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Stabilization of Rice Bran by Infrared Radiation Heating for Increased Resilience and Quality of Rice Bran Oil Production

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ABSTRACT: Rice bran, a by-product of rice milling, is a valuable source of rice bran oil (RBO). However, it is prone to rancidity and must be processed quickly after rice polishing. The researchers found that rice bran stabilization with infrared radiation (IR) at 125 V and 135 V for 5～10 min. The most promising IR treatments were 125 V for 10 min and 135 V for 5 min, which resulted in the lowest lipase activity (93～96% inhibition) and levels of γ-oryzanol and α-tocopherol comparable to those of the untreated control. However, the color of rice bran and RBO based on L*, a*, b*, and total color difference (ΔE) and Gardner-20 mm index darkened. Upon storage of rice bran at 38°C for 8 weeks, the use of these two IR treatments completely inhibited the rise in free fatty acid (FFA) content and peroxide values throughout the storage period. In contrast, the control had a pre-storage FFA more than double that of IR-stabilized rice bran, which further increased during storage and, in the 8th week, was more than 6-fold higher than the pre-storage level. γ-oryzanol and α-tocopherol slightly decreased with storage and their levels did not differ between stabilized and unstabilized rice bran. RBO color darkening was again observed, but the color lightened with storage, especially upon treatment at 135 V for 5 min. In contrast, the color of control RBO darkened with storage. Thus, IR at 135 V for 5 min was the most promising method for rice bran stabilization, based on which commercial IR treatment instruments can be developed.

Keywords: milling waste utilization, oil extraction, *Oryza sativa* L., quality loss

INTRODUCTION

Rice bran is a by-product of rice milling and contains 12～22% oil, 10～15% moisture, 11～17% protein, and 8～17% ash depending on the rice variety, premilling treatment, and milling system (Sharif et al., 2014). Rice bran oil (RBO) is a value-added food and industrial product popular in various countries, such as Thailand, Japan, South Korea, China, India, Indonesia, and Taiwan, as an excellent cooking and salad oil due to its high smoke point and delicate flavor (Pali, 2013; Lai et al., 2019). RBO has many nutritional and health benefits because it is rich in bioactive compounds such as tocopherols, γ-oryzanol, phytosterols, polyphenols, tocotrienol, squalene, and unsaturated fatty acids (Pali, 2013).

Unfortunately, the susceptibility of rice bran to hydrolytic and oxidative rancidity constrains RBO production (Yılmaz, 2016; Yılmaz Tuncel and Yılmaz Korkmaz, 2021). In the intact rice grain, lipases are localized in the testa layer, while the oil is in the aleurone layer and germ. The germ, which consists of 60% lipase, is similarly compartmentalized. However, the enzyme and substrate are brought together during the milling process and their interaction causes hydrolysis of neutral RBO to free fatty acids (FFAs), leading to the development of hydrolytic rancidity. The produced FFAs are subjected to oxidation by endogenous lipoxygenase, leading to rancid off-flavors. These reactions occur rapidly, and the bran becomes unsuitable for RBO processing. This is a particular problem in the cold-pressed RBO industry. Cold-pressed RBO processing requires the rice bran to be pressed within 24 h after rice polishing; if delayed, FFA accumulates, and rancidity rapidly develops. To enable rice bran storage before oil extraction and increased resilience or flexibility in rice bran handling and processing, it is essential to prevent lipid degradation without sacrificing RBO quality.

The problem of rice bran deterioration before oil extraction can be solved in two ways: (1) immediate oil extraction after the polishing process or (2) rice bran stabilization. The first method is very difficult in practice, especially on an industrial scale, due to the laborious and time-consuming handling and transporting of rice bran

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from rice mill to oil mill and because rice milling is not performed every day. It is necessary to collect and store rice bran until a sufficient volume has been obtained to warrant cost effective oil extraction. Thus, rice bran stabilization is a more viable option. There are several approaches to stabilize rice bran, such as microwave heating (Kim et al., 2014), dry heating (Kim et al., 2014), extrusion (Mujahid et al., 2005), ohmic heating (Loypimai et al., 2009), and infrared radiation (IR) heating (Krishnamurthy et al., 2008; Yılmaz et al., 2014; Irakli et al., 2018). The basic drawbacks of the first four of these methods are inhomogeneous heat transfer, an inability to achieve irreversible enzyme inactivation, high cost of operation, and loss of bioactive nutrients (Yılmaz Tuncel and Yılmaz Korkmaz, 2021). IR has been shown to be an efficient and cost effective method for rice bran stabilization without adversely affecting the bioactive compounds when employed under optimum conditions (Yılmaz Tuncel and Yılmaz Korkmaz, 2021). It can effectively transfer heat to rice bran and inhibit lipases at the same time, while also shortening processing times and saving energy (Krishnamurthy et al., 2008). For instance, it was reported that 5-min exposure of Osmancik rice bran to 600 W IR resulted in less than 5% residual FFA content after 165 days of storage (Yılmaz et al., 2014). Moreover, when rice bran was exposed to 700 W IR for 7 min, the amount of stabilized γ-oryzanol and the composition of the FFA did not change, but the tocopherol content decreased compared with that of raw rice bran or three fractions of Baldo rice bran. The shelf life of rice bran was increased to 90 days without remarkable changes in FFA content (Yılmaz, 2016).

It is important to choose the appropriate IR wavelength (IR emitter/source) since this variable affects the intensity of absorption of IR energy by specific food components (Yılmaz Tuncel and Yılmaz Korkmaz, 2021). IR is categorized into three regions, namely, near-IR (short wave), mid-IR (medium-wave), and far-IR (FIR, long wave), corresponding to the spectral ranges of 0.75～1.40, 1.40～3.00, and 3.00～1,000 μm, respectively. A longer IR wavelength could be more effective in rice bran stabilization. Yılmaz Tuncel and Yılmaz Korkmaz (2021) showed that stabilization of rice bran by mid-IR was more effective at retarding the FFA increase than near-IR stabilization. It was further shown that storage in the oil form resulted in greater losses of tocopherol and γ-oryzanol than storage in the bran form.

Against this background, the main objective of our study was to determine the efficacy of IR heating using FIR (3～50 μm) for stabilizing rice bran for a shelf life of at least 1 month without undesirable effects on physicochemical (color- and rancidity-related compounds) and nutritional qualities (α-tocopherol and γ-oryzanol).

MATERIALS AND METHODS

Rice bran samples and reagents

Freshly milled organic rice bran samples (Khao Dawk Mali 105) were collected in sacks from a local rice milling company, Buntharik District, Ubon Ratchathani Province, Thailand, during November to December 2021 and were taken to the Faculty of Agriculture, Ubon Ratchathani Rajabhat University, within 3 h. The rice bran samples were separated into 2 kg vacuum bags, sealed, and kept in a freezer at −20°C before use within 24 h. α-Tocopherol and γ-oryzanol (99% purity) standards were purchased from Merck and Sigma-Aldrich Co., respectively. All other chemicals and reagents were of analytical grade (Sigma-Aldrich Co. or Merck).

IR heating device

Fig. 1 shows the IR heating device characterized as a semi-open stove with curved smooth aluminum (30 cm width and 57 cm length) covered with 2 cm of heat insulation. It was equipped with three IR lamps (750 W providing up to 700°C, diameter of 17 mm, and length of 1,000 mm) (Hi-Den Heattech Co., Ltd.) made of nickel chrome wire and emitting FIR with a wavelength of 3～50 μm. The tube body of the IR lamps was made of carbon silicone ceramic. The distance between the lamps and the surface of the rice bran was set at 6 cm. The IR intensity or output power of the emitters was varied by regulating the voltage through a variac (220 V, 5,000 W, and 20 A) (Hi-Den Heattech Co., Ltd.) adjusting the temperature with a connected IR thermometer (Testo 805i, Testo SE & Co. KGaA).

IR heating experiment

A 2×4 factorial experiment with a completely randomized design (CRD) was conducted with IR power (potential difference) of 125 V (5.4 A) and 135 V (5.8 A) as factor A and IR heating time of 0 (control), 5.0, 7.5, and 10.0 min as factor B. All treatments were replicated three times. The rice bran sample was prepared by sifting through a sieve with a mesh size of 0.2 mm. The sample (54.30 g of rice bran per replicate) was spread on a Teflon sheet and then placed in an aluminum tray (24.5 cm width and 40.5 cm length) with a uniform thickness of 1.5 mm. The IR heating device was switched on at a voltage of 125 V (5.4 A) or 135 V (5.8 A) with an exposure time of 0～10 min as mentioned above until a constant temperature was reached. The distance from the tube surface to the rice bran surface was fixed at 6 cm. During heating, the rice bran was turned over every 30 s. The temperature at the surface of the rice bran was measured every 2 s using an infrared thermometer (Testo 805i). After the IR heating treatment, the rice bran was allowed to cool to room temperature for 10 min before packing in

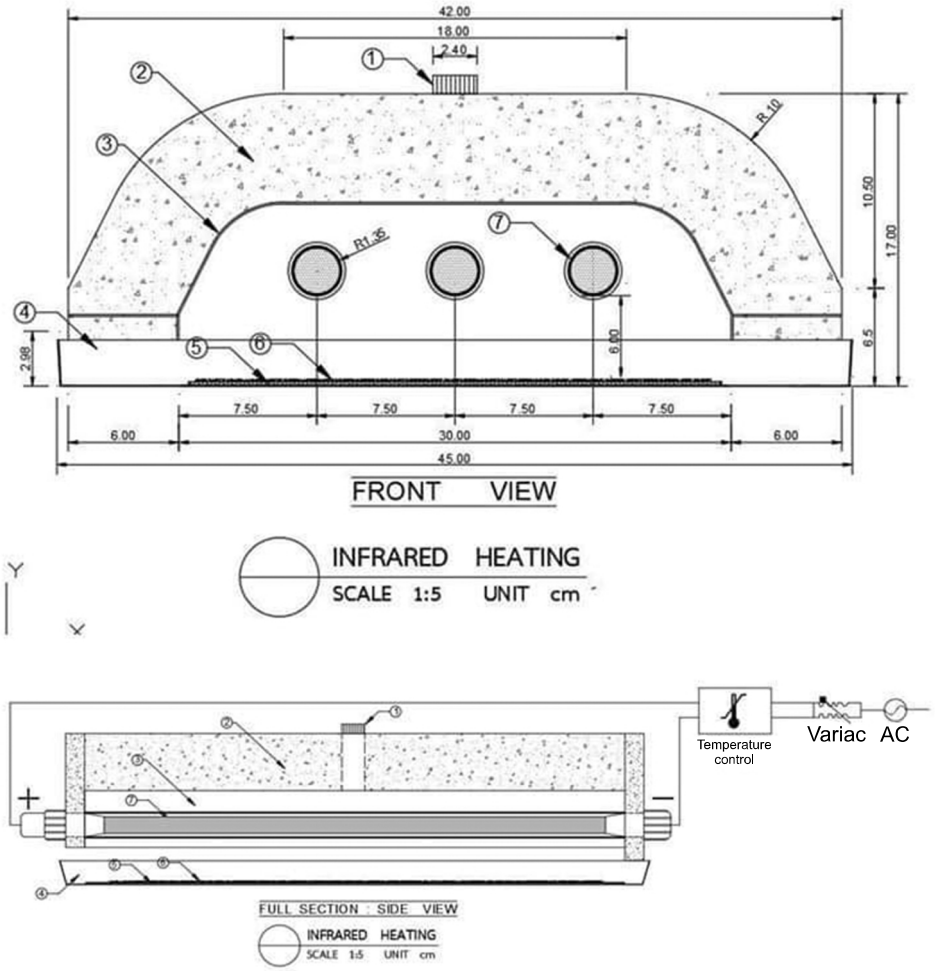

Fig. 1. Schematic of infrared heating device. (1) infrared thermometer, (2) heat insulation, (3) smooth aluminum, (4) aluminum tray, (5) teflon sheet, (6) rice bran, and (7) infrared lamp.

an embossed vacuum bag, which was sealed with a vacuum sealer. All samples were kept at −18°C before RBO extraction for analysis of lipase activity, and physicochemical (color of rice bran and RBO) and nutritional qualities (α-tocopherol and γ-oryzanol) within 24 h. The treatment conditions that showed promise for stabilizing rice bran were chosen based on the following criteria: (1) low lipase activity, (2) residual γ-oryzanol and α-tocopherol contents close to those of the control, (3) low moisture content, and (4) color of RBO similar to that of the control.

Storage experiment

From the preceding experiment, the most promising IR heating conditions for stabilizing rice bran were 125 V for 10 min or 135 V for 5 min. Samples subjected to these treatments were compared to the unheated control in a storage study conducted in a simple CRD. The IR-heated and unheated samples were separately placed in 14.0× 16.5 cm sacks each containing 70 g of rice bran and stored under accelerated aging conditions of 38°C. Every 2 weeks during the 8-week storage period, samples were taken for RBO extraction and analysis. The study aimed to preserve rice bran quality for at least 1 month before RBO extraction.

RBO extraction

Rice bran samples after IR treatment and after every 2 weeks of storage at 38°C were subjected to RBO extraction by the immersion stirring method of Stanojević et al. (2004) and Mingyai et al. (2017). The samples were immersed and stirred in *n*-hexane solvent at a ratio of 1:4 (w/v) for 3 h at room temperature. The mixed slurry was separated on Whatman paper No. 4 (Sigma-Aldrich Co.) under a vacuum. The filtrate as the miscella was centrifuged at 6,000 rpm for 10 min. The supernatant was filtered through Whatman paper No. 4 and transferred to a rotary flask for evaporation to dryness at 50°C. RBO extract was collected and stored at −18°C before analysis within 24 h.

Measurement of parameters

***Lipase activity*:** The lipase activity was determined in the IR heating experiment to determine the most promising IR conditions for stabilizing rice bran. A modified version of the lipase extraction method reported by Prabhu et al. (1999) was applied. Defatting was first performed to remove oil and other lipid substances. A rice bran sample (5 g) was placed in a 50 mL beaker with 15 mL of *n*-hexane and homogenized at 4,000 rpm for 2 min using a

homogenizer (T25 digital ULTRA-TURRAX®, IKA). The mixture was filtered through Whatman paper No. 4 to remove the solvent. Extraction with *n*-hexane was performed three times. The defatted bran was allowed to air-dry for about 1 h to evaporate any *n*-hexane residue. It was then immersed in 0.05 M phosphate buffer of pH 7 (containing 0.5 mM $CaCl_2$) at 10°C for 1 min with constant stirring. The mixture was centrifuged at 5,000 rpm for 20 min at 4°C and the supernatant was obtained, filtered through Whatman paper No. 4, and again centrifuged at 5,000 rpm for 20 min at 4°C. The supernatant was collected as crude lipase extract.

The lipase activity was measured against *p*-nitrophenyl butylate (*p*NPB) following a modified version of the method of Moreno et al. (2021). 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 of Triton X-100 and 0.1 g of gum arabic dissolved in 90 mL of 0.05 M phosphate buffer, pH 7.0. The substrate solutions were prepared by dropwise 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 h at room temperature. For the *p*NPB substrate system, 0.1 mL of appropriately diluted enzyme sample was added to 3.3 mL of substrate solution and incubated at 40°C for 1 h. The enzyme activity was stopped by adding 2 mL of 2-propanol and measured at a wavelength of 410 nm using a spectrophotometer [Genesys G10-S ultraviolet-visible (UV-Vis), Thermo Fisher Scientific]. The same treatment was applied to a sample without any enzyme, which was used as a blank. One lipase unit was defined as the amount of enzyme that liberated 1 mol *p*NPB per min under the assay conditions. The lipase activity was expressed in international units per microliter (IU/μL).

***Moisture content*:** The moisture content of rice bran was determined in the IR heating experiment. This was performed after IR treatment using the method of Ding et al. (2015). Rice bran samples (1 g) were dried in a hot air oven at 130°C until a constant weight was obtained. Results are expressed as a percentage of dry weight basis.

***FFA content*:** FFA contents of RBO were measured titrimetrically following a slightly modified version of the method of Pourali et al. (2009). Briefly, 2 g of RBO was mixed with 50 mL of 95% ethanol and titrated with standard 0.1 N sodium hydroxide in the presence of 1% phenolphthalein indicator. The analysis were conducted in triplicate and the results were calculated as oleic acid equivalents.

***Peroxide value (PV)*:** PV of RBO was determined following the method of Yılmaz Tuncel and Yılmaz Korkmaz (2021) and expressed in meq/kg oil.

***γ-Oryzanol and α-tocopherol contents*:** γ-Oryzanol and α-tocopherol contents of RBO were determined in both IR heating and storage experiments, using a slightly modified version of the method of Tuncel and Yılmaz (2011). The RBO sample (0.2 g) was diluted with 5 mL of isopropanol-methanol (30:70 v/v), filtered through a 0.45 m membrane filter, and dispensed to high performance liquid chromatography vials for separation using a Zorbox Eclipse Plus C18 column (100 mm length, 4.6 mm internal diameter, and 3.5 μm particle size) (Agilent 1100 series, Agilent Scientific Instruments) at 29.5°C with isopropanol-methanol (30:70 v/v) as mobile phase. The isocratic flow was 1.0 mL/min and the injection volume was 20 μL. The UV-Vis (diode array detector) was set at 325 and 292 nm for γ-oryzanol and α-tocopherol, respectively, which were compared to the chromatograms of the standards. Results were expressed as mg/100 g RBO for γ-oryzanol content and g/g RBOs for α-tocopherol content.

***Color*:** The color of rice bran and RBO in the IR heating experiment was measured using a colorimeter (ColorFlex EZ, Hunter Associates Laboratory, Inc.). The CIE color values were recorded as L* (lightness), a* (redness), and b* (yellowness). The total color difference (ΔE) between control and treated samples was also calculated using the following equation: $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. In the storage experiment, RBO color was determined based on E and Gardner-20 mm index, the latter using a ColorQuest®XE (Hunter Associates Laboratory, Inc.) colorimeter with a transmission cell (optically clear glass cell) and an optical path length of 20 mm. It was measured on a regular transmission with Illuminant D65/10°C (HunterLab, 2008). The RBO sample was diluted with hexane at a 1:10 ratio before measurement. The Gardner scale was used to measure the change in color from yellow to brown or red using a scale of 1 to 18: the color is light yellow if close to 1 and brown or red if close to 18.

Statistical analysis

Results were subjected to analysis of variance and comparison of treatment means by Duncan's multiple range test using the Statistix 8.0 program (StatSoft). $P \leq 0.01$ was considered to indicate a significant difference. The results are expressed as mean±standard deviation.

RESULTS AND DISCUSSION

IR heating experiment

***Lipase activity*:** Lipase activity was significantly lower at 135 V than at 125 V and decreased as heating time increased (Table 1). The interaction effects of IR power and heating times were also significant. Lipase activity was lowest upon treatment at 125 V for 10 min and at 135 V for 7.5 min and 10 min (4.56∼5.05 IU/μL), with the values being equivalent to a 96% reduction of lipase activity compared with the control or no IR (125.19 IU/μL). Shorter heating times at 125 V (5.0∼7.5 min) also re-

Table 1. Effect of IR heating on lipase activity, γ-oryzanol, and α-tocopherol contents of RBO after stabilization treatment

Treatment	Lipase activity (IU/μL)	γ-Oryzanol content (mg/100 g RBO)	α-Tocopherol content (μg/g RBO)
IR power (factor A) (V)			
125	45.69±0.34[a]	884.78±10.06[a]	51.12±0.58[a]
135	35.76±0.38[b]	743.49±12.14[b]	42.63±0.84[b]
F-test	**	*	*
Heating time (factor B) (min)			
0	125.19±0.49[a]	902.52±12.36[a]	53.18±1.00[a]
5.0	25.28±0.38[b]	879.29±8.64[a]	50.56±1.12[a]
7.5	7.61±0.25[c]	772.25±10.15[b]	43.82±0.94[b]
10.0	4.80±0.31[d]	702.50±8.64[b]	40.33±0.86[b]
F-test	**	**	**
Interaction (A×B) (V, mim)			
No IR	125.19±0.49[a]	902.52±12.36[a]	53.18±1.00[a]
125, 5.0	41.83±0.46[b]	893.75±7.99[a]	50.62±0.56[a]
125, 7.5	10.67±0.17[c]	875.75±8.88[a]	50.08±0.35[a]
125, 10.0	5.05±0.25[e]	867.08±4.96[a]	50.58±0.96[a]
135, 5.0	8.72±0.29[d]	864.83±3.73[a]	50.49±0.67[a]
135, 7.5	4.56±0.34[e]	668.70±16.83[b]	37.55±1.93[b]
135, 10.0	4.56±0.38[e]	537.92±12.69[c]	30.08±0.64[c]
F-test	**	**	**

Means with different letters (a-e) within a column per factor are significantly different based on Duncan's multiple range test at $P \leq 0.01$.
Significantly different using F-test at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$.
IR, infrared radiation; RBO, rice bran oil.

duced the lipase activity, but not as dramatically as the other treatments. A shorter heating time at 135 V (5 min) resulted in the second lowest lipase activity of 8.72 IU/L, which represented a reduction of approximately 93% compared with the control. This IR treatment with 125 V for 10 min was selected for testing in the storage experiment. Despite the lowest lipase activity, IR heating at 135 V for 7.5～10.0 min was not considered because of its undesirable effects on other parameters (see below).

Inhibition of lipase activity is the basic measure of a stabilization process, which indirectly indicates stable FFA content during storage (Yılmaz, 2016). It could be induced by heating through destruction of the high structural binding force of lipase and unfolding and denaturation of the enzyme. Indeed, Rodchuajeen et al. (2016) reported a reduction of lipase in the rice bran cultivar Khao Dawk Mali 105 exposed to vibrated bed IR heating at 2,000 W and a frequency of 450 rpm. Similarly, Marei et al. (2017) showed that heating rice bran with a hot air oven at 150°C for 10 min completely inhibited lipase activity. Rice bran stabilization by IR heating at 140°C for 15 min also inhibited the lipase activity of Axios-Long A-type rice bran (Yılmaz Tuncel and Yılmaz Korkmaz, 2021).

Lipases also require sufficient moisture content to act as effective catalysts (Hampson and Foglia, 1999); catalytic activity generally increases with increasing water content (Wehtje and Adlercreutz, 1997). Analysis of the moisture content revealed that IR-treated rice bran had a much lower moisture content (0.1～0.2%) than the control (5～6%) (data not shown). This may have contributed to the loss of lipase activity in response to IR heating in addition to the direct effect of IR-induced heating on enzyme inactivation. An earlier study showed that lipase with an initial moisture content of 1.5% was associated with little evidence of hydrolysis, whereas that with moisture content of 5.4～23.5% resulted in the formation of hydrolytic products (Hampson and Foglia, 1999). It has been reported that enzymes require a certain level of water in their structures to maintain their natural conformation (Rezaei et al., 2007) and function effectively; such function is maximized at the optimum level of water, but below, or above this level enzyme activity declines due to loss of enzyme stability.

γ-Oryzanol and α-tocopherol contents: IR power, heating time, and their interaction also significantly affected γ-oryzanol and α-tocopherol contents (Table 1). Specifically, both γ-oryzanol and α-tocopherol contents were lower at 135 V than at 125 V and generally decreased with increasing heating time. The significant interaction effect was mainly due to the marked reduction of γ-oryzanol and α-tocopherol contents at 135 V for 7.5～10.0 min, equivalent to losses of 25% to more than 40% relative to that of the control (Table 2). Other IR treatments, particularly 125 V for 10 min and 135 V for 5 min, had γ-oryzanol and α-tocopherol contents that were statistically comparable to that of the control; losses were only 5% or lower.

γ-Oryzanol and α-tocopherol are strong antioxidants and are of particular scientific interest among all of the bioactive lipophilic compounds present in RBO due to

Table 2. Percent loss of γ-oryzanol and α-tocopherol contents of IR-stabilized rice bran oil relative to the control (no IR heating)

Treatment (V, mim)	Loss of γ-oryzanol content (%)	Loss of α-tocopherol content (%)
125, 5.0	2.06^{c}	4.70^{c}
125, 7.5	2.96^{c}	5.80^{c}
125, 10.0	3.90^{c}	4.83^{c}
135, 5.0	4.15^{c}	5.00^{c}
135, 7.5	25.87^{b}	29.37^{b}
135, 10.0	40.40^{a}	43.43^{a}
F-test	**	**

Means with different letters (a-c) within a column are significantly different based on Duncan's multiple range test at $P \leq 0.01$.
Significantly different using F-test at $^{**}P \leq 0.01$.
IR, infrared radiation.

their potential health benefits (Yılmaz Tuncel and Yılmaz Korkmaz, 2021). They may be affected by IR heating depending on the treatment conditions. At lower rates of IR heating, both compounds were not as markedly affected as in treatments with low IR power (i.e., 125 V) or IR application for a shorter duration, even at higher IR power (i.e., 135 V, 5 min). Previous studies showed that IR treatment did not cause significant differences in γ-oryzanol content (Yilmaz and Tuncel, 2015; Irakli et al., 2018). Similarly, mid-IR did not cause tocopherol loss in rice bran (Yılmaz Tuncel and Yılmaz Korkmaz, 2021), whereas high-heat-emitting near-IR resulted in the loss of up to 50% tocopherol (Yılmaz et al., 2014). Among tocopherols, α-tocopherol is the least stable but comprises 73~81% of the total tocopherol content (Irakli et al., 2018; Yılmaz Tuncel and Yılmaz Korkmaz, 2021). Furthermore, Table 1 shows that the γ-oryzanol contents were much higher than the α-tocopherol contents. The same trend was observed in previous studies (Yılmaz, 2016; Jung et al., 2017). Based on the results and those on lipase activity, the authors selected IR heating at 125 V for 10 min and 135 V for 5 min for further testing in the storage experiment.

***Color of rice bran and RBO*:** The L*, a*, and b* color values and ΔE of rice bran were affected significantly ($P \leq 0.01$) by the different potentials (125 or 135 V), heating times (5~10 min), and their interaction (Table 3). L* decreased while a* values and ΔE increased with IR treatment when compared with the findings in the control. Meanwhile, b* values decreased only when IR was applied at 135 V for 7.5~10.0 min. IR at 135 V for 10 min caused the lowest L* and b* values and highest a* and ΔE. The same trend was obtained for RBO color in terms of b* values, which increased with the increasing rate of IR treatment (Table 4). RBO color measurement using the Gardner −20 mm index showed that rice bran heated at 125 V for 5~10 min and 135 V for 5 min had an average Gardner scale value of 5.20~5.57 (yellow-red), while the control had one of 2.22 (light yellow) (data not shown).

Color is an important quality factor that determines the visual acceptability of RBO. The decrease in L* and increases in a* and ΔE in association with IR treatment indicate that the color of rice bran and RBO darkened. The

Table 3. Effect of IR heating on the color of rice bran after stabilization treatment

Treatment	L*	a*	b*	ΔE
IR power (factor A) (V)				
125	53.13±0.59^{a}	6.50±0.16^{b}	17.67±0.09^{a}	5.92±0.69^{b}
135	44.76±1.13^{b}	6.94±0.61^{a}	15.59±1.38^{b}	14.21±1.47^{a}
F-test	**	*	**	**
Heating time (factor B) (min)				
0	58.54±0.15^{a}	4.45±0.02^{c}	16.78±0.11^{ab}	0.00±0.00^{d}
5.0	54.21±1.46^{b}	6.43±0.33^{b}	17.95±0.09^{a}	4.99±0.97^{c}
7.5	44.36±0.79^{c}	7.75±0.31^{a}	16.69±0.75^{ab}	14.74±0.43^{b}
10.0	38.67±1.04^{d}	8.24±0.15^{a}	15.09±0.75^{b}	20.52±0.75^{a}
F-test	**	**	**	**
Interaction (A×B) (V, mim)				
No IR	58.54±0.15^{a}	4.45±0.02^{d}	16.78±0.11^{ab}	0.00±0.00^{e}
125, 5.0	56.83±0.83^{a}	5.69±0.34^{c}	17.92±0.04^{a}	2.43±0.77^{e}
125, 7.5	51.49±0.80^{b}	7.32±0.08^{b}	17.99±0.12^{a}	7.71±0.74^{d}
125, 10.0	45.64±0.58^{c}	8.51±0.06^{a}	17.99±0.12^{a}	13.53±0.56^{c}
135, 5.0	51.58±2.09^{b}	7.17±0.63^{b}	17.99±0.12^{a}	7.55±2.14^{d}
135, 7.5	37.22±0.77^{d}	8.18±0.84^{ab}	15.40±2.01^{b}	21.77±0.55^{b}
135, 10.0	31.70±1.50^{e}	7.96±0.38^{ab}	12.19±2.01^{c}	27.50±1.70^{a}
F-test	**	**	**	**

Means with different letters (a-e) within a column per factor are significantly different based on Duncan's multiple range test at $P \leq 0.01$.
Significantly different using F-test at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$.
IR, infrared radiation; ΔE, total color difference.

Table 4. Effect of IR heating on the color of rice bran oil after stabilization treatment

Treatment	L*	a*	b*	ΔE
IR power (factor A) (V)				
125	71.94±1.26^{a}	15.20±0.91^{b}	17.40±0.13^{a}	5.92±0.69^{b}
135	55.22±0.80^{b}	24.03±0.94^{a}	15.56±1.39^{b}	14.21±1.47^{a}
F-test	**	**	**	**
Heating time (factor B) (min)				
0	81.47±0.93^{a}	5.10±0.46^{c}	16.79±0.11^{a}	0.00±0.00^{d}
5.0	73.20±1.20^{b}	17.58±0.88^{b}	17.88±0.10^{a}	4.99±0.97^{c}
7.5	52.53±0.85^{c}	27.85±0.61^{a}	16.69±0.75^{a}	14.74±0.43^{b}
10.0	47.08±0.93^{d}	27.92±0.82^{a}	14.56±0.78^{b}	20.52±0.75^{a}
F-test	**	**	**	**
Interaction (A×B) (V, min)				
No IR	81.47±0.93^{a}	5.10±0.46^{d}	16.79±0.11^{ab}	0.00±0.00^{e}
125, 5.0	75.30±1.67^{b}	14.30±1.15^{c}	17.92±0.04^{a}	2.43±0.77^{e}
125, 7.5	66.93±1.01^{d}	20.60±0.53^{b}	17.99±0.12^{a}	7.71±0.74^{d}
125, 10.0	64.07±1.10^{e}	20.80±1.06^{b}	16.92±0.23^{ab}	13.53±0.56^{c}
135, 5.0	71.10±1.01^{c}	20.87±1.03^{b}	17.84±0.16^{ab}	7.55±2.14^{d}
135, 7.5	38.20±0.62^{f}	35.10±0.85^{a}	15.40±2.01^{b}	21.77±0.55^{b}
135, 10.0	30.10±0.75^{g}	35.03±0.95^{a}	12.19±2.01^{c}	27.50±1.70^{a}
F-test	**	**	**	**

Means with different letters (a-g) within a column per factor are significantly different based on Duncan's multiple range test at $P \leq 0.01$.
Significantly different using F-test at $^{**}P \leq 0.01$.
IR, infrared radiation; ΔE, total color difference.

high temperature during IR heating catalyzed nonenzymatic oxidation, resulting in yellow to brown pigmentation (Maillard reaction) (HunterLab, 2008; Rodchuajeen et al., 2016; Marei et al., 2017). Rodchuajeen et al. (2016) and Irakli et al. (2018) showed similar darkening of the color of IR-treated rice bran and RBO. In industrial practice, such darkening is prevented by chemical refining to produce RBO of a lighter color, although this process removes most of the γ-oryzanol (Van Hoed et al., 2010).

Storage experiment

***FFA content*:** Before storage (week 0), FFA content in stabilized rice bran treated at 125 V for 10 min or 135 V for 5 min was less than half that of the unstabilized rice bran sample (Table 5). During storage for 8 weeks, FFA contents in the two IR treatments did not significantly change and were less than 5%, which were acceptable for use in food products for human consumption (Tao et al., 1993; Lakkakula et al., 2004). In contrast, the FFA content of unstabilized rice bran significantly increased with storage and, after 8 weeks, it was more than six times higher than that at week 0. These results are similar to those of previous studies. Irakli et al. (2018) found that IR stabilization of rice bran cv. Axios-Long A type at 140°C for 15 min and then stored at 25~30°C resulted in a slower increase in FFA content than that of unstabilized rice bran. In addition, Yılmaz et al. (2014) reported that the FFA content of rice bran (var. Osmancik) stabilized at IR power of 600 W applied for 5 min remained below 5% for 165 days. Yılmaz (2016) further showed that stabilization at mid-IR power of 700 W for 7 min provided 90 days of shelf life without a notable change in FFA content of the rice bran fraction obtained from the first whitening step.

FFA content is an important indicator for determining whether rice bran stabilization can completely inhibit lipase activity. The comparable levels of FFA in the two IR treatments from week 0 to week 8 suggest that the lipase activity was completely inhibited. Other studies did not obtain this; instead, in one study it was reported that

Table 5. Effect of infrared radiation heating on free fatty acid contents during storage for 8 weeks

Stabilization treatment	Free fatty acid (% as oleic acid)				
	Week 0	Week 2	Week 4	Week 6	Week 8
None, control	4.37±0.75^{eA}	17.24±0.57^{dA}	22.86±0.54^{cA}	26.56±0.07^{bA}	28.61±0.51^{aA}
125 V, 10 min	1.83±0.37^{aB}	2.21±0.29^{aB}	2.16±0.71^{aB}	2.14±0.15^{aB}	2.35±0.16^{aB}
135 V, 5 min	1.77±0.07^{aB}	2.63±0.33^{aB}	2.40±0.37^{aB}	2.35±0.33^{aB}	2.68±0.37^{aB}

Means with different lower-case letters (a-e) within a row and upper-case letters (A, B) within a column are significantly different at $P \leq 0.01$.

Table 6. Effect of infrared radiation heating on peroxide value during storage for 8 weeks

Stabilization treatment	Peroxide value (meq/kg oil)				
	Week 0	Week 2	Week 4	Week 6	Week 8
None, control	0.94 ± 0.08^{dA}	2.31 ± 0.35^{dA}	4.89 ± 0.33^{cA}	8.72 ± 0.55^{bA}	13.71 ± 1.22^{aA}
125 V, 10 min	0.87 ± 0.07^{cA}	0.89 ± 0.05^{cB}	0.91 ± 0.03^{bcB}	1.05 ± 0.08^{abB}	1.08 ± 0.03^{aB}
135 V, 5 min	0.85 ± 0.03^{cA}	0.87 ± 0.03^{cB}	0.90 ± 0.05^{bcB}	1.02 ± 0.07^{abB}	1.04 ± 0.07^{aB}

Means with different lower-case letters (a-d) within a row and upper-case letters (A, B) within a column are significantly different at $P \leq 0.01$.

the FFA content of rice bran, especially stabilized with mid-IR, continued to increase with storage although at a reduced rate compared with that of unstabilized rice bran (Yılmaz Tuncel and Yılmaz Krokmaz, 2021). Elsewhere, slow, and steady increases of FFA in stabilized rice bran samples were observed in studies employing infrared, microwave, and extrusion stabilization procedures (Shin et al., 1997; Ramezanzadeh et al., 2000; Yılmaz et al., 2014).

***PV*:** PV measures the concentrations of peroxides and hydroperoxides formed in the early stages of lipid oxidation, which indirectly reflects the oil freshness. According to the Codex Alimentarius Commission, the acceptable limit of PV for RBO is <10 meq/kg (Patil et al., 2016). The results of the present study showed a trend in PV values similar to that of FFA, except that PV values before storage (week 0) did not differ significantly with treatment (Table 6). During storage, PV in stabilized rice bran treated with 125 V for 10 min or 135 V for 5 min was maintained at about 1.0 meq/kg RBO or lower for 8 weeks. In contrast, unstabilized rice bran showed an increase of PV with storage, which after 8 weeks reached approximately 13.71 meq/kg RBO, exceeding the acceptable limit. These results indicate that IR heating effectively inhibited the enzymes (lipoxygenase and lipase) involved in lipid peroxidation and degradation in stabilized rice bran. However, in an earlier study by Yılmaz Tuncel and Yılmaz Korkmaz (2021) using near- and mid-IR, PV of rice bran did not significantly differ from that of unstabilized rice bran.

***γ-Oryzanol and α-tocopherol contents*:** The contents of γ-oryzanol and α-tocopherol slowly decreased with storage (Fig. 2). They did not vary markedly between IR-stabilized and unstabilized rice bran at any timepoint during the storage period, except at week 2, at which point rice bran stabilized with the two IR treatments had higher α-tocopherol content than that without stabilization treatment. The losses of γ-oryzanol content after 8 weeks of storage were 5.41% for unstabilized rice bran, 4.75% for rice stabilized with 125 V for 10 min, and 4.28% for rice bran stabilized with 135 V for 5 min. Regarding α-tocopherol content, the order of losses after 8 weeks of storage was as follows: unstabilized rice bran (7.99%) > 125 V, 10 min (7.43%) > 135 V, 5 min (7.13%). Again, γ-oryzanol contents were much higher than α-tocopherol contents regardless of the treatment and storage period. These results are consistent with previous findings (Mujahid et al., 2005; Yılmaz et al., 2014; Yılmaz, 2016; Yılmaz Tuncel and Yılmaz Korkmaz, 2021). Irakli et al. (2018) also found an insignificant effect of IR on γ-oryzanol content during the first 4 months of ambient storage, but after 6 months of storage, the level of γ-oryzanol decreased. Meanwhile, Yılmaz (2016) and Irakli et al. (2018) reported that total tocopherol contents of stabilized rice bran were higher than those of unstabilized rice bran, but in the present study, this was obtained only in the second week of storage. Yılmaz Tuncel and Yılmaz Krokmaz (2021) obtained no significant differences in α-tocopherol content of unstabilized and IR-stabilized rice

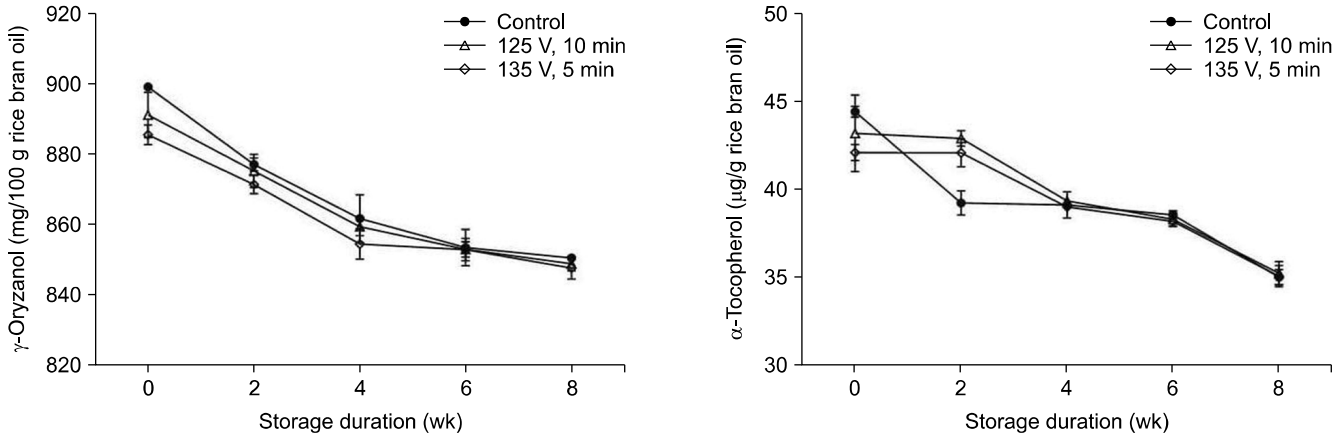

Fig. 2. The effect of infrared radiation heating on γ-oryzanol and α-tocopherol contents during storage for 8 weeks.

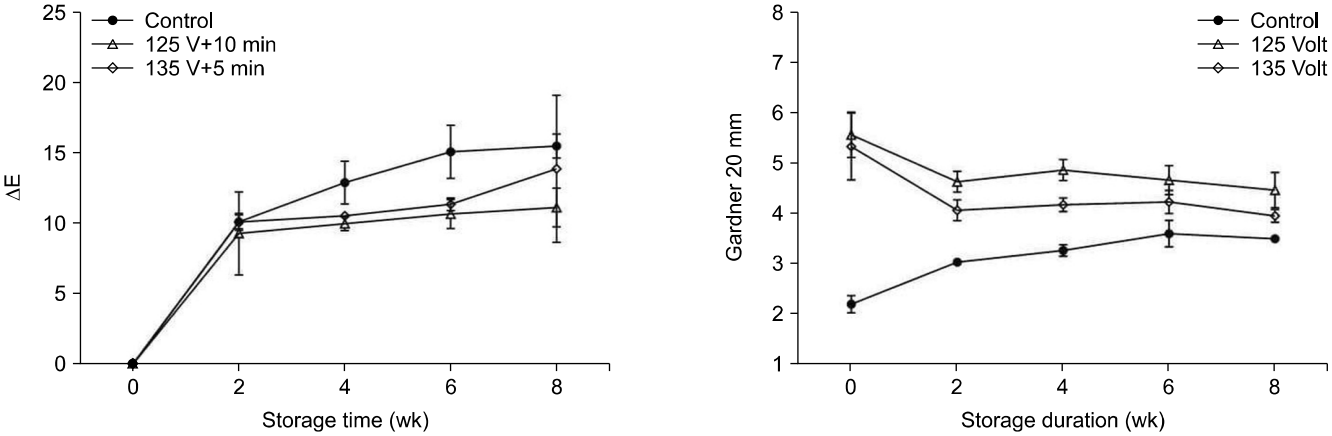

Fig. 3. The effect of infrared radiation heating on total color difference (ΔE) and Gardner-20 mm index during storage for 8 weeks.

bran throughout the storage life ($P>0.05$).

***RBO color*:** ΔE values of RBO from IR-stabilized rice bran were lower than those from unstabilized rice bran (Fig. 3). In terms of the Gardner color scale, RBO from stabilized rice bran had higher scores of 4.03～5.57 (yellow-brown) than that from unstabilized rice bran in week 0. With storage, RBO from stabilized rice bran lightened (decreased Gardner color score) while that from unstabilized rice bran darkened (increased Gardner color score), but the latter still maintained lower scores than the former throughout the storage period. The actual appearance of RBO color is shown in Fig. 4.

The darkened RBO color of IR-stabilized rice bran before the start of storage is similar to that obtained in the IR treatment study and could have been due to the immediate effect of IR heating on nonenzymatic oxidation leading to the formation of colored pigments. These colored pigments are further oxidized into colorless compounds, resulting in lightening of the color as storage progresses. In contrast, unstabilized rice bran was not exposed to heat before storage and, as expected, the color of the extracted RBO was lighter. With storage, FFA content increased due to the lipase activity, as shown in Table 2. Oil or fat oxidation by light, air, or heat produces free radicals that bind with oxygen to form yellow to brown secondary products (Rodchuajeen et al, 2016; Marei et al., 2017). This could explain the darkening of RBO from unstabilized rice bran with the progression of storage.

In conclusion, the stabilization of rice bran by IR heating at a potential of 125 V (5.4 A) for 10 min or 135 V (5.8 A) for 5 min reduced the moisture content, lipase activity, and FFA, and maintained the pre-storage level of FFA throughout the entire 8-week storage period at 38°C without adverse effects. Meanwhile, γ-oryzanol and α-tocopherol contents only slightly decreased during storage, similar to the findings of unstabilized rice bran. However, the color of rice bran and RBO darkened. During storage, the RBO color lightened, eially with IR treatment at 135 V for 5 min, approaching the color of unstabilized rice bran. Hence, IR heating with 135 V for 5 min ap-

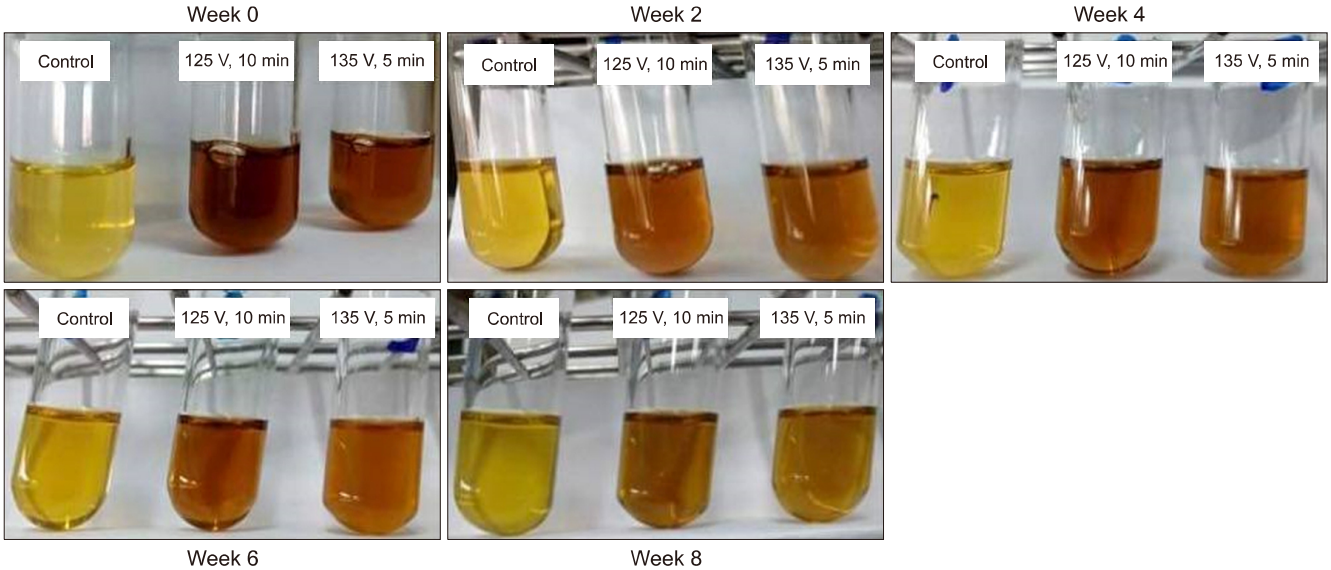

Fig. 4. The appearance of rice bran oil during storage for 8 weeks in response to infrared radiation heating.

pears to be the most promising treatment because it took less time to stabilize the rice bran; γ-oryzanol and α-tocopherol contents in RBO did not differ between IR-stabilized and unstabilized rice bran at any stage during storage; the color of RBO was similar to that of the control; and RBO had FFA of less than 5% and PV of only about 1.0 meq/kg, indicating reduced potential for the development of rancidity for more than 1 month of storage. Future work could optimize the IR treatment procedure using 135 V for 5 min as a baseline. Studies could also look into decolorizing methods to produce light-colored RBO without affecting nutritional properties. Once optimized, a tunnel-oven-type IR treatment instrument could be developed to heat rice bran continuously. The belt to be used must be able to withstand heat of at least 200°C and the instrument should have a feature allowing rice bran to be heated evenly throughout the length of the tunnel, such as installing steel spurs to rake the rice bran over the transverse belt.

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The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: RD. Analysis and interpretation: all authors. Data collection: all authors. Writing the article: all authors. Critical revision of the article: all authors. Final approval of the article: all authors. Statistical analysis: all authors. Obtained funding: all authors. Overall responsibility: all authors.

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