C, 10 s	0.22 $\pm$ 0.09 <sup>d</sup>	12.29 $\pm$ 0.06 <sup>c</sup>	58.18 $\pm$ 0.16 <sup>d</sup>	8.42 $\pm$ 0.01 <sup>f</sup>
5M SRB	Untreated control	1.06 $\pm$ 0.04 <sup>a</sup>	9.78 $\pm$ 0.18 <sup>d</sup>	43.62 $\pm$ 0.06 <sup>f</sup>	14.30 $\pm$ 0.08 <sup>b</sup>
	275°C, 15 s	0.77 $\pm$ 0.04 <sup>b</sup>	8.89 $\pm$ 0.11 <sup>e</sup>	48.68 $\pm$ 0.80 <sup>e</sup>	13.66 $\pm$ 0.03 <sup>c</sup>
	325°C, 10 s	0.40 $\pm$ 0.04 <sup>c</sup>	8.95 $\pm$ 0.02 <sup>e</sup>	42.46 $\pm$ 0.64 <sup>fg</sup>	12.39 $\pm$ 0.18 <sup>d</sup>
	375°C, 10 s	0.28 $\pm$ 0.10 <sup>d</sup>	8.27 $\pm$ 0.01 <sup>f</sup>	41.55 $\pm$ 0.00 <sup>g</sup>	14.66 $\pm$ 0.05 <sup>a</sup>

GAE = gallic acid equivalent; FRAP = ferric reducing antioxidant power; DPPH = 2,2'-diphenyl-1-picrylhydrazyl;  $IC_{50}$  = concentration providing 50% inhibition.

\* a–h = different lowercase superscript letters within the same column are significantly different ( $p < 0.05$ ).

Data = mean  $\pm$  SD of two independent replications ( $n = 6$ ).

2009). Rice bran has numerous applications in food, specifically increasing the nutritional and functional properties (Malekian et al., 2000). Stabilized rice bran is a good source of fat and dietary fiber. Rice bran contains high unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid, which might help lower LDL-cholesterol (Malekian et al., 2000). Soluble dietary fibers in rice bran have been reported to be effective in reducing total blood cholesterol and promoting satiety, whereas insoluble dietary fibers might lower the risk of colon cancer and diverticular disease (Mishra and Chandra, 2012). Certain amounts of rice bran have been applied in food products such as high fiber bread (Hu et al., 2009), biscuits (Mishra and Chandra, 2012), frozen pizza (de Delahaye et al., 2005) and beverages (Faccin et al., 2009). Utilization of stabilized riceberry bran is possible and promising as a functional food ingredient for high fiber, high antioxidant enhancement.

In conclusion, all the SSH conditions decreased the moisture content, PV, FFA and lipase activity in both FRB and 5M SRB. There was rapid moisture reduction to below 5% when the samples were treated for more than 15 s, regardless of the applied temperature. Hydrolysis rancidity and lipid oxidation in riceberry bran could be retarded by using SSH. Several SSH conditions promoted and retained antioxidant activities of FRB and 5M SRB. Even though the long-storage riceberry bran could be treated using SSH, to use riceberry bran as a food ingredient, it should be momentarily stabilized for superior quality. Riceberry bran treated at 275°C for 15 s had a PV below the maximum acceptable level and its antioxidant activities were higher than for untreated riceberry bran. SSH could be potentially used to inhibit rancidity of lipids and to promote antioxidant activities in riceberry bran.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

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