EFFECTS OF INFRARED AND RADIO-FREQUENCY TREATMENTS ON STABILITY, PHYSICO-CHEMICAL PROPERTIES AND BIOACTIVE COMPOUNDS OF RICE BRAN AND CRUDE RICE BRAN OIL

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ABSTRACT

Rice bran (RB) is the most important rice by-product of the rice milling process, which is the conversion of brown rice to white rice, and it contains various bioactive compounds that impart beneficial effects on human and animal health. RB is particularly rich in dietary fiber and essential fatty acids. It also contains significant quantities of starch, protein, vitamins, dietary and minerals. More importantly, rice bran oil (RBO) fractionation contains several important biochemical compounds including tocotrienols, tocopherols, oryzanol, and phytosterol. Hydrolytic rancidity seriously reduces the quality and limits the utilization of RB in foods. Thus, the stabilization of RB has become of great interest to producers. The purposes of this study were 1) to optimize the RB stabilization conditions using the infrared (IR), infrared in a vacuum (IR-VC), infrared combined with hot air (IR-HA), and radio frequency (RF) heat treatments by response surface methodology (RSM) and central composite design (CCD) 2) to investigate the effects of the IR, IR-VC, IR-HA and RF on the physico-chemical properties, stability (FFA, MC, a_w, color, proximate, microbial, and fatty acid composition), bioactive compounds (γ-tocotrienol, δ-tocopherol, and γ-oryzanol), and shelf life of RB during storage and 3) to investigate the effects of IR and RF on the extraction yield, physico-chemical properties, stability, bioactive compounds, and shelf life of RBO during storage.

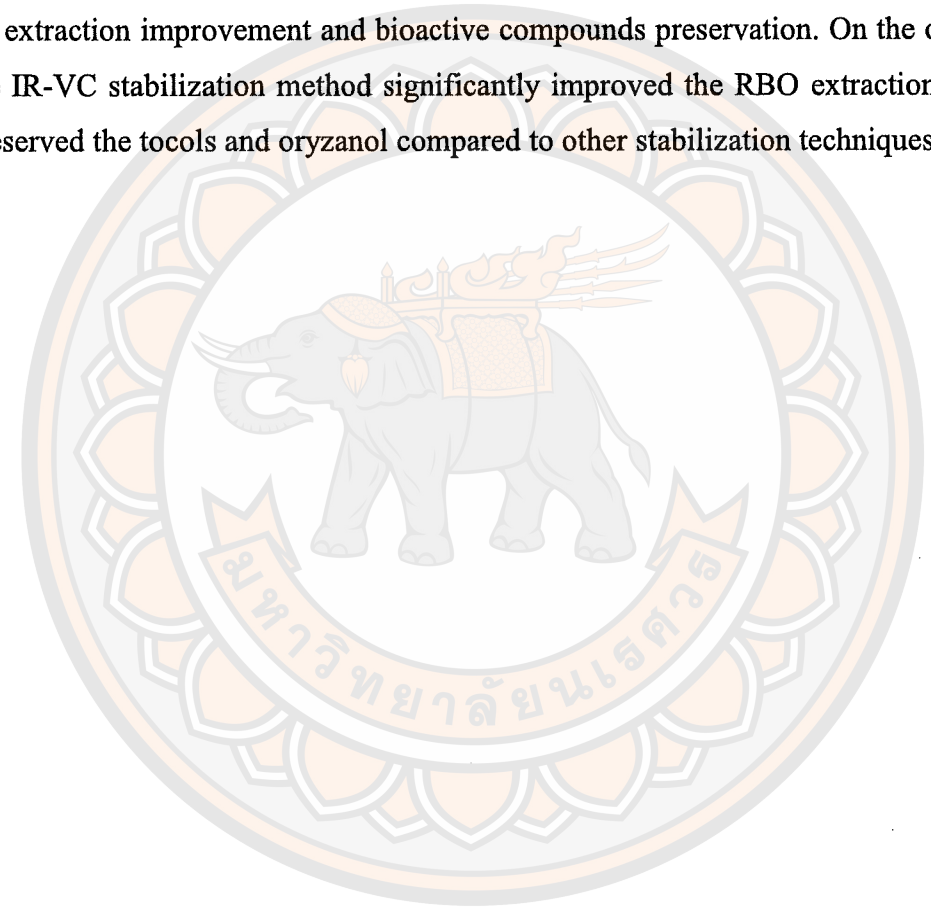
The optimal condition for RB stabilization by IR was found at an IR wattage of 993 W and treatment duration of 598 seconds. In addition, the optimal condition for IR-VC stabilization of RB was an IR wattage of 997 W, treatment duration of 598 seconds, and vacuum strength of 610 mmHg, while that for IR-HA treatment was an IR wattage of 999 W, treatment duration of 598 seconds, and hot air temperature of 72°C. The optimal condition of the RF treatment was found to be at temperature of 130°C, RB moisture content of 10.22%, and treatment duration of 322 seconds. The results of the RSM analysis revealed that the FFA content was more sensitive to infrared wattage and treatment duration than vacuum strength and hot air temperature. The RF and IR-VC generated greater efficiency for the stabilization of the RB. Furthermore, this level of IR-VC treatment successfully reduced the fatty acid hydrolysis while still preserving the bran's beneficial components such as tocotrienol, tocopherol, oryzanol and essential fatty acids. The shelf life of non-stabilized rice bran (NSRB) and stabilized rice bran (SRB) samples was estimated using the Q-10 method, based on the FFA content lower than 5%. NSRB and SRB were stored at 25°C and 35°C for 8 weeks. It was found that the shelf life of SRB by IR, IR-VC, IR-HA, and RF treatments compared to the NSRB were improved by 2.77, 3.73, 2.40, and 5.57 times, respectively.

The highest extraction oil yield (%) by the cold press was found in SRB from IR-VC treatment (8.87±0.38g/100g bran), followed by those obtained from IR (7.10±0.50 g/100g bran), IR-HA (6.97±0.38g/100g bran), RF (5.63±0.15g/100g bran), and NSRBO (5.37±0.38g/100g bran). The acid value (AV) of the NSRBO increased sharply and reached 30.96 mg KOH/g RB oil at the end of storage. The RF treatments were the most effective methods to inhibit rancidity, which provided a lowest AV (14.86 mg KOH/g RB oil) and peroxide value (PV) (6.16 meq.O_2/kg RB oil) during storage. The fatty acid composition of the NSRBO and SRBO obtained by IR, IR-VC, IR-HA, and RF methods and did not change during storage. The IR-VC was effectively maintained tocols and oryzanol in SRBO compared to other techniques, was not significantly different.

Shelf life of NSRBO and SRBO were determined by Q-10 method based on the FFA content lower than 5% at 25°C and 35°C for 8 weeks. The shelf life of SRBO obtained by the IR, IR-VC, IR-HA, and RF treatments were 16.8, 27.4, 13.4, 39.2

days, respectively, while that of NSRBO was 1.7 days when storage at 30°C. The FFA formation rate was estimated by the zero-order kinetic model and it was defined as %FFA/day. The lowest FFA formation rate at 25°C was found in the SRBO by the RF treatment (0.033%/day), while the highest was discovered in the IR-HA treatment (0.075%/day).

In conclusion, RF was the most effective stabilization process to inhibit rancidity in RB and prolong the shelf life of RBO but it was not effective in terms of oil extraction improvement and bioactive compounds preservation. On the other hand, the IR-VC stabilization method significantly improved the RBO extraction yield and preserved the tocols and oryzanol compared to other stabilization techniques.



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CHAPTER I

INTRODUCTION

Background

Thailand is one of the world's largest rice producers and exporters. The approximate production has been currently forecast by the United States Department of Agriculture (USDA) at 18.6 million metric tons (milled basis), and approximately 1.49 million tons of rice bran (RB) are produced in Thailand each year (USDA Foreign Agricultural Services, 2017). RB is the most important rice by-product of the rice milling process, which is the conversion of brown rice to white rice, and it contains various bioactive compounds that impart beneficial effects on human and animal health. RB is particularly rich in dietary fiber and essential fatty acids. It also contains significant quantities of starch, protein, vitamins, dietary minerals and phytic acid, which is an anti-nutrient that prevents nutrient absorption (Kahlon, 2009). More importantly, rice bran oil (RBO) fractionation contains several important biochemical compounds including tocopherols, tocotrienols, oryzanol, and phytosterol (Revilla et al., 2009).

The most important issue for the development of raw RB is rancidity. The causes of rancidity and hydrolytic reactions (hydrolytic rancidity) are lipase enzyme and oxidative reaction with a lipoxygenase enzyme, a catalyst causing an odor and unpleasant taste (Malekian et al., 2000). The rancidity seriously reduces the quality of the RB, especially for RBO manufacturers. Therefore, an appropriate stabilization method of the RB after the milling process is the major key to preserving and prolonging the quality and shelf life of fresh RB by inhibiting enzyme activities that cause rancidity and microbial growth (Ju, & Vali, 2005). According to the literature, lipase and lipoxygenase can be inactivated by chemical, physical and heat treatments. Moreover, RB could be stabilized by various methods including hot air drying, steam, (Juliano, 1985), microwave heating (MH) (Lakkakula et al., 2004; Zigoneanu et al., 2008), extrusion (Kim et al., 1987), ohmic heating, (Loypimai et al., 2009), pH adjustment (Amarasinghe et al., 2009), vapor ethanol treatment (Kumar

et al., 2006), and subcritical water conditions (Pourali et al., 2009). The efficiency of the different methods on the quality of stabilized rice bran (SRB) could be varied. However, many stabilization techniques are not appropriate or not practical for industrial production. Hence, this is a technological challenge to find new techniques for the stabilization of RB that could be applied on an industrial scale while maintaining the quality and nutritional values of the RB.

This study aimed to investigate novel technology using infrared (IR) and radio frequency (RF) to stabilize RB. An important point was that the IR could be found in the air, absorbed by food, and transferred heat to food. Therefore, the surrounding air in the oven chamber of the IR process was not heated (Sakai, & Mao, 2006). The heating process with the IR proved extremely successful with a high-quality product and could maintain the colors and nutrition of the product after drying. Additionally, the heating of the IR could cause an inactivation of chemical changes, destroy enzymes, and inhibit the microorganisms' growth in RB, thus providing efficient heat to maintain any nutrients, especially biochemicals; such as, tocopherol, tocotrienol, oryzanol, etc. (Yılmaz, 2016). Hence, equipment was designed to have compact, functional automation, be easily controlled, and safe (Sakai, & Mao, 2006). This reduced the time, energy consumption, and low capital cost (Yılmaz, 2016). In addition, the RF energy generated the volumetric heating rapidly within the products by the combined effects of the polarization mechanisms of dipole rotation and ionic conduction (Piyasena et al., 2003). The penetration depth of the RF heating was deeper than that of microwave treatments because of its longer wavelength. As such, RF could be a promising technology for food applications because of the associated rapid and uniform heat distribution, large penetration depth, and lower energy consumption (Nelson, & Trabelsi, 2012). The RF treatment for stabilization could inactivate chemical changes, inhibit microorganisms in RB growth, increase the percentage of the yield of RBO by using a cold-press extraction method, maintain the color of RBO and appear to be inferior, as well as create a slight increase in the lipase and lipoxygenase activity in RB (Vearasilp et al., 2015).

As a consequence, the combination of hot air or vacuum conditions with IR was investigated. The optimization of the RB stabilization conditions by both the IR and RF techniques using the central composite design (CCD) were studied. The qualities

of the SRB in comparison with those of fresh RB (control) were investigated in terms of color, a_w, proximate analysis, free fatty acid (FFA), fatty acid composition, and bioactive compounds. The stability of the RB samples was monitored and evaluated for a period of 8 weeks (approximately 60 days). The bioactive compounds remaining in the samples were also determined during storage. The effects of the IR and RF on the extraction yield, qualities of crude RBO, shelf life of RBO, and storage stability of RBO were investigated.

Hypothesis

Heat treatment could inactivate the residual lipase, lipoxygenase and microorganisms; such as, fungi, which were capable of producing lipase inducing the deterioration of the RB and RBO. The combination of the hot air or vacuum conditions with the IR improved the enzyme inhibitory, microorganisms, and maintained the nutrients. In addition, RF was a high efficiency heat treatment used to stabilize the RB due to its rapid heating and high penetration depth. The RB associated with the early and uniform heat distribution from the RF could effectively destroy the lipase and microorganisms. Additionally, the IR and RF could prolong the shelf life of the RB through a lipase activity for inactivation; therefore, the RB could be stored with less than 5% of FFA over 8 weeks. Throughout this period, the FFA, as indicated by the lipase activity and microorganism, should be stable with low MC and low a_w conditions. The color values (L*, a*, b*, and ΔE) of the SRB by the IR and RF would have slight changes. Furthermore, the IR and RF processes might have an inhibitory effect on the browning reaction and preserve bioactive compounds; such as, tocopherol, tocotrienol, and oryzanol. The IR and RF methods increased the crude RBO extraction yield and also maintained the qualities of the crude RBO. The IR and RF could prolong the shelf life of the RBO through the lipase activity for inactivation; therefore, the RBO could be stored with less than 5% of FFA over 8 weeks.

Objectives

1. To optimize the RB stabilization conditions using the IR and RF techniques by RSM and CCD,

2. To investigate the effects of the IR, IR-VC, IR-HA and RF on the physico-chemical properties, stability (FFA, MC, a_w, color, proximate, microbial, and fatty acid composition), bioactive compounds (γ-tocotrienol, δ-tocopherol, and γ-oryzanol), and shelf life of the RB during storage,

3. To investigate the effects of IR and RF on the extraction yield, physico-chemical properties, stability (AV, PV, FFA, MC, a_w, and fatty acid composition), bioactive compounds (γ-tocotrienol, δ-tocopherol, and γ-oryzanol), and shelf life of RBO during storage.

CHAPTER II

LITERATURE REVIEW

Rice bran

The topics of RB include: production of RB, composition of RB, and utilization of RB an as follow:

1. Production of RB

Rice (*Oryza sativa* L.) is an important cereal crop and a staple food for more than half of the world's population (Wani et al., 2012). The global rice production in the year 2010 stood at 696,324,394 tons (FAOSTAT, 2012). Its importance to the world population's dietary requirements is evident from the presence of rice in the diet of a quarter of world's population. Rice is an important energy source with versatile functional properties. It is the major staple food in Thailand and countries in Asia and it is also the main export product of Thailand. The grain production is estimated at 20 million tons per year in Thailand and the milling process creates approximately 7 to 8.5% of weight of a paddy which creates about 1.6 million tons of RB. RB is mainly utilized as a food ingredient and it is also an important source of protein, fiber, essential vitamins, unsaturated fatty acids, minerals and phenolic compounds (Kahlon, 2009). RB from the rice grains undergoes several processing steps before being consumed as food. Rice processing involves various steps like cleaning, hulling and post hulling processing (whitening, polishing and grading). Milling of paddy yields 70% of rice (endosperm) as the major product and byproducts consisting of 20% husk, 8% bran and 2% germ (Van Hoed et al., 2006). RB a by-product of rice milling is obtained from an outer layer of the brown (husked) rice kernel during milling. Of the total weight of rough RB constitutes around 10% (Hu et al., 1996).

Malekian et al. (2000) investigated and reported the proportion of rice and RB by-product of rice milling and it constitutes nearly 7%-8.5% of the total grain. The product fractions from standard milling of rice is shown in Figure 1.

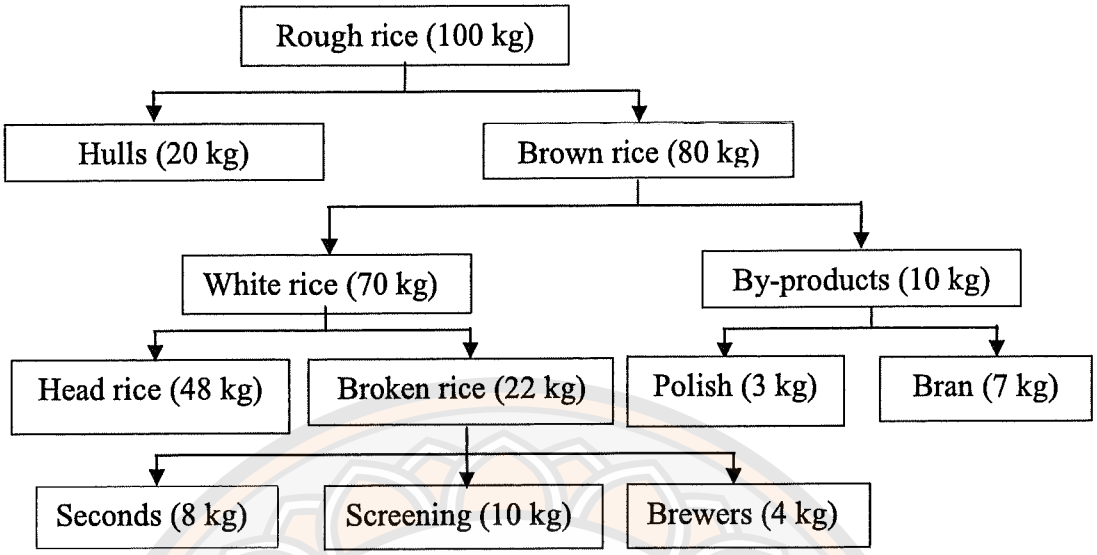


Figure 1 Product fractions from standard milling of rice

Source: Malekian et al., 2000

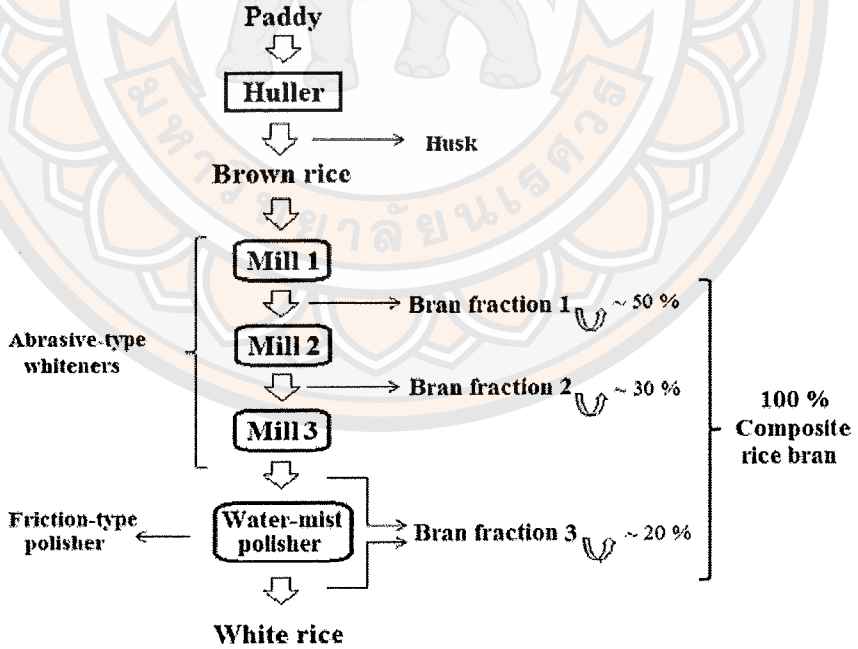


Figure 2 Schematic diagram of rice milling

Source: Yılmaz, 2016

Yılmaz (2016) presented RB from the multi-break milling system which consists of 3 abrasive-type mills (Mill 1, 2 and 3) and a friction-type water-mist polisher as shown in Fig. 2. The white rice is produced from brown rice by removing the bran and germ. The whitening process applies pressure to the rice grain which generates heat, causes cracking and results in low head rice yield. Therefore, brown rice is passed through 2–4 whitening machines, which is the so called multi-break systems, connected in series to avoid overheating. Typically, there are 2 types of whitening machines; abrasive type and friction type. The abrasive-type milling, rice kernel pass between a moving abrasive surface and stationary screen while in friction-type machines, kernels are forced against each other. The friction-type whitener applies more pressure to the grain compared to abrasive-type whiteners and are less preferred. The number, type and the order of location of these machines varies depending on the kernel variety, shape, and the required rice specifications. One of the most common whitening systems was performed. Usually, the whitening process ends up with a polishing step, which provides a shiny, smooth and dust-free final product and also cools the rice. During the polishing step, rice grains are gently polished by friction generally in a humidified atmosphere. Finally, the composite RB is produced with different mills and under current practices, these bran fractions are combined and the total mixture is named as RB. Food Network Solution (n.d.) is divided the level of milled into four levels including 1) extra well milled is milled RB removed until all the grains are extraordinarily white, 2) well milled is the bran removed all the grain looks white, 3) reasonably well milled is RB removed as much grain look white enough, and 4) ordinarily milled is RB removed partially.

Composition of RB

The composition of RB varies with the rice type, climatic, condition and rice processing method. The chemical profiles of RB differ with respect to rice variety. RB is a mixture of RB (brown layer) and germ, these by-product are produced during the milling process in the production of white rice from brown rice. It is a good source of proteins, minerals, fatty acids and dietary fiber (Gul et al., 2015). In addition totocotrienols, fiber and γ-oryzanol, the phenolic acid fraction of RB may also bebeneficial for the treatment of type 2 diabetes mellitus because it regulates blood

glucose level by elevating glucokinase activity and the production of glucokinase in the liver (Jung et al., 2007). RB contains 5–8% of minerals, including iron, phosphorus, and magnesium. Furthermore, it has 11-13% crude protein, approximately 11.5% of fibers and a good amount of oil because it may contain 20% of its weight in oil. The estimated chemical properties of RB were 12.12 % moisture, 12.32% protein, 20.31% fat, 8.73% ash, 17.92% digestible carbohydrate, 28.60% dietary fiber, pH 6.85 and color with L* a* b* are 68.85, 3.49 and 18.07 respectively (Gul et al, 2015). RB has high protein content especially essential amino acids such as lysine and high protein efficiency ratio (PER) by a value of 1.6 to 1.9, compared with casine 2.5 (Saunder, 1985). RB contains important carbohydrates such as 8.7-11.4% hemicellulose, 9-12.8% cellulose, 5% powder and 1% beta-glucan. In addition, RB has 15-23% of fat by a fatty acid, including 12-18% of palmitic acid, 40-50% of oleic acid, 30-35% of linoleic acid, and about 90% FFA of the total fatty acids in the RB. Furthermore, 8-10% of fiber, it is also a good source of vitamins B, minerals such as iron, potassium, calcium, chlorine, magnesium, and manganese, etc. Recent US Department of Agriculture (USDA) reports the results of the study shown that RB can lower blood cholesterol and reduce the risk of heart disease, moreover they found that RB, oat bran, costs less and tastes better than oat bran.

Crude RB fractions were analyzed for their proximate composition by Yılmaz (2016) to understand the basic differences among them. The proximate composition of rice milling fractions from table 1, which shown constituents consist of MC, crude protein, and soluble dietary fiber, which doesn't have the difference ($p > 0.05$) but crude fat, ash, phytate, insoluble dietary fiber and total dietary fiber were different in the difference fraction. The first fraction of milling contains crude fat, ash, phytate, insoluble dietary fiber and total dietary fiber more than 2 or 3 times the fraction step. But in reality, all bran step should be released and kept together to collect in store.

Table 1 Proximate composition of rice milling fractions

Constituents	Bran fraction 1	Bran fraction 2	Bran fraction 3
Moisture (%)	10.23±0.14^{ns}	10.46±0.08^{ns}	10.31±0.01^{ns}
Crude protein (%)	15.50±0.27^{a}	16.08±0.01^{a}	16.48±0.07^{a}
Crude fat (%)	21.74±0.29^{a}	20.14±0.37^{b}	18.15±0.13^{c}
Ash (%)	12.19±0.18^{a}	11.23±0.01^{b}	10.02±0.03^{c}
Phytate (mg/g)	45.84±0.11^{a}	45.64±0.13^{a}	42.47±0.06^{b}
Soluble dietary fiber (%)	6.53±0.25^{ns}	6.45±1.43^{ns}	4.93±1.44^{ns}
Insoluber dietary fiber (%)	35.66±0.29^{a}	20.70±0.80^{b}	11.13±1.09^{c}
Total dietary fiber (%)	42.19±0.55^{a}	27.15±0.62^{b}	16.06±2.53^{c}

Note: Means followed by different letters within each row are significantly different ($p < 0.05$)

Source: Yılmaz, 2016

Moongngarm et al. (2012) found proximate composition of crude RB fractions from *Khao dok mali* 105 (Thailand cultivar), these results is shown on table 2, which noted the significant highest δ-tocopherol and γ-tocopherol content (%dry weight) in *Khao dok mali* 105 (non-waxy rice) were in germ but γ-oryzanol content significant highest in bran layer. Similar, Faria et al. (2012) reported the nutritional composition of RB submitted to different stabilization procedures (WRB: whole rice bran; MRB: whole RB treated in microwave oven; RRB: whole RB roasted on conventional stove) are show on table 3.

Table 2 Chemical compositions and phytochemical content of bran layer, RB, and germ (%dry weight) in *Khao dok mali* 105 (non-waxy rice)

Compositions	Bran layer	RB	Germ
Yield (% of whole grain)	14.89±0.47^{b}	17.64±0.72^{a}	2.81±0.11^{c}
Color	Light brown	Light brown	Light brown
Fat	12.45±0.23^{c}	18.80±0.51^{b}	21.58±0.92^{a}
Protein	10.90±0.09^{c}	13.66±0.14^{b}	15.27±0.32^{a}
Carbohydrate	45.31±1.00^{a}	40.63±0.08^{b}	30.96±0.61^{c}
Fiber	13.51±2.08	12.48±0.12	9.52±1.73
Ash	12.18±0.10^{a}	10.65±0.28^{b}	6.94±0.05^{c}
Phenolic compounds (mg/g)	1.64±0.11^{a}	1.57±0.07^{a}	0.40±0.04^{b}
Phytic acid (mg/g)	63.88±0.34^{a}	50.68±0.86^{b}	37.92±0.37^{c}
Gamma-oryzanol (mg/g)	5.50±0.08^{a}	3.50±0.03^{b}	1.75±0.01^{c}
Sigma-tocopherol (mg/g)	32.77±0.75^{c}	46.12±1.42^{b}	62.52±2.02^{a}
Gamma-tocopherol (mg/g)	46.63±1.52^{c}	40.94±1.82^{b}	51.59±1.66^{a}

Note: Means followed by different letters within each row are significantly different ($P < 0.05$)

Source: Moongngarm et al., 2012

Table 3 Nutritional composition of RB samples subjected to two different heat stabilization procedures

Components (in 100 g)	**Bran type (dry weight basis)**		
	WRB	**MRB**	**RRB**
Moisture (g)	8.41±0.2^{a}	6.28±0.10^{Ab}	5.14±0.10^{Bb}
Mineral Fraction (g)	8.13±0.04^{a}	6.98±0.04^{Bb}	8.52±0.02^{Aa}
Total lipid (g)	17.87±0.30^{b}	20.05±0.40^{Aa}	18.34±0.10^{Ab}
Proteins (Gn x 5.95)	16.61±0.20^{b}	19.38±0.30^{Aa}	18.93±0.20^{Ab}
Insoluble fiber (g)	22.67±0.41^{b}	0.74±0.24^{Ab}	0.11±0.07^{Bb}
Soluble fiber (g)	1.48±0.02^{a}	0.74±0.24^{Ab}	0.11±0.01^{Bb}

Table 3 (cont.)

Components (in 100 g)	Bran type (dry weight basis)		
	WRB	**MRB**	**RRB**
Total dietary fiber (g)	24.15^{b}	25.38^{Aa}	20.45^{Bc}
Available Carbohydrate (g)	33.24^{b}	28.21^{Aa}	33.76^{Cb}
Energy	423.19^{b}	447.21^{Aa}	437.06^{Ba}
Calcium (ug/g)	438 ± 41^{b}	733 ± 186^{Aa}	n.d.
Iron (ug/g)	94 ± 0.0^{b}	115 ± 7^{Aa}	115 ± 7^{Ab}
Sodium (ug/g)	17 ± 2^{a}	12 ± 1^{Bb}	15 ± 2^{Ab}
Zinc (ug/g)	72 ± 5^{b}	140 ± 10^{Aa}	84 ± 7^{Ba}
Potassium (ug/g)	11.293 ± 0.960^{b}	5.418 ± 0.305^{Bc}	15.824 ± 0.355^{Aa}
16:0 palmitic acid (g)	2.73 ± 0.34^{b}	2.85 ± 0.01^{Ab}	2.46 ± 0.01^{Cb}
18:0 steric acid (g)	0.37 ± 0.02^{b}	0.51 ± 0.00^{Aa}	0.34 ± 0.00^{Cb}
18:1 oleic acid (g)	6.86 ± 0.03^{b}	8.14 ± 0.03^{Aa}	6.91 ± 0.02^{Bb}
18.2 linoleic acid (g)	6.35 ± 0.29^{b}	6.85 ± 0.03^{Aa}	6.84 ± 0.02^{Aa}
18:3 alpha-linoleic acid (g)	0.26 ± 0.03^{b}	0.20 ± 0.00^{Cb}	0.27 ± 0.00^{Aa}

Note: *results in triplicate, and dietary fiber in quadruplicate, for each repetition.

** WRB: whole RB; MRB: whole RB treated in microwave oven; RRB: whole RB roasted on conventional stove.

***Calculated by difference: total carbohydrate (100 g – total in g proteins, lipids and mineral fraction) – total dietary fiber. Each value represents the mean and ± standard deviation of triplicates of three repetitions; Comparison between the untreated and the stabilized samples (microwave oven and conventional stove) was performed by ANOVA followed by Tukey's test or by Kruskal-Wallis test using the software STATISTICA (2007).

****Values followed by different lower case letters on same line indicate statistically significant difference between treated samples and control ($p<0.05$); Values followed by different upper case letters on same line indicate statistically significant difference between treated samples ($p<0.05$). n.d = not detected

Source: Faria et al., 2012

Chemical characteristics and functional properties of RB are presented in table 4. RB is potentially a valuable source of natural bioactive compounds such as antioxidants, including anthocyanins, flavonoids, polymeric carbohydrates, phenolic acid, cinnamic acids, and steroidal compounds. Full-fat RBO includes an array of bioactive phytochemicals such as oryzanol, phytosterols, and tocotrienols. The proportion of these phytochemicals varies with the type of rice cultivar (Iqbal et al., 2005).

Revilla et al. (2009) found that the use of biochemicals from extracting from RB using enzymes were tocopherols, tocotrienols, oryzanol, and phytosterol. There was reduced cholesterol both in human and in vivo studies. The health benefit biochemicals in RB are shown in table 5.

Table 4 Qualitative of bioactive compounds present in RB

Anthocyanins and Flavonoids	Polymeric carbohydrates	Phenolic and Cinnamic acids	Steroidal compounds
Anthocyanin monomers,	Arabinoxylans	Caffeic acid	Acetylated steyrl
dimmers and polymers	Glucans	Coumaric acid	glucosides
Apigenin	Hemicellulose	Catechins	Cycloartenol ferulate
Cyaniding glucoside		Ferulic acid	Campesterol ferulate
Cyanidin rutinoside		Gallic acid	24-
Epicatechins		Hydroxybenzoic acid	methylenecycloartenol
Hesperetins		Methoxycinnamic acid	ferulate
Isohamnetins		Sinapic acid	γ-oryzanol
Luteolin		Syringic acid	β-sistosterol ferulate
Peonidin glucoside		Vanillic acid	Tocopherol
Tricin			Tocotrienol

Source: Iqbal et al., 2005

Table 5 The health benefits biochemical in RB

phytosterols	(mg/kg)	Tocopherols	(mg/kg)	Tocotroenols	(mg/kg)	γ-Oyzanol
Campesterol	648±23	α-Tocopherol	44±3	α-Tocotrienol	25±2	1260±50
Stigmasterol	414±29	ß-Tocopherol	10±2	ß -Tocotrienol	-	-
ß- Stitosterol	1518±98	γ -Tocopherol	38±4	γ -Tocotrienol	62±4	-
δ-Avenasterol	115±9	α-Tocopherol	7±1	δ-Tocotrienol	87±5	-
δ-Avenasterol	845±63	-	-	-	-	-
δ-Avenasterol	524±50	-	-	-	-	-
Total	4084±98	-	99±7	-	174±10	1260±50

Source: Revilla et al., 2009

The health benefit biochemicals in RB are founded phytosterols, tocopherols, tocotrienols, and γ-oryzanol with the ability to reduce cholesterol in the blood and antioxidant. These compounds in RBO depend on many factors, such as extraction method, stabilization method, stored method, and purification method. Nevertheless, these pure substances may be added into RBO to enhance the benefits of them. Nutritional studies in animals and humans have shown cholesterol-lower potential for RB and RB fractions. Among compounds whose hypocholesterolemic activity has been demonstrated in animal and/or human subjects are rice waxes, oryzanol (ferulic acid esters of triterpene alcohols), hemicelluloses, neutral detergent fiber fractions, proteins, and oil components. The products of fractions from standard milling of RB acids such as oleic, linoleic, and linolenic acid, are presented in RBO for lowered LDL-cholesterol when replacing saturated fat (Malekian et al., 2000).

Utilization of RB

Each year, 90% of the RB produced in the world is utilized cheaply as a feedstock for cattle and poultry. The remainder is used for extraction of RBO (Schramm et al, 2007). Fractionation of RB is an important process in industrial processing and involves the conversion of RB into various parts containing more of desirable components than undesirable ones. Fractionation of RB allows for the

selective use of portions of the RB and is advantageous for that less rice bran needs to be processed for obtaining components of interest (Schramm et al., 2007). The increasing demand for RB and RBO as healthy functional food ingredients has led to the emergence of defatted RB as a potential by-product obtained as RB meal after the extraction of oil from RB (Chan et al., 2013).

Defatted RB contains important nutrients including high- quality proteins and nutraceutical properties (Saunders, 1990). The biochemical compounds extracted from RB also contains phytochemicals with potent antioxidant activities such as tocopherol, tocotrienols, and γ-oryzanol (Akihisa et al., 2000). RBO is an excellent source of nutritionally beneficial compounds and chemo-preventive properties. RBO also contains a range of fats, with 47% of its fats monounsaturated, 33% polyunsaturated, and 20% saturated. A major RB fraction contains 12–13% oil and highly unsaponifiable components (4.3%), this fraction contains tocotrienols, γ -oryzanol, and β-sitosterol. RBO has been reported to have hypolipidemic, antiatherogenic, and antidiabetic properties (Chen and Cheng, 2006).

RB is the richest source of oryzanol and owing to its beneficial effects on human health has generated global interest in developing facile methods for its separation. Degumming has been reported to remove 1.1% while dewaxing has been reported to remove 5.9% of oryzanol (Zullaikah et al., 2009). However, alkali treatment removes 93–94.6% oryzanol from crude RBO (Van Hoed et al., 2006). Other methods for isolation of oryzanol from crude RBO include a combination of methods such as solid-liquid extraction (leaching) and crystallization or liquid-liquid extraction (Zullaikah et al., 2009).

Nutraceuticals including phytochemicals are perceived as offering opportunities for improving human health (Camire et al., 2003; Gul et al., 2015). Natural products obtained from plants have been used for the prevention and treatment of diseases in humans and animals (Bagchi, 2006). Phytochemicals have been interested in researchers in the recent past because of their potential to counter various diseases. RB contains phytochemicals with promising health benefits (Jariwalla, 2001). Some of the important bioactive components presented in RB are presented including anthocyanins and flavonoids, polymeric carbohydrates, phenolic and cinnamic acids, and steroidal compounds, these also rich in natural antioxidants for a

role in reducing the risk of chronic diseases (Thanonkaewa et al., 2012). Moreover, phytosterols occur in RB commonly known as oryzanol which various studies indicate that rice bran is rich in γ-oryzanol. Oryzanol is a mixture of ferulic acid esters of triterpene alcohols and sterols. It has several biological and physiological effects, such as serving as an antioxidant (Dave, & White, 1991) and anti-blood cholesterol-lowering agent (Seetharamaiah, & Chandrasekhara, 1989). Antioxidant activities reported the oryzanol and some vitamin in RB protect cells from the oxidative damage of plasma very-low-density lipoprotein, cellular proteins, and DNA (Xu et al., 2001). Oryzanol has been shown to inhibit tumor promotion and reported to diversion health effects including hypolipidemic effect, growth promotion, and stimulation of the hypothalamus (Seetharamaiah, & Chandrasekhara, 1989). In addition, oryzanol has been shown to inhibit tumor promotion inhibit tumor growth in tumor-bearing mice by the induction of natural killer (NK) activity, inactivation of macrophages, and inhibition of angiogenesis (Kim et al., 2012). There are effective in reduction of serum cholesterol levels (Sugano, & Tsuji, 1997). It also can be used to treat nerve imbalance and menopausal disorders (Nakayama et al., 1987). RB protein is uniquely nutritional value and nutraceutical properties (Saunders, 1990). It is a hypoallergenic food ingredient (Helm, & Burks, 1996) and anticancer activity agent (Kawamura et al., 1993). RB tocotrienol is a function to improve the utilization of whole-body glucose and insulin sensitivity in diabetic mice (Fang et al., 2010).

The high fiber content in RB can also slow down the absorption of glucose in the human digestive systems. There is lowering blood sugar levels (Jenkins, & Jenkins, 1985). Antioxidants can help in slowing the onset of diabetes, Alzheimer's disease, and play a role in the prevention of coronary heart diseases and cancer (Adom, & Liu, 2002). Tocotrienols have been shown to address free radicals in cell membranes and help in the prevention of coronary artery disease. Furthermore, oryzanol has been shown to lower blood cholesterol and reduce levels of cholesterol in the liver. In addition, tocotrienols, fiber, and γ-oryzanol, the phenolic acid fraction of RB may be beneficial for the treatment of type 2 diabetes mellitus because it regulates blood glucose level by elevating glucokinase activity and the production of glucokinase in the liver (Jung et al., 2007). Various studies have shown that antioxidants reduce oxidative damage to biomolecular structures that play a role in the

prevention of chronic diseases (Rohrer, & Siebenmorgen, 2004). RB is gaining importance commercially due to the beneficial nutritive and biological effects.

Previous numbers of studies have been carried out to evaluate RB as a functional ingredient in various foods to improve nutritional quality. RB has high dietary fiber and has therapeutic potential, in addition, it can contribute to the development of value-added foods or functional foods that currently are in high demand. RB has great potential in the food industry, especially in the development of functional foods such as functional bakery products, bread, cookies, pizza, beverages, tuna oil, milk powder, and ground beef, etc, for functional and nutritional purposes. Enrichment of bakery products with RB and its effects are shown in table 6.

Table 6 Enrichment of bakery products with RB and its effects

Product enriched	Purpose of addition	Inference	Reference
Pizza with high content dietary fiber SRB flour	Effect of chemical and functional properties of storage frozen pizza	Pizza dough with enrichment level of 5% was stable during 60 days at -18°C	De Delahaye et al. (2005)
Gluten-free bread with dietary fiber fraction	Quality improvement	Acceptable structure and textural quality and sensory acceptance increased and shelf life extended	Phimoisiripol, Mukprasert and Schoenlechmer (2012)
Pan bread with defatted rice bran	Enrichment with fiber and minerals	- Quality improved (high dough yield, attractive crumb and crust) by 5% addition of DRB - High fiber and high mineral contents reported	Ajmal et al. (2006)
Bread with full fat and defatted rice bran	Effect on functional properties	- Loaf volume increased with full fat and decreased with defatted rice bran - No significant effect on texture	Lima, Guaraya, and Champagne (2002)

Table 6 (Cont.)

Product enriched	Purpose of addition	Inference	Reference
Cookies (Wheat flour with rice bran)	Effect on proteins	Increased levels of lysine and dietary fiber	Sharma and Chauhan (2002)
Banana muffins and peanut butter cookies with defatted rice bran and oil	To replace margarine and flour	- Rice bran fraction can be used to produce low fat high fiber products - Tenderness increased with rice bran fractions and decreased with rice bran fraction	Kennedy et al. (1996)
Bread with rice bran hemicellulose	Effect on chemical and functional properties	- Enrichment resulted in higher water binding and swelling capacity - Significantly reduced loaf volume and increased firmness of breads	Hu et al. (2009)
Cookies	Fiber and mineral enrichment	- Supplementation improved dietary fiber content and mineral profile - Defatted rice bran can be substituted up to20% in wheat flour	Sharif, Butt, Anjum and Na-waz (2009)

Table 6 (Cont.)

Product enriched	Purpose of addition	Inference	Reference
Pasta	- Effect of enrichment on the color, cooking, sensory quality and shelf life of enriched pasta - Effect on textural and antioxidant properties	- Pasta with added rice brans had higher dietary fiber and protein contents - Rice bran up to 15% level did not affect the physico-chemical, cooking and sensory quality of pasta. - Noodles with added rice bran had lower cohesiveness and higher polyphenols, flavonoids and anthocyanins and higher antioxidant activity. - Pasta supplemented with rice bran was highly acceptable up to 4 months of storage	Kaur, Sharma, Nagi, and Dar (2012) Kong et al. (2012)

Source: Gul et al., 2015

The color, flavor, protein extractability, solubility, water, and fat absorption, emulsifying and foaming capacity of RB property have demonstrated improvements that further enlighten us on the potential use of RB in foodstuffs (Barber et al., 1981). The physiological benefits provided by RB as a high fiber food can give texture, gelling, thickening, emulsifying, and stabilizing properties to certain foods (Dreher, 1987). Foods coated with SRB absorb less fat during frying while the small amount of fat found naturally in RB. The fiber can act as a carrier for flavors (Hammond, 1994).

Fermented RB contains many essential amino acids for humans which use as a new nutritional food adjunct (Kim et al., 2001). RB contains about 10–15% of high-quality proteins (Fabian, & Ju, 2011). A protein for mutation-based on RB can help overcome protein-related nourishment disorders. Additionally, the unique hypoallergenic

and anticancer properties make RB proteins superior to cereal proteins (Fabian, & Ju, 2011). However, the development of RB as a medicinal food or dietary supplement is yet in its infancy.

RB has a high content of proteins, minerals, fiber, and fatty acids. It can serve as an important raw material for the development of nutraceuticals and functional foods including bread, corn flakes, ice cream, pasta, noodles, flake products, and zero-trans-fat shortening. RB health benefits associated with the consumption of RB, detailed in vivo studies are recommended to create a database. Furthermore, the comparative analysis of the shelf life achieved by stabilization with various techniques is an interesting area of research. There are predictions regarding the best procedure for stabilization to enhance the supplementation of RB in various food systems.

Deterioration of RB

RB has great potential to be a supplementary source of many nutrients. The most important issue for the exploitation of raw RB is the rancidity from fat in RB. It also limits the use of RB as food and animal feed. The instability in the RB rancidity due to the reaction of hydrolytic rancidity and oxidative rancidity (Figure 3). Usually, the fat in paddy and brown rice are relatively high stable. Lipase in grain still in the cells of the seed coat (tegmen) while fat is mostly stored in the aleurone layer and germ which two different location. The milling process is making lipase causing rancidity because it breaking down cell membranes kernels of rice and the lipase contacted to fat in rice. The milling process is caused by the degradation of fats occurs fatty acid and antioxidant in RB lipases such as phospholipases and esterases (Takano, 1993). Lipase in RB molecular mass of 40 kDa (kDa) has the pH optimum working at 7.5 to 8.0 and temperature of 37°C. Lipase will split the ester bond in position 1,3 of fatty acids (FAs) (Aizono et al., 1971).

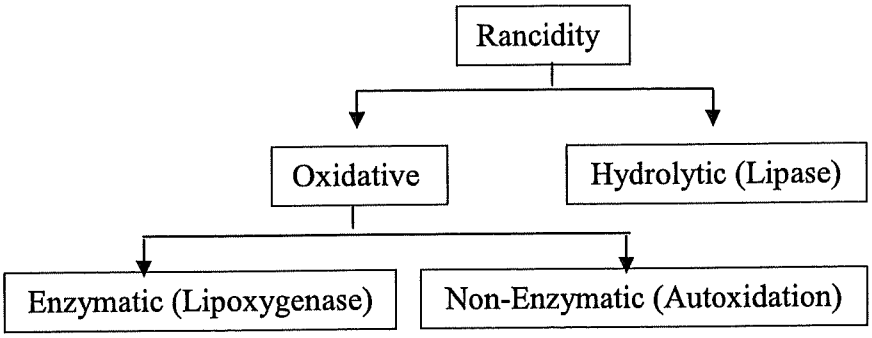


Figure 3 Types of rancidity

Source: Malekian et al., 2000

A major component of cell membranes spherosome was biodegradable, phosphatidic acid by phospholipase D and spherosomes to melt away then triglycerols. There has been located in the membrane will flow out and be with enzymes. Lipase is decay-causing triglyceride to FFA. RB an contains 12-23% crude fat, which depends on source, size of a grain, manufacturer and variety of rice. The hydrolytic rancidity and oxidative rancidity on RB have occurred immediately after the milling process. The rapid deterioration of fat in raw RB by lipase is faster than the oxidative rancidity process by lipoxygenase. Both reactions are still introduced to consumers. The nature of the lipase is hydrolyzed triglycerides in the RB. It is also the primary fat in RB and occurs FFA. The FFA content in rice bran makes higher acidity with lower pH and occurs new substances. This reaction will affect as well as change the properties of RB such as color and taste, etc. The reacting of hydrolytic rancidity will occur more or less depending on the type of lipase in RB, which contains several species and certain types of specific triglycerides in the RB and has the ability to separate bonding one or three molecules of triglyceride well. The kind of lipase, the reaction rancid also depends on the storage conditions, packaging and type of packaging methods which will help slow the deterioration of the lipase (Takano, 1993).

The reactions of rancidity from oxidative rancidity will be the reaction of oxygen with unsaturated Fas associated with free radicals begin (the radical may be

obtained from the decomposition of carbon bonds in substances such as aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactone (Frankel, 1982) with violent reactions and reactions continuous chain causes hydrogen an oxidant (H•) resulting free radicals, unstable fat (L•) reacts with oxygen to form a free radical peroxy (LOO•) in this process, free radicals (LOO•) unsaturated FAs react more in the form of hydroperoxide in fat (LOOH) (Fig. 4). These reactions are a similar auto-oxidation reaction. However, the reaction of lipoxygenase can act more rapidly than naturally auto-oxidation reaction and be more specific in terms of the final product. The radical may be obtained from the decomposition of carbon bonds in substances such as aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactone (Frankel, 1982).

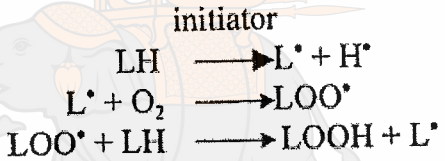


Figure 4 Reaction of oxidation FAs in RB

Source: Malekian et al., 2000

Deterioration of RB caused by oxidative rancidity involves the oxidation of fat in the RB and oxygen molecules. The reaction in the double bonds of unsaturated FAs is catalyzed by oxygen, free radicals and metal ions, such as iron, copper, cobalt ions or light radiation and lipoxygenase (Barnes, & Galliard, 1991). These also depend on the composition of FAs (Nawar, 1985). Lipoxygenase is a general enzyme and found in many plants especially legumes and grains such as rye, wheat, oats, barley, and corn, etc. (Tappel, 1963). Lipoxygenase unlike lipase which is will be accelerated by water inthe product (Barnes, & Galliard, 1991). Lipoxygenase is high effectively in the presence of oxygen and especially the unsaturated FAs and has many double bonds. The double bonds of esters and acetic acid with glycerol unsaturated FAs contain cis-cis 1,4, pentadiene at a double bond in the carbon 6-10 from methyl last (Shastry, & Rao, 1975). There are also changes in taste and smell in food. The reaction

with unsaturated FAs is lipoxygenase workings this particular little-published data on the role of lipoxygenase in RB, especially during storage. Lipase and lipoxygenase in plants and animals will start working when the tissue of animals and plants damaged, cracked or torn. The enzyme in fat begin working, the FAs and FFA are saturated with oxygen to react hydrogen peroxide (Hydroperoxides). Lipoxygenase reaction can be explained in equation as follows.

$$\text{Unsaturated fatty acid} + O_2 \xrightarrow{\text{lipoxygenase}} \text{peroxide derivative of unsaturated fatty acid}$$

Lipoxygenase causes off-flavours and taste which are a significant major problem of maintaining stabilize RB and FA composition containing lipids. The inhibition of lipoxygenase from many researchers who study the inactivation of lipoxygenase by method investigate it, including additional antioxidant such as pyrocatechol, homocatechol, propylgallate, nordihydroguariaretic, resorcinol acid, phioroglucinol, hydroquinone, butylated hydroxyanisole and flavonoids etc., pH adjustment and heat treatment (O'Connor, & O'Brien, 1991).

Mostly, the precursors of rancidity RB are essential FAs including linoleic acid, linoleic acid, and arachidonic acid. These acids are deteriorated by enzymatic changes to hydroperoxides with oxygen. In addition, these are generally accepted as the main causes for the change of taste contributed to the storage of RB. The cause of the deterioration of RB by lipase and lipoxygenase are the temperature of storage and the type of packaging. The RB should be stored in sealed bags. There is in order to have a shelf life longer than at atmosphere. However, the method of keeping the RB in a vacuum environment to prevent deterioration from lipase and lipoxygenase are interesting to be used to control aerobic microbiology. Lipase and highly content fat in RB are contributed significantly to the quality of rice. The condition of storage RB in room temperature for a long period and the time of lag between production are caused the degradation of triglyceride in oil, which causes chemical flavors and odors such as al-aldehyde and ketone, etc. There are important problems for the RB industry because it affects the quality of commercial storage and shelf life of RB. In addition, certain enzymes, such as lipoxygenase and peroxidase contained in the RB are caused by the

deterioration. In addition, this is a lesser degree compared to the lipase given and may be a reaction such as a lipid peroxidation reactions by various enzymes. The lipase, lipoxygenase, and peroxidase are the main cause of degradation in RB. The main changes in rancidity, oxidation, and rancidity degradation have affected the quality of rice.

In industry, the production of RB should have FFA content no more than 5% and in RBO product to not have more than 10 % unfit for human consumption (Tao et al., 1993 as cited by Kim, S-M. et al., 2014). RB is not immediately extracted to RBO. It will be decomposed into FFA and glycerol by enzymatic hydrolysis. Thus, in the manufacture of oil-based raw materials should have a low FFA. However, due to problems caused by enzymatic hydrolysis of RB, transportation problems of RB and production facility cause delay. Therefore, it is necessary to inactivate the enzyme that causes rancidity in the RB immediately after the milling process, to maintain the shelf life of RB before production of RBO and another product. As well as to solve the problem of transporting rice to the production.

Stabilization of RB

The step of enzyme control or treatment procedures for stabilization of RB and rapidly extraction RB after milling are an effective method for the control of enzymes and control effect of the chemical reaction of rancidity in RB (Ju and Vali, 2005). The quality of RB can be destroyed by heat during an adequate time. The stabilization of RB by various methods, these have been studied extensively in several decades by physical and chemical method are followed.

1. Physical methods

A stabilization of RB by physical methods, such as drying with hot air drying, steam (Juliano, 1985), MH (Lakkakula et al., 2004; Zigoneanu et al., 2008), extrusion (Kim et al., 1987), ohmic heating (Lypamai et al, 2015), and subcritical water condition (Pourali et al., 2009). Some methods have been used to maintain stability in commercial rice. The most popular technique used to stabilize the RB commercially are hot air and MH techniques. The hot air method is an effective way to stabilizing RB but it is often difficult to stabilize and it uses prolonged and intense heat. The normal stabilization with hot air method is operated at high temperature and

long time (30-60 minutes) of processing. It can damage the valuable component in RB. The other techniques are specifically advantaged each other. The success technique must be inhibited or inactivate activity of lipase, lipoxygenase or other enzymes to effect a permanent rancidity. As a result, the % yield of RBO extraction is the highest, both in terms quality of RB by the nutritional value contained in the original RB (Orthoefer, 2005), which is also environmentally friendly. The methods of RB stabilization by physical methods are as followed.

Kim et al. (2014) studied various heat treatments on rancidity and some bioactive compounds of RB including dry-heating (DH), freeze-drying followed by dry-heating (FDDH), MH (MH), autoclaving (AC), and ethanol vapor (EV) treatment were applied to RB and their effects on the storage stability at room temperature were evaluated. At first, the color of SRB that were studied from various heat treatment is shown in table 7. The color values (L*, a* and b*) of the heat-treated RB samples found that the brightness (L*) of RB after all treatment decreased but redness (a*) and yellowness (b*) of RB after all treatment increased. Similarly, the decrease in brightness (L*) of RB with the increase in heating temperature by DH and FDDH methods has been reported. This result could be due to the formation of some products from the Maillard reaction induced by the heat treatment. The degree of color change by FDDH treatment was much lower than that by DH. It indicates that freeze-drying prior to the heat treatment was effective in retarding the browning reaction. Among the tested treatments, the maximum value of DE (10.7) was observed in RB with MH treatment for 5 min and the minimum value of DE (1.4). There was recorded in the RB treated with EV for 20 min. The microwave-heated sample (5 min) might have generated an excessive thermal energy and the browning reaction became prevalent. The ethanol vapor treatment least the color change, possibly because the vapor treatment was done at a lower temperature than that with the direct heat treatments. Moreover, ethanol vapor might have an inhibitory effect on the browning reaction. Moreover, they were studied compare FFA in the rice bran on a hot destination and retained for 24 weeks and shown that RB's FAs increased steadily from 2.14% to 19.81% as shown in Figure 5.

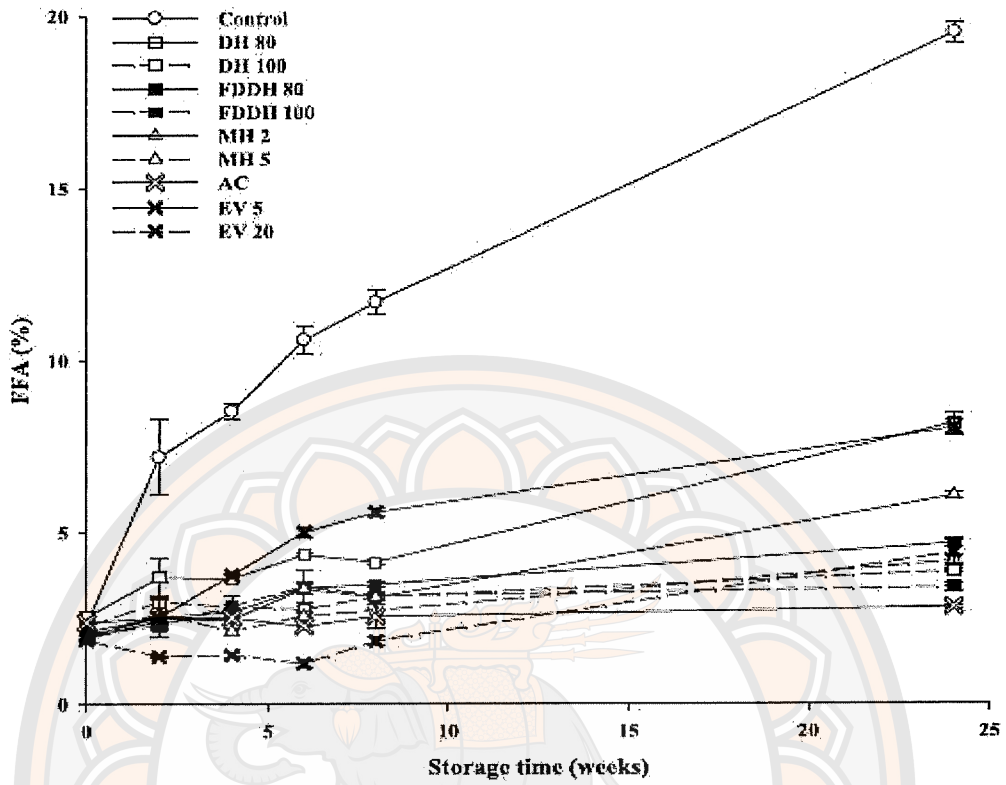


Figure 5 FFA content in the RB treated by various method

Source: Kim et al., 2014

Table 7 Color of RB treated by difference thermal processes and by EV

Treatment	Color values			
	L*	a*	b*	ΔE
Control	69.2±0.3^{a}	3.8±0.1^{g}	17.87±0.3^{h}	-
Dry heating (DH)				
80 °C	66.0±0.3^{e}	4.8±0.1^{g}	19.5±0.1^{d}	3.7±0.2^{d}
100 °C	63.4±0.1^{h}	6.5±0.1^{g}	21.0±0.2^{a}	7.1± 0.1^{b}
Freeze-drying follows by dry-heating (FDDH)				
80 °C	67.4±0.4^{cd}	4.2±0.2^{f}	18.4±0.2^{fg}	1.9±0.4^{f}
100 °C	66.8±0.4^{d}	4.5±0.2^{e}	18.7±0.1^{ef}	2.6±0.5^{e}
MH (MH)				
2 min	65.3±0.3^{f}	4.8±0.1^{d}	19.3±0.2^{d}	4.2±0.2^{d}
5 min	59.5±0.6^{i}	7.8±0.2^{a}	20.0±0.3^{c}	10.7±0.5^{a}
Autoclave (AV)	63.9±0.3^{g}	5.5±0.2^{c}	20.0±0.1^{b}	5.8±0.8^{c}
Ethanol vapor (EV)				
2 min	67.8±0.1^{bc}	4.3±0.2^{f}	18.3±0.1^{g}	1.5±0.1^{f}
5 min	68.5±0.4^{b}	4.3±0.1^{f}	18.9±0.2^{e}	1.4±0.1^{f}

Remark: *L* ,a* ,b* and ΔE represent lightness, color in the region of green to red, color in the region of blue to yellow and total color difference between the control and treated rice bran. Data represents means of three measurements (n=3) ± standard deviations. Values with different superscripts within the same column are significantly different ($p < 0.05$).

Source: Kim et al., 2014

The FFA contents in the RB treated by dry Heating (DH), freeze-Drying followed by dry heating (FDDH), microwave Heating (MH), autoclaving (AC), or ethanol vapor exposure (EV), and then stored for 24 weeks at room temperature. DH was done at either 80 °C or 100°C, and MH was done for 2 or 5 min. AC was done at 121°C for 20 min. EV exposure was done for 5 or 20 min. The FFA content in the heat-treated RB samples as compared with the control RB during the 24 weeks of storage is shown in Figure 5. The FFA content continuously increased as the storage

time increased. The increase in FFA content for the control sample was much higher than those for the treated RB samples, indicating that the treatments were effective in retarding the enzymatic degradation. RB contains not only inherent enzymes but microorganisms such as fungi which are capable of producing enzymes inducing the deterioration of RBO (Jayaramanet, & Kalyanasundaram, 1994). The dry heating might be effective in reducing the MC and more importantly in inactivating the residual enzymes and microorganisms, resulting in the retarded FFA formation during storage, with DH and FDDH method which had 5.12-6.22% and 3.06-3.40% MC, respectively. The enhanced inhibitory treatment itself might provide an additional effect on the inactivation of enzymes and microorganism when to compare with the NSRB and other types of heat treatment including MH, AC, and EV appeared as effective as the direct dry-heating. The FFA content increased to 4.15% when MH was treated for 5 min, and to 4.39% when EV was treated for 20 min. After an AC treatment (121°C, 20 min), the FFA content in RB was rarely changed during storage (2.34-2.81%), indicating that the enzymes responsible for the FFA formation had been fully inactivated. Stabilization of RB by inactivating the residual enzymes is critical for the utilization of RB for human consumption. The FFA content of the untreated RB was higher than 5% after an ambient storage for 1 week. The FFA content of 5% or lower was desirable, but the bran containing more than 5% FFA was considered not suitable for human consumption. Among the treatments tested, the FFA content in RB after storage for 24 weeks at room temperature was less than 5% when RB was treated by DH at 100°C, FDDH at 80°C or 100°C, MH for 5 min, AC, and EV for 20 min.

According to Mujahid et al. (2005) reported the roasting method was effective to SRB for 180 days of storage but it can not destroy microbial contamination (Jayaramanet et al., 1994). The SRB storage at low temperatures may be available for an extended period of time. However, the low-temperature methods of storage SRB may not be sufficient to inhibit the lipase, as can be seen from the increase of fatty acid storage period because when the optimum conditions for the enzymes activity in RB that was not destroyed, which indicated with increased FFA contents (Ramezanzadeh et al, 1999). An experimental treatment RB by using MH at 850 W for 3 minutes and maintained for 16 weeks at room temperature compared with maintained at 4-5°C. It had increased over the amount of FFA about 2.5 times from

4-5°C. At the temperature 4-5°C, there was not significant changes in FFA but may not prevent rancidity caused by oxidation of bran (Tao et al., 1993). The SRB storage at room temperature should complete inhibit enzymes and destroy bacteria. The study extracted RBO obtained from SRB by Thanonkaewa et al. (2012), reported the effect on stabilization methods of RB carried out by using hot air, baking, steaming and MH to the quality of RBO. The effects of domestic heating on the MC of RB and extraction yield of cold-pressed RBO were shown in table 8.

The NSRB had 14.56 g/100 g bran MC. The data show that domestic heating could reduce the MC of RB. The MC of steaming, hot air, microwave and roasting of SRB were 11.41, 4.58, 4.05 and 2.12 g/100 g bran, respectively. Oil extraction yield from SRB increased according to the application of hot air, roasting and MH ($P < 0.05$). The oil extraction yield of stabilized RBO by HA, MW, roasting and steaming were 5.53, 4.81, 4.77 and 3.41 g/100 g bran, respectively. The stabilization of RB by steaming had no significant difference in oil extraction yield from NSRB ($p < 0.05$). The low MC in the SRB can make them more brittle and therefore can achieve a greater rupture of tissue and increase the extraction of oil during the mechanical pressing (Uquiche et al., 2008). The heating may be effect to the cellular wall, which results in greater porosity. It also could vaporize the water of the RB microstructure, increasing the pressure in its interior; its release causes the disintegration of the material. The cell membrane was broken and improves the efficiency of the pressing extraction of oil from oilseeds, enabling the passage of oil from the cell membrane (Uquiche et al., 2008).

Table 8 Effect of stabilization of RB by domestic heating on MC of RB and extraction yield of cold-pressed RBO.

Stabilization methods	MC (g/100 g bran)	Extraction yield (g/100 g bran)
Non-stabilized	14.56 ± 0.08^{a}	3.29 ± 0.23^{c}
Hot air	4.58 ± 0.51^{c}	5.53 ± 0.16^{a}
Roasting	2.13 ± 0.04^{e}	4.77 ± 0.30^{b}
Steaming	11.41 ± 0.04^{b}	3.41 ± 0.14^{c}
Microwave	4.05 ± 0.13^{d}	4.81 ± 0.24^{b}

Note: Values (means±SD) with different index letters are statistically significantly different ($P < 0.05$)

Source: Thanonkaewa et al., 2012

Thanonkaewa et al. (2012) reported the purpose of the stabilization method of RB to inactivate enzyme activity is the most important factor in RBO extraction. Poor or no stabilization causes the increase in AV, PV and FFA content and affects on the extraction process, oil quantity, and qualities. The chemical property including AV, FFA and PV were the parameters used for determination of the chemical quality of cold-pressed RBO from different stabilization RB method, is shown in table 9.

Table 9 Chemical properties of RBO from different stabilization RB methods

Stabilization methods	Chemical property		
	AV (mg KOH/g oil)	FFA (%)	PV (mg Eqv/kg oil)
Non-stabilized	11.11±0.84[a]	5.58±0.42[a]	18.85+0.45[a]
Hot air	6.98±0.31[cd]	3.51±0.16[cd]	12.13±0.22[d]
Roasting	7.56±0.03[c]	3.80±0.01[c]	15.18±0.50[c]
Steaming	9.01±0.40[b]	4.53±0.20[b]	17.16±0.59[b]
Microwave	6.30±0.55[d]	3.17±0.27[d]	11.72±0.59[d]

Note: Values (means±SD) with different index letters are statistically significantly different ($P < 0.05$).

Source: Thanonkaewa et al., 2012

Generally, the content of AV, FFA and PV were positively related to the activity of enzyme lipase. AV can be used for a purity check of oil and may have already started decomposition reactions. Although the refined oils were largely devoid of FFA, considerable amounts may be present in crude oils. Hydroperoxides were the primary products of autoxidation which in themselves are odorless. These were decomposed leads to the formation of a wide range of carbonyl compounds, hydrocarbons, furans and other products that contribute to the stale flavor of foods and may also be involved in biological oxidation (Frankel, 1991).

Steaming, roasting, DH and MH method could retard the forming of AV, FFA and PV compared with NSRB. Cold- pressed RBO of DH and MH had lower AV, FFA, and PV than that of roasting and steaming methods, respectively. However, there was no significant difference between the RB samples treated with DH and MH ($p<0.05$). Stabilization of RB with DH and MH were effective methods for controlling enzyme activity in RB. According to CODEX standards for edible fat and oil (CODEX STAN 210, 1999), the maximum level of AV and PV of cold pressed oil are 4.0 mg KOH/g oil and 15 milliequivalents of active oxygen/kg oil, respectively.

According to Tao et al. (1993), RBO with over 5% FFA was considered unsuitable for human consumption. These results shown that stabilized RBO by DH and MH had 6.30-6.98 mg KOH/g oil AV, 3.17-3.51% FFA and 11.72-12.13 milliequivalents of active oxygen/kg oil PV. The SRB with DH and MH had PV lower than that of CODEX standards and had less than 5% FFA but had a little higher AV than that of CODEX standards. Therefore, the SRB by DH and MH could be applied to produce the cold pressed RBO for human consumption. But it may need to find some process to reduce AV to reach the CODEX standard.

According to Revilla et al. (2009) reported RB contains several classes of antioxidants, including phenolic compounds, tocols, and γ-oryzanol. Reportedly, antioxidants are protective against oxidative damage, which has been implicated in a range of diseases, including cancer and cardiovascular disease. These also one of the principal ingredients that protect food quality by preventing oxidative deterioration of lipids. Cold-pressed edible seed oils may be preferred by consumers because the cold pressing procedure involves neither heat nor chemicals, and may increase the retention of beneficial phytochemicals (Lutterodt, et al., 2015). It was well accepted that antioxidants may be protected important cellular components such as DNA and membrane lipids from oxidative damage and suppressed the pathology of cancer, cardiovascular diseases, and other aging-associated health problems. RBO rich in natural antioxidants may play a role in reducing the risk of chronic diseases. Phytochemical contents of stabilized cold-pressed RBO as compared to NSRBO are displayed in table 10.

Table 10 Effect of stabilization of RB by domestic heating on phytochemical content of cold-pressed RBO

Stabilization methods	Phytochemical contents		
	Total phenolic (mg FAE/g oil)	Flavonoid (mg CE/g oil)	γ-oryzanol (g/100 g oil)
Non- stabilized	11.59±0.89^{c}	9.04±0.12^{b}	2.03±0.05^{c}
Hot air	15.70±3.35^{a}	11.81±1.20^{a}	2.30±0.08^{a}
Roasting	13.71±2.53^{b}	11.75±0.28^{a}	2.24±0.04^{ab}
Steaming	13.65±1.79^{b}	10.01±0.69^{b}	2.16+0.05^{b}
Microwave	16.27±1.10^{a}	12.18±0.65^{a}	2.25+0.02^{ab}

Note: Values (means ± SD) with different index letters are statistically significantly different ($P < 0.05$).

Source: Thanonkaewa et al., 2012

The results of table 10 show the total phenolic contents ranging from 11.59 to 16.27 mg FAE/g oil, flavonoid contents ranging from 9.04 to 12.18 mg CE/g oil and γ-oryzanol contents ranging from 2.03 to 2.25 g/100 g oil. SRB with domestic heating had yielded significantly higher amounts of total phenolic compounds, flavonoid content, γ-oryzanol than NSRB ($P < 0.05$). However, steaming stabilized RBO had no significant difference in flavonoid contents compared with RBO of NSRB ($P>0.05$). MW and HA heating stabilized RBO contained higher contents of total phenolic compounds than roasting and steaming methods. Cold-pressed RBO of HA heating had the highest content of γ-oryzanol but there was not a significant difference in MW, Roasting and Steaming method ($p < 0.05$) RBO contains a significant amount of natural phytochemicals such as oryzanol, tocopherols, and tocotrienols have been reported as the strongest antioxidants in RB (Lai et al., 2009).

In most cases, the highest efficiency of methods for stabilization of RB was suggested by domestic heating. This could be applied to RBO extraction prior to pressing to improve oil extraction yield, qualities, and antioxidant properties of cold-pressed RBO. HA and MH were the most effective methods for stabilization of rice bran with a high extraction yield, and lowering of AV, FFA, and PV. Those heating methods also provided higher contents of total phenolic compounds, flavonoid, and γ-oryzanol while increasing the antioxidant activities of RBO. The production of RBO in food manufacturing industry should maintain quality because it affects the value of products. Thus, the food industry from RB had to be eliminated or prevent the deterioration of the RB. The key was degradation by enzymes lipase elevations and lipoxygenase together perfectly at the same time to preserve nutrients in RB. The heat treatment for stabilization of RB had several different methods intended to inhibit or inactivate lipase activity. The majority of this process involved a heating process by drying or heating in the presence of moisture. The use of chemicals and radiation results in a stabilization of RB but the result was not satisfactory for the food industry and health hazards. The industry for a common stabilize RB by a heating process is a thermal break or damage to the useful component of RB, removal of MC from RB at high levels and could not destroy and prevent the reactivated the enzyme completely. According the study of Barber and Benedito de Barber (1980) reported, the RB with moisture may be more effective to heating than the dry RB but the heating process with steam less successful destroyed enzyme and the results will still not be satisfactory.

2. Chemical methods

Several whole RB stabilization methods have been reported in the literature. These had reported stabilization method used the physical method with thermal treatment such as MH, extrusion, HA, roasting, etc. In addition, the methods to RB stabilization by chemical method such as acidification (Amarasinghe et al., 2009) and some alcohol (Kim et al., 2014) as shown follow.

2.1 Acidification

Gopinger et al. (2015) reported whole RB stabilization by using a short-chain organic acids which were acetic acid and propionic acid mixture on the proximal composition, colorimetric profile, gross energy, lipids acid and lipid oxidation product in RB storage. Whole RB treated with organic acids and stored for

120 days exhibited lower quality, which had the highest gross energy values, lower lipid acidity increases, less primary and secondary lipid oxidation product formation. It maintained yellow color after storage for 120 days. This study has shown applying an acetic and propionic acid mixture conserved the RB well. Especially, the effect of storage time as a function of organic acid used on the RB lipid acidity rate increased significantly ($p < 0.05$) during RB treated control sample. It was found that the RB treated with organic acid exhibited less variation in lipid acidity during storage, which ranged from 63.06 to 101.08 mg of NaOH/100 g at the beginning and after 120 days of storage, respectively, compared with the control. The control exhibited a 63.06-120.96 mg increased in NaOH/100 g at beginning and after 120 days of storage, respectively. The acidity rate increased indicates FAs hydrolysis through enzymatic activity, mainly lipase activity, which breaks FAs ester links with glycerol. Acidification by acetic and propionic acid mixture, which demonstrated the benefits of using organic acids to maintain lipid acidity during storage. A conclusion of Gopinger et al. (2015) study, adding 2% of acetic and propionic acid mixture to whole RB stored over 120 days aided in preserving the qualities, which was demonstrated by higher gross energy values, smaller lipid acidity increases, less primary and secondary lipid oxidation product formation, and yellow color maintenance.

2.2 Alcohol

Champagne et al. (1992) reported stabilizing brown rice by ethanol vapors. They found brown rice could be stabilized to lipolytic hydrolysis by exposure to vapors from boiling aqueous ethanol (EtOH). During 6 months of storage at 36°C, FFA increased little or none in brown rice kernels treated with EtOH vapors for 3-10 min. Flours produced from treated kernels had low residual lipase activity. Treated kernels and flours prepared from them were more susceptible to oxidative deterioration than untreated kernels and flours, as indicated by increases in conjugated diene hydroperoxide content during storage. EtOH vapor treatment lowered the MC of the 12.8% moisture brown rice kernels approximately 1.5% loss of kernel oil was less than 3%. The MC of 8% kernels was not changed, and no oil was extracted by the EtOH vapor treatment. Thiamin and tocopherols were not lost in EtOH vapor-treated kernels. Thermal curves of treated and untreated kernels obtained by differential

scanning calorimetry indicated no starch gelatinization in the treated kernels. EV treatment of brown rice kernels reduced microbial populations to very low levels.

Kim et al. (2014) reported SRB by EV exposure at 2 and 5 min, they found that the ethanol vapor treatment was least effective in the color change, possibly because the EV treatment was done at a lower temperature than that with the direct heat treatments. Moreover, EV might have an inhibitory effect on the browning reaction and are displayed in table 11.

Table 11 Color of RB treated by EV

Treatment	Color values			
	L*	a*	b*	ΔE
Control	69.2 ± 0.3^{a}	3.8 ± 0.1^{g}	17.87 ± 0.3^{h}	-
EV				
2 min	67.8 ± 0.1^{bc}	4.3 ± 0.2^{f}	18.3 ± 0.1^{g}	1.5 ± 0.1^{f}
5 min	68.5 ± 0.4^{b}	4.3 ± 0.1^{f}	18.9 ± 0.2^{e}	1.4 ± 0.1^{f}

Note: *L* ,a* ,b* and ΔE represent lightness, color in the region of green to red, color in the region of blue to yellow and total color difference between the control and treated RB. Data represents means of three measurements (n=3) ± standard deviations. Values with different superscripts within the same column are significantly different ($p < 0.05$).

Source: Kim et al., 2014

Moreover, Kim et al. (2014) reported the EV exposure treatment was least effective in the color change, possibly because the vapor treatment was done at a lower temperature than that with the direct heat treatments. In addition, EV might have an inhibitory effect on the browning reaction. The enhanced inhibitory effect by EV compared with MH. The FFA content increased from 2.14% to 4.15% when MH was treated for 5 min, and the FFA contents increased from 2.14% to 4.39% when EV was treated for 20 min indicating that the enzymes responsible for the FFA formation had

been fully inactivated. Among the treatments tested in this study had the FFA content in the RB after storage for 24 weeks at room temperature less than 5% when the RB was treated by DH at 100°C, freeze dry and dry heat at 80°C or 100°C, MH for 5 min, Autoclave, and EV for 20 min.

Emerging technology of RB stabilization

Besides, the methods to stabilize RB included a physical method such as roasting, steaming, HA, autoclave, MW, etc, and a chemical method such as acidification and EV. The emerging technology of RB stabilization is interested such as extrusion heating, IR, and RF methods are suggested. The principle works and the advantages of the emerging technology of RB stabilization are as followed.

1. **Extrusion method**

Extrusion technology, well-known in the plastics industry, has now become a widely used technology in the agri-food processing industry, where it is referred to as extrusion-cooking. It has been employed for the production of so-called engineered food and special feed. Generally speaking, extrusion-cooking of vegetable raw materials deals with extrusion of ground material at baro thermal conditions. The food materials are heated to its melting point or plasticating point with the help of shear energy, exerted by the rotating screw, and additional heating of the barrel. These changes rheological properties of food on conveyed under high pressure through a die or a series of dies and the product expands to its final shape. The products of extrusion technology have very different physical and chemical properties compared with other methods. Extrusion cooking is a modern high-temperature-short-time (HTST) processing technology, gaining ground in certain industries for various reasons. It offers several advantages over other types of cooking processes, such as faster processing times and a significant reduction in energy consumed, which consequently results in lower prices for the final products. In recent days, the products of extrusion are of major importance in the food and feed industries. Extruders can be used for a wide range of traditional (conventional) food products, as well as in the production of numerous new products (cereal baby food, confectionery, breakfast cereals, snack foods, bakery products, flavors, pasta, pet food, and meat products) (Wiedemann, & Strobel 1987). An extruder represents a very complex bioreactor in which, various

types of food raw materials with different MC and viscosities are treated, under high temperatures, short residence times, high pressures, and very strong shear forces. During any extrusion process, the treatment of the material consists of mixing, mass kneading, heating, and shearing, and finally extrusion through a die appropriately designed to form and dry the product under expansion and rapid fall in pressure (Akdogan, 1999). The qualities of extrudate produced are determined using different methods according to their applicability in a variety of food industry sectors. Sensory qualities including aspects of the food product can adequately be evaluated by the consumer. These include color, size, shape, taste, odor, and structure. Such qualities vary considerably among products, significantly affecting their status and success. Evaluation of sensory characteristics is a difficult task, and some of the characteristics are very hard to estimate the screws of a twin-screw extruder are built up modularly. A series of unit operations can ideally be combined in a downstream process. There is already done in the plastics industry and a common approach to change and vary the screw configuration until the desired product is reached. A unit operation is realized by a discrete element or a combination of screw elements, e.g. feeding, conveying, mixing retaining functions are possible. The classical twin-screw element shapes are well described in the literature. Kohlgrüber (2007) reported an overview of the available patents concerning screw element geometry. Screw elements are primarily defined by their number of flights. In this work always two flight elements are used, i.e. conveying and kneading elements, as classical screw elements and the newer combine mixer elements were applied. Besides, extrusion cooking is a popular technology in food processing, especially in the processing of fiber-rich products as it can change the compositional and physicochemical properties of food materials, including dietary fiber (Vasanthan et al, 2002 as cited in Dang, & Vasanthan, 2017).

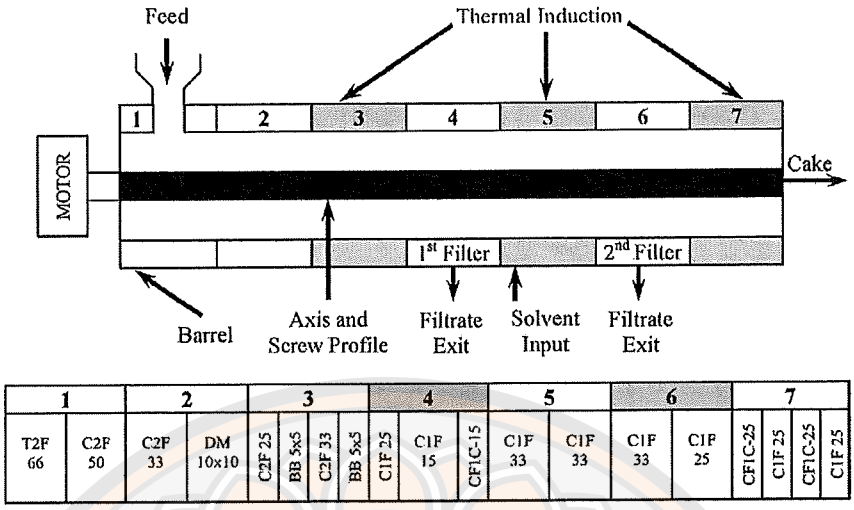


Figure 6 Schematic representation of the Clextral BC45 twin-screw extruder configuration and the screw profile applied for sunflower oil extraction through combined thermomechanical pressing and solvent extraction

Source: Kartika et al., 2010 as cited in Uitterhaegen, & Evon, 2017

2. IR method

IR is electromagnetic waves with wavelengths between radio waves and visible (Figure 7). A wavelength of 700 nm to 1 mm with a frequency of 430 Terahertz (1012 Hz) to 300 gigahertz (109 Hz) and energy protons at 1.24 meV to 1.7 meV with the same frequency in the MW at a temperature between -200 ° C to 4000 ° C to emit infrared radiation. Although it is not a pure spectrum and includes some wavelengths overlap each other, IR radiation is classified into 3 regions, namely, near-IR (NIR), medium-IR (MIR), and far-IR (FIR) corresponding to the spectral ranges of 0.78–1.4, 1.4–3, and 3–1000 lm, respectively (Sakai, & Hanzawa, 1994). The unique properties of IR as a deviation in the electromagnetic field is the based on the frequency properties. The frequency of IR has the high effect on high energy. The classification energy of IR divided 3 type as figure follows. IR occurs from changes in the energy levels of electrons in the atom or molecule. There is a transfer of energy by not needing an intermediary and it has a maximum speed of light and occurs in a vacuum. The heat transfer is caused by the release of radiation

from objects such as the difference between temperature itself and the temperature around. The energy of IR is electromagnetic waves.

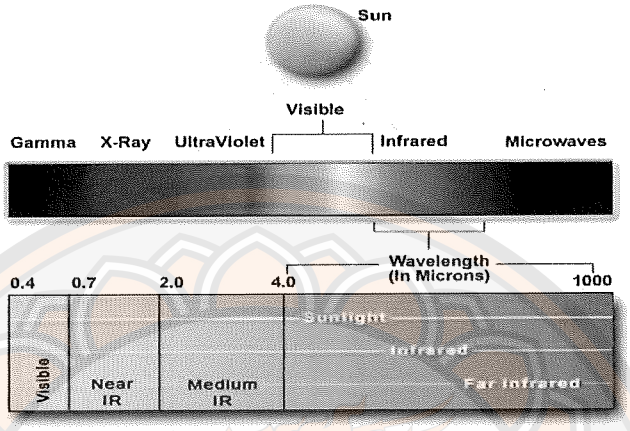


Figure 7 The classification energy of IR

Source: Far-IR from the energy of the sun, n.d.

Figure 7 is separated by the wavelength to 3 levels. Short-wave infrared (NIR) with wavelengths between 0.78 to 1.4 micrometers, medium wave IR (MIR) with wavelengths between 1.4 to 3 micrometers and long-wave infrared rays (FIR) with a wavelength between 3-1000 microns. The thermal processes of IR in the food industry are FIR to often use in the form of heating (Sakai, & Mao, 2006). The wavelengths between 2.5 to 30 micrometers are suitable for heat in the food industry (Shimizu, & Igarashi, 1991). IR through the air and absorbed by food and it is also transferring heat to the food. Thus, it has effectively reduced time, energy consumption, and cost. The important concept of process is the air in an oven of the IR chamber not heated hence, the surrounding air continues at normal temperature levels. It is possible to design equipment to compact and highly functional automation. This can be controlled by easy and safe thermal control directly as required, etc. (Sakai, & Mao, 2006).

The application of IR in the food industry involves heating food using (FIR) more than NIR because the food will absorb FIR energy efficient and it makes

the temperatures between 600-950 K (Sakai, & Hanzawa, 1994). The characteristics of heat from the IR is heating effectively food with less time and energy than the traditional process. The FIR to the cooking container found reduced heat source consumption of about 55 % than gas (Sheridan, & Shilton, 1999). The FIR takes less time, the product has a shorter time, no was the loss of nutrients and higher quality product. It also found that the FIR oven is better in terms of energy costs (Sasaki, 1992).

Drying process with IR heating is preferred for vegetable products because it makes a high-quality product, especially maintains the colors and nutritrients of vegetables after drying and inactivated chemical changes in chlorophyll or carotenoids, which is also caused by thermal and oxidation during drying. The study of Itoh, & Han (1994) were reported rate of degradation of β-carotene and chlorophyll present in vegetables. These were heated by solar light, hot air, NIR and FIR at 60°C. They found the pigment was destroyed irreversibly by ultra-violet radiation (solar light) and the lowest rate of deterioration of ß-carotene and chlorophyll A by FIR radiation. This suggested an advantage to use this method as a heat source for drying vegetables. IR can be penetrate food to a depth of 1 to 18 mm. The energy transmitted through the food. The factor to reduce the absorption of energy in the food is the depth of penetration or thickness of food's skin. The application of Infrared heat expects to increase in popularity as a way to preserve the nutritional value, good physical property and good taste of food. It also uses less energy than the traditional method.

Yılmaz (2016) reported SRB by using the IR method. The objective was to study the feasibility of using IR in the treatment of SRB by using FAs content indicated and effects on biochemicals in RB such as gamma, oryzanol, and tocopherol contents. The 4 factors various in this study were 1) bran fraction of RB 2) the power of IR at 500, 600 and 700 watt 3) the period of time process was 3.0, 5.5 and 7.0 minutes and 4) the time of storage were 15, 30, 45, 60, 75 and 90 days. The SRB from final bran fraction of milling rice and polish was higher FFA content than the first and second steps. The researcher suggested not taking the rice bran from the final step to combine with the first two steps. The first and second-step milling rice can reduce the FFA contents in RB approximately 80%. They used raw materials to treat and stabilize

the RB by infrared radiation. The stabilization at 700 W of MIR power for 7.0 min and provided 90 days of shelf life was a notable change in FFA. After storing for 90 days, the FFA in SRB had 6% which beyond the CODEX of fats and oils (Codex Standards for Fats and Oils from Vegetable Sources, ed). The oil must contain FFA less than 5%.

The studies on the important bioactive compounds in RB, such as tocopherol and oryzanol in the SRB for storage of 90 days. Total tocopherol and γ-oryzanol contents of SRB fractions were higher than their crude counterparts. The result was shown in table 12. The condition of SRB with MIR at 700 Watts IR power for 7.0 min had a shelf life of 3 months. The most important bioactive compounds without causing damage in the first whitening of RB fractions were obtained tocopherols and oryzanol. In this condition, the total tocopherol content was increased but the γ-oryzanol content was decreased in crude bran fractions as the milling progressed ($p < 0.05$). However, tocopherol content was no longer different from the stabilization procedure at 700W for 7.0 min, while γ-oryzanol content kept the decreasing trend even after the stabilization at any condition ($p > 0.05$).

In recent years, IR-VC drying has been investigated as a potential method for obtaining high quality dried food products, including fruits, vegetables and grains. IR-VC drying combines the advantages of both IR heating and vacuum drying. The low temperature and fast mass transfer conferred by vacuum combined with rapid energy transfer by IR heating generates very rapid, low temperature drying and thus it has the potential to improve energy efficiency and product quality (Giri, & Prasad, 2007 as cited in Salehi, & Kashaninejad, 2018). It is evident that increase in vacuum pressure enhanced the drying rate. This was expected because as vacuum pressure increases, there is an accelerated removal of moisture buildup in the chamber, which consequently enhanced the drying process. In combined IR-VC drying, the most important effect is to cause an increase in drying rate (Salehi, & Kashaninejad, 2018). The general improved quality of the dried products using this IR-VC dryer may be due to the combined effects of increased pressure gradient between the inner and outer layers of the lemon as well as the low temperatures maintained throughout the drying process (Salehi, & Kashaninejad, 2018). Therefore, the IR-VC made more efficient heat transfer. In order to destroy the enzyme and microorganisms in RB deteriorated completely, causing pH, AV, FFA and PV are lower than the standard CODEX.

The providing efficient heat is maintained nutrients, especially biochemicals, such as tocopherol, tocotrienol, oryzanol, and phytosterol, etc. Infrared heating may be used as a therapy to enhance the stability of RB as an alternative.

Table 12 γ-Oryzanol content of SRB fractions

IR power (W)	Process time (min)	γ-Oryzanol content (mg/kg)		
		Bran fraction 1	Bran fraction 2	Bran fraction 3
500	3.0	2387.1±27.0	1492.0±30.0	895±57.3
	5.5	2443.2±44.4	1992.0±301.0	1031.0±103.0
	7.0	2509.0±149.0	2233.0±588.0	911.4±22.5
600	3.0	2614.0±107.0	1602.3±31.8	925.2±59.3
	5.5	2470.0±243.0	1755.0±142.0	883.6±52.2
	7.0	2388.4±43.1	1626.0±136.0	857.4±26.5
700	3.0	2485.7±18.9	1672.8±40.1	952.6±74.8
	5.5	2511.7±96.5	1625.7±99.2	871.5±3.1
	7.0	2726.4±17.5	1695.4±22.3	882.1±9.2

Source: Yılmaz, 2016

The interested study on title stabilization of RB and its effect on bioactive compounds content, antioxidant activity and storage stability during IR radiation heating by Irakli, et al (2018) was investigated the stabilization optimized condition by IR in 12 treatments. The IR treatments were namely, T1 (100°C, 5 min), T2 (100°C, 10 min), T3 (100°C, 15 min), T4 (100°C, 20 min), T5 (120°C, 5 min), T6 (120°C, 5 min), T7 (120°C, 15 min), T8 (120°C, 20 min), T9 (140°C, 5 min), T10 (140°C, 10 min), T11 (140°C, 15 min) and T12 (140°C, 20 min). Untreated raw RB was used as a control. The results of was shown in table 13. The FFA content of raw RB measured immediately after laboratory milling, increased from 4.4% to 62.8% after 6-months of storage (table 13). All IR treated RB samples were observed lower FFA contents than control after 6-months of storage and the most sufficient stabilization IR treatments for inhibiting hydrolytic rancidity of RB during 6-months storage were T11 and T12, both of them at high temperature (140°C) at 15 and 20 min exposure to IR, respectively. Furthermore, it was also observed that process time for 15 and 20 min insignificantly

affected the FFA content at all IR treatments except T1-T4 (100°C). Consequently, the optimal time process for IR stabilization of RB was 15 min at 140°C as longer IR exposure time caused undesirable burning of the RB.

The influences of different IR treatments on the color changes of RB are shown in table 14. All color values (L*, a* and b*) were affected significantly ($p < 0.05$) by IR. It was obvious that all treated RB samples exhibited significantly lower L* values but higher a* and b* values in comparison with those of the control. The same trend was observed as IR temperature increased in the same duration time, most probably due to the formation of brown polymers from the Maillard reaction induced by the heat treatment. A dramatic increase of browning was evident at 140°C for 15 and 20 min during IR heating. The DE value expressed as a single value in color difference of L*, a* and b*, also significantly increased as the IR temperature increase. The T12 (140°C, 20 min) sample exhibited the darkest brown color, as observed from the lowest L* value, but the highest a* and DE* values among all treatments.

Table 13 Effect of IR treatment on FFA contents of RB during storage at room temperature

Trials	FFA (as oleic acid), %[b]					
	1 mouth	2 mouth	3 mouth	4 mouth	5 mouth	6 mouth
Control[a]	41.2±1.7^{a}	53.5±2.1^{a}	56.1±2.8^{a}	59.4±1.1^{a}	60.5±2.1^{a}	62.8±1.7^{a}
T1	23.0±2.9^{b}	31.0±1.2^{b}	37.4±1.1^{b}	45.1±1.3^{b}	49.6±0.8^{b}	52.1±1.5^{b}
T2	16.8±1.6^{c}	26.0±2.8^{bc}	30.1±4.2^{b}	34.1±1.3^{c}	39.7±1.9^{c}	44.0±1.4^{c}
T3	22.5±3.6^{b}	25.1±4.2^{cd}	27.3±2.8^{c}	31.1±1.4^{c}	33.8±0.8^{d}	37.2±1.1^{d}
T4	16.3±0.4^{c}	17.2±1.8^{e}	20.0±0.4^{d}	20.9±2.0^{e}	22.7±1.0^{e}	25.2±1.2^{e}
T5	9.6±0.6^{de}	20.1±0.4^{de}	21.5±0.8^{d}	27.4±0.8^{d}	32.6±0.9^{d}	37.1±1.3^{d}
T6	12.2±2.8^{cd}	16.5±2.1^{e}	20.0±0.5^{d}	26.2±1.5^{d}	31.1±1.2^{d}	34.5±2.2^{d}
T7	8.3±0.1^{de}	8.8±0.8^{f}	11.0±0.3^{e}	11.1±1.5^{fg}	11.8±0.4^{gd}	12.1±0.7^{g}
T8	8.1±1.3^{de}	7.7±0.8^{f}	9.0±0.1^{ef}	10.3±0.2^{fgh}	10.9±0.1^{g}	11.4±0.2^{g}
T9	7.7±1.5^{de}	9.8±0.4^{f}	10.4±0.5^{ef}	13.8±1.1^{f}	15.9±1.3^{f}	17.7±1.0^{f}
T10	7.1±1.4^{e}	8.7±0.4^{f}	8.2±0.3^{ef}	8.3±0.1^{ghi}	10.4±0.3^{g}	12.8±0.5^{g}
T11	6.4±0.5^{e}	6.6±0.5^{f}	6.7±0.6^{ef}	6.8 ±0.3^{hi}	7.2±0.3^{h}	7.3±0.3^{h}
T12	6.1±0.8^{e}	6.1±0.1^{f}	6.2±0.2^{f}	6.3±0.1^{i}	6.5±0.2^{h}	6.7±0.4^{h}

Note: [a]FFA of RB at 0 month was 4.4±0.7%

[b]values were mean± standard deviation.

Means with same superscript letter within the same line are not significantly different ($p > 0.05$).

Source: Irakli et al., 2018

Table 14 Effect of IR treatment on color of RB

Treatments	color			
	L*	a*	b*	△E
control	73.30^{a}	3.27^{i}	15.37^{j}	-
T1	74.07^{a}	3.57^{h}	20.69^{i}	3.90^{h}
T2	73.74^{b}	3.61^{h}	21.11^{h}	4.25^{g}
T3	73.04^{bc}	3.89^{g}	22.6^{2e}	5.40^{f}
T4	70.74^{e}	4.92^{d}	24.77^{d}	7.45^{d}
T5	73.63^{bc}	3.60^{h}	20.62^{i}	3.90^{h}
T6	73.43^{bc}	3.70^{h}	20.70^{i}	4.00^{h}
T7	69.30^{f}	5.90^{c}	25.47^{c}	8.50^{c}
T8	68.07^{g}	6.37^{b}	26.13^{b}	9.40^{b}
T9	71.92^{d}	4.26^{f}	21.66^{g}	5.10^{g}
T10	71.14^{e}	4.65^{e}	22.32^{f}	5.80^{e}
T11	64.99^{h}	7.89^{a}	27.27^{a}	11.60^{a}
T12	64.68^{h}	7.78^{a}	27.26^{a}	11.71^{a}

Note: Means with same superscript letter within the same line are not significantly different ($p > 0.05$).

Source: Irakli et al., 2018

3. RF method

Hou et al. (2016) reported a principle of RF, one of the thermal treatments using electromagnetic energy. Any material with polarized molecules and charged ions were subjected to an electromagnetic field that rapidly changes direction. The heating of RF was occurring as polarized molecules and charged ions interacted with the alternating electromagnetic field, resulting in friction as they rotated and moved (Barber, 1983). The higher frequency of the alternating field made the energy imparted greater to the material until the high frequency rotating molecules cannot keep up with the external field due to lattice limitations (Zhao et al., 2000). The RF could not apply frequencies between 10 and 300 MHz and specifically allocated to be 13.56, 27.12,

and 40.68 MHz because it avoided disturbing communication systems of the US Federal Communications Commission (FCC). Many factors influencing the RF including, the dielectric properties of products and distribution of electromagnetic fields, and the thermal energy converted from electromagnetic energy, etc. RF energy was generated while heating volumetrically and rapidly within products by the combined effects of polarization mechanisms of dipole rotation and ionic conduction (Piyasena et al., 2003). Most of the agricultural products were acted as an electric capacitor to store electrical energy and also as a resistor to transform electric energy into thermal energy, thereby heating the products. (Nelson, & Trabelsi, 2012). In general, the dielectric constant and loss factor of agricultural products increases with increasing temperature in a certain frequency, which causes those agricultural products to absorb more electromagnetic energy as their temperatures increase (Hossan, & Dutta, 2012). The portion of products at higher temperatures tends to absorb more energy for accelerating the temperature differences, which is called the thermal runaway effect. On the other hand, thermal energy is converted from electromagnetic energy. The water in a product is evaporated during the heating process. Less energy is absorbed in the higher temperature part than in the lower temperature one. This is termed as a temperature leveling effect. Therefore, it is essential that the electric field in RF units should be uniform to ensure even heating. The temperature distribution within a material is seldom as predictable as that for a conventional process. The heat distribution and penetration studies are not straightforward in a dielectric heating process. Since, the dielectric properties of air are totally different from those of products, the density of pulverized or granular materials have a notable effect on the dielectric properties. The electromagnetic waves strike an object, part of the waves is reflected, the remaining part penetrates into the material, and is gradually absorbed. Theoretically, the penetration depth of a sample is defined as the depth below the surface of a material where the power density of a perpendicularly impinging. The penetration depth of a material is inversely proportional to the frequency as the dielectric properties are fixed. The penetration depth of RF heating is deeper than that of MW treatments because of its longer wavelength. The agricultural products with high MC, the dielectric constant and the loss factor are relatively high, resulting in small penetration depth. The penetration

depth of dry products is large, providing potential to treat large thicknesses of the products in RF systems. A free-running oscillator RF system widely used in the industry is described (Figure 8). The target material placed between top and bottom electrodes is moved on a conveyor belt to simulate continuous processes and acts as a capacitor to store electrical energy and a resistor to transfer electric energy to thermal energy. Moving the top electrode is used to change the electrode gap, and thus regulate RF heating rate.

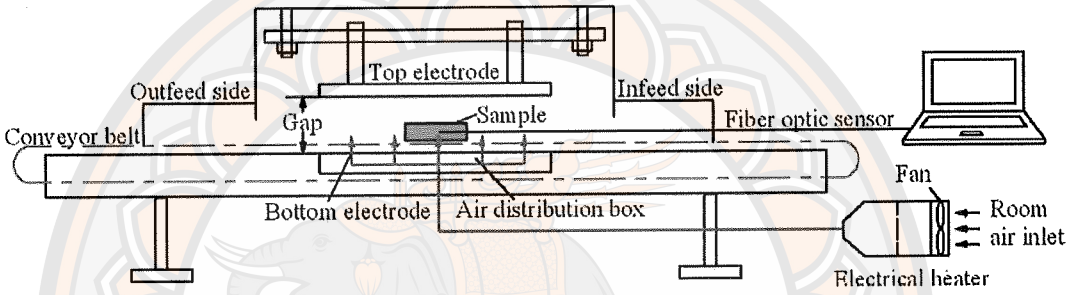


Figure 8 Schematic view of the free-running oscillator 6 kW, 27.12 MHz RF system showing the plate electrodes, conveyor belt, the HA system and the fiber optic sensors

Source: Hou et al., 2014 as cited in Hou et al., 2016

RF of dry products is important to improve the heating uniformity of sample before developing an effective RF heating treatment. Heating uniformity index of almonds, beans, and walnuts was greatly reduced by using forced hot air, movement, and mixing of the samples during RF heating (Wang et al., 2010; Gao et al., 2010; Jiao et al., 2012). The same methods were used to improve a heating uniformity of chestnuts during RF (Hou et al., 2014). After achieving satisfactory heating uniformity, the protocol usually consists of RF heating to a target temperature, holding for pre-determined time with HA, and cooling by forced room air in a single layer. Samples quality was not affected by the RF treatments because quality parameters of treated dry samples are not significantly different from those of controls (Gao et al.,

2010; Hou et al., 2014). The RF treatment protocols have been applied in industrial scales and hold potential for commercial applications.

RF is a promising technology for food applications because of the associated rapid and uniform heat distribution, large penetration depth and lower energy consumption. RF heating has been successfully applied for drying, baking and thawing of frozen meat and in meat processing. However, its use in continuous pasteurization and sterilization of foods is rather limited. During RF heating, heating is generated within the product due to molecular friction resulting from oscillating molecules and ions caused by the applied alternating electric field. The RF heating is influenced principally by the dielectric properties of the product when other conditions kept constant (Piyasena et al., 2003).

However, its commercial use in disinfestations for postharvest agricultural products is rather limited. The minimum temperature–time combinations that result in 100% mortality for insects show that the fifth-instar navel orange worm is the most thermos tolerant insect in walnuts and used as target insect for RF treatment validation studies. Then, the infected walnuts were heated to 55°C by a 27.12 MHz pilot scale RF system and holding in HA at least 5 min, resulting in 100% mortality of the fifth-instar navel orange worm. Furthermore, the rancidity, sensory qualities and shell characteristics of treated walnuts are not affected by the RF treatment. Finally, Wang et al. (2007) use two 27 MHz, 25 kW industrial RF systems to simulate the industrial processing and evaluate the energy efficiency and cost of RF treatment. An electrode gap (28.0 cm) was chosen based on the electric current and heating time, conveyor speed is set to 57 m/h, and one mixing of walnuts between continuous RF treatments. After RF treatments, average and minimum walnut temperatures were 60°C and 52°C, respectively. Then the walnuts were held for 5 min by hot air at 60°C, resulting in complete control of target insects without negative effects on walnut quality after stored 20 days at 35°C for simulating 2 year storage under commercial conditions at 4°C.

The reported of Jiao et al. (2012), They used a 27.12 MHz, 6 kW RF unit with a forced hot air system to conduct industrial scale-up studied on disinfecting lentils. Based on the electric current and heating time, an electrode gap was set at 14.0 cm with a conveyor speed of 7.5 m/h. To accomplish 100% cowpea weevil mortality, the RF treatment protocol was developed with forced HA to heat lentils to 60°C for 10

min, followed by forced ambient air cooling for 20 min. These results have shown the quality such as MC, color, and germination of lentils were acceptable. The average heating efficiency of the RF system was 76.5% with a throughput of 208.7 kg/h. The scale-up applications indicated that an industrial-scale RF process provides a promising physical treatment as an alternative to chemical fumigation.

Suchada et al. (2015) reported, RF treatment for stabilizing extracted *Perilla frutescent* (L) Britt oil at a low temperature of 50°C for any treating period was increased the oil recovery. However, the quality of oil appeared to be inferior, as it contained high levels of hydrogen peroxide, peroxide value, and TBA value. There was not surprising because it was a slight increase in lipoxygenase activity during the initial period of RF treatment and the lipase activity was significantly affected the oil quality. In contrast to this result on high treating temperature over 80°C and 10 minutes duration, the low activation of lipase occurred. The result is shown in table 15.

Therefore, the RF stabilizing technique was provided the high oil quality, less lipid peroxidation and lower energy consumed than conventional drying. Consequently, it seems imperative that further work needs to be carried out to find the optimum RF treating condition on the inactivation of lipase and lipoxygenase, which were responsible for FFA release and the subsequent peroxidation leading to rancidity of oil. The RF heat treatment had an effect on the enzymes activities. On treating temperature over 80°C for 10 minutes, the low activation of lipase occurred. The better oil quality which had a low level of hydrogen peroxide, PV and TBA value were achieved.

Table 15 Total antioxidant activity and enzymatic activity of *Perilla frutescens* (L.) Britt grain under various RF heating treatment

Treatment	Total antioxidant activity (IC50)	Enzymatic activity			
		Lipase (U g^{-1} dry matter)	LOX (U g^{-1} dry matter)	APX (µmol min^{-1} 100 mg-1 protein)	SOD[1] (Δactivity mg^{-1}protein)
Grain moisture content (%-db)					
10	62.658 ± 0.6165b	92 ± 0.16c	15.316 ± 1.2330b	18.390 ± 0.0854a	9.945 ± 0.0991a
14	63.312 ± 0.4103a	94 ± 0.08b	16.546 ± 1.0449a	18.390 ± 0.0483a	9.839 ± 0.0685b
18	63.319 ± 0.7262a	100 ± 0.11a	16.624 ± 0.8206a	18.313 ± 0.0725b	9.835 ± 0.0642b
RF treated temperature (°C)					
50	68.035 ± 7.250b	122 ± 0.04a	20.167 ± 0.665	19.410 ± 2.165a	10.340 ± 1.401a
60	67.402 ± 6.549b	120 ± 0.11b	19.350 ± 5.973	18.440 ± 1.561bc	10.215 ± 1.276a
70	66.562 ± 6.797b	119 ± 0.12c	19.957 ± 2.536	18.292 ± 1.619bc	10.164 ± 0.977ab
80	72.390 ± 8.605a	117 ± 0.13d	19.780 ± 3.067	18.632 ± 1.830b	9.690 ± 1.1933b
90	71.594 ± 6.162a	115 ± 0.04e	18.968 ± 3.951	17.930 ± 1.830c	9.090 ± 1.2475c
RF treated duration (min)					
1	65.598 ± 7.356b	120 ± 0.08a	19.752 ± 3.568	18.799 ± 2.059a	9.924 ± 1.317
3	69.136 ± 7.180a	120 ± 0.08a	19.424 ± 5.426	18.945 ± 1.804a	9.927 ± 1.201
5	69.792±7.151a	118 ± 0.04b	19.860 ± 3.264	18.928 ± 1.480a	9.760 ± 1.388
7	70.211±6.870a	118 ± 0.18b	19.674 ± 3.454	18.415 ± 1.917a	10.120 ± 1.363
10	71.247±7.668a	117 ± 0.45c	19.511 ± 3.455	17.618 ± 1.742b	9.768 ± 1.249
The conventional heating techniques (110°C for 30 minutes)					
	68.425	118	18.667	18.762	9.987

Note: APX: ascorbate peroxidase, LOX: lipoxygenase, SOD: superoxide dismutase

Values are means f standard deviations

Source: Suchada et al., 2015

Theory of RSM

RSM is a collection and multivariate statistical techniques useful for developing, improving and optimizing processes, i.e., to discover the conditions in which to apply a procedure in order to obtain the best possible response in the experimental region studied. This methodology involves the design of experiments and multiple regression analysis as tools to assess the effects of two or more independent variables on dependent variables (Myers et al., 2009). This performance measure or quality characteristic is called the response (output variable). It is typically measured on a continuous scale, although attribute responses, ranks and sensory response are not unusual. The most applications of RSM will involve more than one response. The input variables are called independent variables and they are subject for purposes of a test or an experiment. The RSM is equipped with statistical tools to determine the significance of a factor over a response. The evaluation of factors using the RSM uses experimental design in order to distribute the selected variables within the boundaries of the design. The response of experiment can be presented graphically, either in the contour plot or three dimension that help visualize the shape of the response (Myers et al., 2009; Khuri, & cornell, 1996).

One additional advantage is the possibility of evaluating the interaction effect between the independent variables on the response. This technique is based on the fit of a polynomial equation to the experimental data to describe the behavior of a set of data. In this way, a mathematical model which describes the studied process is generated. The objective is to simultaneously optimize the levels of the studied variables to attain the best possible performance of the process (Myers et al., 2009). Recently, the researchers have been published on the application of RSM to optimize extraction bioactive compounds and antioxidant activities. Bachir-Bey et al. (2014) studied the optimum conditions for extracting total phenolic compounds and antioxidant activity from fresh dark fig (*Ficus carica* L.) whereas Yuan et al. (2015) investigated the methanol concentration, extraction time, and liquid/solid ratio as factors and eight bioactive compounds contents as responses. Şahin et al. (2013) was determined HCl concentration between 0.41 and 0.44 mol/L, methanol volume between 55% and 59% (v/v), extraction temperature between 64 and 70°C, extraction time between 101 and 107 min for extraction of phenolic content and antioxidant

capacity from *Artemisia absinthium*. Yuan et al. (2012) was determined energy density between 20 and 40 (Watt/ml), citric acid concentration between 0.2 and 0.6 mol/dm^3, liquid:solid ratio between 1:15 and 1:20, extraction time between 20 and 60 second for extraction of anthocyanins yield from grape peel. Ranic et al., (2014) was studied extraction process from espresso spent coffee grounds that is a waste material abundantly produced by restaurants and cafeterias using RSM with microwave-assisted extraction. They found that the reduced time of extraction, low power and medium liquid to solid ratio while using minimal concentration of ethanol, the polyphenols extract with high antioxidant activity can be achieved.

1. The Box–Behnken Design

Another class of response surface designs are called Box-Behnken designs (BBD) that provides three levels for each factor (-1, 0, 1) and consists of a particular subset of the factorial combinations from the 3k factorial design. Some Box–Behnken designs are rotatable, but, in general, this design is not always rotatable. They are very useful in the same setting as the central composite designs. Their primary advantage is in addressing the issue of where the experimental boundaries should be, and in particular to avoid treatment combinations that are extreme. By extreme, the corner points and the star points are thinking, which are extreme points in terms of region in the experiment. The BBD avoids all the corner points, and the star points. Therefore, the BBD is popular in industrial research because it is an economical design and requires only three levels for each factor (Myers et al., 2009; Khuri, & cornell, 1996).

However, both the CCD and the BBD can work but they have different structures, so if the experimental region is such that extreme points are a problem then there are some advantages to the BBD. Two important models are commonly used in RSM. These are special cases of model (1) and include the first-degree model (d = 1), and the second-degree model (d = 2)

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \varepsilon \qquad (1)$$

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=1}^{3} b_{ij} X_i X_j + \varepsilon \qquad (2)$$

Where Y represent the response variables, b_0 is a constant, b_i , b_{ii} and b_{ij} are the linear, quadratic and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables (Myers et al., 2009).

2. The CCD

The CCD is one of the most commonly used response surface designs for fitting second-order models. The factorial portion is used to fit all linear and interaction terms. The axial points provide additional levels of the factor for purposes of estimation of the quadratic terms. This is perhaps the most popular of all second-order designs. This design consists of the following three portions:

2.1 A complete (or a fraction of) 2k factorial design whose factors' levels are coded as −1, 1. This is called the factorial portion.

2.2 An axial portion consisting of 2k points arranged so that two points are chosen on the axis of each control variable at a distance of α from the design center (chosen as the point at the origin of the coordinates system). The values of α (or the axial parameter) and is chosen so that the CCD can acquire certain desirable properties.

3. n_0 center points.

Thus, the total number of design points in a CCD is n = 2k + 2k + n0. For example, a CCD for k = 2, α = √2, n0 = 2 has the form (Myers et al., 2009; Khuri, 2005).

CHAPTER III

RESEARCH METHODOLOGY

Materials

The fresh RB (Thai jasmine rice cultivar) used in this study was supplied by Sawangtawarn enterprises, rice milling factory located in Kamphaeng Phet Province, Thailand. The 3 step of milling provided: the rough milling, fine milling, and water mist polisher were conducted to the milling process. Immediately after milling, the RB was packed into an aluminum foil bag and stored at -18 °C until the experiment was carried out. The fresh RB was analyzed for its FFA, MC, a_w, color, chemical composition, tocotrienol, tocopherol, oryzanol, and microbiological content.

Chemicals

1. Acetonitrile (RCI Labscan, Thailand)
2. Chloroform (RCI Labscan, Thailand)
3. Ethanol (Merck, Germany)
4. Diethyl ether (Merck, Germany)
5. Hexane (RCI Labscan, Thailand)
6. Methanol (Merck, Germany)
7. Potassium hydroxide (RCI Labscan, Thailand)
8. Isopropanol (Merck, Germany)
9. Potassium iodide (Sigma aldrich, USA)
10. Starch (Merck, Germany)
11. Sodium thiosulfate (Merck, Germany)
12. Acetic acid (Merck, Germany)
13. γ-Tocotrienol (Sigma aldrich, USA)
14. α-Tocopheral, (Sigma aldrich, USA)
15. γ-Oryzanol (Sigma aldrich, USA)

Apparatuses

1. Analytical balance 2 decimals (Metteler Toledo, PB3002-S, Switzerland)
2. Analytical balance with 4 decimal points (Mettler, model AB 204-5, Switzerland)
3. Centrifuge (Hettich Zentrifugen, model Universal 32 R, Germany)
4. High performance liquid chromatography (HPLC) (Perkin Elmer series 200, USA)
5. Hot air oven (Memmert, UNE 500, Germany)
6. Magnetic stirrer (Nuova, model Thermolyne, USA)
7. Micro pipet (Rainin, U.S.A)
8. Orbital shaker (Gemmy, model VRN-480, USA)
9. Rotary evaporator (Buchi, model V-500, Switzerland)
10. Vacuum pump (Buchi, model V-500, Switzerland)
11. Vortex mixer (Vortex, model Genie 2, USA)
12. Water activity (Aqualab, model Series 3TE, USA)
13. Water bath (Memmert, model Wb22, Germany)
14. IR heating, IR stabilization system was used to stabilize the RB fractions. It is a closed iron chamber with polymer enamel consisted of two MIR emitters (Heraeus-Noblelight, 600 mm, 230 V, 1000 W, Hanau, Germany). The chamber has a loading unit which enables the bran to spread out uniformly and in the form of a thin layer (thickness is approximately 0.5 cm) on the black chamber. Distance between the emitters and the sample was maintained constant at 15 cm throughout the experiments. The schematic diagram of IR, IR-VC and IR-HA heating is shown in Fig. 9.
15. RF heating, RF heating stabilization procedure was carried out using a 3 kW parallel-plate RF unit operating at 40 MHz (SO6B, Strayfield International, Wokingham, U.K.). This equipment of the parallel plate electrode separation from 12 cm using the top electrode (40 cm×83 cm) so as to regulate the RF power. A rectangular polycarbonate (PC) container (inner: 21 cm×14 cm×7.5 cm) with perforated (Φ 1.5 mm) side walls and bottoms was used to hold samples for RF heating. The schematic diagram of RF stabilization process is shown in Figure 10.

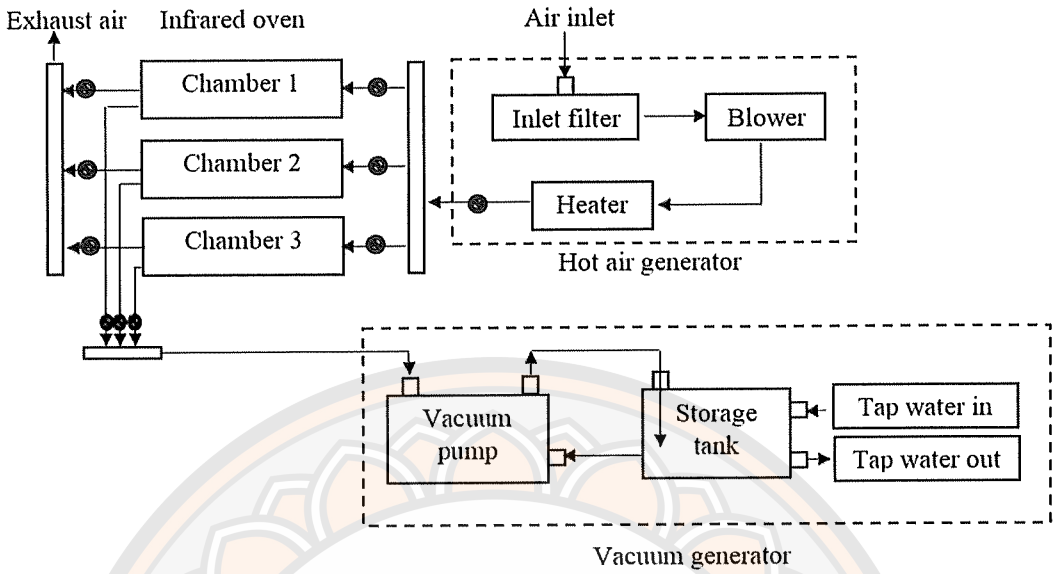


Figure 9 Schematic diagram of IR, IR-VC and IR-HA stabilization process

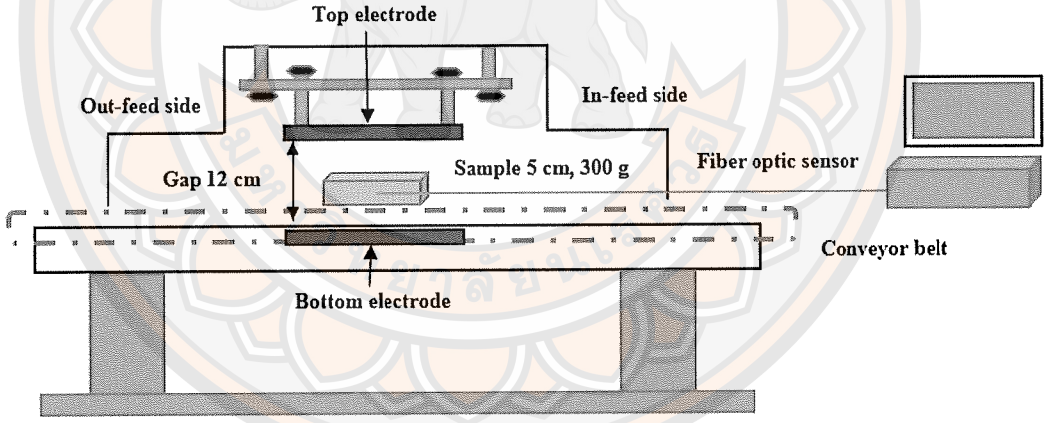


Figure 10 Schematic diagram of RF stabilization process

Scope of the study

The research methodology divided into four sections as follows,

Section 1 Fresh rice bran analysis

The color (L* a* b* and ΔE), proximate composition (MC, fat, protein, ash, and fiber), FFA content, FA composition, tocopherol, tocotrienol, oryzanol, and microbial analysis (TPC, yeast and mold) of fresh RB were analyzed.

Section 2 RB stabilization and optimization

IR, IR-VC, IR-HA an RF heat treatment were investigated for FFA, MC and a_w in stabilized rice bran. Application of RSM for optimization of lowest FFA, MC and a_w in stabilized rice bran using IR heat treatment (IR with parameter i.e., IR wattage, treatment duration, vacuum strength and hot air temperature) and RF heat treatment (RF with parameter i.e., treatment temperature, treatment duration, and MC of sample). The best-stabilized condition was selected according to be % yield and used in the next section.

Section 3 Storage stability

The NSRB and SRB (the optimal condition in section 2 i.e., IR, IR-VC, IR-HA and RF heat treatment method) were stored at 35°C for 8 weeks. The samples were collected each week and FFA content, MC and a_w analysis, shelf-life and FFA formation rate in RB were investigated. The USRB and SRB samples were collected at 0, 4, and 8 weeks for determination of proximate composition, FA compositon, tocotrienol, tocopherol, oryzanol, and microbial analysis.

Section 4 Extraction yield, qualities, and stability of crude RBO

The NSRB and IR, IR-VC, IR-HA and RF stabilization RB sample were extracted by cold-pressing method. The RBO of NSRB and SRB samples were stored at 35°C for 8 weeks. The samples were collected each week for the FFA content analysis, shelf-life and FFA formation rate in RBO were investigated. The NSRBO and SRBO were collected at 0, 4, and 8 weeks for PV, FA profile, tocopherol, tocotrienol, and oryzanol analysis.

The conclusion of the study scope of this study is shown in schematic diagram of experimental sections 1 to 4 (Figure 11).

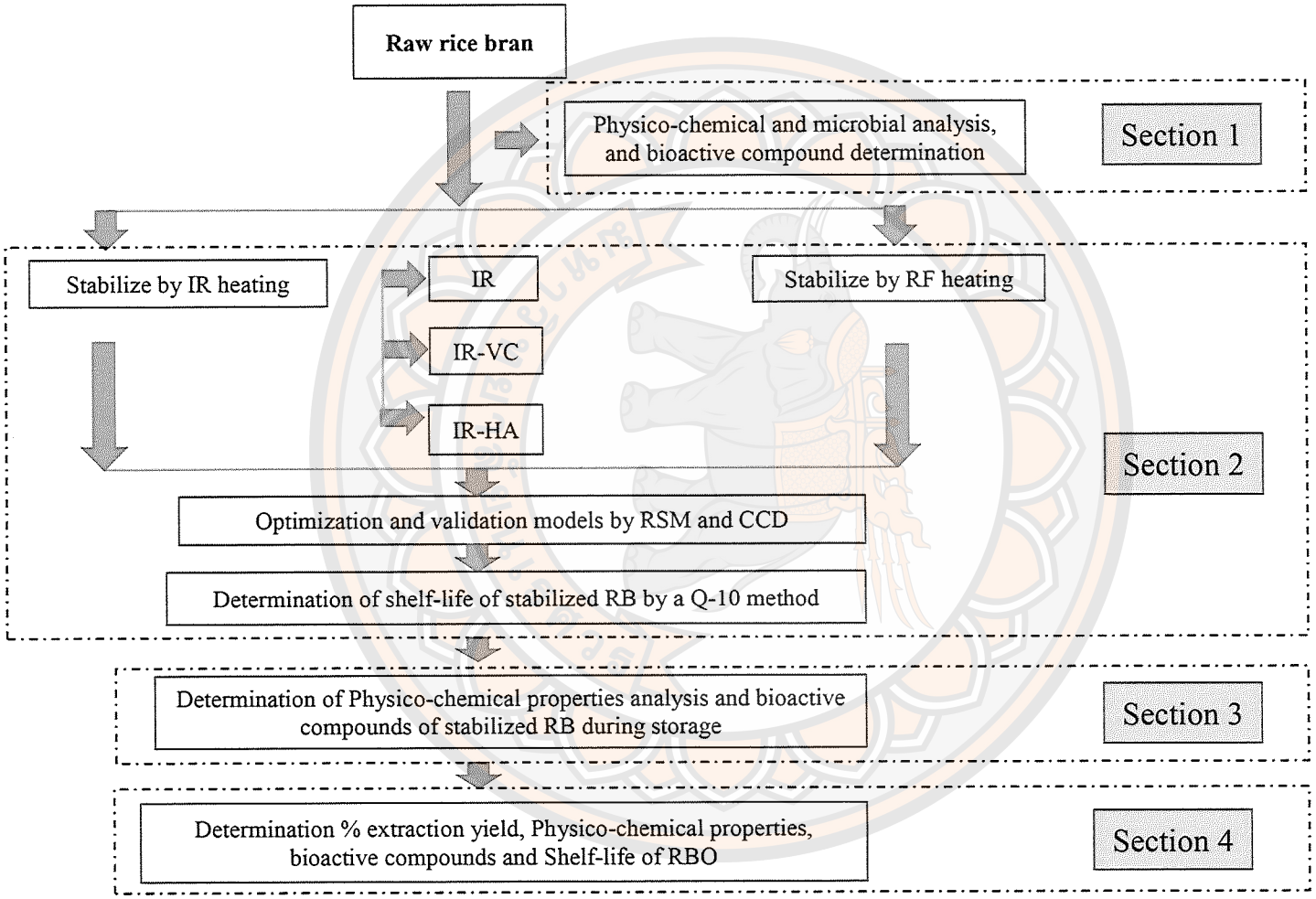


Figure 11 Schematic diagram of experimental scope

Research methodology

Section 1 Fresh rice bran analysis

1. Fresh RB analysis

The fresh RB was analyzed for its

1.1 Physical property

1.1.1 Color L* a* b* and ΔE

The sample was tested for color brightness (L*, a* and b*) (ColorFlex, HunterLab, USA) and the ΔE was calculated as Eq. 3.

1.1.2 $\Delta E = [(L_1^*-L_2^*)^2+(a_1^*-a_2^*)^2+ (b_1^*-b_2^*)^2]^{1/2}$. (3)

1.2 Chemical analysis

1.2.1 The proximate composition including MC, fat, protein, ash and fiber were determined followed AOAC (1995) method. The sample underwent proximate analysis, crude fat, protein, ash, and dietary fiber content were determined by methods 30-10, 46-12, 08-01 and 32-07 of AOAC (2000), respectively. All measurements were performed in triplicate and expressed as dry weight.

1.2.2 The MC of sample was determined by an AOAC (2000) method.

1.2.3 The a_w of sample was analyzed using an AquaLab water activity meter (Decagon, USA). All the data from the quantitative analysis was presented on a dry basis.

1.2.4 FFA of sample was analyzed follow AOCS (2004) method (Appendix 1; 1.1 FFA analysis method).

1.2.5 FA composition of sample was analyzed follow AOAC (2012) method (Appendix 1; 1.2 FA profile analysis method).

1.2.6 Tocols and oryzanol contents of sample were determined using the methods of Pestana-Bauer, V.R. et al. (2012)(Appendix 1; 1.3 Tocols and oryzanol content analysis method.

2. Microbial analysis

The TPC and yeast&mold content of the SRB and USRB were analyzed using the BAM (2000) method.

Section 2 RB stabilization and optimization

1. RB stabilization and optimization by IR, IR-VC and IR-HA

1.1 Pre-treatment RSM

RSM, which was originally described by Box and Wilson (1951), was applied in order to determine the experiment conditions. The 2 independent variables X_1 (IR wattage, W) and X_2 (treatment duration, seconds) and their 5 variation levels for the experiment of IR are shown in table 16. The 3 independent variables X_1 (IR wattage, W), X_2 (treatment duration, seconds) and X_3 (vacuum strength, mmHg) and their five variation levels for the experiment of IR-VC are shown in table 17. The 3 independent variables X_1 (IR wattage, W), X_2 (treatment duration, seconds) and X_4 (hot air temperature, °C) and their 5 variation levels for the experiment of IR-HA are shown in table 18.

Table 16 Independent variables and their levels, from the CCD of IR stabilization method

Variables	Name (units)	Levels				
		-1.414	-1	0	1	-1.414
X_1	IR wattage (W)	600	660	800	940	1000
X_2	Treatment duration (seconds)	360	395	480	565	600

Table 17 Independent variables and their levels, from the CCD of IR-VC stabilization method

Variables	Name (units)	Levels				
		-1.682	-1	0	1	1.682
X_1	IR wattage (W)	600	680	800	920	1000
X_2	Treatment duration (seconds)	360	410	480	550	600
X_3	Vacuum strength (mmHg)	450	490	550	610	650

Table 18 Independent variables and their levels, from the CCD of IR-HA stabilization method

Variables	Name (units)	Levels				
		-1.682	-1	0	1	1.682
X_1	IR wattage (W)	600	680	800	920	1000
X_2	Treatment duration (seconds)	360	410	480	550	600
X_4	hot air temperature (^oC)	60	64	70	76	80

1.2 RB stabilization and optimization

The model construction and data analysis were carried out using Design-Expert software (version 6.0.8, Stat-Ease Inc., Minneapolis, MN). The completed design consisted of 11 experimental treatments of IR, 17 experiments of IR-VC and 17 experiments of IR-HA were performed in triplicate.

The rice bran was stabilized by IR, IR-VC and IR-HA. For each of the treatments (table 19, 20 and 21 respectively), 300 g of the RB was placed in a uniform layer in a round, black, non-stick coated metal container with a diameter of 16 cm. The thickness of the sample layer was 5 mm, so that the bran could absorb the IR radiation evenly. The irradiated rice bran was divided up 100 g samples (to form treatment triplicates), and each sample was sealed in its own 250 ml polyethylene bag and then stored in an incubator, at one of the various temperatures specified in the experiment. The 11 treatments of stabilized RB were in storage for 8 weeks at 35°C, and each week a 10 g sample of each treatment was removed for testing. Each removed samples was tested to determine its FFA content, MC and a_w. At week 0, an initial test of each treatment, un-stored, was also conducted.

Table 19 Coded values and actual values of each independent variables applied in CCD of IR stabilization method

Experiment no.	Coded levels		Actual levels	
	X_1	X_2	X_1 (Watts)	X_2 (second)
1	0.000	0.000	800	480
2	1.414	0.000	1000	480
3	0.000	0.000	800	480
4	1.000	1.000	940	565
5	-1.414	0.000	600	480
6	-1.000	1.000	660	565
7	0.000	0.000	800	480
8	-1.000	-1.000	660	395
9	1.000	-1.000	940	395
10	0.000	1.414	800	600
11	0.000	-1.414	800	360

Table 20 Coded values and actual values of each independent variables applied in CCD of IR-VC stabilization method

Experiment no.	Coded levels			Experiment levels		
	X_1	X_2	X_3	X_1 (Watts)	X_2 (second)	X_3 (mmHg)
1	-1.000	1.000	-1.000	681	550	490
2	0.000	0.000	-1.682	800	480	450
3	1.000	1.000	-1.000	919	550	490
4	1.682	0.000	0.000	1000	480	550
5	0.000	0.000	1.682	800	480	650
6	0.000	0.000	0.000	800	480	550
7	-1.000	-1.000	-1.000	681	410	490
8	0.000	0.000	0.000	800	480	550

Table 20 (cont.)

Experiment no.	Coded levels			Experiment levels		
	X_1	X_2	X_3	X_1 (Watts)	X_2 (second)	X_3 (mmHg)
9	1.000	-1.000	-1.000	919	410	490
10	1.000	-1.000	1.000	919	410	610
11	0.000	1.682	0.000	800	600	550
12	1.000	1.000	1.000	919	550	610
13	0.000	-1.682	0.000	800	360	550
14	-1.682	0.000	0.000	600	480	550
15	0.000	0.000	0.000	800	480	550
16	-1.000	-1.000	1.000	681	410	610
17	-1.000	1.000	1.000	681	550	610

Table 21 Coded values and real values of each independent variables applied in CCD of IR-HA stabilization method

Experiment no.	**Coded levels**			**Actual levels**		
	X_1	**X_2**	**X_4**	**X_1 (Watts)**	**X_2 (second)**	**X_4 (^oC)**
1	0.000	1.682	0.000	800	600	70
2	0.000	0.000	0.000	800	480	70
3	0.000	0.000	0.000	800	480	70
4	0.000	-1.682	0.000	800	360	70
5	-1.000	1.000	-1.000	680	550	64
6	0.000	0.000	-1.682	800	480	60
7	-1.000	-1.000	1.000	680	410	76
8	-1.000	-1.000	-1.000	680	410	64
9	0.000	0.000	1.682	800	480	80
10	1.682	0.000	0.000	1000	480	70

Table 21 (cont.)

Experiment no.	Coded levels			Actual levels		
	X_1	X_2	X_4	X_1 (Watts)	X_2 (second)	X_4 (°C)
11	-1.682	0.000	0.000	600	480	70
12	-1.000	1.000	1.000	680	550	76
13	1.000	1.000	-1.000	920	550	64
14	1.000	-1.000	-1.000	920	410	64
15	1.000	1.000	1.000	920	550	76
16	1.000	-1.000	1.000	920	410	76
17	0.000	0.000	0.000	800	480	70

1.3 Post-treatment RSM

RSM after treatment, the p and r^2 values from a quadratic model of RSM for SRB after 8 weeks of storage at 35°C, choose the significance of FFA, MC, and a_w are highest (i.e. lowest *p*-value) during weeks to investigate. In order to build a model that can validate the experiment results, the actual experimental data was fitted to a second-order polynomial model, and the regression coefficients were obtained.

The generalized second-order polynomial model used in the response surface analysis is as following Eq. 4.

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} X_i X_j \qquad (4)$$

where b_0, b_i, b_{ii}, and b_{ij} are the regression coefficients for the intercept, linear, quadratic and interaction terms, respectively, and X_i, and X_j were the independent variables. The statistical analysis system of Design-Expert is used to generate response surfaces and contour plots while holding 2 variables constant in the second-order polynomial model.

Data on various treatment parameters (e.g. treatment duration, IR wattage, etc.) and data on the resulting experimental data for FFA content, MC and a_w

are fed into a prediction model. The prediction model was validated by putting the data for the various treatments into the prediction model again and comparing the experimental data with the predicted data under the same conditions. The criterion for fitting the efficiency data to the model was the average mean deviation (%E) as shown in Eq.5.

$$E(\%) = \frac{1}{n_e} \sum_{i=1}^{n} \left\| \frac{V_E - V_P}{V_E} \right\| \times 100 \quad (5)$$

Where n_e was the number of experiment data, V_E was the experimental value and V_P was the predicted value (Hossain et al., 2012).

The optimal conditions for stabilizing rice bran with IR, IR-VC and IR-HA were determined numerically with the optimizer module in Design-Expert by looking for the week with the most significant FFA, MC, and a_w results (i.e. minimum p values). The conditions for optimizing MC and a_w were also determined by numerical optimization. Finally, a sample of rice was treated with IR, IR-VC and IR-HA using the optimized conditions recommended by the Design-Expert, and the treated sample was then studied to find its storage stability and shelf-life

2. RB stabilization and optimization by RF

2.1 Pre-treatment RSM

The 3 independent variables RF treated temperature (X_1) treatment duration (X_2) and rice bran sample MC (X_3) and their 5 variation levels for the experiment are shown in table 22. The model construction and data analysis were carried out using Design-Expert software (version 6.0.8, Stat-Ease Inc., Minneapolis, MN). The completed design consisted of 17 experimental treatments (table 23) were performed in triplicate.

Table 22 Independent variables and their levels, from the CCD of RF stabilization method

Variables	Name (units)	Levels				
		-1.682	**-1**	**0**	**1**	**1.682**
X_1	RF treated temperature (°C)	90	98	110	122	130
X_2	Treatment duration (second)	180	253	360	467	540
X_3	Rice bran moisture content (%)	10	12	15	18	20

2.2 RB stabilization and optimization

The RB was stabilized using RF. For each of the 17 treatments (table 23), 300 g of the RB was placed in a uniform layer in a round, plastic container with a diameter of 16 cm. The thickness of the sample layer was 5 cm, so that the bran could absorb the RF evenly. The irradiated RB was divided up 100 g samples (to form treatment triplicates), and each sample was sealed in its own polyethylene bag and then stored in an incubator, at one of the various temperatures specified in the experiment. The 17 treatments of SRB were in storage for 8 weeks at 35°C, and each week a 10 g sample of each treatment was removed for testing. Each removed sample was tested to determine its FFA content, MC, and a_w. At week 0, an initial test of each treatment, un-stored, was also conducted.

Table 23 Coded values and actual values of each independent variables applied in CCD of RF stabilization method

Experiment no.	Coded levels			Actual levels		
	X_1	X_2	X_3	X_1 (°C)	X_2 (second)	X_3 (%)
1	0.000	-1.682	0.000	110	180	15
2	-1.000	-1.000	-1.000	98	253	12
3	0.000	0.000	1.682	110	360	20
4	0.000	0.000	0.000	110	360	15
5	-1.682	0.000	0.000	90	360	15
6	1.000	-1.000	-1.000	122	253	12
7	-1.000	1.000	-1.000	98	467	12
8	1.682	0.000	0.000	130	360	15
9	0.000	0.000	-1.682	110	360	10
10	-1.000	1.000	1.000	98	467	18
11	1.000	-1.000	1.000	122	253	18
12	0.000	0.000	0.000	110	360	15
13	1.000	1.000	-1.000	122	467	12
14	0.000	0.000	0.000	110	360	15
15	1.000	1.000	1.000	122	467	18
16	0.000	1.682	0.000	110	540	15
17	-1.000	-1.000	1.000	98	253	18

2.3 Post-treatment RSM

In order to build a model that can validate the experiment results, the actual experimental data is fitted to a second-order polynomial model, and the regression coefficients are obtained. The generalized second-order polynomial model used in the response surface analysis was as following Eq. 4.

Data on various treatment parameters (RF treated temperature, treatment duration, and sample MC) and data on the resulting experimental data for FFA content, MC and a_w were fed into a prediction model. The prediction model was validated by putting the data for the various treatments (17 treatments set up by RSM) into the prediction model again and comparing the experimental data with the

predicted data under the same conditions. The criterion for fitting the efficiency data to the model was the average mean deviation (%E) following Eq. 5.

The optimal conditions for SRB with RF were determined numerically with the optimizer module in Design-Expert software by looking for the week with the most significant FFA, MC, and a_w results (i.e. minimum *p*-values). The conditions for optimizing MC and a_w were also determined by numerical optimization. Finally, a sample of RB was treated with RF using the optimized conditions recommended by the Design-Expert software, and the treated sample was then studied to find its %yield, storage stability, and shelf-life.

Section 3 Storage stability

The NSRB and SRB (the best condition in section 2 i.e. IR, IR-VC, IR-HA and RF method)) were stored at 35°C for 8 weeks. The samples were collected each week during that period for the determination of FFA content, MC and a_w of each sample and shelf-life and FFA formation rate in RB were investigated. The USRB and SRB sample were collected at 0, 4 and 8 weeks that period for determination of proximate composition, microbial analysis, fatty acid profile, tocopherol, tocotrienol, and oryzanol content.

1. Storage stability

1.1 FFA content

NSRB and SRB were stored at 35°C for 8 weeks, and samples were collected each week during that period. The FFA content of each sample was determined using a standard titration method. (Appendix 1; 1.1 FFA analysis method)

1.2 MC and a_w

NSRB and SRB were stored at 35°C for 8 weeks, and samples were collected each week during that period. MC of each sample is determined by an AOAC (2000) method. The a_w of each sample were analyzed using an AquaLab water activity meter (Decagon, USA). All the data from the quantitative analysis was presented on a dry basis.

1.3 Color measurement

NSRB and SRB were tested for color brightness (L*, a* and b*) (ColorFlex, HunterLab, USA) every week during the 8 weeks of storage, and the ΔE was calculated as: $\Delta E = [(L_1^*-L_2^*)^2+(a_1^*-a_2^*)^2+ (b_1^*-b_2^*)^2]^{1/2}$.

1.4 Proximate analysis

NSRB and SRB underwent proximate analysis 3 times, at weeks 0, 4, and 8. Crude fat, protein, ash, and dietary fiber content were determined by methods 30-10, 46-12, 08-01 and 32-07 of AOAC (2000) respectively. All measurements were performed in triplicate and expressed as dry weight.

1.5 Microbial analysis

The TPC and yeast/mold content of the NSRB and SRB were analyzed using the BAM (2000) method.

1.6 FA composition

The FA composition of the NSRB and SRB were determined by GC-FID followed analysis method in Appendix 1, 2 (FA composition analysis method).

1.7 Tocols and oryzanol

Tocols (γ-Tocotrienol, α-tocopheral) and γ-oryzanol content of the NSRB and SRB were determined using the methods of Pestana-Bauer, V.R. et al. (2012) with some modifications followed in appendix 1, 3 (tocols and oryzanol content analysis method).

2. Shelf life and FFA formation rate

2.1 Predicting shelf life

NSRB and SRB were stored at 25°C, 35°C, and 45°C for 8 weeks, and samples were collected each week during that period. The shelf-life of each sample was estimated using the Q-10 method, based on the FFA content did not over 5%. FFA content of each sample was determined using a standard titration method (Kim, et al., 2014; AOCS, 2004). The results were plotted in a graph, with storage duration on the x-axis and %FFA content on the y-axis, using Microsoft Excel (2010). Using the scatter chart tool of Excel, added trending linearity equation to display linear equation on the chart. The estimating the shelf-life calculations were based on linear regression, $y = mx + c$ when y was 5 %FFA content and x was shelf-life of RB storage. The shelf-life predicted of NSRB and SRB was calculated following Eq. 6.

$$Q\text{-}10^{(\Delta/10)} = \text{Shelf-life time at T / Shelf-life at T+10} \qquad (6)$$

The shelf-life prediction at room temperature (30°C) of NSRB and SRB was calculated following Eq. 7.

$$Q\text{-}10^{(\Delta/10)} = \text{time prediction / time accelerated condition} \quad (7)$$

Where: Δ was mean the difference temperature of prediction and experiment

2.2 FFA formation rate

FFA formation rate was estimated by the zero order kinetic model (Steele, 2004) following Eq. 8.

$$A_S = A_0 - k_s t_s \text{ or } k_S = (A_0 - A_s)/t_s \quad (8)$$

Where; A_s: FFA content of rice bran at end of shelf life (5%)

A_o: The initial FFA content of rice bran

k_s : The FFA formation rate

t_s : The shelf life

Section 4 The effects of IR, IR-VC, IR-HA and RF stabilization methods on crude RBO yield, qualities and storage stability

The optimized stabilization RB conditions by IR, IR-VC, IR-HA, and RF heat treatment were extracted by screw-press and investigated % yield of crude RBO. The NSRBO and SRBO samples were stored at 35°C for 8 weeks. The samples were collected each week during that period for the determination of FFA content, AV and PV of each sample. Shelf-life and the formation of FFA in RBO were also investigated. The NSRBO and SRBO sample were collected at 0, 4, and 8 week period for determination of PV, FA composition, tocotrienol, tocopherol, and oryzanol content.

1. RBO yield

The NSRBO and SRBO were obtained by pressing 5 kg with a screw type expeller (Karaerler NF500, Turkey). This operation was carried out 3 times and the extracted oil was weighed. Fine particles in the pressed oil were separated by centrifugation at 9000 rpm (7.245g) for 6 min (Hettich Zentrifugen Universal 32R,

Germany). The supernatant was filtered with a double layer of What-man filter paper no.1. The oil extraction yield was defined as grams of RBO per hundred grams of RB (g RBO /100 g RB).

2. Storage stability

2.1 AV

NSRBO and SRBO were stored at 35°C for 8 weeks, and samples were collected each week during that period. The AV of each sample was determined using a standard titration method (AOAC, 1999) were shown in appendix 1, 4 AV analysis method.

2.2 PV

NSRBO and SRBO underwent proximate analysis 3 times, at weeks 0, 4, and 8. PV was determined by methods IUPAC 2.501 (as amended), AOCS Cd 8b - 90 (97) or ISO 3961: 1998, which shown in appendix1, 5 peroxide value analysis method.

2.3 FA composition

The FA composition of the NSRBO and SRBO were determined by GC-FID analysis method, which shown in appendix 1, 2) FA composition analysis method.

2.4 Tocopheral, Tocotrienol and Oryzanol

α-Tocopheal, γ-tocotrienol (Tocols) and γ-oryzanol content of the NSRBO and SRBO were determined using HPLC methods, which shown in appendix 1, 3 tocols and oryzanol content analysis method.

3. Shelf life and FFA formation rate

3.1 Predicting shelf life

NSRBO and SRBO were stored at 25°C and 35°C for 8 weeks, and samples are collected each week during that period. The shelf life of each sample was estimated using the Q-10 method, based on the FFA content did not over %5. The estimating the shelf-life calculations were based on linear regression: $y = mx + c$, when y was 5 %FFA content and x was shelf-life of RBO storage. The shelf-life predicted of NSRBO and SRBO was calculated following Eq. 9.

$$Q\text{-}10^{(\Delta/10)} = \text{Shelf-life time at T / Shelf-life at T+10} \quad (9)$$

The shelf-life prediction at room temperature (30°C) of NSRBO and SRBO was calculated following Eq. 10.

$$Q\text{-}10^{(\Delta/10)} = \text{time prediction / time accelerated condition} \quad (10)$$

Where: Δ was mean the difference temperature of prediction and experiment

3.2 The FFA formation rate

The FFA formation rate was estimated by the zero order kinetic model (Steele, 2004) following Eq. 11.

$$A_S = A_0 - k_S t_S \text{ or } k_S = (A_0 - A_s)/t_s \quad (11)$$

Where; A_s: FFA content of rice bran oil at end of shelf life (5%)

A_o: The initial FFA content of rice bran oil

k_s : The formation rate of FFA content

t_s : The shelf-life

Statistical analysis

All data are expressed as the means of triplicate determinations with individual duplication (n = 6). Analysis of variance (ANOVA) was carried out using SPSS software, version 14.0 (SPSS Inc., Chicago, USA), and the determination of significant differences among treatment means was done by Duncan's multiple range tests ($p \leq 0.05$).

CHAPTER IV

RESULTS AND DISCUSSIONS

This chapter provides the results and discussions of the study which were divided into 4 sections as follows:

Section 1 Fresh RB analysis

The qualities of this study's fresh RB shows in table 24. The color of the fresh RB was light brown (L* =69.55, a*=7.25 and b*=19.09), similar to the light brown color reported by Moongngarm et al. (2012) for their fresh RB (Khao Dok Mali 105 cultivar from Thailand).

The natural a_w value of the NSRB was 0.636, which was low enough to somewhat inhibit bacteria growth but less effective at inhibiting yeast and mold. Enzyme activity was certainly possible at this a_w level (Fennema, 1976). The measured content of the crude ash, crude protein, crude fat and crude fiber were 6.81%, 12.26%, 14.86%, and 4.37% (dry basis), respectively. These values were different from the results of Moonggarm et al. (2012), who reported values of 10.65%, 13.66%, 18.80%, and 12.48%, respectively for RB from Thailand. This was also different from the results of Yilmaz (2016), who reported values of 10.02-12.19%, 15.50-16.48%, 18.15-21.74%, and 16.06-42.19%, respectively for RB from Turkey. These differences were to be expected since the composition of fresh RB naturally varies depending on such factors as the rice cultivar and the particular milling and manufacturing processes employed. The γ-tocotrienol, α-tocopherol and γ-oryzanol contents of the fresh RB were 331 mg/kg, 104.37mg/kg and 994.87mg/kg (dry basis), respectively compared with Revilla et al. (2009), who reported values of 62 mg/kg, 44 mg/kg and 1260 mg/kg, respectively, and Moongnarm et al. (2012), who reported 46.12 mg/kg, 40.92 mg/kg, and 3,500 mg/kg, respectively. Pestana-Bauer et al. (2012) reported (only) α-tocopherol and γ-oryzanol contents of 161mg/kg and 1,240 mg/kg, respectively. These differences in the results reflected how the composition of FRB naturally varies by cultivar, source, production, and processing. The milling process is particularly

influential in affecting the RB composition, as it would vary greatly from one mill to the next (Yilmaz, 2016). The RB composition, in turn, would affect the hydrolytic deterioration behavior of the RB. Initial microbial testing of the untreated fresh RB showed a TPC level of $7.86\pm0.54\times10^3$ CFU/g and a yeast & mold level of $3.20\pm0.50\times10^2$ CFU/g.

Table 24 Color and composition of fresh rice bran

Item	Value	Unit
Color		
L*	69.55±0.02	-
a*	7.25±0.04	-
b*	19.09±0.06	-
Chemical composition		
a_w	0.636±0.008	-
Moisture	7.79±0.03	% (db)
Ash	6.81±0.11	% (db)
Protein	12.26±0.05	% (db)
Fat	14.86±0.93	% (db)
Fiber	4.37±0.08	% (db)
Carbohydrate	53.91±0.00	% (db)
FFA	1.38±0.04	% (as Oleic acid, db)
Tocols and oryzanol		
γ-Tocotrienol	331.20±6.05	mg/kg (db)
α-Tocopherol	104.37±0.99	mg/kg (db)
γ-Oryzanol	994.87±11.05	mg/kg (db)
Microbiological		
TPC	$7.86\pm0.54\times10^3$	CFU/g
Yeast and mold	$3.20\pm0.50\times10^2$	CFU/g

Section 2 RB stabilization and optimization

RB stabilization and optimization by IR heat treatments (IR, IR-VC, and IR-HA) and RF heat treatment were investigated for the FFA content, MC and a_w in SRB. The application of RSM and CCD for the optimization of the lowest FFA, MC, and a_w in SRB were used for the IR heat treatment (IR with parameters, i.e., IR wattage, treatment duration, vacuum strength, and hot air temperature) and RF heat treatment (RF with parameters, i.e., RF treatment temperature, treatment duration, and RB moisture content). The best stabilized condition was selected and applied in sections 3 and 4. The results of the data of the experiments and discussion are as follows.

1. RB stabilization and optimization by IR method

1.1 Pre-treatment RSM

The average and standard deviation of the %FFA content, MC, and a_w results in the SRB each week from the experiments during the storage duration. These shows in tables 25-27, respectively. This was put on the RSM program to analyze the F-value, *p*-value, lack of fit, R^2 value, and %CV.

1.2 RSM after treatment

The highest significance of the indicated result (%FFA, %MC, and a_w) in the storage week in the SRB treatment was the target of the investigation. The sample of storage week with the highest F-value and lowest *p*-value was selected, while the lack of fit should not be significant, the R^2 values should be high (> 0.90), and the %CV should be low (<10%). Table 25 shows the quadratic model of the RSM for the FFA content in the SRB of each week; a total of 8 weeks storage at 35°C. The significance of the FFA content was highest (i.e. lowest *p*-value) during week 4, where the F-value, *p*-value, lack of fit, R^2, and %CV were 5.37, 0.0440, 04643, 0.8431, and 4.91, respectively. The low coefficient of the variation (%CV=4.91) clearly indicated the high precision and reliability of the experimental values (Krishma et al., 2013).

Table 25 FFA content in SRB by IR method and the analysis of variance table of ANOVA for RSM each weeks from experiments

Experiments no.	Levels		FFA (% as Oleic acid)								
	Infrared wattage (W)	Treatment duration (second)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	800	480	1.99±0.04	3.89±0.08	4.84±0.12	5.51±0.04	6.00±0.07	7.00±0.07	7.19±0.07	7.45±0.04	7.50±0.04
2	1000	480	1.97±0.04	3.61±0.04	4.06±0.07	4.30±0.04	5.27±0.04	5.55±0.04	6.47±0.04	6.57±0.01	7.10±0.07
3	800	480	1.90±0.03	3.01±0.04	3.70±0.07	3.73±0.07	4.13±0.04	4.58±0.11	5.44±0.04	5.63±0.07	5.73±0.09
4	940	565	2.02±0.05	3.54±0.02	4.13±0.04	4.34±0.04	5.13±0.07	5.27±0.07	6.04±0.07	5.82±0.08	6.69±0.04
5	600	480	1.76±0.04	3.18±0.04	3.56±0.05	3.87±0.07	4.41±0.07	5.06±0.02	5.54±.0.11	5.49±0.04	6.02±0.11
6	660	565	1.88±0.04	3.77±0.07	3.99±0.04	4.84±0.04	5.25±0.07	4.77±0.05	6.57±0.04	6.50±0.04	6.78±0.12
7	800	480	1.76±0.07	3.30±0.05	3.92±0.07	4.32±0.07	4.98±0.11	5.55±0.07	5.85±0.07	6.28±0.07	6.52±0.04
8	660	395	1.97±0.07	3.04±0.11	3.47±0.07	4.01±0.07	4.84±0.04	5.27±0.04	5.59±0.04	5.92±0.11	6.38±0.07
9	940	395	2.02±0.04	3.56±0.06	3.85±0.04	4.30±0.04	5.17±0.02	5.06±0.12	5.97±0.07	6.40±0.12	6.57±0.04
10	800	600	1.88±0.08	3.51±0.07	3.56±0.11	4.18±0.04	4.79±0.07	5.17±0.07	5.51±0.04	5.97±0.04	6.50±0.07
11	800	360	1.80±0.04	2.99±0.05	3.06±0.04	3.80±0.12	4.15±0.11	4.84±0.04	4.91±0.03	5.63±0.07	5.80±0.07
	F-value		0.88	1.20	1.26	3.05	5.37	1.49	1.77	3.15	2.80
	p-value		0.5549	0.4095	0.4042	0.1229	0.0440*	0.3373	0.2734	0.1168	0.1417
	Lack of Fit		0.8447	0.7457	0.7622	0.3269	0.4643	0.1732	0.5835	0.9504	0.6835
	R^2		0.4676	0.5536	0.5568	0.7533	0.8431	0.5977	0.6388	0.7590	0.7367
	%CV		5.03	7.59	7.83	5.62	4.91	5.75	7.18	4.57	4.84

Table 26 MC in SRB by IR method and the analysis of variance table of ANOVA for RSM each weeks from experiments

Experiments no.	Levels		Moisture content (%)								
	Infrared wattage (W)	Treatment duration (second)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	800	480	6.33±0.25	5.80±0.21	6.22±0.09	5.44±0.05	6.00±0.05	6.49±0.09	5.69±0.15	6.18±0.09	6.65±0.19
2	1000	480	4.40±0.13	4.19±0.05	4.91±0.19	4.20±0.07	5.17±0.13	5.47±0.07	4.66±0.03	5.23±0.15	5.39±0.28
3	800	480	5.30±0.38	4.68±0.16	5.11±0.18	4.79±0.09	5.77±0.03	5.92±0.06	5.16±0.07	5.52±0.04	5.93±0.42
4	940	565	4.57±0.16	3.94±0.27	4.65±0.07	4.18±0.10	4.83±0.07	5.27±0.11	4.55±0.19	5.06±0.21	5.26±0.32
5	600	480	5.68±0.19	5.52±0.10	5.76±0.09	5.35±0.08	5.87±0.15	6.39±0.05	5.50±0.18	6.07±0.22	6.33±0.28
6	660	565	4.67±0.20	4.76±0.18	5.36±0.06	5.00±0.03	5.28±0.14	6.16±0.09	5.09±0.12	5.69±0.07	6.10±0.25
7	800	480	4.70±0.11	4.48±0.07	5.28±0.09	4.43±0.10	5.17±0.12	5.88±0.09	4.84±0.04	5.07±0.14	5.74±0.17
8	660	395	6.19±0.07	5.61±0.08	6.28±0.20	5.27±0.10	6.23±0.12	6.34±0.06	5.60±0.12	5.87±0.26	6.47±0.15
9	940	395	4.92±0.22	5.76±0.06	5.41±0.16	4.82±0.09	5.62±0.16	5.84±0.12	5.13±0.15	5.51±0.14	5.85±0.04
10	800	600	4.30±0.72	3.94±0.06	4.81±0.16	4.32±0.02	5.07±0.10	5.42±0.04	4.81±0.06	5.10±0.20	5.37±0.12
11	800	360	5.90±0.26	5.62±0.18	5.93±0.28	5.21±0.12	5.91±0.08	6.11±0.12	5.49±0.08	6.24±0.69	5.86±0.24
	F-value		2.88	3.32	3.16	3.03	4.07	5.88	3.12	1.59	2.89
	p-value		0.1363	0.1071	0.1163	0.1247	0.0719	0.0371*	0.1186	0.3123	0.1346
	Lack of Fit		0.9947	0.9035	0.9918	0.9848	0.9702	0.9870	0.9977	0.9091	0.9455
	R^2		0.7422	0.7683	0.7595	0.7517	0.8027	0.5977	0.7574	0.6135	0.7429
	%CV		10.23	10.26	7.11	6.93	5.15	5.75	5.33	7.07	5.56

Table 27 The a_w in SRB by IR method and the analysis of variance table of ANOVA for RSM each weeks from experiments

Experiments no.	Levels		a_w								
	Infrared wattage (W)	Treatment duration (second)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	800	480	0.403±0.003	0.465±0.003	0.510±0.001	0.434±0.002	0.522±0.001	0.621±0.001	0.550±0.002	0.540±0.002	0.548±0.002
2	1000	480	0.346±0.001	0.386±0.003	0.438±0.001	0.372±0.001	0.482±0.001	0.486±0.003	0.531±0.002	0.505±0.004	0.531±0.002
3	800	480	0.342±0.001	0.404±0.003	0.460±0.002	0.391±0.001	0.526±0.004	0.520±0.001	0.540±0.002	0.516±0.001	0.535±0.002
4	940	565	0.331±0.001	0.354±0.005	0.433±0.003	0.341±0.002	0.454±0.002	0.486±0.002	0.521±0.001	0.498±0.002	0.511±0.003
5	600	480	0.405±0.002	0.463±0.003	0.502±0.002	0.403±0.002	0.499±0.001	0.521±0.001	0.547±0.001	0.523±0.002	0.535±0.003
6	660	565	0.361±0.002	0.423±0.003	0.463±0.003	0.366±0.001	0.476±0.003	0.517±0.001	0.538±0.002	0.519±0.001	0.531±0.001
7	800	480	0.353±0.002	0.394±0.002	0.480±0.002	0.406±0.004	0.486±0.003	0.512±0.003	0.523±0.002	0.517±0.001	0.526±0.002
8	660	395	0.415±0.002	0.424±0.001	0.514±0.002	0.394±0.003	0.504±0.002	0.530±0.003	0.544±0.003	0.527±0.001	0.532±0.003
9	940	395	0.397±0.001	0.385±0.003	0.489±0.003	0.365±0.002	0.484±0.004	0.512±0.003	0.524±0.005	0.517±0.001	0.530±0.002
10	800	600	0.305±0.001	0.357±0.001	0.430±0.002	0.395±0.004	0.476±0.003	0.500±0.002	0.524±0.002	0.510±0.001	0.530±0.002
11	800	360	0.478±0.002	0.443±0.003	0.500±0.002	0.373±0.004	0.517±0.003	0.515±0.002	0.520±0.003	0.523±0.002	0.534±0.002
	F-value		0.88	1.20	1.26	3.05	2.53	1.49	1.77	3.15	2.80
	p-value		0.5549	0.4095	0.4042	0.1229	0.1652	0.3373	0.2734	0.1168	0.1417
	Lack of Fit		0.8447	0.7457	0.7622	0.3269	0.8228	0.1732	0.5835	0.9504	0.6835
	R^2		0.4676	0.5536	0.5568	0.7533	0.7171	0.5977	0.6388	0.7590	0.7367
	%CV		5.03	7.59	7.83	5.62	3.41	5.75	7.18	4.57	4.84

The lowest *p*-value of the MC as seen in table 26 was 0.0371 at week 5, hereupon the F-value, lack of fit, R^2, and %CV were 5.88, 0.9870, 0.5977, and 5.75, respectively. The non-significance for the a_w in table 27 with the lowest *p*-value was at week 4, and the *p*-value, F-value, lack of fit, R^2, and %CV were 0.1652, 0.8228, 0.7171, and 3.41, respectively. However, the main indicator for the analysis of variance (ANOVA) table for the response surface quadratic model each week from the experiments was the FFA, which was significantly the lowest at week 4. The week 4 values were acceptable, applicable, and accurate for use in the polynomial model to optimize the IR conditions. The ANOVA of the response surface quadratic model of the FFA content, MC, and a_w were investigated together with the F-value, *p*-value, and R^2 at week 4 of the storage of the SRB. The results are shown in table 28.

Table 28 Variable and factor's F, *p*-value and R^2 in term of % FFA content, MC and a_w of ANOVA analysis for a RSM at week 4 by IR stabilization method

Variables /Factors	Responses					
	FFA		MC		a_w	
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Model	5.37	0.0444*	4.07	0.0749^{ns}	3.53	0.1652^{ns}
X_1	14.23	0.0130*	6.46	0.0518^{ns}	1.92	0.2248^{ns}
X_2	8.09	0.0361*	13.19	0.0150^{*}	5.93	0.0590^{ns}
X_1X_2	2.28	0.1914^{ns}	0.079	0.7902^{ns}	0.004	0.9550^{ns}
X_1^2	0.16	0.7060^{ns}	0.31	0.6010^{ns}	3.80	0.1087^{ns}
X_2^2	3.35	0.1267^{ns}	0.47	0.5233^{ns}	2.39	0.1822^{ns}
Lack of Fit	1.29	0.4643^{ns}	0.071	0.9702^{ns}	0.31	0.8228^{ns}
R^2	0.8431		0.8027		0.7171	
Adj. R^2	0.6861		0.6053		0.4341	

Note: X_1 = infrared wattage, X_2 = treatment duration

*= significant $p<0.05$, ^{ns}= non-significant

To evaluate the significance of any regression model in predicting the effects of a set of independent variables on the response variables, the F-value test

has to be conducted. The F-distribution test was a probability distribution test used to compare variance by examining their ratios. The F-ratio value in the ANOVA table in this study, therefore, was the ratio of the model mean square to the appropriate error mean square (table 28). Krishma et al. (2013) reported that the larger the ratio, the larger the F-value, and the more likely that the variance distributed by the observed models would be statistically larger than the random error. The F-value reported for each response variable was 5.37, 4.07, and 3.53, for the FFA, MC, and a_w, respectively. The large F-values reported for the model coefficients indicated that the variation in the mineral content of the IR treatment could be explained by the regression models. It was also clear that the linear and interaction terms were highly significant. The *p*-values in this study were 0.0444, 0.0749, and 0.1652, respectively. This indicated that the models of the FFA content were significant. The lack of fit indicated the variation of the data around the fitted model. If the model was significant, then it did not fit the data well (Ghafari et al., 2009; Han et al., 2016).

The lack of fit of the FFA, MC, and a_w presented in table 28 displayed non- significance (0.4643, 0.9702, and 0.8228, respectively); hence, the IR wattage responses suggested the quadratic polynomial models could be affected and used to predict the models. These results corresponded with the findings of González-Centeno et al. (2014), who reported that the significant regression and non-significant lack of fit of a model would fit to the experimental data, which were effectively predicted within the system.

The R^2 values for the FFA, MC, and a_w model were found as 0.8431, 0.8027and 0.7171, respectively. This indicated the variability of the parameters by the models, which demonstrated the prediction values by the quadratic polynomial equations and showed a strong correlation with the actual experimental values with Pearson's correlation coefficient (Hossain et al., 2012). These models were well-adapted to respond and effectively predict the FFA, MC, and a_w from the SRB. In addition, the adjusted determination coefficient (Adj-R^2) for the FFA and MC was 0.6861 and 0.6053, respectively. Thus, this further confirmed that the variable was adequate and strengthened the general availability and accuracy of the polynomial model (Han et al., 2016). The Adj-R^2 of the a_w value was 0.4341, which was particularly low for strengthening the general availability and accuracy of the polynomial model.

The FFA regression equation in the SRB relating to the actual levels of the infrared wattage (X_1) and treatment duration (X_2) is shown in Eq. 18.

$$FFA = - 4.01898 + 0.011806(X_1) + 0.024811(X_2) - 0.000004(X_1)(X_2) - 0.0000076(X_1)^2 - 0.000025(X_2)^2 \quad (18)$$

The three-dimensional response surface plots were represented by the regression equation indicating the relationship between the dependent and independent variables. The response surface plots had differing shapes indicating whether the variables or mutual interactions were significant or not (Liu et al., 2013). The FFA response surface plots based on Eq. 18 are shown in Figure 11.

The IR wattage and treatment duration affected the FFA content surface plot that showed an increase in the IR wattage and decrease in the treatment duration of the FFA content. This finding was similar to that reported by Irakli et al. (2018); Yamaz et al. (2018); Yılmaz (2016), who observed that the obtained high IR wattage and treatment duration decreased the FFA content. A similar study was reported by Malekian et al. (2000) that IR wattage had a dominant effect on the FFA content of the RB and that the FFA content decreased with the increasing IR wattage almost linearly from 200 W to 700 W, corresponding to the FFA contents of 28.91-10.26%, respectively. In the current study, the treatment time significantly affected the FFA content ($p < 0.01$). A short treatment time was found to be insufficient to inactivate the lipase. The FFA content decreased with the increasing treatment times from 360 to 600 seconds at the same IR wattage. The experiment did not use treatment times longer than 600 seconds because above 600 seconds at 1,000 W, there were undesirable changes in the RB such as, browning of the color and a cooked/burnt-like odor (Malekian et al., 2000).

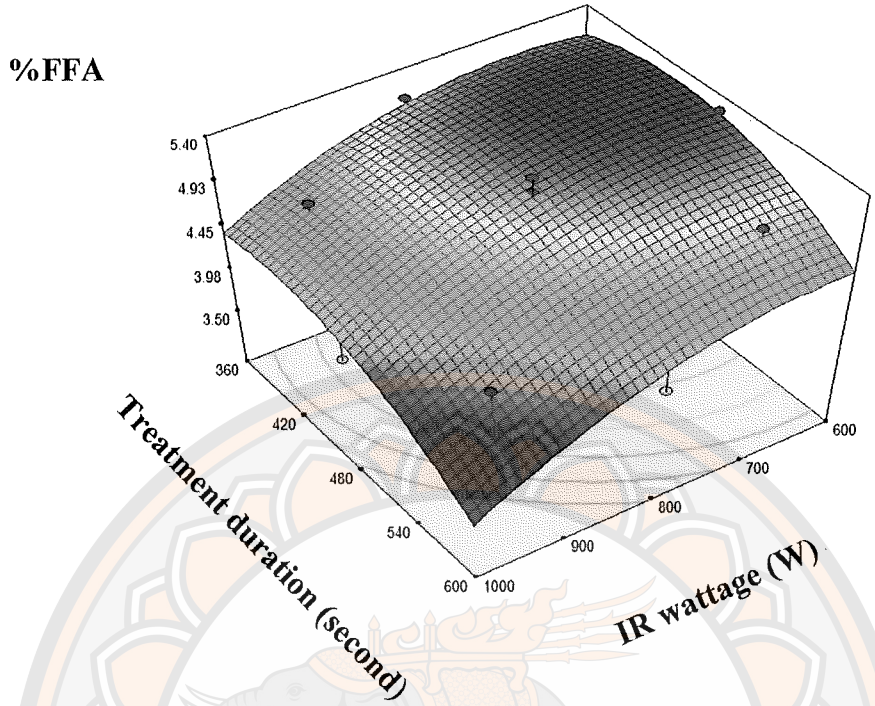


Figure 12 Response surface and contour plots for FFA in SRB by IR method as a function of treatment duration and IR wattage

The IR wattages variable of the IR wattage non-significantly influenced the MC with a *p*-value of 0.0519. Still, the treatment duration variable was significant to the MC with a *p*-value of 0.0150. The treatment duration significantly reduced the MC in the SRB. However, all interactions and quadratic coefficient terms were not significant since the *p*-values were less than 0.05. The second-order polynomial equation of the MC was shown in Eq. 19.

$$MC = 5.67644 + 0.00194(X_1) + 0.00397(X_2) + 0.0000034(X_1)(X_2) - 0.00000336(X_1)^2 - 0.0000114(X_2)^2 \quad (19)$$

The three-dimensional surface and contour plots for the effects of the factors on the MC in the SRB based on Eq. 19 shows in Figure 13.

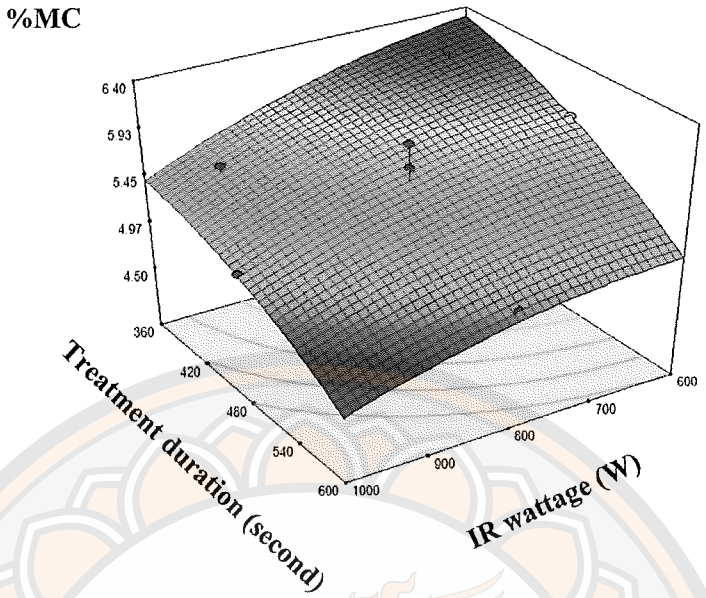


Figure 13 Response surface and contour plots for MC in SRB by IR method as a function of treatment duration and IR wattage

The IR wattage and treatment duration affected by the MC surface plot showed that an increase in the IR wattage and treatment duration decreased the MC. These results were in agreement with Wang et al. (2017), who found that after the IR heating, the tempering treatment significantly improved the moisture removal during natural cooling. For one-pass drying, the moisture removal during natural cooling increased with the rise of the tempering time. Thus, the tempering process reduced the moisture gradient in the rice kernels and allowed the moisture to equilibrate before the rice kernels were cooled. Therefore, the tempering process was a critical step to increase the moisture removal during the cooling of rough rice. This confirmed that IR heating followed by tempering and natural cooling could achieve the efficiency of high heating and high drying for RB.

The a_w linear coefficients of the independence variable in the SRB from the dependence variable (model, IR wattage: X_1, and treatment duration: X_2) were non-significant with *p*-values of 0.1652, 0.2248 and 0.0590, respectively. The quadratic term coefficients of X_1X_2, X_1^2, X_2^2, and X_3^2 were non-significant ($p > 0.05$). The experimental data allowed the development of mathematical equations resulting

in the predicted results. The second-order polynomial equation of a_w was shown in Eq. 20.

$$a_w = -0.17110 + 0.00107(X_1) + 0.00132(X_2) - 0.00000004(X_1)(X_2) - 0.00000069(X_1)^2 - 0.0000015(X_2)^2 \quad (20)$$

The three-dimensional surface and contour plots for the effects of the factors on the a_w in the SRB based on Eq. 20 shows in Figure 14, and the figure shows the effect of the IR wattage and treatment duration on the a_w.

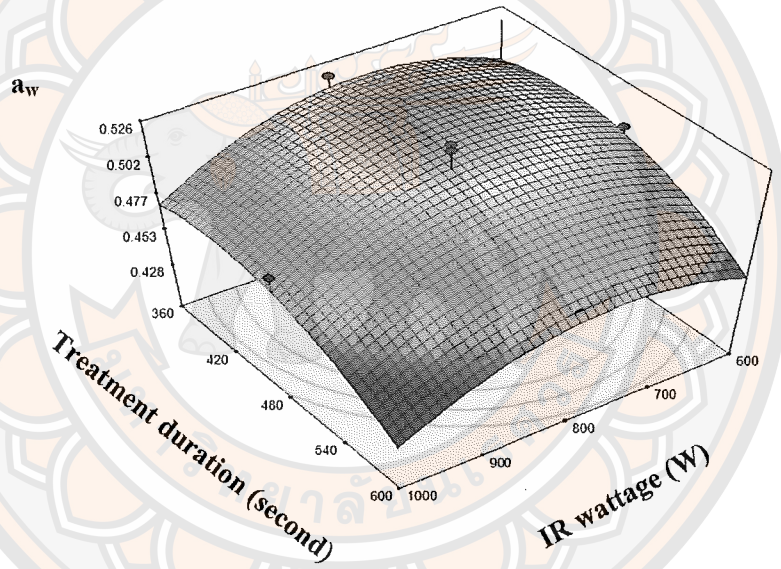


Figure 14 Response surface and contour plots for a_w in SRB by IR method as a function of treatment duration and IR wattage

The IR wattage and treatment duration affected the a_w, which the surface plot showed that an increase in the IR wattage and treatment duration decreased the a_w. However, the IR wattage and treatment duration did not significantly affect the a_w (p-values = 0.1652 and 0.2248, respectively). Figure 14 shows the highest IR wattage at 1000 watts, and the 600-second treatment duration produced the lowest a_w.

The validation of the prediction equation of the FFA, MC, and a_w obtained from the RSM was investigated by the RB samples from Sawangthawarn

Enterprises, Kamphaeng-Phet province, Thailand. A new lot of RB samples was used to be stabilized using IR with various conditions. The high condition (1,000 W of IR wattage and 600 seconds of treatment duration), medium condition (800 W of IR wattage and 480 seconds of treatment duration), and low conditions (600 of IR wattage and 360 seconds of treatment duration) were validated and determined the FFA, MC and a_w.

The results of the validation with the FFA, MC, and a_w had a high difference between the experimental and predicted values. It also had a high %E, which the FFA %E values were 37%, 29%, and 32% at a low, medium, and high condition, respectively. The MC %E values were 32%, 23%, and 26% at a low, medium, and high condition, respectively. The a_w %E values were 25%, 18%, and 21% at a low, medium, and high condition, respectively. This was very high and non-acceptable for the validation of the prediction of the equation tolerances. The different sampling time (approximately three months), different MC, and different composition in the RB samples resulted in an unsuccessful validation of the stabilization process. Therefore, only the calculation reports of the results using the experimental results to compare them with were used to predict the values (Table 29).

The experimental data allowed the development of mathematical equations (Eq. 18-20) to show the predicted data results of the FFA, MC, and a_w using the RSM model (table 29). The prediction model was validated by putting the data for the various treatments into the prediction model again and comparing the experimental data with the predicted data under the same conditions. The criterion for fitting the efficiency data of the FFA, MC, and a_w model calculated the %E differences between the experimental and predicted data (Eq. 2) (Hossain et al., 2012).

Table 29 Experimental design of CCD, actual experiment value, and predicted value of FFA content, MC and a_w of SRB by IR method

Experi-ments no.	Factors		Responses					
	X_1	X_2	FFA		MC		a_w	
	Infrared wattage (W)	Treatment duration (second)	Experi-mental	Predi-cted	Experi-mental	Predi-cted	Experi-mental	Predi-cted
1	800	480	5.27	5.18	6.00	5.66	0.522	0.516
2	1000	480	4.13	4.42	5.17	5.17	0.482	0.478
3	800	480	5.13	5.18	5.77	5.66	0.526	0.516
4	940	565	4.41	4.28	4.83	4.94	0.454	0.471
5	600	480	5.25	5.33	5.87	5.89	0.499	0.499
6	660	565	4.98	5.01	5.28	5.36	0.476	0.487
7	800	480	4.84	5.18	5.17	5.66	0.486	0.516
8	660	395	5.17	5.32	6.23	6.17	0.504	0.511
9	940	395	4.79	4.78	5.62	5.58	0.484	0.498
10	800	600	4.15	4.53	5.07	4.99	0.476	0.476
11	800	360	5.10	5.10	5.91	6.01	0.517	0.513
	%E		3.08		2.38		1.93	

Table 29 shows the %E value, which was an indication of the predicted values in close agreement with the experimental values. This had variations between the predicted and experimental values obtained for the FFA content, and the MC and a_w were within an acceptable error range as depicted by the average mean deviation (E%). Therefore, the predictive performance of the established model may be considered acceptable. Hossain et al. (2012) studied the optimization of the ultrasound-assisted extraction (UAE) of antioxidant compounds from marjoram (*Origanum majorana* L.) using RSM. The study showed the %E between the predicted and experimental values of the parameters tested at optimal UAE conditions compared to the conventional solid/liquid extraction values. The average mean deviation between the predicted and experimental values of the optimal UAE had a 0.45-1.55 %E. In the current study, the calculated %E differed from the experimental data in which the FFA, MC, and a_w were 3.08, 2.38, and 1.93, respectively. The different

process time (approximately eight hours) and the unstable instrument over a long time of the experiment resulted in an unsuccessful validation of the stabilization process. The experiment shown in this study had a higher %E than Hossain et al. (2012).

1.3 Optimization of RB stabilization by IR

The FFA content was the main factor of the RSM software to suggest the optimum conditions for the SRB by the IR treatment method. The lowest FFA content as the most important criterion combined the MC and a_w to the RSM software to give the most and completed optimized conditions. In the RSM software, the designation weight of the indicators here was the FFA MC, and a_w should be co-determined. The FFA was used as the most important indicator; the level of importance was *****, while the MC and a_w were less influential than the FFA; the level of importance was ***.

The combination of the optimized conditions of the FFA content, MC and a_w (at week 4) as calculated by the RSM in Design-Expert software is shown in table 30. The optimum values of the process variables were obtained for the maximum desirability function of 1.000, 1.000, and 1.000, respectively, which the optimal condition of the IR wattage and treatment duration were 993 W and 598 seconds, respectively. The quantitative value of the optimized conditions for the FFA content, MC, and a_w at this condition were 3.59%, 4.58%, and 0.432, respectively. The desirability profiles for the optimum conditions suggested by the RSM are shown in table 30. The selected optimization condition depended on the desirability value from each suggested solution.

The desirability values showed that the selected conditions were suitable for the optimum responses (FFA, MC, and a_w) of the IR wattage and treatment duration. Therefore, the suggested IR wattage and treatment duration was 998 W and 593 seconds, respectively. The desirability of the suggested optimized condition may depend on the data obtained from the 11 experimental designs and/or from the analysis of each response. However, the desirability of all the recommended solutions was really strong, as the value was 1.000. The optimal condition should be chosen with the result of desirability above 0.8 (Nazmi, & Sarbon, 2019).

Table 30 Optimized conditions for IR stabilization in term of FFA content, MC and a_w

Variables	Optimized conditions for FFA content (%)	Optimized conditions for MC (%)	Optimized conditions for a_w	Optimized conditions for % FFA, MC (%) and a_w
IR wattage (W)	993	980	994	993
Treatment duration (seconds)	599	596	593	598
Quantities of dependent variables as predicted by RSM software	3.67 (% as Oleic acid)	4.63 (%)	0.434	3.59%, 4.58%, 0.432
Desirability	1.000	1.000	1.000	1.000

Note: The importance of % FFA, MC, and a_w with optimization combination calculated by RSM in Design- Expert were *****, ***, and *** respectively

2. RB stabilization and optimization by IR-VC method

2.1 Pre-treatment RSM

The current study of the RSM with three independent variables comprised X_1 (IR wattage: W), X_2 (treatment duration: seconds), and X_3 (vacuum strength: mmHg). The completed design consisted of 17 experimental treatments. The %FFA content, MC, and a_w results in the SRB each week from the experiments on the storage duration shows in tables 30-32, respectively.

The highest significance of the indicated result (%FFA, %MC, and a_w) at the storage week in the SRB treatment was the target for the investigation and selected for the next section (storage stability study). The storage week with the highest F-value and lowest *p*-value was most important, as that week should have a non-significant lack of fit, the highest R^2 values, and lowest %CV. Table 33 shows the quadratic model of the RSM for the SRB after eight weeks of storage at 35°C for the FFA content. The significance of the FFA content was the highest (i.e. lowest *p*-value) during week 4, which showed the F-value, *p*-value, lack of fit, R^2, and %CV of 9.46, 0.0036, 0.4685, 0.9241, and 9.15, respectively.

Table 31 FFA content in SRB by IR-VC method and the analysis of variance table of ANOVA for RSM each weeks from experiments

Experiments no.	Levels			FFA content (% as oleic acid)								
	Infrared wattage (W)	Treatment duration (second)	Vacuum strength (mmHg)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	680	550	490	1.38±0.04	2.52±0.15	2.63±0.07	2.90±0.04	3.32±0.04	3.32±0.04	3.76±0.04	3.93±0.04	4.19±0.04
2	800	480	450	1.52±0.08	2.33±0.11	2.40±0.08	2.73±0.04	2.90±0.04	3.09±0.04	3.45±0.07	3.64±0.04	4.27±0.04
3	920	550	490	1.54±0.11	1.71±0.07	1.42±0.07	1.66±0.04	1.73±0.04	1.85±0.07	1.82±0.04	2.30±0.07	2.56±0.04
4	1000	480	550	1.38±0.04	1.69±0.04	1.42±0.07	1.61±0.04	1.88±0.04	1.97±0.04	1.97±0.04	2.44±0.07	2.61±0.07
5	800	480	650	1.40±0.08	1.92±0.14	2.11±0.08	1.90±0.04	2.28±0.07	2.30±0.04	2.59±0.07	3.16±0.07	3.28±0.04
6	800	480	550	1.35±0.12	2.73±0.04	2.16±0.04	2.78±0.00	2.99±0.07	3.30±0.04	3.21±0.04	4.03±0.07	4.10±0.07
7	680	410	490	1.50±0.07	2.78±0.14	3.11±0.08	2.66±0.04	3.09±0.04	3.28±0.07	3.19±0.04	4.00±0.04	4.00±0.08
8	800	480	550	1.59±0.11	2.82±0.04	2.59±0.11	3.11±0.04	3.44±0.04	3.99±0.07	3.36±0.04	4.31±0.07	4.31±0.07
9	920	410	490	1.90±0.04	2.87±0.04	2.49±0.07	2.68±0.04	2.68±0.04	3.13±0.07	2.90±0.04	4.19±0.08	4.07±0.04
10	920	410	610	1.85±0.07	2.59±0.04	1.97±0.08	2.21±0.07	2.75±0.04	3.06±0.07	2.49±0.04	2.73±0.07	3.33±0.04
11	800	600	550	1.78±0.07	2.33±0.04	2.54±0.04	1.83±0.04	2.11±0.04	2.28±0.07	1.82±0.04	3.04±0.04	2.97±0.04
12	920	550	610	1.69±0.04	1.95±0.04	1.97±0.11	1.59±0.04	1.95±0.04	1.99±0.07	3.55±0.04	2.30±0.07	2.76±0.04
13	800	360	550	1.69±0.01	2.78±0.07	2.99±0.07	3.39±0.04	3.58±0.04	3.30±0.04	3.91±0.04	4.29±0.04	4.41±0.04
14	600	480	550	1.73±0.04	2.99±0.07	3.16±0.04	2.87±0.04	3.54±0.04	3.82±0.04	3.74±0.07	4.36±0.04	4.55±0.04
15	800	480	550	1.73±0.04	3.04±0.04	2.75±0.04	3.09±0.04	3.23±0.04	3.35±0.07	3.55±0.04	4.31±0.07	4.27±0.04
16	680	410	610	1.88±0.04	3.11±0.04	3.18±0.04	3.11±0.04	3.32±0.04	3.28±0.07	3.76±0.04	4.36±0.04	4.43±0.04
17	680	550	610	1.71±0.07	2.25±0.04	2.44±0.08	2.40±0.04	3.51±0.04	2.33±0.04	2.83±0.04	3.48±0.04	3.64±0.04

Table 31 (cont.)

Experiments no.	Levels			FFA content (% as oleic acid)								
	Infrared wattage (W)	Treatment duration (second)	Vacuum strength (mmHg)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
F-value				0.51	3.21	5.72	7.43	9.46	5.77	7.34	6.23	6.04
p-value				0.8304	0.0690	0.0158	0.0075	0.0036	0.0154	0.0078	0.0124	0.0135
Lack of Fit				0.4771	0.1810	0.6317	0.2921	0.4685	0.6677	0.5020	0.1270	0.0747
R^2				0.3950	0.8051	0.8803	0.9052	0.9241	0.8812	0.9041	0.8890	0.8860
%CV				13.23	12.33	11.66	11.01	9.15	11.80	10.49	10.63	9.21

Table 32 MC in SRB by IR-VC method and the analysis of variance table of ANOVA for RSM each week from experiments

Experiments no.	Levels			Moisture content (%)								
	Infrared wattage (W)	Treatment duration (second)	Vacuum strength (mmHg)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	680	550	490	6.10±0.42	6.22±0.02	6.03±0.09	6.16±0.12	6.08±0.07	6.03±0.02	6.17±0.10	6.23±0.04	6.03±0.07
2	800	480	450	5.09±0.25	5.11±0.19	5.09±0.07	5.08±0.04	5.54±0.08	5.54±0.04	5.38±0.15	5.62±0.01	5.48±0.02
3	920	550	490	2.73±0.17	2.46±0.16	2.70±0.06	3.38±0.07	3.67±0.11	3.94±0.07	3.81±0.01	4.11±0.06	4.29±0.11
4	1000	480	550	2.57±0.20	2.38±0.15	2.34±0.03	3.01±0.15	3.25±0.20	3.20±0.23	3.57±0.17	3.94±0.11	3.78±0.03
5	800	480	650	3.59±0.04	3.51±0.08	3.81±0.06	3.75±0.13	4.39±0.03	4.31±0.13	4.71±0.25	5.02±0.01	4.65±0.05
6	800	480	550	5.31±0.56	5.29±0.44	4.77±0.22	5.17±0.21	5.29±0.19	5.62±0.14	5.29±0.09	5.56±0.07	4.81±0.06
7	680	410	490	6.01±0.22	5.75±0.28	5.13±0.30	5.31±0.08	5.63±0.06	5.14±0.08	5.47±0.09	5.90±0.11	5.75±0.03
8	800	480	550	5.84±0.40	5.82±0.06	5.94±0.11	5.65±0.28	5.36±0.10	5.14±0.11	5.73±0.11	5.58±0.02	5.85±0.07
9	920	410	490	5.01±0.45	4.77±0.10	4.69±0.12	4.93±0.21	4.62±0.06	4.55±0.15	4.94±0.09	4.89±0.05	5.10±0.09
10	920	410	610	4.33±0.53	4.08±0.08	4.11±0.07	4.35±0.09	4.00±0.01	4.05±0.05	4.75±0.05	4.35±0.06	4.40±0.08
11	800	600	550	3.09±0.12	3.07±0.05	3.34±0.22	3.56±0.07	3.50±0.03	3.60±0.20	4.58±0.10	4.54±0.18	4.20±0.19
12	920	550	610	2.85±0.21	2.83±0.06	2.54±0.13	3.26±0.12	3.40±0.05	3.18±0.25	3.46±0.04	3.86±0.16	4.03±0.14
13	800	360	550	6.46±0.35	6.48±0.08	6.27±0.16	6.21±0.11	6.18±0.07	5.80±0.33	6.28±0.08	6.03±0.07	5.99±0.18
14	600	480	550	6.74±0.67	6.56±0.10	6.31±0.08	6.09±0.13	6.21±0.10	5.87±0.33	6.05±0.11	5.97±0.16	5.94±0.01
15	800	480	550	5.81±0.24	5.61±0.31	5.19±0.29	5.21±0.20	5.55±0.20	4.79±0.12	5.63±0.19	5.49±0.03	5.38±0.21
16	680	410	610	5.40±0.15	5.18±0.09	5.26±0.03	5.06±0.10	5.23±0.10	5.31±0.04	5.54±0.17	5.37±0.03	5.22±0.14
17	680	550	610	4.00±0.32	4.18±0.06	4.38±0.07	4.43±0.04	4.85±0.12	4.22±0.29	4.86±0.11	4.83±0.02	4.72±0.09

Table 32 (cont.)

Experiments no.	Levels			Moisture content (%)								
	Infrared wattage (W)	Treatment duration (second)	Vacuum strength (mmHg)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
F-value				12.15	10.40	7.93	7.38	7.48	6.32	15.33	10.80	4.65
p-value				0.0012	0.0027	0.0062	0.0076	0.0073	0.0119	0.0008	0.0024	0.0276
Lack of Fit				0.2223	0.1547	0.5876	0.1996	0.0584	0.4703	0.4016	0.9770	0.7552
R^2				0.9398	0.9304	0.9106	0.9047	0.9058	0.8905	0.9517	0.9328	0.8566
%CV				10.81	11.99	12.66	10.28	9.58	9.85	5.64	5.82	8.42

Table 33 The a_w in SRB by IR-VC method and the analysis of variance table of ANOVA for RSM each week from experiments

Experiments	Levels			Water activity (a_w)								
no.	Infrared wattage (W)	Treatment duration (second)	Vacuum strength (mmHg)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	680	550	490	0.302±0.005	0.291±0.002	0.266±0.003	0.475±0.003	0.438±0.012	0.520±0.001	0.515±0.002	0.520±0.001	0.515±0.002
2	800	480	450	0.222±0.004	0.259±0.002	0.237±0.003	0.422±0.002	0.397±0.005	0.474±0.002	0.471±0.002	0.511±0.001	0.474±0.003
3	920	550	490	0.098±0.002	0.135±0.002	0.150±0.002	0.288±0.001	0.310±0.006	0.390±0.003	0.407±0.001	0.443±0.002	0.423±0.002
4	1000	480	550	0.089±0.002	0.121±0.002	0.126±0.002	0.293±0.001	0.294±0.004	0.333±0.002	0.378±0.002	0.439±0.002	0.398±0.001
5	800	480	650	0.173±0.004	0.175±0.002	0.220±0.002	0.309±0.002	0.330±0.009	0.382±0.002	0.467±0.002	0.513±0.002	0.466±0.003
6	800	480	550	0.165±0.003	0.187±0.002	0.241±0.002	0.434±0.002	0.420±0.002	0.529±0.002	0.517±0.004	0.546±0.002	0.463±0.003
7	680	410	490	0.258±0.004	0.243±0.002	0.232±0.002	0.396±0.003	0.392±0.008	0.449±0.002	0.468±0.001	0.506±0.001	0.498±0.001
8	800	480	550	0.263±0.005	0.243±0.002	0.241±0.002	0.387±0.003	0.422±0.001	0.487±0.001	0.474±0.003	0.527±0.004	0.481±0.001
9	920	410	490	0.210±0.003	0.210±0.002	0.230±0.001	0.357±0.003	0.393±0.004	0.420±0.003	0.473±0.002	0.530±0.002	0.452±0.001
10	920	410	610	0.162±0.003	0.190±0.001	0.190±0.002	0.342±0.001	0.347±0.013	0.412±0.002	0.468±0.004	0.440±0.003	0.440±0.002
11	800	600	550	0.109±0.002	0.137±0.002	0.169±0.001	0.276±0.001	0.299±0.009	0.405±0.010	0.389±0.002	0.467±0.005	0.436±0.002
12	920	550	610	0.120±0.003	0.136±0.002	0.143±0.001	0.279±0.002	0.285±0.003	0.363±0.003	0.329±0.002	0.483±0.002	0.518±0.002
13	800	360	550	0.292±0.006	0.284±0.002	0.265±0.001	0.431±0.001	0.421±0.005	0.463±0.003	0.465±0.002	0.504±0.003	0.528±0.001
14	600	480	550	0.280±0.004	0.281±0.001	0.261±0.001	0.404±0.001	0.420±0.001	0.469±0.002	0.474±0.001	0.482±0.002	0.524±0.001
15	800	480	550	0.248±0.002	0.231±0.001	0.222±0.001	0.378±0.001	0.407±0.001	0.461±0.002	0.475±0.001	0.419±0.001	0.503±0.002
16	680	410	610	0.229±0.003	0.222±0.002	0.221±0.002	0.321±0.002	0.374±0.003	0.422±0.003	0.461±0.001	0.454±0.001	0.524±0.002
17	680	550	610	0.161±0.003	0.179±0.002	0.194±0.001	0.295±0.002	0.375±0.001	0.362±0.002	0.434±0.002	0.452±0.001	0.537±0.002
F-value				7.12	7.01	6.23	5.46	10.51	6.43	10.22	0.53	5.16
p-value				0.0085	0.0089	0.0124	0.0179	0.0026	0.0113	0.0029	0.8139	0.4043
Lack of Fit				0.0498	0.0562	0.1707	0.4754	0.1113	0.7569	0.7693	0.9283	0.1742
R^2				0.9018	0.9001	0.8890	0.8753	0.9311	0.8921	0.9293	0.4062	0.6112
%CV				16.77	12.68	10.29	9.41	5.45	6.46	4.37	9.13	9.32

The most important consideration for the optimal stabilization condition of the RB by IR-VC technique was the FFA. In week 4, there was the most obvious difference (highest significant or lowest *p*-value). However, the experiment considered the MC value and a_w, which had to be consistent with the FFA value. Table 33 shows the MC analysis of the ANOVA for the response surface quadratic model for each week. From the value of the experiments, it was found that the lowest *p*-value was at week 6, but the most important indicator was the FFA; hence, the MC had lower importance than the FFA, and the significance of the FFA content was the highest during week 4. Therefore, at Week 4, the *p*-value, F-value, lack of fit, and %CV were 0.0073, 7.48, 0.0584, 0.9058, and 9.58, respectively. As such, these were accepted for selection to investigate the storage stability in the next section.

The a_w value had the highest significance in week 4 similar to the FFA content. The *p*-value, F-value, lack of fit, R^2, and %CV were 0.0026, 10.51, 0.1113, 0.9311, and 5.45, respectively. This confirmed the high significant obvious difference at week 4 for the stabilization of the RB of IR-VC method. Therefore, the three responses (IR wattage, treatment duration, and vacuum strength) suggested quadratic polynomial models that could be effectively used to predict the models. These results corresponded with the findings of González-Centeno et al. (2014), who reported that the significant regression and non-significant lack of fit of the model would fit to the experimental data that were effectively predicted within the system. The response from the variables and factors of the F-value, *p*-value, and R^2 in terms of % FFA content, MC and a_w of the RSM after the selection of the week 4 storage SRB IR-VC method are shown in table 34.

Table 34 The variable and factor's F, *p*-value and R^2 in term of % FFA content, MC and a_w at week 4 of storage time by IR-VC method

Variables /Factors	Responses					
	FFA		MC		a_w	
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Model	9.46	0.0036**	7.48	0.0073**	10.51	0.0026***
X_1	69.56	0.0004****	41.30	0.0004****	36.98	0.0005****
X_2	26.14	0.0014***	12.23	0.0100**	16.53	0.0048***
X_3	1.99	0.2010^{ns}	6.68	0.0363*	12.47	0.0096**
X_1X_2	2.64	0.1463^{ns}	1.51	0.2590^{ns}	11.21	0.0123*
X_1X_3	1.46	0.2663^{ns}	0.31	0.5923^{ns}	0.030	0.8665^{ns}
X_2X_3	1.53	0.2564^{ns}	0.13	0.7267^{ns}	0.18	0.6882^{ns}
X_1^2	5.87	0.0459^{ns}	3.67	0.0968^{ns}	10.26	0.0150*
X_2^2	3.15	0.1191^{ns}	2.60	0.1511^{ns}	9.09	0.0195*
X_3^2	8.91	0.0204*	1.73	0.2294^{ns}	7.96	0.0257*
Lack of Fit	1.39	0.4685^{ns}	16.42	0.0584^{ns}	8.28	0.1113^{ns}
R^2	0.9241		0.9058		0.9311	
Adj R^2	0.8264		0.7847		0.8426	

Note: X_1 = infrared wattage, X_2 = treatment duration, X_3 = vacuum strength
*= significant *p*-value <0.05, **= significant *p*-value <0.01,
= significant *p*-value <0.005, *= significant *p*-value <0.001, ^{ns}= non-significant

This represented the variability of the parameters by the models that indicated the predicted values, which were obtained by the quadratic polynomial equations that had a strong correlation with the actual experimental values with Pearson's correlation coefficient (Hossain et al., 2012). The *p*-value of the models of the FFA, MC, and a_w were 0.0036, 0.0073, and 0.0026, respectively. This demonstrated that the response was well-adapted and affected the prediction of the FFA, MC, and a_w of the SRB samples. In addition, the coefficient of determination (R^2) for the FFA, MC, and a^w was 0.9241, 0.9058, and 0.9311, respectively. The Adj-R^2 for the FFA, MC, and a_w was 0.8264, 0.7847, and 0.8426, respectively, which further confirmed that the general availability and accuracy of the polynomial model were adequate (Han et al., 2016). The FFA content

regression equation of the SRB relating to the actual levels of the IR wattage (X_1), treatment duration (X_2), and vacuum strength (X_3) was shown in Eq. 21.

$$FFA = -31.36+0.017(X_1) + 0.049(X_2) + 0.069(X_3) - 0.000017(X_1)(X_2) + 0.000015(X_1)(X_3) - 0.000026(X_2)(X_3) - 0.000013(X_1)^2 - 0.000026(X_2)^2 - 0.000064(X_3)^2 \quad (21)$$

The FFA response surface plots based on Eq. 21 shows in Figure 15. The infrared wattage and treatment duration affected the FFA content, while the vacuum strength variable was constantly fixed at the respective zero levels (550 mmHg) (Figure 15a). The IR wattage significantly affected the FFA content (p-value = 0.0004), whereas it decreased while increasing the IR wattage. The treatment time significantly affected the FFA content (p-value < 0.0014). The short treatment time was found to be insufficient to inactivate the lipase. The FFA content decreased with the increasing treatment times (360, 480, or 600 seconds) at the same IR wattage level.

The maximum experiment treatment duration used in this study was 600 seconds. Since the pretreatment experiment showed that the treatment duration above this level (at 1,000 W of IR wattage), the SRB was undesirably changed; such as, a dark color and cooked/burnt-like odor. However, varying the vacuum pressure of the experiment within the range of 450-650 mmHg did not affect the SRB's FFA content; likewise, the vacuum pressure of 450 mmHg was sufficient for this process. The IR wattage and vacuum strength affected the FFA content, while the treatment duration variable was fixed at the respective zero levels (480 seconds) (Figure 15b).

The surface plot showed that an increase in the IR wattage decreased the FFA content, while the vacuum strength did not affect the FFA content (the IR wattage variable was fixed at the respective zero levels: 800 watts) (Figure 15c). The plot showed that an increase in the treatment duration and vacuum strength decreased the FFA content.

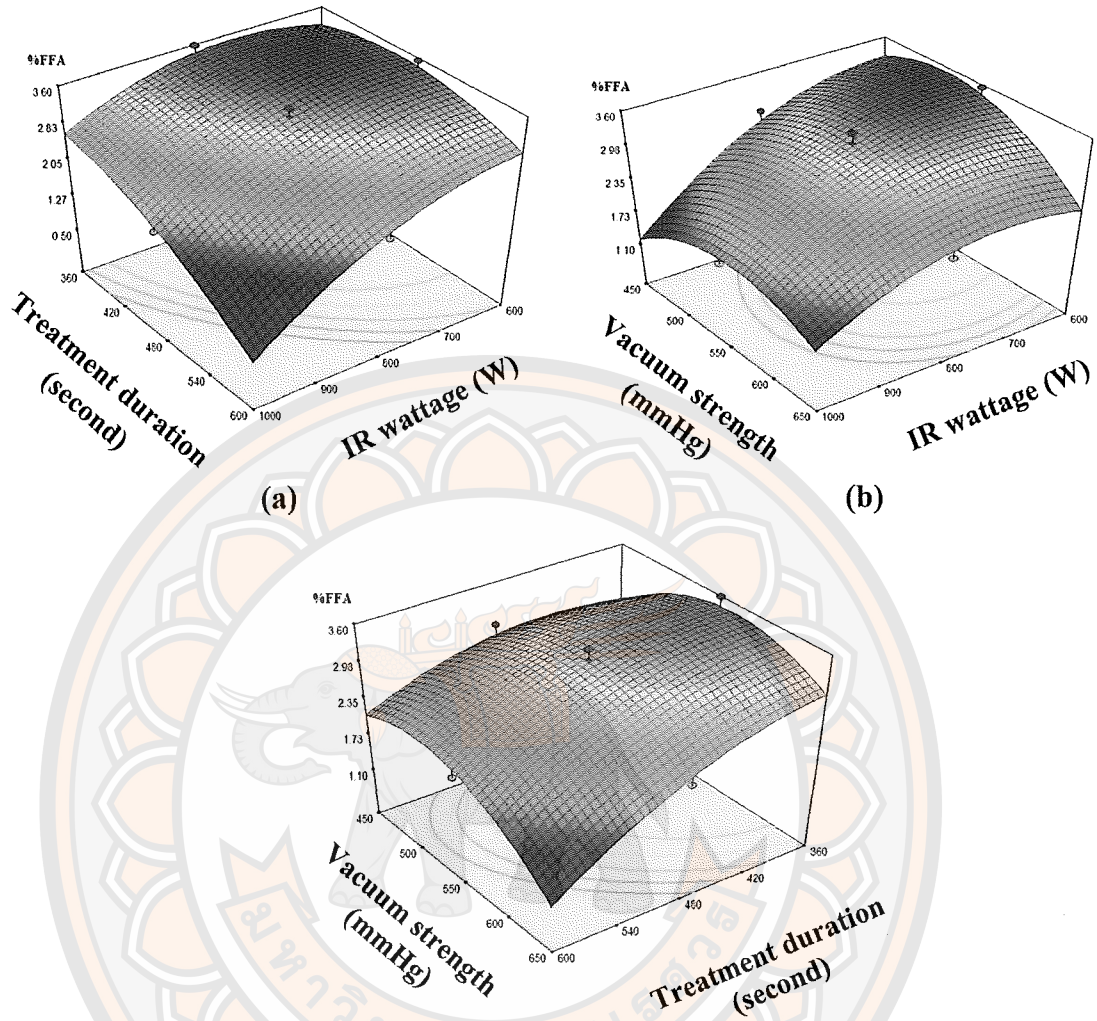


Figure 15 **Response surface and contour plots for FFA content in SRB by IR-VC method as a function of treatment duration and IR wattage (a), vacuum strength and infrared wattage (b), and vacuum strength and treatment duration (c)**

The IR wattage, treatment duration, and vacuum strength significantly affected the MC of the RB. However, all interactions and quadratic coefficient terms were not significant since the *p*-values were less than 0.05. The second-order polynomial equation of the MC shows in Eq. 22.

$$MC = -28.34 + 0.028(X_1) + 0.063(X_2) + 0.047(X_3) - 0.000024(X_1)(X_2) + 0.000013(X_1)(X_3) - 0.000014(X_2)(X_3) - 0.000019(X_1)^2 - 0.000044(X_2)^2 - 0.000052(X_3)^2 \quad (22)$$

The three-dimensional surface and contour plots of the effects of the factors on the MC of the SRB based on Eq. 22 shows in Figure 16. The IR wattage and treatment duration affected the MC, while the vacuum strength variable was fixed as constant at the respective zero levels (550 mmHg) (Figure 15a). The surface plot of Figure 16a demonstrated that as the IR wattage and treatment duration increased, the MC decreased. This was expected because as vacuum pressure increases, there would be an accelerated removal of moisture build-up in the chamber, which would consequently enhance the drying process. In the combined infrared-vacuum drying, the most important effect would cause an increase in the drying rate (Salehi, & Kashaninejad, 2018). An increase in the IR wattage reduced the MC of the samples, while the vacuum strength in a range of 450 to 650 mmHg did not affect the MC (Figure 16b). Longer treatment duration and high vacuum strength also significantly reduced the MC of the RB (Figure 16c).

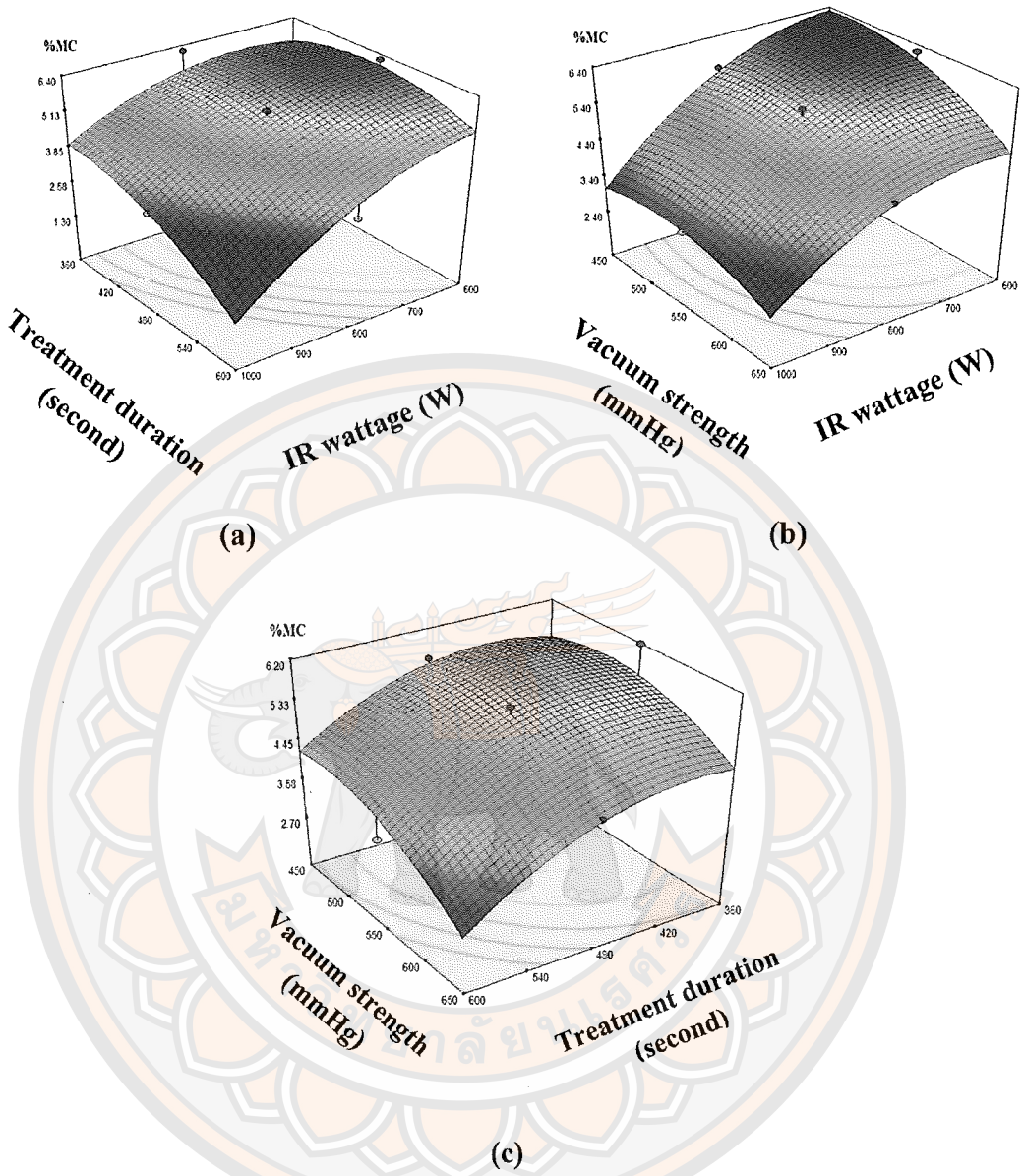


Figure 16 Response surface and contour plots for MC in SRB by IR-VC method as a function of treatment duration and IR wattage (a), vacuum strength and infrared wattage (b), and vacuum strength and treatment duration (c)

The a_w linear coefficients of the independence variable in the SRB from the dependence variable (model; IR wattage: X_1, treatment duration: X_2, and vacuum strength: X_3) were significant with *p*-values of 0.0026, 0.0005, 0.0048 and 0.0096, respectively. The quadratic term coefficient of X_1X_2, X_1^2, X_2^2, and X_3^2 were significant (*p*-value $\leq$ 0.05) with *p*-values of 0.0123, 0.0150, 0.0195 and 0.0257,

respectively. The experimental data allowed the development of mathematical equations resulting in the predicted results. The second-order polynomial equation of the a_w shows in Eq. 23.

$$a_w = - 3.396 + 0.003179(X_1) + 0.005816(X_2) + 0.005167(X_3) - 0.000002857(X_1)(X_2) + 0.0000001736(X_1)(X_3) - 0.0000007143(X_2)(X_3) - 0.000001364(X_1)^2 - 0.000003597(X_2)^2 - 0.000004807(X_3)^2 \quad (23)$$

The three-dimensional surface and contour plots for the effects of the factors (IR wattage, treatment duration, and vacuum strength) on the a_w in the SRB based on Eq. 23 is shown in Figure 17.

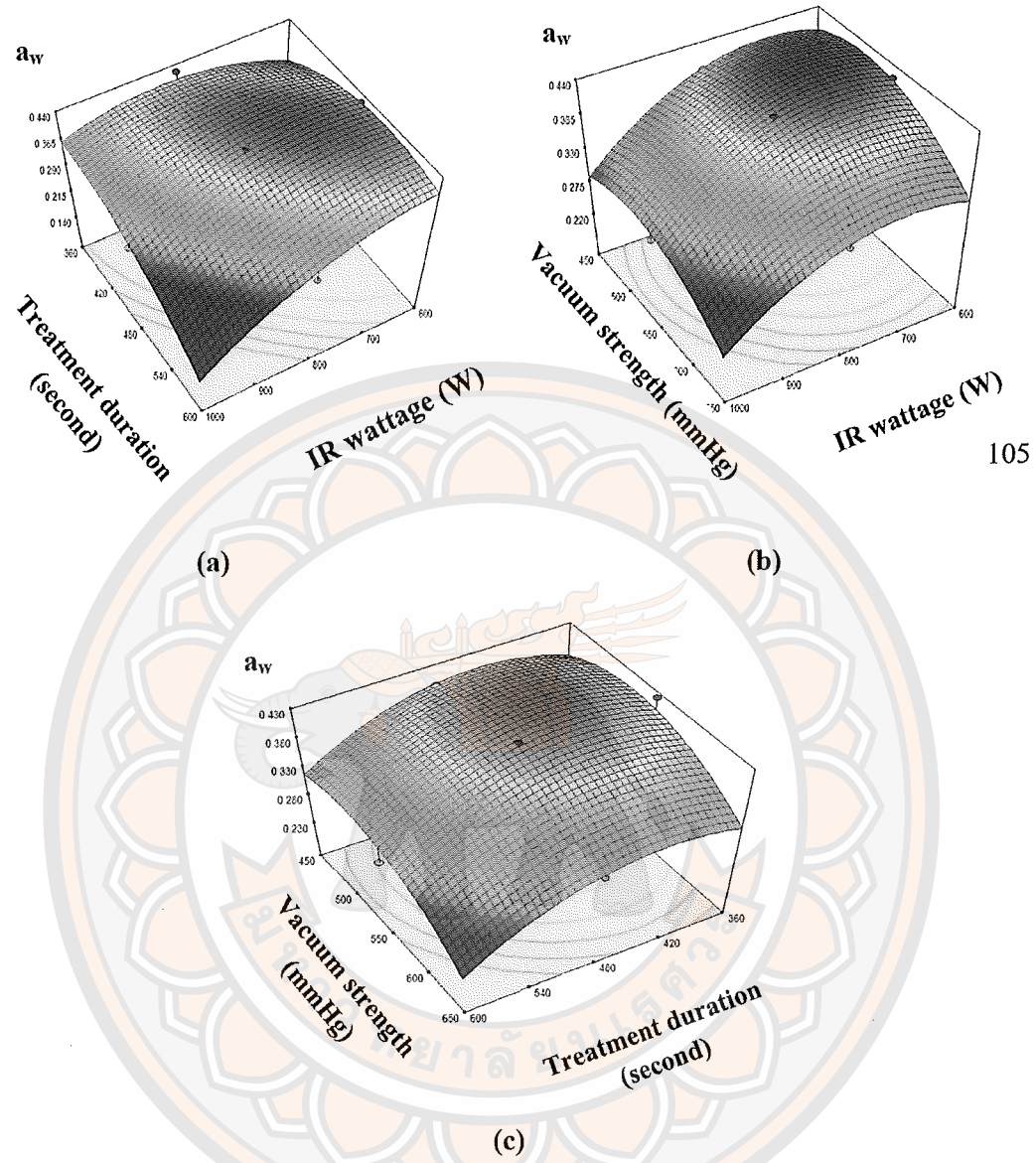


Figure 17 Response surface and contour plots for a_w in SRB by IR-VC method as a function of treatment duration and infrared wattage (a), vacuum strength and IR wattage (b), and vacuum strength and treatment duration (c)

The IR wattage and treatment duration affected the a_w, while the vacuum strength variable was a fixed constant at the respective zero level (550 mmHg) (Figure 17a). It could be seen that an increase in the IR wattage and treatment duration significantly reduced the a_w values. The surface plot showed that an increase

in the IR wattage and heating time decreased the a_w of the RB samples (Figure 17b and Figure 17c).

The experimental data allowed for the development of the mathematical Eq. 21-23 and showed the FFA, MC and a_w predicted data results in table 35. The prediction model was validated by entering the data for the various treatments into the prediction model again and comparing the experimental data with the predicted data under the same conditions as presented in table 35. The criterion for fitting the efficiency data of the FFA, MC, and a_w model was calculated as the %E difference between the experimental and predicted data (Eq. 2) (Hossain et al., 2012). The FFA, MC, and a_w predicted by the RSM model are shown in table 35, which calculated the %E difference from the experimental data as 12.62, 7.37, and 8.90, respectively.

The combination of the optimized conditions of the FFA content, MC, and a_w (at week 4) were calculated by the RSM in Design-Expert software (table 36). The optimum values of the process variables were obtained for the maximum desirability function of 1.000, 1.000, and 1.000 respectively, which were the IR wattage=997 W, treatment duration=598 seconds, and vacuum strength=610 mmHg. The optimized condition with a combination of the FFA content, MC, and a_w at this condition was 0.33%, 1.00%, and 0.117, respectively.

Table 35 Experimental design of CCD, actual experiment value, and predicted value of FFA content, MC and a_w in SRB by IR-VC method

Experi-mental no.	Factors			Responses					
	X_1 Infrared wattage (W)	X_2 Treatment duration (second)	X_3 Vacuum strength (mmHg)	FFA		MC		a_w	
				Experi-mental	Predi-cted	Experi-mental	Predi-cted	Experi-mental	Predi-cted
1	680	550	490	2.90	3.22	6.16	5.91	0.475	0.420
2	800	480	450	2.73	2.75	5.08	5.61	0.422	0.400
3	920	550	490	1.66	1.84	3.38	3.80	0.288	0.303
4	1000	480	550	1.61	1.98	3.01	3.57	0.293	0.305
5	800	480	650	1.90	2.42	3.75	4.51	0.309	0.335
6	800	480	550	2.78	3.22	5.17	5.58	0.434	0.416
7	680	410	490	2.66	3.41	5.31	6.27	0.396	0.410
8	800	480	550	3.11	3.22	5.65	5.58	0.387	0.416
9	920	410	490	2.68	2.61	4.93	4.97	0.357	0.389
10	920	410	610	2.21	2.86	4.35	4.62	0.342	0.358
11	800	600	550	1.83	2.24	3.56	4.18	0.276	0.326
12	920	550	610	1.59	1.64	3.26	3.21	0.279	0.260
13	800	360	550	3.39	3.44	6.21	5.70	0.431	0.420

Table 35 (cont.)

Experi-mental no.	Factors			Responses					
	X_1 Infrared wattage (W)	X_2 Treatment duration (second)	X_3 Vacuum strength (mmHg)	FFA		MC		a_w	
				Experi-mental	Predi-cted	Experi-mental	Predi-cted	Experi-mental	Predi-cted
14	600	480	550	2.87	3.43	6.09	6.10	0.404	0.417
15	800	480	550	3.09	3.22	5.21	5.58	0.378	0.416
16	680	410	610	3.11	3.22	5.06	5.55	0.321	0.375
17	680	550	610	2.40	2.59	4.43	4.95	0.295	0.373
		%E		12.62		7.37		8.90	

Table 36 Optimized condition for IR-VC stabilization method in term of FFA content, MC and a_w

Variables	Optimized conditions for FFA content (%)	Optimized conditions for MC (%)	Optimized conditions for a_w	Optimized conditions for combination of % FFA, MC (%), and a_w
IR wattage (W)	995	954	997	997
Treatment duration (second)	593	599	522	598
Vacuum strength (mmHg)	628	650	644	610
Quantities of dependent variables as predicted by RSM	0.21 (% as Oleic acid)	1.09	0.192	0.33 (% as Oleic acid), 1.00, 0.117
Desirability	1.000	1.000	1.000	1.000

Note: The importance of optimization combination of % FFA, MC, and a_w were *****, ***, and *** respectively

3. Rice bran stabilization and optimization by IR-HA

3.1 Pre-treatment RSM

The RSM comprised three independent variables of X_1 (IR wattage: W), X_2 (treatment duration: seconds), and X_4 (hot air temperature). The completed design consisted of 17 experimental treatments that determined the %FFA content, MC, and a_w in the SRB each week of the storage duration. The results of the %FFA, MC, and a_w are shown in tables 37-39, respectively.

The highest significance of the indicated result (%FFA, %MC, and a_w) in the storage week of the SRB treatment was the target of the investigation. The storage week with the highest F-value and lowest *p*-value was selected for the study variable and the factors of the F-value, *p*-value, and R^2 in terms of the %FFA content, MC, and a_w, respectively. This presented the variability of the parameters by the models, which indicated that the predicted values obtained by the quadratic polynomial equations had a correlation with the actual experimental values. The calculated %E between the actual experiment and predicted values from the equation models were used for the storage stability study in the next section.

Table 37 FFA content in SRB by IR-HA method and the analysis of variance table of ANOVA for RSM each week from experiments

Experiments no.	Levels			%FFA								
	Infrared wattage (W)	Treatment duration (second)	Hot air temperature (°C)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	800	600	70	1.47±0.04	2.52±0.04	3.30±0.07	4.13±0.04	5.15±0.04	5.39±0.04	6.76±0.14	6.66±0.08	7.05±0.14
2	800	480	70	1.54±0.04	2.44±0.04	3.20±0.07	4.77±0.04	5.44±0.04	5.91±0.07	6.71±0.08	6.66±0.08	7.38±0.08
3	800	480	70	1.45±0.04	2.75±0.04	3.87±0.04	4.53±0.04	5.27±0.07	5.77±0.07	6.23±0.08	6.82±0.30	7.10±0.08
4	800	360	70	1.54±0.04	3.32±0.08	4.56±0.08	5.39±0.08	6.76±0.12	6.53±0.04	7.91±0.14	7.96±0.08	8.25±0.22
5	680	550	64	1.59±0.04	3.04±0.04	4.08±0.04	4.65±0.04	5.03±0.04	5.96±0.04	6.90±0.14	7.48±0.14	7.91±0.14
6	800	480	60	1.57±0.07	2.85±0.07	3.85±0.11	4.32±0.07	5.29±0.08	5.77±0.07	6.71±0.08	7.48±0.14	7.91±0.14
7	680	410	76	1.54±0.04	3.16±0.04	3.68±0.04	4.58±0.04	5.48±0.07	6.30±0.07	6.86±0.08	7.29±0.08	7.57±0.08
8	680	410	64	1.54±0.08	2.90±0.08	4.06±0.07	4.56±0.08	5.41±0.07	6.24±0.04	7.33±0.14	7.57±0.08	7.57±0.08
9	800	480	80	1.54±0.04	2.80±0.08	3.94±0.08	4.39±0.08	6.03±0.08	6.10±0.04	6.66±0.08	7.72±0.08	7.53±0.08
10	1000	480	70	1.57±0.07	2.47±0.04	3.04±0.04	3.89±0.04	4.82±0.04	5.10±0.04	5.70±0.08	6.57±0.08	6.57±0.17
11	600	480	70	1.57±0.07	2.75±0.08	4.06±0.07	4.56±0.08	5.60±0.04	6.34±0.07	6.76±0.14	7.19±0.14	7.67±0.08
12	680	550	76	1.54±0.04	2.73±0.04	3.68±0.04	4.68±0.04	5.65±0.04	5.48±0.07	6.95±0.08	7.24±0.08	7.72±0.08
13	920	550	64	1.54±0.08	2.85±0.07	2.85±0.04	4.72±0.07	4.72±0.04	5.48±0.07	7.00±0.08	6.93±0.08	7.43±0.08
14	920	410	64	1.54±0.04	2.49±0.07	3.66±0.07	4.79±0.07	4.87±0.08	6.24±0.04	6.09±0.08	6.90±0.14	6.86±0.22
15	920	550	76	1.54±0.04	2.63±0.07	2.97±0.011	3.66±0.07	4.82±0.04	5.43±0.011	5.61±0.14	6.76±0.14	6.81±0.08
16	920	410	76	1.47±0.04	2.90±0.04	3.42±0.04	4.30±0.04	5.10±0.08	5.96±0.04	6.56±0.08	7.00±0.08	7.38±0.46
17	800	480	70	1.54±0.04	2.92±0.07	3.77±0.08	4.37±0.07	5.15±0.04	5.48±0.07	6.42±0.17	7.43±0.22	7.29±0.08

Table 37 (cont.)

Experiments no.	Levels			%FFA								
	Infrared wattage (W)	Treatment duration (second)	Hot air temperature (°C)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
F-value				0.63	1.84	3.01	2.28	1.48	5.58	2.20	3.02	2.24
p-value				0.7492	0.2167	0.0801	0.1451	0.3102	0.0169*	0.1553	0.0793	0.1507
Lack of Fit				0.7553	0.7443	0.6916	0.3018	0.0756	0.4015	0.2107	0.5531	0.1250
R^2				0.4456	0.7029	0.7947	0.7456	0.6552	0.8777	0.7389	0.7955	0.7419
%CV				2.76	7.18	8.65	6.55	8.26	4.69	6.44	5.86	4.52

Table 38 MC in SRB by IR-HA method and the analysis of variance table of ANOVA for response surface quadratic model each week from experiments

Experiments no.	Levels			Moisture content								
	Infrared wattage (W)	Treatment duration (second)	Hot air temperature (°C)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	800	600	70	5.00±0.07	5.63±0.18	5.09±0.07	5.77±0.26	5.47±0.11	5.62±0.36	5.87±0.28	6.13±0.13	5.73±0.30
2	800	480	70	5.26±0.16	5.28±0.07	4.86±0.11	6.39±0.41	5.43±0.03	5.65±0.05	6.03±0.09	6.21±0.25	6.12±0.28
3	800	480	70	4.79±0.25	6.24±0.11	5.29±0.28	5.88±0.16	5.70±0.22	5.74±0.19	5.80±0.24	6.84±0.08	5.98±0.29
4	800	360	70	6.61±0.08	7.33±0.10	6.30±0.23	7.02±0.01	6.54±0.12	6.86±0.44	6.96±0.17	6.56±0.11	6.68±0.22
5	680	550	64	5.57±0.23	6.09±0.11	5.35±0.29	6.27±0.43	5.56±0.09	6.10±0.54	5.94±0.06	6.15±0.18	6.02±0.26
6	800	480	60	5.30±0.24	6.02±0.19	5.43±0.29	5.89±0.30	5.72±0.20	6.59±0.08	6.15±0.21	6.54±0.50	6.36±0.29
7	680	410	76	5.32±0.05	6.05±0.13	5.60±0.24	5.79±0.18	5.47±0.12	6.18±0.30	6.16±0.23	6.52±0.44	6.42±0.09
8	680	410	64	6.21±0.24	6.54±0.14	6.01±0.09	6.03±0.20	6.14±0.30	6.07±0.09	6.28±0.34	6.77±0.30	6.40±0.11
9	800	480	80	5.62±0.18	5.32±0.07	5.29±0.04	5.62±0.18	6.06±0.35	6.30±0.09	5.77±0.12	6.49±0.40	6.42±0.49
10	1000	480	70	3.99±0.20	5.00±0.30	4.30±0.08	5.05±0.16	4.22±0.11	5.47±0.19	5.36±0.20	5.43±0.14	5.63±0.17
11	600	480	70	6.81±0.05	5.77±0.23	6.11±0.09	6.07±0.20	5.70±0.30	6.63±0.30	6.07±0.22	6.37±0.41	6.32±0.18
12	680	550	76	5.07±0.05	5.46±0.16	5.08±0.05	5.86±0.11	5.38±0.20	6.05±0.47	6.23±0.47	5.59±0.11	6.23±0.19
13	920	550	64	3.79±0.14	5.29±0.22	4.01±0.15	6.20±0.17	4.89±0.26	5.66±0.12	5.97±0.17	5.59±0.20	6.14±0.04
14	920	410	64	4.46±0.07	5.23±0.10	5.07±0.10	6.18±0.12	5.14±0.12	6.15±0.10	5.24±0.14	6.21±0.13	5.62±0.31
15	920	550	76	4.13±0.10	4.94±0.10	4.27±0.26	4.96±0.10	5.32±0.02	4.80±0.13	5.51±0.12	5.73±0.17	5.89±0.50
16	920	410	76	4.46±0.15	5.71±0.03	4.99±0.14	6.10±0.16	5.64±0.23	6.40±0.07	6.25±0.26	6.01±0.15	6.10±0.03
17	800	480	70	4.99±0.04	5.56±0.12	5.53±0.18	5.84±0.18	5.34±0.19	5.44±0.13	5.64±0.11	6.17±0.35	6.04±0.15

Table 38 (cont.)

Experiments no.	Levels			Moisture content								
	Infrared wattage (W)	Treatment duration (second)	Hot air temperature (°C)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
F-value				2.71	3.81	6.11	2.57	3.81	4.03	6.35	3.46	2.29
p-value				0.1014	0.0459	0.0131	0.1133	0.0457	0.0398	0.3526	0.0581	0.1439
Lack of Fit				0.0924	0.8133	0.6211	0.4730	0.2834	0.1622	0.1900	0.7366	0.0674
R^2				0.7768	0.8304	0.8871	0.7677	0.8305	0.8382	0.6351	0.8163	0.7463
%CV				12.43	6.58	6.14	5.81	5.08	5.26	6.19	4.40	3.70

Table 39 The a_w in SRB by IR-HA method and the analysis of variance table of ANOVA for RSM each weeks from experiments

Experiment no.	Levels			a_w								
	Infrared wattage (W)	Treatment duration (second)	Hot air temperature (°C)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	800	600	70	0.339±0.001	0.275±0.005	0.294±0.003	0.321±0.002	0.327±0.004	0.331±0.003	0.363±0.003	0.342±0.001	0.374±0.001
2	800	480	70	0.322±0.001	0.262±0.001	0.301±0.001	0.345±0.004	0.338±0.005	0.338±0.003	0.374±0.002	0.346±0.002	0.376±0.001
3	800	480	70	0.309±0.002	0.306±0.006	0.313±0.003	0.328±0.002	0.338±0.002	0.339±0.005	0.348±0.001	0.348±0.002	0.362±0.001
4	800	360	70	0.391±0.013	0.381±0.001	0.325±0.003	0.361±0.004	0.382±0.002	0.349±0.002	0.389±0.002	0.376±0.001	0.396±0.001
5	680	550	64	0.335±0.001	0.321±0.005	0.343±0.002	0.334±0.003	0.354±0.002	0.344±0.004	0.380±0.002	0.354±0.004	0.372±0.001
6	800	480	60	0.295±0.003	0.303±0.003	0.314±0.004	0.322±0.002	0.386±0.002	0.331±0.002	0.373±0.004	0.353±0.002	0.385±0.002
7	680	410	76	0.301±0.004	0.327±0.044	0.321±0.002	0.328±0.001	0.336±0.003	0.338±0.006	0.365±0.002	0.357±0.002	0.378±0.001
8	680	410	64	0.359±0.002	0.333±0.002	0.346±0.001	0.330±0.001	0.360±0.002	0.345±0.003	0.386±0.001	0.366±0.001	0.379±0.001
9	800	480	80	0.348±0.001	0.300±0.004	0.317±0.003	0.321±0.001	0.356±0.004	0.337±0.006	0.362±0.001	0.365±0.003	0.372±0.001
10	1000	480	70	0.246±0.004	0.273±0.004	0.235±0.002	0.306±0.001	0.314±0.013	0.305±0.005	0.346±0.001	0.345±0.005	0.346±0.001
11	600	480	70	0.382±0.006	0.301±0.003	0.364±0.003	0.340±0.001	0.349±0.001	0.350±0.013	0.368±0.001	0.366±0.001	0.381±0.001
12	680	550	76	0.270±0.001	0.276±0.001	0.312±0.001	0.315±0.001	0.330±0.002	0.315±0.003	0.372±0.002	0.350±0.002	0.368±0.002
13	920	550	64	0.265±0.003	0.278±0.001	0.271±0.001	0.326±0.002	0.320±0.021	0.319±0.002	0.368±0.003	0.343±0.002	0.364±0.002
14	920	410	64	0.288±0.002	0.286±0.010	0.313±0.003	0.334±0.003	0.316±0.002	0.342±0.004	0.360±0.002	0.357±0.002	0.351±0.001
15	920	550	76	0.275±0.004	0.265±0.005	0.286±0.013	0.287±0.002	0.320±0.009	0.300±0.004	0.355±0.002	0.355±0.004	0.361±0.001
16	920	410	76	0.273±0.004	0.299±0.001	0.305±0.003	0.325±0.002	0.336±0.004	0.342±0.003	0.384±0.004	0.357±0.004	0.364±0.002
17	800	480	70	0.307±0.004	0.303±0.004	0.323±0.009	0.317±0.002	0.326±0.004	0.319±0.005	0.352±0.001	0.354±0.002	0.356±0.001
F-value				1.47	3.16	4.62	2.63	2.17	2.40	1.63	2.86	2.09
p-value				0.3113	0.0716	0.0280	0.1082	0.1599	0.1311	0.2672	0.0897	0.1718
Lack of Fit				0.0337	0.7577	0.2937	0.7465	0.1668	0.5658	0.7773	0.2705	0.5916
R^2				0.6547	0.8526	0.8558	0.7715	0.7361	0.7550	0.6764	0.7864	0.7287
%CV				11.96	6.67	5.17	3.56	4.13	3.41	3.03	1.82	2.70

Table 37 shows the quadratic model of the RSM for the SRB after 8 weeks of storage at 35°C for the FFA content. The significance of the FFA content was the highest (i.e., the lowest *p*-value). During week 5, the F-value, *p*-values, lack of fit, R^2, and %CV were 5.58, 0.0169, 0.4015, 0.8777, and 4.69, respectively. The most important consideration was the FFA. The most obvious difference was found at week 5. However, the experiment considered the MC value and the a_w value, which had to be consistent with the FFA value. Table 38 shows the MC analysis of ANOVA for the response surface quadratic model for each week from the experiments' value, and the lowest *p*-value was found at week 5. The *p*-value, F-value, lack of fit, R^2, and %CV were 0.0398, 4.03, 0.1622, 0.8382, and 5.26, respectively. The a_w value had the highest significance at week 2, which the *p*-value, F-value, lack of fit, R^2, and %CV were 0.0280, 4.62, 0.2937, 0.8558, and 5.17, respectively.

Therefore, the three responses (IR wattage, treatment duration, and hot air temperature) suggested quadratic polynomial models that could be effectively used to predict the models at week 5. The significant regression and non-significant lack of fit of the model would fit the experimental data that were effectively predicted within the system.

The response from the RSM software of the variable and factors of the F-value, *p*-value, and R^2 in terms of the % FFA content, MC and a_w of the SRB (IR-HA method) sample of week 5 are shown in table 40. The SRB sample (storage duration of five weeks) was used in the polynomial model to optimize the IR-HA conditions for the SRB based on the FFA content (table 40). The high F-values (5.58) and low *p*-values (0.0169) for all responses indicated that the models were highly significant.

Table 40 The variable and factor's F, *p*-value and R^2 in term of % FFA content, MC and a_w at week 5 of storage time of IR-HA method

Variables /Factors	Responses					
	FFA		MC		a_w	
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Model	5.58	0.0169*	4.30	0.0398*	2.40	0.1311^{ns}
X_1	14.97	0.0061**	8.22	0.0241*	7.50	0.0290^{*}
X_2	27.18	0.0012***	13.55	0.0079**	8.09	0.0249^{*}
X_3	1.12	0.3252^{ns}	0.79	0.4022^{ns}	1.17	0.3156^{ns}
X_1X_2	1.86	0.2147^{ns}	5.00	0.0605^{ns}	1.64	0.2407^{ns}
X_1X_3	1.22	0.3059^{ns}	0.57	0.4763^{ns}	0.28	0.6115^{ns}
X_2X_3	2.64	0.1484^{ns}	2.03	0.1968^{ns}	1.64	0.2407^{ns}
X_1^2	0.078	0.7876^{ns}	0.64	0.4486^{ns}	0.47	0.5134^{ns}
X_2^2	0.60	0.4626^{ns}	2.40	0.1651^{ns}	0.40	0.5471^{ns}
X_3^2	0.44	0.5300^{ns}	5.27	0.0553^{ns}	0.00	0.9968^{ns}
Lack of Fit	1.76	0.4015^{ns}	5.45	0.1622^{ns}	1.01	0.5658^{ns}
R^2	0.9241		0.9058		0.9311	
Adj R^2	0.8264		0.7847		0.8426	

Note: X_1 = infrared wattage, X_2 = treatment duration, X_4 = hot air temperature
*= significant $p<0.05$, **= significant $p<0.01$, ***= significant $p<0.005$,
^{ns}= not significant

The models were well-adapted to the response and were effective to predict the FFA, MC, and a_w of the SRB samples with the coefficient of determination (R^2) for 0.9241, 0.9058, and 0.9311, respectively. Moreover, the Adj-R^2 for the FFA, MC, and a_w were 0.8264, 0.7847, and 0.8426, respectively, which further confirmed that the general availability and accuracy of the polynomial model were adequate (Han et al., 2016). The FFA regression equation of the SRB relating to the actual levels of the IR wattage (X_1), treatment duration (X_2), and hot air temperature (X_4) was shown in Eq. 24.

$$FFA = -6.36493 + 0.018006(X_1) + 0.0.02115(X_2) + 0.072174(X_4) - 0.0000156(X_1)(X_2) - 0.000147(X_1)(X_4) - 0.000372(X_2)(X_4) - 0.0000016(X_1)^2 + 0.0000124(X_2)^2 + 0.0015(X_4)^2 \quad (24)$$

The FFA response surface plots based on Eq. 24 are shown in Fig. 18.

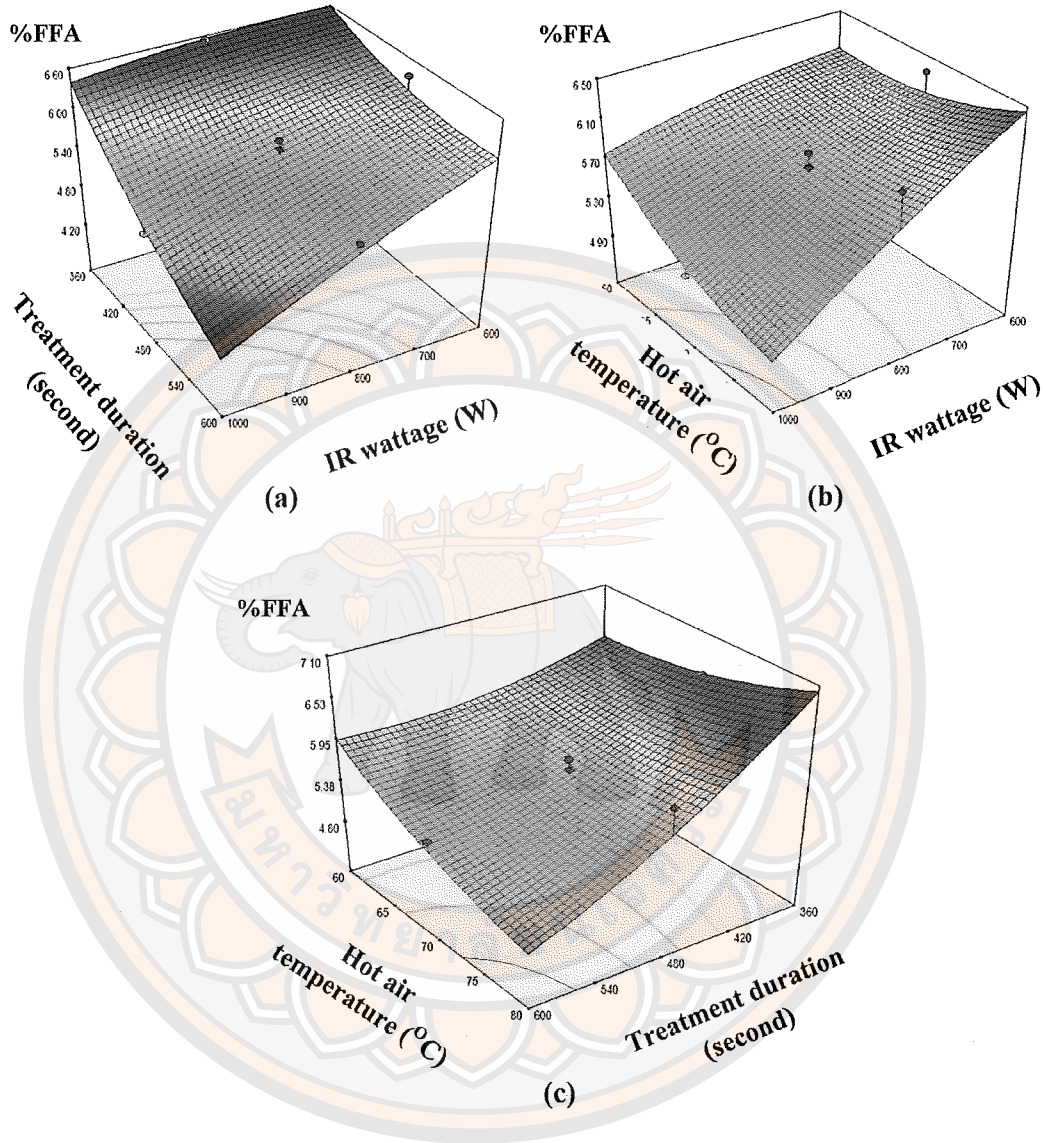


Figure 18 **Response surface and contour plots for FFA content in SRB by IR-HA method as a function of treatment duration and IR wattage (a), hot air temperature and IR wattage(b), and hot air temperature and treatment duration (c)**

The IR wattage and treatment duration affected the FFA content, while the hot air temperature variable was constantly fixed at the respective zero levels (70°C) (Figure 18a). A similar study was conducted by Malekian et al. (2000) in which the IR wattage had a dominant effect on the FFA content of the RB. The FFA content decreased with the increasing IR wattage almost linearly from 200 W to 700 W, which corresponded with the FFA contents of 28.91-10.26%, respectively.

The treatment time significantly affected the FFA content (p-value=0.0012), and the short treatment time was found to be insufficient to inactivate the lipase. The FFA content also decreased with the increasing treatment times at the same IR wattage. However, varying the hot air temperature of the experiment within the range of 60-80°C did not affect the SRB's FFA content (p-value=0.3252), as it had a low influence on the FFA content related to the lipase activity, so it did not promote or refute the SRB process between the IR and hot air because the IR was radiation heat-transfer, but the hot air was convective heat-transfer and the IR was promoted in a vacuum condition. In recent years, IR-VC drying has combined the advantages of both IR heating and vacuum drying. The low temperature and fast mass transfer conferred by the vacuum combined with the rapid energy transferred by the IR heating has generated very rapid, low-temperature drying; thus, it has the potential to improve energy efficiency and product quality (Giri, & Prasad, 2007 as cited in Salehi, & Kashaninejad, 2018).

Therefore, the IR in a vacuum made greater efficient heat transfer than IR combined with hot air. In order to destroy the enzyme and microorganisms in the RB, IR heating was used as a therapy to enhance the stability of the RB as an alternative. The IR wattage and hot air temperature affected the FFA content, while the treatment duration variable was fixed at the respective zero levels (480 seconds) (Figure 18b). The surface plot showed that an increase in the IR wattage decreased the FFA content, while the hot air temperature had a low effect on the FFA content, and the IR wattage variable was fixed at the respective zero levels (800 watts) (Figure 18c). The plot showed that an increase in the treatment duration and hot air temperature decreased the FFA content.

The IR wattage and treatment duration significantly affected the MC of the SRB, but the hot air temperature did not affect the MC. However, all interactions and quadratic coefficient terms were not significant since the *p*-values were less than 0.05. The second-order polynomial equation of the MC was shown in Eq. 25.

$$MC = 19.46766 + 0.011825(X_1) + 0.018144(X_2) - 0.58832(X_3) - 0.0000296(X_1)(X_2) - 0.000116319(X_1)(X_3) - 0.000378(X_2)(X_3) - 0.00000530(X_1)^2 + 0.0000287(X_2)^2 + 0.00607(X_3)^2 \quad (25)$$

The three-dimensional surface and contour plots of the effects of the factors on the MC of the SRB based on Eq. 25 were shown in Figure 19.

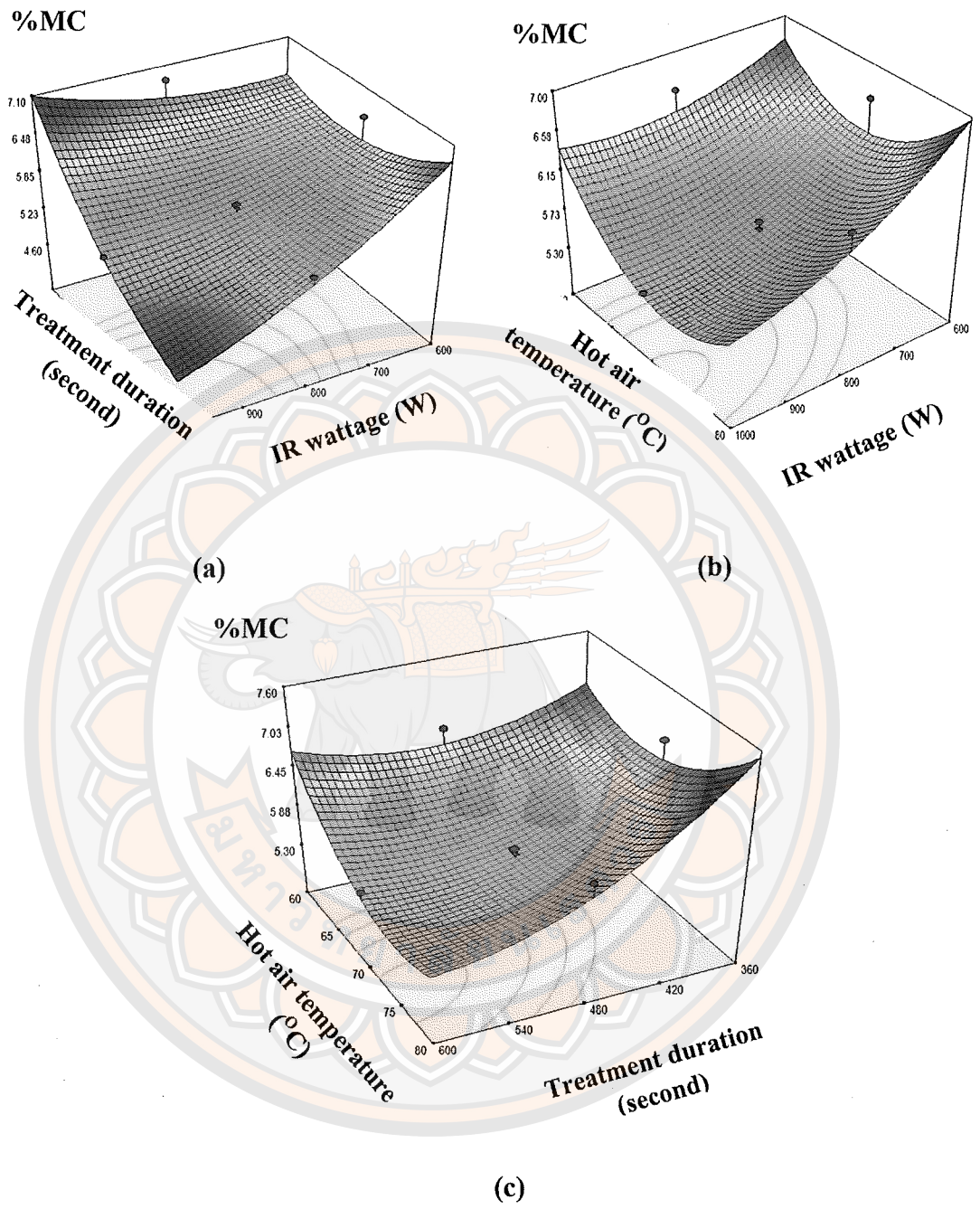


Figure 19 Response surface and contour plots for MC in SRB by IR-HA method as a function of treatment duration and IR wattage (a), hot air temperature and IR wattage (b), and hot air temperature and treatment duration (c)

The IR wattage, treatment duration, and hot air temperature affected the MC of the SRB with the *p*-values of 0.0241 and 0.0079, respectively, but the hot air temperature did not have any significance (*p*-value=0.4022). The MC response surface plots based on Eq. 25 were presented in Fig. 19. The IR wattage and treatment duration affected the MC, while the hot air temperature variable was fixed as constant at the respective zero levels (70°C) (Figure 18a). The surface plot of Figure 18a demonstrated that as the IR wattage and treatment duration increased, the MC decreased. An increase in the IR wattage apparently reduced the MC of the samples, while the hot air temperature in a range of 60-80°C did not affect the MC (Figure 19b). Longer treatment duration and high hot air temperature also significantly reduced the MC of the RB (Figure 19c).

The IR wattage and treatment duration were significant with *p*-values of 0.0290 and 0.0249, respectively, as for the a_w, the hot air temperature did not have any significance (*p*-value=0.311). However, all interactions and quadratic coefficient terms were not significant since the *p*-values were less than 0.05. The second-order polynomial equation of a_w was shown in Eq. 26.

$$a_w = - 0.00236 + 0.0002777(X_1) + 0.000815(X_2) + 0.00299778(X_3) - 0.00000061(X_1)(X_2) + 0.0000029519(X_1)(X_3) - 0.0000122(X_2)(X_3) - 0.000000163(X_1)^2 + 0.00000042(X_2)^2 - 0.000000393(X_3)^2 \quad (26)$$

The a_w response surface plots based on Eq. 26 shows in Figure 20. The IR wattage and treatment duration affected the a_w, while the temperature variable was a fixed constant at the respective zero level (70°C) (Figure 20a). Thus, it could be seen that an increase in the IR wattage and treatment duration significantly reduced the a_w values. The surface plot showed that an increase in the IR wattage and heating time decreased the a_w of the RB samples (Figure 20b and Figure 20c).

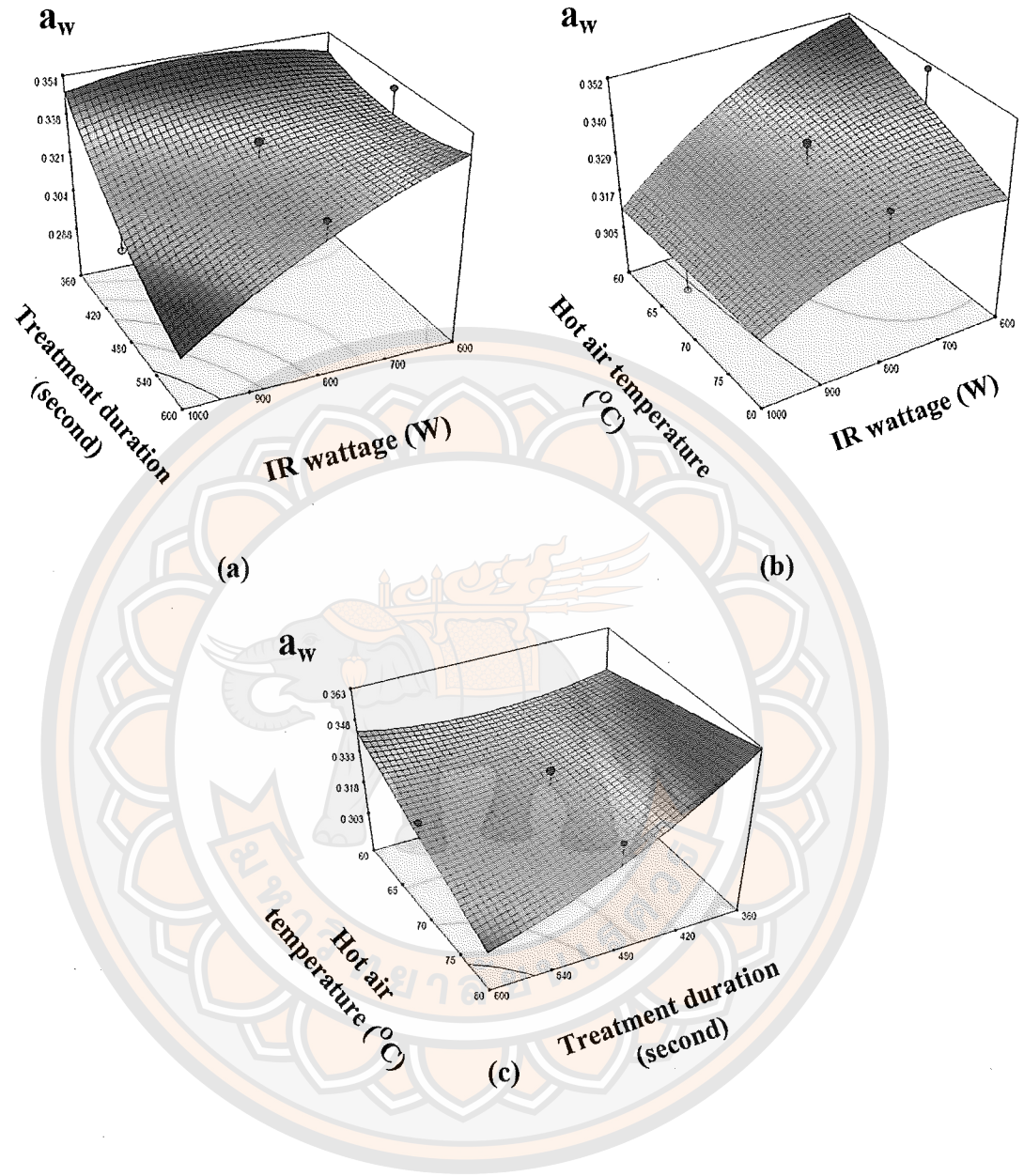


Figure 20 **Response surface and contour plots for a_w in SRB by IR-HA method as a function of treatment duration and IR wattage (a), hot air temperature and IR wattage (b), and hot air temperature and treatment duration (c)**

The prediction equation models of the FFA, MC, and a_w as stabilization by IR-HA calculated by the RSM were validated by entering the data for the various treatments into the prediction model again and comparing the experimental

data with the predicted data under the same conditions (Table 42). The criterion for fitting the efficiency data of the FFA, MC, and a_w models was calculated as the %E difference between the experimental and predicted data (Eq. 2) (Hossain et al., 2012). The FFA, MC, and a_w predicted by the RSM model are shown in table 42, which calculated the %E difference of the FFA, MC, and a_w from the experimental data as 0.15, 0.10, and 0.14, respectively.

The optimized conditions of the FFA content, MC and a_w, as calculated by the RSM in Design-Expert software, are shown in table 43. The optimum values of the process variables obtained the FFA, MC, and a_w for the maximum desirability function of 1.000, 1.000, and 1.000, respectively, in which the optimization condition for the combination was mainly IR wattage = 999 W, treatment duration = 596 seconds, and hot air temperature = 72°C. The attained values of the FFA, MC, and a_w under these conditions were 4.22 % as oleic acid, 4.68%, and 0.286, respectively.

Table 41 The experimental design of CCD, actual experiment value, and predicted value of FFA, MC and a_w in RB at week 4 of storage time by IR-HA method

Experi-mental no.	Factors			Responses					
	X_1 Infrared wattage (W)	X_2 Treatment duration (second)	X_3 Hot air temperature (mmHg)	FFA		MC		a_w	
				Experi-mental	Predi-cted	Experi-mental	Predi-cted	Experi-mental	Predi-cted
1	800	600	70	5.39	5.23	5.62	5.50	0.331	0.324
2	800	480	70	5.91	5.71	5.65	5.62	0.338	0.333
3	800	480	70	5.77	5.71	5.74	5.62	0.339	0.333
4	800	360	70	6.53	6.54	6.86	6.57	0.349	0.353
5	680	550	64	5.96	5.96	6.10	6.39	0.344	0.348
6	800	480	60	5.77	6.00	6.59	6.36	0.331	0.338
7	680	410	76	6.34	6.51	6.18	6.53	0.338	0.344
8	680	410	64	6.24	6.15	6.07	6.20	0.345	0.344
9	800	480	80	6.10	5.72	6.30	6.10	0.337	0.327
10	1000	480	70	5.10	5.18	5.47	5.42	0.305	0.312
11	600	480	70	6.34	6.11	6.63	6.24	0.350	0.340
12	680	550	76	5.48	5.69	6.05	6.09	0.315	0.326

Table 41 (cont.)

Experi-mental no.	Factors			Responses					
	X_1 Infrared wattage (W)	X_2 Treatment duration (second)	X_3 Hot air temperature (mmHg)	FFA		MC		a_w	
				Experi-mental	Predi-cted	Experi-mental	Predi-cted	Experi-mental	Predi-cted
13	920	550	64	5.48	5.35	5.66	5.57	0.319	0.316
14	920	410	64	6.24	6.06	6.15	6.37	0.342	0.334
15	920	550	76	4.53	4.66	4.80	4.93	0.300	0.304
16	920	410	76	5.56	6.00	6.40	6.37	0.342	0.342
17	800	480	70	5.48	5.71	5.44	5.62	0.319	0.333
		%E		0.15		0.10		0.14	

Table 42 Optimized conditions for IR-HA stabilization method in term of FFA content, MC and a_w

Variables	Optimized conditions for % FFA	Optimized conditions for MC	Optimized conditions for a_w	Optimized conditions for combination of % FFA, MC, and a_w
IR wattage (W)	995	997	998	999
Treatment duration (second)	586	573	597	596
Hot air temperature (°C)	78	72	79	72
Quantities of dependent variables as predicted by RSM software	3.85 (% as Oleic acid)	4.63 (%)	0.275	4.22 (% as Oleic acid), 4.68, 0.286
Desirability	1.000	1.000	1.000	1.000

Note: The importance of optimization combination of % FFA, MC, and a_w were *****, ***, and *** respectively

2. RB stabilization and optimization by the RF

2.1 Pre-treatment RSM

The RSM comprised three independent variables of X_1 (RF treated temperature: °C), X_2 (treatment duration: seconds), and X_3 (RB moisture content: %). The completed design consisted of 17 experimental treatments that determined the FFA content, MC, and a_w in the SRB each week for the storage duration. The results of the %FFA content, MC, and a_w are shown in tables 43- 45, respectively.

Table 43 shows the FFA analysis of ANOVA for the response surface quadratic model of each week. From the value of the experiments, the lowest *p*-values were found at weeks 4 and 5 (0.0003), and the F-value at week 4 (21.28) was higher than week 5 (21.75). Table 44 shows the MC analysis of ANOVA for the response surface quadratic model of each week from the value of the experiments. The lowest *p*-value was found at Week 4 (p-value<0.0001). The a_w value (table 45) shows the lowest p-value at week 6 (*p*-value<0.0001). Thorough consideration of the *p*-value from the RSM software of each week of stabilizing the

RB storage duration by the RF treatment with the FFA content, MC, and a_w indicated that the conclusion of the selected week for continuing the experiment was week 4.

Therefore, the three responses (sample processing temperature, treatment duration, and RB moisture content) suggested quadratic polynomial models at week 4 that could be effectively used to predict the models. The significant regression and non-significant lack of fit of the model would fit to the experimental data that were effectively predicted within the system.

Table 43 FFA content in SRB by RF method and the analysis of variance table of ANOVA for RSM each week from experiments

Experiments no.	RF treated temperature(°C)	Levels		%FFA								
		Treatment duration (second)	Rice bran moisture content (%)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	110	180	15	1.52±0.04	1.85±0.07	2.44±0.04	2.87±0.04	3.20±0.04	3.30±0.04	3.66±0.04	3.80±0.04	3.85±0.07
2	98	253	12	1.97±0.08	4.79±0.04	6.36±0.04	7.33±0.07	7.71±0.07	8.21±0.04	8.62±0.04	8.74±0.04	8.92±0.04
3	110	360	20	1.54±0.04	1.59±0.04	1.90±0.04	2.04±0.04	2.16±0.04	2.49±0.07	2.47±0.07	2.75±0.04	2.59±0.04
4	110	360	15	1.59±0.04	1.92±0.07	2.33±0.04	2.71±0.07	2.75±0.07	3.09±0.04	3.73±0.04	3.63±0.02	4.01±0.04
5	90	360	15	1.90±0.04	4.22±0.04	5.77±0.07	6.62±0.07	6.79±0.07	7.55±0.07	7.74±0.07	7.79±0.04	7.88±0.07
6	122	253	12	1.61±0.04	1.50±0.07	1.64±0.07	1.73±0.08	1.73±0.08	1.73±0.04	1.92±0.04	1.90±0.04	2.16±0.04
7	98	467	12	1.57±0.07	2.99±0.07	3.99±0.07	4.56±0.07	5.15±0.07	5.58±0.04	5.53±0.04	5.84±0.07	5.58±0.04
8	130	360	15	1.73±0.04	1.52±0.04	1.52±0.04	1.66±0.04	1.64±0.04	1.69±0.04	1.90±0.04	1.88±0.07	2.02±0.04
9	110	360	10	1.80±0.0	1.76±0.04	2.14±0.07	2.37±0.04	2.61±0.04	3.06±0.07	3.30±0.07	3.30±0.04	3.44±0.07
10	98	467	18	1.71±0.07	2.42±0.07	3.11±0.04	3.80±0.11	3.47±0.11	4.39±0.04	4.65±0.04	4.75±0.04	4.89±0.04
11	122	253	18	1.78±0.07	1.66±0.04	1.92±0.07	1.85±0.07	2.09±0.07	1.97±0.04	2.09±0.04	1.90±0.04	1.99±0.07
12	110	360	15	1.80±0.11	1.61±0.04	1.95±0.07	2.06±0.07	1.97±0.07	2.02±0.04	2.23±0.04	2.75±0.04	2.54±0.11
13	122	467	12	1.57±0.07	1.73±0.04	1.92±0.07	1.85±0.07	1.92±0.07	1.88±0.04	2.09±0.04	2.06±0.07	2.23±0.04
14	110	360	15	1.95±0.04	1.76±0.04	2.16±0.04	2.40±0.08	2.42±0.08	3.18±0.04	3.28±0.07	2.94±0.04	3.18±0.07
15	122	467	18	1.92±0.07	1.88±0.04	2.04±0.04	1.85±0.07	2.09±0.07	2.02±0.04	2.16±0.04	2.33±0.04	2.56±0.04
16	110	540	15	1.52±0.04	1.85±0.07	2.44±0.04	2.87±0.04	3.20±0.04	3.30±0.04	2.47±0.04	2.59±0.04	2.75±0.04
17	98	253	18	1.92±0.07	3.20±0.07	4.08±0.04	4.72±0.04	5.20±0.07	5.44±0.04	5.53±0.04	5.89±0.04	5.98±0.07

Table 43 (cont.)

Experiments no.	Levels			%FFA								
	RF treated temperature(°C)	Treatment duration (second)	Rice bran moisture content (%)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	F-value			0.75	11.99	16.01	18.00	21.28	21.75	14.50	20.81	18.72
	p-value			0.6657	0.0018	0.0007	0.0005	0.0003	0.0003	0.0010	0.0003	0.0004
	Lack of Fit			0.3295	0.1219	0.1133	0.2415	0.3537	0.5694	0.3423	0.6836	0.7109
	R^2			0.4900	0.9391	0.9537	0.9586	0.9647	0.9655	0.9491	0.9640	0.9601
	%CV			9.32	16.43	16.93	17.41	16.01	16.27	19.80	16.06	16.08

Table 44 MC in SRB by RF method and the analysis of variance table of ANOVA for RSM each week from experiments

Experiment no.	Levels			Moisture content (%)								
	RF treated temperature(°C)	Treatment duration (second)	RB moisture content (%)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	110	180	15	4.05±0.16	3.61±0.12	4.09±0.06	4.00±0.18	4.36±0.22	3.95±0.07	4.10±0.12	3.90±0.11	4.42±0.09
2	98	253	12	11.31±0.20	10.71±0.13	9.90±0.32	8.99±0.17	8.20±0.27	7.88±0.09	7.73±0.12	7.37±0.20	7.50±0.09
3	110	360	20	2.75±0.12	3.11±0.07	3.34±0.10	3.91±0.15	3.51±0.08	3.61±0.04	3.56±0.06	3.42±0.10	3.95±0.09
4	110	360	15	3.58±0.06	3.62±0.10	3.79±0.19	4.02±0.05	3.59±0.13	3.83±0.27	4.04±0.26	3.80±0.14	4.79±0.03
5	90	360	15	14.12±0.59	14.51±0.38	11.06±0.08	10.75±0.08	9.30±0.17	8.55±0.03	8.37±0.10	7.82±0.03	7.83±0.04
6	122	253	12	0.81±0.17	0.58±0.03	1.45±0.10	1.90±0.07	1.83±0.04	1.88±0.04	1.99±0.19	2.19±0.08	3.64±0.12
7	98	467	12	6.58±0.02	6.30±0.11	6.15±0.12	5.87±0.28	5.84±0.15	5.41±0.10	5.47±0.10	5.24±0.12	5.68±0.17
8	130	360	15	0.48±0.22	0.48±0.08	1.77±0.11	1.41±0.06	1.78±0.09	1.83±0.03	2.21±0.14	2.10±0.03	2.80±0.07
9	110	360	10	3.04±0.14	3.10±0.10	3.57±0.10	3.44±0.11	3.33±0.13	3.75±0.07	3.70±0.06	3.43±0.14	4.02±0.18
10	98	467	18	6.02±0.18	5.91±0.26	5.87±0.05	5.88±0.15	5.47±0.15	5.21±0.11	5.70±0.10	4.90±0.16	5.61±0.18
11	122	253	18	1.19±0.17	1.07±0.02	1.59±0.09	1.83±0.08	2.15±0.15	2.81±0.05	2.39±0.09	2.24±0.08	3.26±0.10
12	110	360	15	1.67±0.24	1.79±0.11	1.97±0.08	2.22±0.18	2.29±0.07	2.31±0.10	2.64±0.01	2.67±0.06	3.51±0.17
13	122	467	12	0.20±0.06	0.48±0.13	0.91±0.24	1.48±0.10	1.71±0.10	1.68±0.05	1.83±0.16	2.25±0.10	3.10±0.15
14	110	360	15	2.85±0.04	2.83±0.09	3.57±0.22	3.42±0.12	3.30±0.05	3.74±0.07	3.62±0.08	2.84±0.23	3.61±0.09
15	122	467	18	2.31±0.26	1.42±0.07	1.67±0.24	2.67±0.34	2.64±0.12	2.38±0.04	2.57±0.06	2.58±0.10	3.24±0.15
16	110	540	15	1.26±0.19	1.96±0.08	2.39±0.14	2.54±0.18	2.78±0.21	2.41±0.19	2.87±0.04	3.02±0.10	3.88±0.10
17	98	253	18	8.36±0.14	7.27±0.17	7.11±0.18	6.60±0.11	6.11±0.13	6.36±0.12	6.11±0.12	5.64±0.08	6.00±0.16

Table 44 (cont.)

Experiment no.	Levels			Moisture content (%)								
	RF treated temperature(°C)	Treatment duration (second)	RB moisture content (%)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	F-value			59.87	31.87	41.47	34.19	47.39	28.43	32.64	36.22	17.37
	p-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0005
	Lack of Fit			0.8855	0.5838	0.9660	0.9348	0.9528	0.9799	0.9540	0.9508	0.9268
	R^2			0.9875	0.9462	0.9816	0.9778	0.9839	0.9734	0.9767	0.9790	0.9071
	%CV			16.30	22.31	15.04	14.40	10.83	12.89	11.26	10.12	10.45

Table 45 The a_w in SRB by RF method and the analysis of variance table of ANOVA for RSM each weeks from experiments

Experiment no.	Levels			a_w								
	RF treated temperature (°C)	Treatment duration (second)	Rice bran moisture content (%)	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
1	110	180	15	0.355±0.006	0.334±0.004	0.441±0.002	0.393±0.003	0.415±0.002	0.443±0.001	0.468±0.002	0.425±0.003	0.402±0.002
2	98	253	12	0.999±0.004	0.998±0.003	0.999±0.002	0.817±0.005	0.729±0.002	0.711±0.002	0.693±0.002	0.625±0.003	0.563±0.002
3	110	360	20	0.255±0.006	0.295±0.005	0.374±0.005	0.359±0.002	0.370±0.002	0.409±0.002	0.411±0.002	0.394±0.003	0.441±0.002
4	110	360	15	0.284±0.007	0.401±0.004	0.380±0.006	0.361±0.002	0.358±0.001	0.391±0.001	0.424±0.003	0.407±0.002	0.453±0.002
5	90	360	15	0.998±0.003	0.999±0.002	0.999±0.004	0.996±0.005	0.881±0.002	0.783±0.008	0.743±0.003	0.675±0.002	0.648±0.002
6	122	253	12	0.089±0.002	0.121±0.002	0.173±0.004	0.231±0.004	0.243±0.002	0.262±0.003	0.305±0.003	0.307±0.004	0.438±0.002
7	98	467	12	0.660±0.004	0.592±0.003	0.562±0.004	0.524±0.003	0.504±0.002	0.485±0.003	0.477±0.003	0.467±0.002	0.522±0.002
8	130	360	15	0.088±0.003	0.097±0.004	0.079±0.002	0.184±0.005	0.223±0.002	0.263±0.004	0.309±0.002	0.317±0.002	0.371±0.001
9	110	360	10	0.229±0.005	0.247±0.002	0.344±0.003	0.290±0.004	0.313±0.002	0.389±0.002	0.375±0.002	0.332±0.001	0.424±0.001
10	98	467	18	0.620±0.003	0.572±0.003	0.572±0.001	0.528±0.002	0.502±0.011	0.477±0.002	0.516±0.003	0.452±0.001	0.508±0.001
11	122	253	18	0.143±0.007	0.173±0.003	0.210±0.002	0.202±0.005	0.262±0.002	0.411±0.012	0.298±0.002	0.302±0.003	0.377±0.001
12	110	360	15	0.163±0.003	0.202±0.004	0.205±0.002	0.206±0.005	0.265±0.002	0.277±0.002	0.362±0.003	0.335±0.001	0.460±0.002
13	122	467	12	0.074±0.006	0.103±0.004	0.125±0.002	0.167±0.003	0.226±0.005	0.235±0.002	0.263±0.004	0.307±0.002	0.357±0.002
14	110	360	15	0.337±0.005	0.329±0.004	0.365±0.004	0.320±0.002	0.332±0.002	0.411±0.002	0.453±0.002	0.363±0.002	0.436±0.004
15	122	467	18	0.112±0.003	0.168±0.001	0.186±0.003	0.266±0.004	0.312±0.003	0.293±0.002	0.341±0.001	0.316±0.003	0.411±0.002
16	110	540	15	0.109±0.002	0.197±0.003	0.252±0.002	0.255±0.003	0.338±0.002	0.288±0.002	0.363±0.002	0.381±0.001	0.508±0.005
17	98	253	18	0.877±0.005	0.723±0.004	0.666±0.003	0.562±0.002	0.545±0.002	0.543±0.002	0.545±0.003	0.457±0.001	0.527±0.002
F-value				19.72	18.78	23.73	22.62	26.72	13.30	30.02	16.86	6.61
p-value				0.0004	0.0004	0.0002	0.0002	0.0001	0.0013	<0.0001	0.0006	0.0105
Lack of Fit				0.4873	0.6784	0.7922	0.7807	0.5987	0.8085	0.8881	0.6077	0.2177
R^2				0.9621	0.9602	0.9683	0.9668	0.9717	0.9447	0.9747	0.9559	0.8948
%CV				25.93	23.23	18.51	16.24	11.56	13.14	7.46	8.66	8.25

In addition, table 46, shows the quadratic model of the RSM for the SRB after 8 weeks of storage at 35°C. The model of responses of the FFA content was significant with a *p*-value of 0.0003. The low *p*-value of the RF treated temperature was < 0.0001 and the *p*-value of the treatment duration was 0.0237. The RB moisture content (10-20%) was not significant with the *p*-value of 0.0562. The RF treated temperature and treatment duration affected the RB RF stabilized treatment method more than the RB moisture content, which was the difference. Vearasilp et al. (2015) reported that the RB moisture content in *Perilla frutescens* L. highland oil seed increased the RF stabilization effectively.

The experiment considered the MC, which had to be consistent with the FFA value and a_w. Table 46 shows the MC analysis of ANOVA for the response surface quadratic model for each week. From the value of the experiments, it was found that the F-value, *p*-value, lack of fit, R^2, and %CV at Week 4 were 47.39, <0.0001, 0.9528, 0.9839, and 10.83, respectively. The a_w value had the highest significance at Week 6, and the F-value, *p*-value, lack of fit, R^2, and %CV were 26.72, 0.0001, 0.5987, 0.9717, and 11.56, respectively. The MC and a_w had a high significant difference the same as the *p*-value of the FFA content, and the RB moisture content did not affect the stabilized treatment by the RF method.

The R^2 values for the FFA, MC, and a_w models were 0.9647, 0.9839, and 0.9717, respectively. The high significant difference (lowest *p*-value) of the FFA content, MC, and a_w at week 4, which the three responses as the RF treated temperature, treatment duration, and RB moisture content, created the quadratic polynomial models that were effectively used to predict the models.

After the selection of the week 4 storage of the SRB (RF method), the response from the variable and factors of the F-value, *p*-value and R^2 in terms of the % FFA content, MC and a_w of RSM shows in Table 46.

Table 46 The variable and factor's F, *p*-value and R^2 in term of FFA content, MC and a_w at week 4 of storage time by RF method

Variables /Factors	Responses					
	FFA		MC		a_w	
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Model	21.28	0.0003****	47.39	<0.0001****	26.72	0.0001****
X_1	133.90	<0.0001****	347.74	<0.0001****	167.06	<0.0001****
X_2	8.29	0.0237*	10.85	0.0132*	4.52	0.0710^{ns}
X_3	5.22	0.0562^{ns}	0.28	0.6101^{ns}	0.0075	0.9333^{ns}
X_1X_2	9.18	0.0191*	7.52	0.0288*	5.27	0.0554^{ns}
X_1X_3	10.18	0.0152*	9.12	0.0194*	4.92	0.0620^{ns}
X_2X_3	0.19	0.6782^{ns}	3.60	0.0997^{ns}	3.60	0.0994^{ns}
X_1^2	23.85	0.0018***	46.03	0.0003****	33.08	0.0007****
X_2^2	2.53	0.1555^{ns}	1.96	0.2038^{ns}	1.58	0.2485^{ns}
X_3^2	0.53	0.4897^{ns}	0.90	0.3734^{ns}	0.13	0.7275^{ns}
Lack of Fit	2.10	0.3587^{ns}	0.17	0.9528^{ns}	0.91	0.5987^{ns}
R^2	0.9647		0.9839		0.9717	
Adj. R^2	0.9194		0.9631		0.9353	

Remark: X_1 = RF treated temperature, X_2 = treatment duration, X_3 = RB moisture content

*= significant $p<0.05$, **= significant $p<0.01$, ***= significant $p<0.005$, ****= significant $p<0.001$

^{ns}= non-significant

Table 46 presents the variability of the parameters by the models, which indicated that the predicted values obtained by the quadratic polynomial equations had a strong correlation with the actual experimental values with Pearson's correlation coefficient (Hossain et al., 2012). These models were well adapted to the response and were effective to predict the equation of the FFA, MC, and a_w of the SRB by the RF method for the stabilization of the RB. The FFA regression equations of the SRB relating to the actual levels of the RF treated temperature (X_1), treatment duration (X_2), and RB moisture content (X_3) were calculated using Eq. 27.

$$FFA = 137.57378 - 1.72948(X_1) - 0.071554(X_2) - 2.42022(X_3) + 0.00044(X_1)(X_2) + 0.016688(X_1)(X_3) + 0.0002514(X_2)(X_3) + 0.005378(X_1)^2 + 0.0000216(X_2)^2 + 0.012842(X_3)^2 \quad (27)$$

The three-dimensional response surface plots represented the regression equation that indicated the relationships between the dependent and independent variables. The different shapes demonstrated whether the variables or mutual interactions were significant or not (Liu et al., 2013). The FFA response surface plots based on Eq. 27 shows in Figure 21. The RF treated temperature (X_1) and treatment duration (X_2) strongly affected the FFA content, while the RB moisture content variable was constantly fixed at the respective zero level (15%) (Figure 21a).

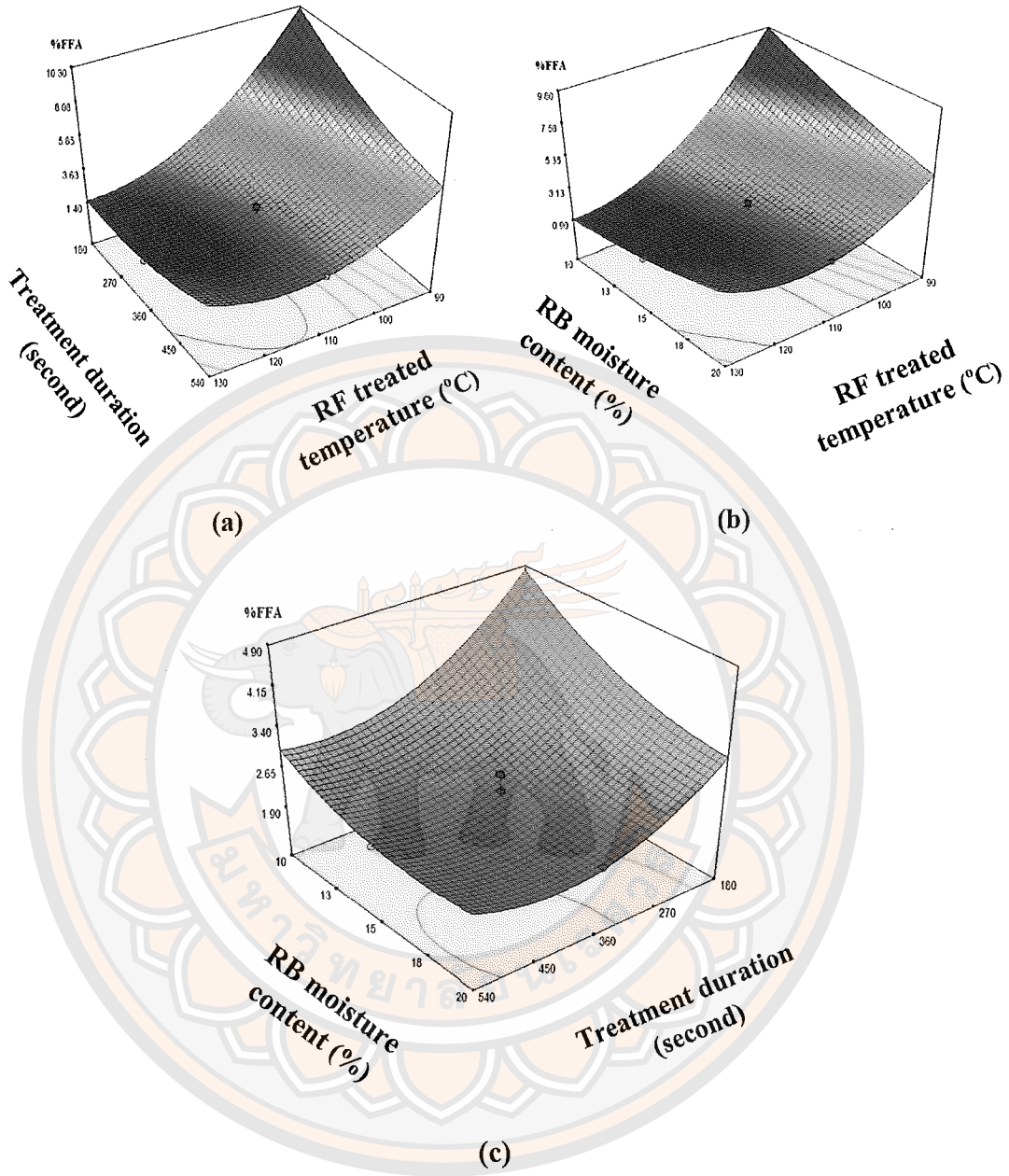


Figure 21 Response surface and contour plots for FFA content in SRB by RF method as a function of RF treated temperature and treatment duration (a), RB moisture content and RF treated temperature (b), and RB moisture content and treatment duration (c)

The FFA content was decreased while it increased in the RF treated temperature, RF treatment duration, and RB moisture content. This finding was similar to that reported by Vearasilp et al. (2015), who observed an increase in the RF treated temperature, RF treatment duration and RB moisture content that was able to decrease the lipase activity of the SRB. The FFA content was related to the lipase activity, and the decreased lipase activity caused a decrease in the FFA content in the SRB. In the current study, the RF treated temperature and RF treatment duration significantly affected the FFA content (p-value < 0.0001). The RF treated temperature was found to have a strong effect on inactivating the lipase or destroying the lipase activity. Additionally, the FFA content was decreased with an increase in the RF treated temperature, while there was an increase in the RF treatment duration (in this study: between 180-540 seconds) that was able to decrease the FFA content. Figure 21b shows the effect of the RF treated temperature and RB moisture content on the FFA content, while the treatment duration variable was constantly fixed at the respective zero levels (360 seconds). It was found that the RF treated temperature affected the SRB's FFA content, but the RB moisture content did not significantly affect the FFA content. This finding was not similar to that reported by Vearasilp et al. (2015), who observed that an increase in grain MC (between 10-18%: db) increased the lipase activity (92-100 Ug-1 dry matter); this may be due to the different raw materials of the grain and RB in this study. The three-dimensional surface and contour plots for the effects of the RB moisture content and treatment duration on the FFA content in the SRB shows in Figure 21c. The FFA content decreased with the increasing RF treatment duration, while the RB moisture content was not significant.

The RF treated temperature and treatment duration significantly affected the MC of the RB, while the RB moisture content did not affect the MC of the RB. However, the X_2X_3, X_2^2, and X_3^2 interactions and quadratic coefficient terms were not significant since the p-value was more than 0.05. The second-order polynomial equation of MC shows in Eq. 28.

$$MC = 144.93794 - 1.86584(X_1) - 0.065151(X_2) - 2.21097(X_3) + 0.00033(X_1)(X_2) + 0.013117(X_1)(X_3) + 0.000915(X_2)(X_3) + 0.006207(X_1)^2 + 0.0000158(X_2)^2 + 0.013917(X_3)^2 \quad (28)$$

The three-dimensional surface and contour plots of the effects of the factors on the MC of SRB based on Eq. 28 shows in Figure 22. The RF treated temperature and treatment duration affected the MC of the SRB, while the RB moisture content did not affect the MC of the SRB. The MC response surface plots based on Eq. 28 are presented in Figure 22. Figure 22a shows the effect of the RF treated temperature and treatment duration on the MC, while the RB moisture content was fixed as constant at the respective zero levels (15%). The high RF treated temperature and treatment duration had a high efficiency to decrease the MC. The surface plot of Figure 22b demonstrates the RF treatment temperature and RB moisture content, while the treatment duration was continuously constant at the respective zero levels (360 seconds). The MC decreased with the increasing RF treatment temperature, but the RB moisture content did not affect the MC in the SRB. Figure 22c shows the effect of the treatment duration and RB moisture content on the MC in the SRB. It was especially found that the treatment duration was affected by the MC, but the RB's MC did not affect the MC in the SRB by the RF treatment.

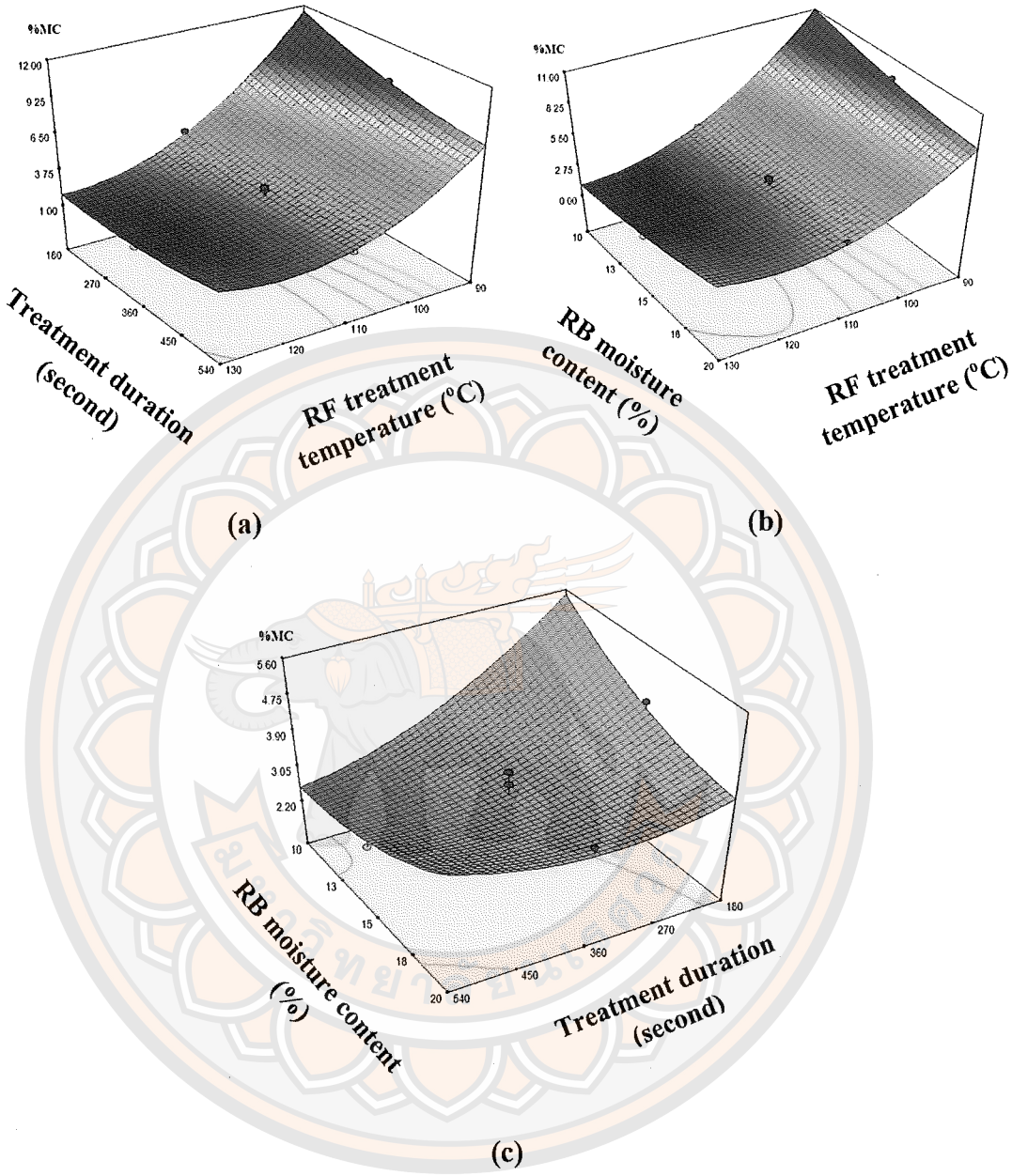


Figure 22 Response surface and contour plots for MC in SRB by RF method as a function of RF treated temperature and treatment duration (a), RB moisture content and RF treated temperature (b), and RB moisture content and treatment duration (c)

The RF treated temperature and treatment duration significantly affected the a_w in the SRB, while the RB moisture content did not affect the MC of the RB (p-value = 0.9333). Additionally, the X_1X_2, X_1X_3, X_2X_3, X_2^2, and X_3^2 interactions and quadratic coefficient terms were not significant since the p-values were more than 0.05. The second-order polynomial equation of a_w shows in Eq. 29.

$$a_w = 12.50777 - 0.16407(X_1) - 0.006061(X_2) - 0.16502(X_3) + 0.00002956(X_1)(X_2) + 0.0010288(X_1)(X_3) + 0.000097816(X_2)(X_3) + 0.00056167(X_1)^2 + 0.0000015176(X_2)^2 + 0.00056679(X_3)^2 \quad (29)$$

The response surface plots based on the a_w (Eq. 29) are depicted in Figure 23. The RF treated temperature and treatment duration affected the a_w, while the RB's MC was a fixed constant at the respective zero levels (15%) (Figure 23a). Figure 23a shows that the RF treated temperature and treatment duration significantly reduced the a_w values. The surface plot showed that an increase in the RF treatment temperature decreased the a_w, while an increase in the RB moisture content increased the a_w in the SRB (Figure 23b). Figure 23c shows the surface plot between the treatment duration and RB's MC. There was an increase in the treatment duration that reduced the a_w, but an increase in the RB's MC could increase the a_w.

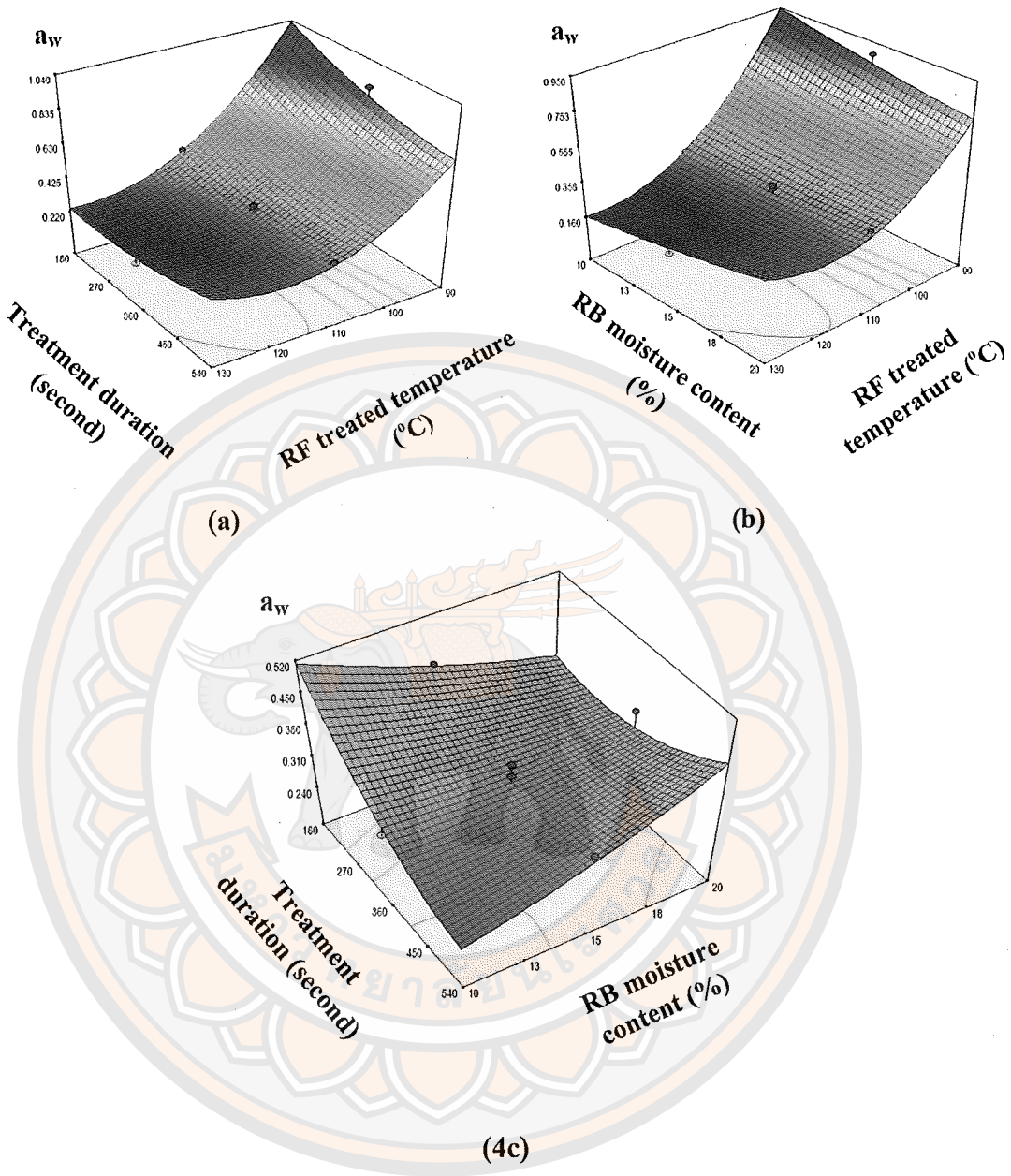


Figure 23 **Response surface and contour plots for a_w in SRB by RF method as a function of RF treated temperature and treatment duration (a), RB moisture content and RF treated temperature (b), and RB moisture content and treatment duration (c)**

The predicted equation model of the FFA, MC, and a_w as stabilization by RF were calculated by the RSM and validated by entering the data for the various treatments into the prediction model again and comparing the experimental data with the predicted data under the same conditions (Table 47). The criterion for fitting the efficiency data of the FFA, MC and a_w models was calculated as the %E difference between the experimental and predicted data (Eq. 2) (Hossain et al., 2012). The FFA, MC and a_w predicted by the RSM model shows in table 47, which calculated the %E difference of the FFA, MC, and a_w from the experimental data, which were 0.60, 0.96 and 9.62, respectively.

Table 47 The experimental design of CCD, actual experiment value, and predicted value of FFA, MC and a_w in RB at week 4 of storage time by RF method

Experi-mental no.	Factors			Responses					
	X_1	X_2	X_3	FFA (%)		MC (%)		a_w	
	Sample temperature (°C)	Treatment duration (second)	Sample moisture (%)	Experi-mental	Predi-cted	Experi-mental	Predi-cted	Experi-mental	Predi-cted
1	110	180	15	3.20	3.74	4.36	4.20	0.393	0.414
2	98	253	12	7.71	7.12	8.20	8.08	0.817	0.728
3	110	360	20	2.16	2.12	3.51	3.25	0.359	0.336
4	110	360	15	2.75	2.35	3.59	3.01	0.361	0.320
5	90	360	15	6.79	7.25	9.30	9.19	0.996	0.833
6	122	253	12	1.73	1.48	1.83	1.86	0.231	0.232
7	98	467	12	5.15	5.01	5.84	5.85	0.524	0.536
8	130	360	15	1.64	1.75	1.78	1.80	0.184	0.256
9	110	360	10	2.61	3.21	3.30	3.47	0.290	0.332
10	98	467	18	3.47	3.31	5.47	5.36	0.528	0.527
11	122	253	18	2.09	1.87	2.15	2.09	0.202	0.245
12	110	360	15	1.97	2.35	2.29	3.01	0.206	0.320
13	122	467	12	1.92	1.63	1.71	1.32	0.167	0.191
14	110	360	15	2.42	2.35	3.30	3.01	0.320	0.320
15	122	467	18	2.09	2.34	2.64	2.72	0.266	0.331
16	110	540	15	2.33	2.36	2.78	2.85	0.255	0.324
17	98	253	18	5.20	5.10	6.11	6.42	0.562	0.593
%E				0.60		0.96		9.62	

The optimized conditions of the FFA content, MC and a_w were calculated by the RSM in Design-Expert software (table 48). The optimum values of the FFA, MC, and a_w of the process variables were obtained for the maximum desirability function of 1.000, 1.000, and 1.000, respectively. The RF's SRB optimized condition was RF treated temperature = 130°C, treatment duration = 322 seconds, and rice bran moisture content = 10.22%. The quantities of the dependent variables as predicted by the RSM software of the FFA, MC, and a_w under these conditions were 0.85 %, 1.10%, and 0.164, respectively.

Table 48 Optimized conditions for RF stabilization method in term of FFA content, MC and a_w

Variables	Optimized conditions for FFA content	Optimized conditions for MC	Optimized conditions for a_w	Optimized conditions for combination of FFA content, MC, and a_w
RF treated temperature (°C)	129	126	121	130
Treatment duration (second)	299	366	537	322
RB moisture content (%)	11.81	10.95	10.33	10.22
Quantities of dependent variables as predicted by RSM software	0.99 (% as Oleic acid)	1.16 (%)	0.167	0.85 (% as oleic acid), 1.10, 0.164
Desirability	1.000	1.000	1.000	1.000

Note: The importance of optimization for combination of % FFA, MC, and a_w were *****, ***, and *** respectively

Conclusion of section 2

The conclusion of the optimized conditions of stabilization by the IR, IR-VC, IR-HA, and RF methods as a combination of the FFA content, MC, and a_w by the RSM in Design-Expert software based on the most significant difference week of the storage time is shown in table 49. The optimal condition for RB stabilization by IR was found in an IR wattage of 993 W and treatment duration of 598 seconds. In addition, the optimal condition of IR-VC stabilization of RB was an IR wattage of 997 W, treatment duration of 598 seconds, and vacuum strength of 610 mmHg, while that for IR-HA treatment was an IR wattage of 999 W, treatment duration of 598 seconds, and hot air temperature of 72°C. Hence, the IR-VC and RF stabilized RB methods were effective in stabilizing the RB because of the low FFA content, MC, and a_w.

The optimal stabilized condition of the IR, IR-VC, IR-HA, and RF stabilized treatment methods were selected to be used in the next section (storage stability). The NSRB and SRB (the best conditions in Section 2, i.e., IR, IR-VC, IR-HA, and RF stabilization methods) were stored at 35°C for 8 weeks. The samples were collected each week during that period for the determination of the FFA content, MC and a_w of each sample, and the shelf life and FFA formation rate in the RB were investigated. The NSRB and SRB samples were collected at Weeks 0, 4 and 8, which was the period for the determination of the proximate composition, microbial analysis, fatty acid composition, tocopherol, tocotrienol, and oryzanol content.

Table 49 Optimized conditions for IR, IR-VC, IR-HA, and RF stabilization methods in term of FFA content, MC and a_w

Stabilization method	Most significantly difference week	optimized conditions stabilization						Optimization condition quantitative		
		IR wattage (W)	Treatment duration (second)	Vacuum strength (mmHg)	Hot air temperature (°C)	RF treated temperature (°C)	RB moisture content (%)	FFA (% as Oleic acid)	MC (%)	a_w
IR	4	993	598	-	-	-	-	3.59	4.58	0.432
IR-VC	4	997	598	610	-	-	-	0.33	1.00	0.117
IR-HA	5	999	596	-	72	-	-	4.22	4.68	0.286
RF	4	-	322	-	-	130	10.22	0.85	1.10	0.164

Section 3 Storage stability

1. Storage stability of the SRB by the IR method

1.1 FFA storage stability

The NSRB and SRB by IR method were stored at 35°C for 8 weeks and the FFA content was monitored every week. The most important measure of the stabilization process was its ability to inhibit the lipase activity in RB resulting in a lower FFA content during storage. Refining of the crude oil with more than 10% of the FFA was considered uneconomical, as RBO normally contains 1.5-2.0% of FFA immediately after milling (Malekian et al., 2000).

Less than 5% of the FFA of RB is desirable in crude oil for economic refining purposes (Enochian et al., 1980). According to Tao et al. (1993, as cited in Kim et al., 2014), the FFA content of 5% or lower is desirable, but RB containing more than 5% of FFA is considered not suitable for human consumption. Lakkakula et al. (2004, as cited in Irakli et al., 2018) similarly reported that SRB by IR stabilization had a lower FFA content than NSRB after 6 months of storage, as the RB sample was exposed to a relatively high temperature of IR for a longer period of time, and a decrease in the FFA content was noticed. The SRB was proved to be ineffective even after 1 month of storage, as the FFA content of the RB exceeded the maximum value (10%), which was considered to be acceptable for human consumption. Yilmaz (2016) also reported that RB stabilized with the IR process at 700 W for 7 minutes showed a slow rising of the FFA from an initial content of 4.86% to 5.75% after 60 days of storage. In the current study, the FFA content of the SRB by IR stabilized the method obtained at an optimal condition (993 watts of IR wattage and 598 seconds of treatment duration) and was compared with the NSRB.

The initial FFA content of the NSRB sample, which was measured immediately after milling, was comparable with that of the SRB (Figure 24). However, the FFA of the NSRB increased from initially 1.88% to 11.19% after 8 weeks of storage, while a steady increase in the FFA level was observed in the SRB that increased from initially 1.93 % to 7.45 % after 8 weeks. It is important to note that at the end of the 8 weeks of storage, the FFA of the SRB was about 7.45 %, which was a non-effective range for oil extraction (Enochian et al., 1980 as cited in Malekian et al., 2000). The FFA content of the NSRB was always higher than that of the SRB throughout the studied

storage period. The reason for this was that the IR stabilization effectively inhibited the lipase activity and delayed the hydrolytic rancidity resulting in a much lower rate of increase in the FFA value compared to that of the NSRB sample.

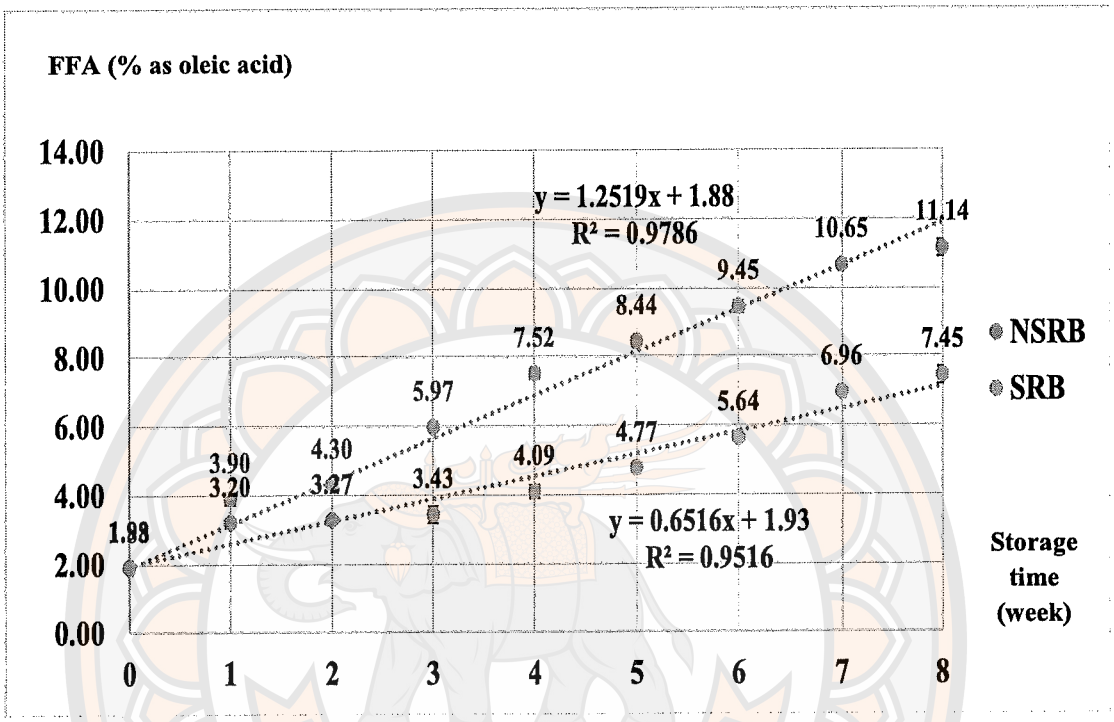


Figure 24 FFA content of NSRB and SRB by IR method during storage

1.2 MC, a_w, and color storage stability

The MC and a_w were also affected by the IR treatments that were related to the FFA results. RB contains lipase and also contains microorganisms; for example, fungi, which are capable of producing enzymes, such as lipase that can induce the deterioration of RBO. The effects of the SRB's a_w between 0.147-0.361 (table 50) on the enzyme activity was low, the bacteria were unable to grow, and the yeast and mold grew at a slight rate. These were located in zone II (0.25-0.80) in the safety control of the a_w graph (Fennema, 1976). Kim & Lim (2014) and Kim et al. (2014) reported that reducing the MC could deactivate the enzymes and microorganisms in the RB. Kim and Lim (2014) reported that the low MC in the RB had a slower rate of the formation of the FFA during storage than a high MC in the RB.

In the current study, the effects of the SRB's MC on the NSRB and SRB by IR method were between 5.83-7.02 and 1.26-3.92, respectively, which IR that the SRB could deactivate more enzymes and microorganisms than the NSRB. The MC and a_w values (Table 50) increased, respectively in the MC and a_w of the SRB, which could be a result of the moisture absorption phenomenon from the surrounding environment during storage even though it was stored in sealed polyethylene (PE) bags. Once the equilibrium stage was achieved, the constant MC was investigated.

The color results are presented in table 50. The SRB was darker than the NSRB sample with regard to the lower lightness (L*) value. The a*, b* and ΔE values of the SRB were also found to be higher than that of the NSRB. Aliva and Silva (1999) similarly observed that the heat treatment of RB led to a decrease in the L* values. Irakli et al. (2018) reported the SRB color could be due to the Maillard reaction occurring during the heat treatment. The same trend was observed as the IR temperature increased in the same duration of time, which was most probably due to the formation of brown polymers from the Maillard reaction induced by the heat treatment (Miranda et al., 2009). The SRB indicated an intense yellow color, which could be due to the intensity of the intermediate stages of the Maillard reaction, and could partly be due to the thermal oxidation of the RBO (Irakli et al., 2018).

Table 50 MC, a_w, color and ΔE changed in NSRB and SRB by IR method during storage

Sample	Storage week	MC (%)	a_w	L*	a*	b*	ΔE
NSRB	0	5.84 ± 0.15^{f}	0.410 ± 0.003^{j}	67.45 ± 0.03^{e}	3.67 ± 0.01^{cd}	19.84 ± 0.06^{ab}	0.00 ± 0.00^{a}
	1	6.05 ± 0.11^{g}	0.424 ± 0.001^{k}	68.38 ± 0.27^{f}	4.59 ± 0.22^{g}	19.99 ± 1.52^{bc}	1.68 ± 0.46^{e}
	2	7.02 ± 0.15^{k}	0.430 ± 0.001^{l}	68.52 ± 0.06^{fg}	3.89 ± 0.12^{c}	20.14 ± 0.17^{bcd}	0.65 ± 0.12^{b}
	3	6.66 ± 0.05^{j}	0.445 ± 0.005^{m}	69.55 ± 0.07^{j}	3.42 ± 0.01^{bc}	19.18 ± 0.20^{a}	2.45 ± 0.23^{f}
	4	6.21 ± 0.18^{h}	0.446 ± 0.002^{m}	65.99 ± 0.04^{c}	4.30 ± 0.04^{fg}	19.60 ± 0.35^{ab}	0.80 ± 0.06^{bc}
	5	6.06 ± 0.06^{g}	0.429 ± 0.002^{l}	68.99 ± 0.01^{hi}	2.88 ± 0.19^{a}	20.28 ± 0.33^{bcd}	1.68 ± 0.27^{e}
	6	6.35 ± 0.11^{hi}	0.432 ± 0.003^{l}	68.76 ± 0.04^{gh}	3.36 ± 0.08^{bc}	20.29 ± 0.15^{bcd}	1.03 ± 0.14^{cd}
	7	6.46 ± 0.06^{ij}	0.444 ± 0.004^{m}	69.24 ± 0.02^{i}	3.40 ± 0.16^{bc}	19.79 ± 0.19^{ab}	1.67 ± 0.06^{e}
	8	5.83 ± 0.08^{f}	0.441 ± 0.015^{m}	69.28 ± 0.06^{i}	3.19 ± 0.09^{b}	20.09 ± 0.22^{bc}	1.86 ± 0.19^{e}
SRB	0	1.26 ± 0.04^{a}	0.147 ± 0.002^{a}	64.84 ± 0.03^{a}	4.55 ± 0.12^{g}	21.43 ± 0.03^{e}	5.11 ± 0.20^{i}
	1	1.90 ± 0.06^{b}	0.195 ± 0.004^{b}	65.25 ± 0.30^{b}	4.08 ± 0.27^{ef}	21.24 ± 0.13^{e}	3.58 ± 0.38^{h}
	2	3.23 ± 0.09^{d}	0.233 ± 0.003^{c}	65.31 ± 0.02^{b}	4.39 ± 0.02^{fg}	21.39 ± 0.01^{e}	3.80 ± 0.02^{h}
	3	3.25 ± 0.12^{d}	0.249 ± 0.004^{d}	65.98 ± 0.02^{c}	4.30 ± 0.04^{fg}	20.71 ± 0.05^{cde}	1.68 ± 0.03^{e}
	4	2.84 ± 0.11^{c}	0.273 ± 0.004^{e}	65.99 ± 0.04^{c}	4.32 ± 0.12^{fg}	20.88 ± 0.18^{de}	1.87 ± 0.17^{e}
	5	3.01 ± 0.12^{c}	0.293 ± 0.002^{f}	66.35 ± 0.55^{d}	3.93 ± 0.54^{de}	21.12 ± 0.49^{e}	1.79 ± 0.21^{e}
	6	3.85 ± 0.21^{e}	0.315 ± 0.004^{g}	65.74 ± 0.04^{c}	4.32 ± 0.09^{fg}	21.35 ± 0.18^{e}	2.89 ± 0.29^{g}
	7	3.92 ± 0.20^{e}	0.366 ± 0.004^{i}	66.60 ± 0.06^{d}	4.34 ± 0.21^{fg}	20.87 ± 0.16^{de}	1.18 ± 0.12^{d}
	8	3.38 ± 0.10^{d}	0.361 ± 0.015^{h}	66.43 ± 0.03^{d}	4.08 ± 0.08^{ef}	21.45 ± 0.10^{e}	1.97 ± 0.19^{e}

Note: Values are expressed as means ± standard deviation. Values for the same rice bran fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($P < 0.05$)

1.3 Proximate and microbial analysis

The NSRB and SRB by the IR method were stored for 0, 4 and 8 weeks to analyze their proximate composition and understand their basic differences (Table 51). The ash, protein, fat, fiber, and carbohydrate content (dry basis) increased as a result of the IR stabilization. Additionally, it was observed that the protein content of the SRB was significantly higher than the fresh counterpart. The fiber content decreased that could probably be explained by the IR treatment disintegrating the long-chain fiber molecule, thereby a decrease in the molecular weight of the fiber and change in the fiber's characteristic was generated or initiated.

Table 51 RB proximate and microbial analysis in NSRB and SRB by IR method during storage

Analysis	Week 0		Week 8		% changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Proximate (g/100g dry basis)						
carbohydrate	50.88 ± 0.50^{a}	52.89 ± 0.99^{b}	50.40 ± 0.21^{a}	50.70 ± 0.14^{a}	-0.94	-4.41
fat	16.54 ± 0.24^{b}	15.61 ± 0.57^{a}	16.83 ± 0.34^{b}	17.00 ± 0.27^{b}	+1.75	+8.90
protein	14.58 ± 0.06^{a}	14.67 ± 0.15^{ab}	14.83 ± 1.32^{ab}	14.91 ± 0.19^{b}	+1.71	+1.64
fiber	9.47 ± 0.49^{b}	8.32 ± 0.22^{a}	9.46 ± 0.33^{b}	8.71 ± 0.11^{a}	-0.11	+4.69
ash	8.52 ± 0.34^{ns}	8.51 ± 1.24^{ns}	8.47 ± 0.11^{ns}	8.68 ± 0.07^{ns}	-0.59	+2.00
	Average % changed				+0.36	+2.56
	Total % changed				+1.82	+12.8
Microbial (CFU/g)						
TPC	$5.15\times10^{3}\pm0.11$	$4.45\times10^{3}\pm0.06$	$3.00\times10^{4}\pm0.04$	$6.45\times10^{3}\pm0.05$	+482.5	+44.9
Yeast & mold	$1.65\times10^{3}\pm0.03$	$3.40\times10^{2}\pm0.03$	$2.45\times10^{3}\pm0.03$	$4.25\times10^{2}\pm0.03$	+48.48	+25.0
	Average % change				+266	+35.0
	Total % change				+531	+69.9

Note: Values are expressed as means ± standard deviation. Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

The TPC of the NSRB and SRB by the IR method are displayed in Table 51. The TPC in the NSRB showed 10 times more significant growth than the SRB after 8 weeks of storage. The yeast&mold in the SRB were detected 2 times to be lower than the NSRB in the 8 weeks of storage. The SRB sample showed a high stability against the microbial growth during the eight weeks of storage. The TPC, yeast& mold in this study were increased but within the standard level for the duration of the storage ($<10^4$ CFU/g). The combination of the IR wattage and treatment duration inhibited the growth of the microbes, especially those that produced lipases; such as, *Pseudomonas sp, fluorescens, Burkholderia cenocepacia,* Acinetobacter junii, Rhizobium miluonense, Pseudoxanthomonas sp., and *Staphylococcus sp.* (Aguilera, & Stanley, 1999). The main reason was that the low MC and a_w values in the SRB retarded the microbial growth and made the bran more stable along with the storage duration.

1.4 FA composition storage stability

The FA composition of the RB samples is shown in table 52. The NSRB contained approximately 4.16% of saturated and 12.42% of unsaturated FA (6.12% monounsaturated and 6.30 polyunsaturated), while the SRB contained approximately 4.36% of saturated and 13.03% of unsaturated FA. The SRB samples possessed more saturated, monounsaturated, polyunsaturated, and total unsaturated FA content than the NSRB. Kim et al. (2014) stated that the mixture of compounds; such as, tocols and policosanols could exist in the RB by binding to a single component; such as, proteins and lipids. Free tocols, policosanols, and other compounds that could be released during the heat treatments increased the lipids and FA contents in the RB.

In the current study, the high molecular weight of the FA in the NSRB could bind proteins or lipids. The combination of the IR wattage and treatment duration disintegrated the binding of the FA to protein or lipid linkages and released lipid compounds. On the other hand, saturated, monounsaturated, polyunsaturated, and total unsaturated FA content was decreased in both the NSRB and SRB after storage. The monounsaturated, polyunsaturated, and total unsaturated FA may have undergone oxidation with the hydronium ion changing the double bond into a single bond (Fennema, 1996). Moreover, the quantities of omega-6 and omega-9 in the SRB samples were higher than in the NSRB, but all displayed a declining trend during storage.

The results indicated that the effect of the IR in the RB could significantly change the FA profile content ($p < 0.05$), but a different result had been already observed by Yilmaz et al. (2014). Ramezanzadeh et al. (2000) also mentioned that after MW, RB could be stored at 4-5°C for up to 16 weeks without any adverse effect on the quality of the proximate and FA composition. Similarly, no significant reduction of main FA in wheat germ stabilized with IR heating was found by Li et al. (2016).

During the 8 weeks of storage, the FA profile content in the NSRB displayed a higher change than the SRB, which was a result of the IR treatment. The average percentage of the changed FA profile in the NSRB decreased by 3.76%, while the SRB decreased by 0.26%. It was found that the IR treatment was effective for stabilizing the FAs content.

Table 52 FA composition in NSRB and SRB by IR method during storage

Fatty acid composition	Week (g/100g)				% Changed	
	Week 0		Week 8			
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Myristic acid (C14:0)	0.08	0.08	0.07	0.08	-12.5	0
Palmtic acid (C16:0)	3.40	3.56	3.18	3.51	-6.47	-1.40
Heptadecanoic acid (C17:0)	ND	ND	ND	ND	-	-
Stearic acid (C18:0)	0.36	0.37	0.34	0.38	-5.55	+2.70
Arachidic acid (C20:0)	0.15	0.15	0.15	0.16	0	+6.66
Heneicosanoic acicd (C21:0)	ND	ND	ND	ND	-	-
Behenic acid (C22:0)	0.06	0.07	0.06	0.07	0	0
Lignoceric acid (C24:0)	0.10	0.11	0.10	0.11	0	0
Saturated fat	4.16	4.36	3.92	4.32	-5.77	-0.92
Palmitoleic acid (C16:1n7)	0.03	0.03	0.03	0.03	0	0
Trans-9-Eladic acid	ND	ND	ND	ND	-	-
(C18:1n9t)	5.98	6.25	5.77	6.13	-3.51	-1.92
Cis-9-Oleic acid (C18:1n9c)						
Cis-11-Ecosenoic acid	0.09	0.10	0.09	0.10	0	0
(C20:1n11)						
Monounsaturated fatty acid	6.12	6.39	5.91	6.46	-3.43	+1.09
Trans-Linolelaidic acid	ND	ND	ND	ND	-	-
(C18:2n6t)						
cis-9,12-Linoleic acid	6.06	6.39	5.58	6.24	-7.92	-2.35
(C18:2n6)						

Table 52 (cont.)

Fatty acid composition	Week (g/100g)				% Changed	
	Week 0		Week 8			
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Gamma-Linoleic acid	ND	ND	ND	ND	-	-
(C18:3n6)	0.22	0.23	0.19	0.22	-13.63	-4.35
Alpha-Linoleic acid (C18:3n3)						
cis-11,14-Eicosadienoic acid (C20:2)	ND	ND	ND	ND	-	-
cis-8,11,14-Eicosadienoic acid (C20:3n6)	ND	ND	ND	ND	-	-
Polyunsaturated Fatty acid	6.30	6.63	5.79	6.47	-8.09	-0.02
Unsaturated fat	12.42	13.03	11.70	12.94	-5.79	-0.69
Trans fat	ND	ND	ND	ND	-	-
Omega 3	0.22	0.23	0.19	0.22	-13.64	-4.35
Omega 6	6.07	6.39	5.59	6.24	-7.91	-2.35
Omega 9	5.99	6.26	5.78	6.33	-3.51	+1.12
Total % changed					-97.72	-6.78
Average % changed					-3.76	-0.26

Note: ND = not detected

1.5 Tocols and oryzanol storage stability

Tocols, which include tocopherols and tocotrienols, have beneficial effects generally believed to be due to their antioxidant action inhibiting lipid peroxidation in biological membranes (Kim et al., 2014). In addition to their antioxidant activity, tocols confer other health benefits, including modulating degenerative diseases like cancer and cardiovascular disease, while lowering blood cholesterol levels (Qureshi et al., 1997). Tocols and oryzanols are the main antioxidants present in RB. The antioxidant activity of oryzanols is almost 10 times higher than that of tocopherols, while tocotrienols have 40-60 times greater antioxidant power than those of tocopherols in different biological systems (Abdel-Aal, & Hucl, 1999 as cited in Iqbal et al., 2005). The level and composition of tocols in rice is significantly influenced by the origin of the rice and also by its genotype and growing environment (Lloyd et al., 2000). Yilmaz et al. (2016) showed that medium wave IR radiation at the above conditions did not cause any tocopherol loss in RB.

In the current study, an increase in γ-tocotrienol up to approximately 16% was observed in the SRB that was stabilized at optimal IR radiation (993 W) for a long period of time (598 seconds) (298.97±28.7mg/kg dry basis in the NSRB to 347.07±4.40mg/kg dry basis in the SRB) in Week 0.

The γ-tocotrienol content in the NSRB and SRB by IR method decreased during storage, while the γ-tocotrienol content in the NSRB decreased at a faster rate than the SRB. The δ-tocopherol content did not experience a significant loss in the SRB that was stabilized at optimal IR radiation (50.07±0.45mg/kg dry basis in the NSRB and 50.97±0.12mg/kg dry basis in the SRB) in week 0, but had a significant decrease during storage (46.20±1.63mg/kg dry basis in the NSRB and 46.50±0.46mg/kg dry basis in the SRB). Yilmaz et al. (2016) similarly reported that the tocopherol content of the RB was not significantly different after the IR stabilization procedure at 700 W for 7.0 minutes ($p > 0.05$). Excessive heating might decompose some of the heat-sensitive tocols and reduce the total content of the tocols.

Yilmaz et al. (2016) reported that the stabilization parameters; such as, IR power and process time (500-700W and 3.0-7.0 minutes) did not significantly affect the oryzanol content of the processed bran samples ($P > 0.05$), but in the current study (Table 53), the optimal IR power and process time increased the γ-oryzanol up to approximately 8.9% in the SRB that was stabilized at optimal IR radiation (993 W) for a long period of time (598 seconds) (1476.27±77.41mg/kg dry basis in the NSRB to 1607.50±61.99 mg/kg dry basis in the SRB) in week 0. The γ-oryzanol significantly decreased during the storage time and the γ-oryzanol content in the NSRB decreased at a faster rate than that from the SRB.

The resulted of the tocotrienol, tocopherol, and oryzanol IR treatment of the RB significantly affected the content in the NSRB and SRB during the 8 weeks of storage (Table 4.31). Thanonkaew et al. (2012) showed that the oryzanol content of the RB stabilized with hot air (at 150°C for 10 minutes), roasting (at 150°C for 10 minutes), steaming (at 130°C for two minutes), or MW (800 W, 2450 MHz) (at 150°C for three minutes), which was significantly higher than that of crude bran ($p< 0.05$). Kim, et al. (2014) also reported that the oryzanol content in RB treated with dry heat (80°C and 100°C for one hour), MW (1,200 W) (100% power for 2 and 5 minutes), or autoclave (121°C for 20 minutes) was relatively higher than the NSRB, and excessive

heating could destroy the heat-sensitive oryzanol and reduce the total content of the oryzanol.

During the 8 weeks of storage, the tocols and oryzanol content in the NSRB experienced higher changes than the SRB treated by IR. The average percentage of the changed tocols and oryzanol content in the NSRB decreased by 20.05%, while the SRB decreased by 6.89%. It was found that the IR treatment was effective for stabilizing the tocols and oryzanol content.

Table 53 Tocols and oryzanol content in NSRB and SRB by IR method during storage

Analysis	Week 0		Week 4		Week 8		% Changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB	NSRB	SRB
Tocols and Oryzanol (mg/kg dry basis)								
γ-Tocotrienol	298.97±28.71^{b}	347.07.±4.40^{c}	246.37±18.83^{a}	336.83±1.21^{c}	221.87±18.40^{a}	329.43±6.54^{c}	-25.79	-5.08
α-Tocopherol	50.07±0.45^{cd}	50.97±0.12^{d}	48.77±0.42^{b}	47.50±0.69^{ab}	46.20±1.63^{a}	46.50±0.46^{a}	-7.73	-8.77
γ-Oryzanol	1476.27±77.41^{c}	1607.50±61.99^{d}	1258.13±30.39^{b}	1520.07±77.29^{cd}	1082.90±73.14^{a}	1497.77±13.81^{cd}	-26.62	-6.83
			Average % changed				-20.05	-6.89
			Total % changed				-60.14	-20.68

Note: Values are expressed as means ± standard deviation.

Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

1.6 Shelf life of NSRB and SRB by IR method

The formation of FFA plays an important role in creating deteriorative changes and affects the quality of the RB. Consequently, the application of an accurate kinetic model to understand and predict the formation of the FFA could be useful in determining the RB rancidity rate and feasibility of the full utilization of RB (Wang et al., 2017).

The shelf life of the NSRB and SRB as a result of the IR method, samples stored at various temperatures of 25°C, 35°C, and 45°C were evaluated using the FFA value as an indicator (Table 54). Linear regression (R^2) was created as a linear modeling approach to demonstrate the relationship between the FFA (%) and storage time (seconds) in the RB samples (table 54). High R^2 values were observed in both the NSRB (storage at 25°C, 35°C, and 45°C were 0.9757, 0.9859, and 0.9919, respectively) and SRB (storage at 25°C, 35°C, and 45°C were 0.9538, 0.9517, and 0.9753, respectively) at all the studied storage temperatures. These high ranges of the linear regression enabled the storage time to be calculated with a high degree of accuracy.

The actual shelf life values of the SRB samples at 25°C, 35°C, and 45°C compare with NSRB were improved for 3.68, 2.09, and 1.50 times, respectively. The actual predicted shelf life of the NSRB and SRB by IR method at 30°C was 16.1 and 44.7 days, respectively. To further describe the effect of IR heating (optimized condition) on the inactivation of lipase in the RB and estimate the storage time in which the concentration of the FFA was maintained at below 5%. The kinetics of the formation of the FFA were investigated at different storage temperatures (25°C, 35°C, and 45°C). The kinetics were determined by the regression relationships of the kinetic data.

A zero-order kinetic equation was used to predict the daily rate of the FFA increase in the RB. Table 54 shows that the initial FFA contents (A_0) of the NSRB and SRB were 1.88% and 1.93%, respectively. At all three storage temperatures, the FFA formation rate (k_S) in the SRB was lower than that in the NSRB. Within the three storage temperatures, lower temperatures corresponded to lower k_S, and the lowest k_S (0.190%) was found in the SRB stored at 25°C. Wang et al. (2017) reported the kinetics of the formation of the FFA and estimated storage time for

single and multiple IR/tempering treatments for different MC at ambient conditions of temperature (21±1°C) of simultaneous rough rice drying and RB stabilization using IR radiation heating. They found, for example, an increase in the tempering time from one to five hours that resulted in the formation rate of the FFA experiencing an increase in the RB of the rough rice drying samples before being milled by IR heating with the MC of 25.54 g moisture/100 g dry solid. This was a decrease from 0.2022% per day (RB from rough rice non-stabilized before milling) to 0.0834% per day (IR heating process) for the one-pass drying samples.

Moreover, with an increase in the initial MC, the lipase activity reduced further under one- and two-pass dryings (IR heating process). At 4 hours of tempering time, the k_S decreased from 0.149% per day with 20.02 g of moisture/100 g dry solid to 0.036% per day with the initial MC of 32.54 g moisture/100 g dry solid. The corresponding values under the two-pass drying were 0.023% per day. The obtained results showed that the simplified kinetics model could successfully describe and predict the k_S in the course of the RB storage. Thus, this model could be used by food industries to prepare an accurate manufacturing plan for processing RB into edible oil before its deterioration.

Table 54 Shelf-life and FFA formation rate of NSRB and SRB by IR method

Condition		Linear equation	R^2	Initial FFA content (A_0)	Final FFA content (A_S)	Actual shelf-life in days, %FFA ≤ 5%	FFA formation rate (k_s)	Predicted shelf-life in days, 30°C
Sample	Storage temperature (°C)							
NSRB	25	Y=1.0118x+2.6293	0.9757	1.88	5.00	16.4	0.190 %	
	35	Y=1.1677x+2.3571	0.9859	1.88	5.00	15.8	0.197 %	16.1
	45	Y=1.4105x+2.1936	0.9919	1.88	5.00	13.9	0.224 %	
SRB	25	Y=0.3957x+1.5807	0.9538	1.93	5.00	60.5	0.051 %	
	35	Y=0.6573x+1.8973	0.9517	1.93	5.00	33.0	0.093 %	44.7
	45	Y=0.8305x+2.5169	0.9753	1.93	5.00	20.9	0.147 %	

2. Storage stability of the SRB by the IR-VC method

2.1 FFA storage stability

The NSRB and SRB were stored at 35°C for 8 weeks, and the FFA content was monitored every week. The FFA content of the SRB obtained at the optimal conditions by the IR-VC stabilization method was compared with the NSRB. The FFA of the NSRB increased from initially 1.75% to 7.26% after 8 weeks of storage, while a steady increase in the FFA level was observed in the SRB sample that increased from initially 1.50% to 3.24% after 8 weeks of storage (Figure 25). It is important to note that at the end of week 8 of storage, the FFA of the SRB was approximately 3.24%, which was in the suitable range for oil extraction and was considered acceptable for human consumption.

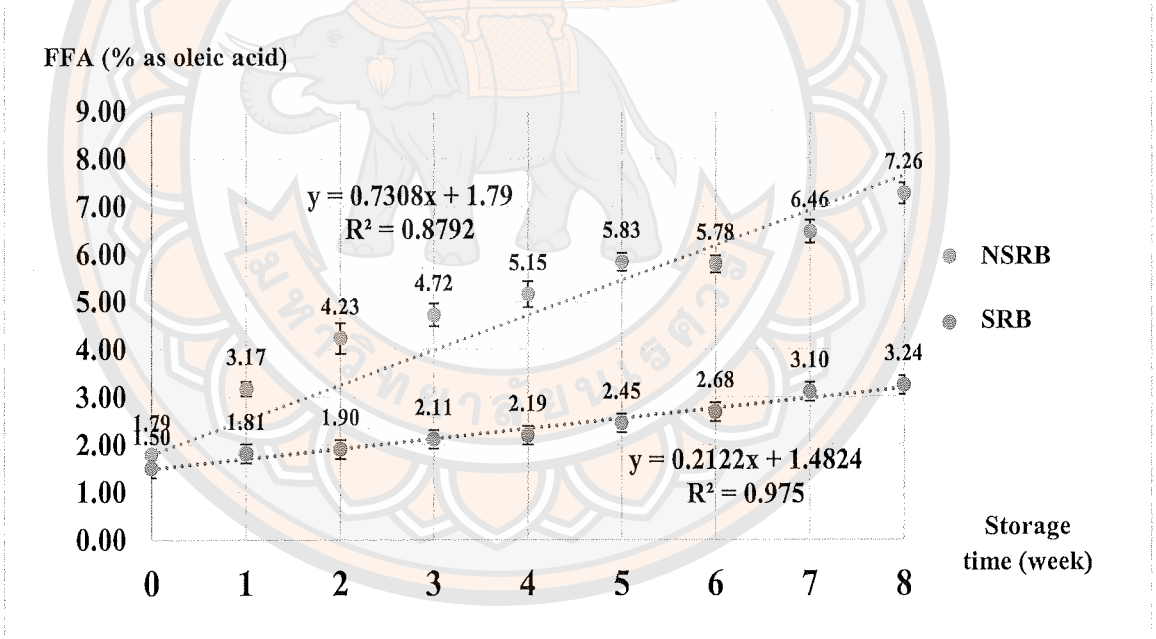


Figure 25 FFA content of NSRB and SRB by IR-VC method during storage

The FFA content of the NSRB was always higher than that of the SRB throughout the studied storage period. The reason for this was that IR-VC drying combined the advantages of both IR heating and vacuum drying. The low temperature and fast mass transfer conferred by the vacuum combined with the rapid energy transfer by IR heating generated very rapid, low temperature drying; thus, it had the potential to improve the energy efficiency and product quality (Giri, & Prasad, 2007

as cited in Salehi, & Kashaninejad, 2018). It was evident that an increase in the vacuum pressure enhanced the drying rate. This was expected because as the vacuum pressure increased, there was an accelerated removal of the moisture build-up in the chamber, which consequently enhanced the drying process.

In the combined IR-VC drying, the most important effect was to cause an increase in the drying rate (Salehi, & Kashaninejad, 2018). Therefore, the IR in the vacuum made greater efficient heat transfer. In order to destroy the enzymes and microorganisms in the RB to completely deteriorate causing the AV and PV to be lower than the standard CODEX (4.0 mg KOH/g oil and 15 meq/kg by cold-pressed method, respectively), IR-VC heating was used as a therapy to enhance the stability of the RB as an alternative. This could effectively inhibit the lipase activity and delay the hydrolytic rancidity resulting in a much lower rate of the increase in the FFA value compared to that of the NSRB sample.

2.2 MC, a_w, and color storage stability

The effects of the SRB's a_w between 0.122-0.495 (Table 55) on the enzyme activity were low, the bacterial growth was not present, and the yeast& mold showed a slight growth rate. These were located in zone II (0.25-0.80) in the safety control of the a_w graph (Fennema, 1976), which indicated the low enzyme activity and microbial growth rate in this study. Kim et al. (2014) reported that reducing the MC could deactivate the enzymes and microorganisms in RB, and this resulted in a slower rate of the formation of the FFA during storage. Reducing the a_w was also found to have a similar effect.

Thanimkar et al. (2019) investigated the vacuum infrared (IR-VC) and vibratory bed assisted vacuum infrared (VC-VIR) drying of *Cissus quadrangularis* Linn. (CQ) and found that for the VC-VIR drying of CQ, the VC-VIR drying of 5 mm of CQ provided the highest maximum drying rate of 0.258 g water/g dry matter per minute. The vacuum operation contributed to improved effective moisture diffusivity (Deff). The low MC and a_w was important to prevent the deterioration of the RB. The MC and a_w of the NSRB sample gradually decreased during the storage time, while both values of the SRB were found to increase. These respective increases in the MC and a_w of the SRB could be the result of moisture absorption from the surrounding environment during storage even though it was stored

in sealed PE bags. Once the equilibrium stage was achieved, the constant MC was investigated.

The color results are presented in table 55. The SRB was darker than the NSRB sample with regard to the lower lightness (L*) value. The a* value in the SRB (all samples in each week) was higher than the NSRB. This inferred that the RB changed to a red color as a result of the IR-VC treatment, but b* (or yellow-blue color) did not have any significant changes between the SRB and NSRB. The ΔE value of the SRB (4.52-7.10) was also found to be higher than that of the NSRB (0-0.73). Similarly, Irakli et al. (2018) reported that the SRB color could be due to the RB being formed during the Maillard reaction that occurred during the heat treatment. This affected the L*, a*, and ΔE values in the RB as a result of the IR-VC treatment.

The IR temperature was increased in the same duration of time, which was most probably due to the formation of brown polymers from the Maillard reaction induced by the heat treatment (Miranda et al., 2009). The SRB showed an intense yellow color, which could be due to the intensity of the intermediate stages of the Maillard reaction and could be partly due to the thermal oxidation of the RBO (Irakli et al., 2018).

Table 55 MC, a_w, color and ΔE changed in NSRB and SRB by IR-VC method during storage

Sample	Storage week	MC (%)	a_w	L*	a*	b*	ΔE
NSRB	0	8.37 ± 0.02^{k}	0.546 ± 0.012^{j}	69.53 ± 0.03^{gh}	2.75 ± 0.04^{bc}	19.09 ± 0.06^{cd}	0.00 ± 0.00^{a}
	1	6.97 ± 0.28^{j}	0.511 ± 0.024^{i}	69.83 ± 0.03^{hi}	2.63 ± 0.02^{ab}	18.48 ± 0.03^{ab}	0.19 ± 0.01^{abc}
	2	7.12 ± 0.14^{j}	0.496 ± 0.014^{f}	68.83 ± 0.13^{e}	2.83 ± 0.04^{cd}	18.75 ± 0.06^{bc}	0.78 ± 0.15^{abc}
	3	7.05 ± 0.51^{j}	0.494 ± 0.019^{h}	69.36 ± 0.05^{g}	2.99 ± 0.37^{d}	20.07 ± 0.69^{gh}	1.47 ± 0.22^{d}
	4	6.81 ± 0.14^{ij}	0.463 ± 0.034^{d}	69.26 ± 0.05^{fg}	2.75 ± 0.04^{bc}	19.66 ± 0.06^{ef}	0.57 ± 0.04^{ab}
	5	7.00±0.28j	0.483 ± 0.028^{h}	69.01 ± 0.02^{f}	2.79 ± 0.02^{bc}	19.85 ± 0.04^{fg}	0.94 ± 0.05^{bc}
	6	6.81 ± 0.14^{ij}	0.498 ± 0.021^{i}	69.85 ± 0.01^{i}	2.69 ± 0.01^{abc}	19.53 ± 0.04^{ef}	0.21 ± 0.02^{ab}
	7	6.44 ± 0.02^{h}	0.512 ± 0.024^{j}	69.29 ± 0.04^{g}	2.74 ± 0.07^{bc}	19.51 ± 0.03^{ef}	0.46 ± 0.06^{ab}
	8	6.11 ± 0.07^{h}	0.503 ± 0.023^{j}	70.25 ± 0.02^{j}	2.53 ± 0.02^{a}	18.30 ± 0.07^{a}	0.53 ± 0.05^{c}
SRB	0	1.57 ± 0.11^{a}	0.122 ± 0.021^{a}	66.04 ± 0.01^{ab}	2.86 ± 0.03^{cd}	20.20 ± 0.02^{ghi}	9.17 ± 0.04^{i}
	1	2.64 ± 0.21^{b}	0.233 ± 0.021^{b}	66.67 ± 0.02^{c}	3.68 ± 0.02^{f}	19.07 ± 0.03^{cd}	5.98 ± 0.04^{f}
	2	2.94 ± 0.09^{bc}	0.230 ± 0.032^{b}	66.39 ± 0.01^{c}	3.80 ± 0.03^{fg}	19.38 ± 0.01^{de}	7.15 ± 0.04^{g}
	3	3.12 ± 0.31^{cd}	0.333 ± 0.018^{c}	65.99 ± 0.24^{ab}	4.04 ± 0.04^{h}	20.81 ± 0.49^{j}	11.56 ± 0.91^{j}
	4	3.40 ± 0.12^{d}	0.340 ± 0.012^{cd}	66.26 ± 0.08^{bc}	3.98 ± 0.01^{h}	20.48 ± 0.03^{ij}	8.88 ± 0.36^{i}
	5	3.99 ± 0.22^{e}	0.404 ± 0.022^{e}	65.98 ± 0.06^{a}	3.97 ± 0.02^{h}	20.57 ± 0.03^{ij}	10.10 ± 0.19^{j}
	6	4.05 ± 0.19^{ef}	0.464 ± 0.019^{fg}	66.56 ± 0.04^{c}	3.98 ± 0.10^{h}	20.29 ± 0.11^{hi}	7.52 ± 0.20^{h}
	7	4.44 ± 0.21^{fg}	0.482 ± 0.021^{h}	66.27 ± 0.07^{ab}	3.92 ± 0.06^{gh}	20.27 ± 0.02^{hi}	8.49 ± 0.34^{i}
	8	4.51 ± 0.23^{g}	0.495 ± 0.023^{h}	67.60 ± 0.18^{a}	3.49 ± 0.05^{e}	18.18 ± 0.17^{a}	3.47 ± 0.32^{e}

Note: Values are expressed as means ± standard deviation. Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

2.3 Proximate and microbial analysis

The NSRB and SRB samples stored for 0, 4 and 8 weeks were analyzed for their proximate composition in order to understand their basic differences (Table 56). The ash, protein, fat, fiber, and carbohydrate content (dry basis) all increased as a result of the IR-VC stabilization. Only the MC decreased because of the evaporation of water. Additionally, it was observed that the fiber content of the SRB was significantly higher than that of the fresh counterpart. This fiber content increase could likely be explained by the IR-VC treatment deteriorating the cell walls, thereby releasing the fiber of the cell walls.

The TPC of the NSRB and SRB are displayed in table 56. The IR-VC treatment significantly reduced the TPC, yeast & mold. The TPC, yeast & mold in the SRB had significant growth during storage, but were still within the standard level of food for the storage duration ($<10^4$ CFU/g). The average percentage change of the TPC, yeast & mold in the NSRB and SRB during the storage time was +4.96% and +1.43%, respectively. This displayed that the IR-VC treatment effectively reduced and inhibited the TPC, yeast and mold in the initial time, and the SRB samples showed a high stability of microbial growth during the storage time than the NSRB.

Table 56 RB proximate and microbial analysis in NSRB and SRB by IR-VC method during storage

Analysis	Week 0		Week 8		%Changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Proximate (g/100g dry basis)						
Carbohydrate	53.60±1.52^{ns}	53.39±1.16^{ns}	53.49±0.29^{ns}	53.00±0.51^{ns}	-0.20	-0.73
Fat	16.83±0.88^{ns}	16.90±0.26^{ns}	17.00±0.39^{ns}	17.65±0.27^{ns}	+1.01	+4.44
Protein	13.95±0.06^{ns}	14.50±0.83^{ns}	13.95±0.08^{ns}	14.02±0.09^{ns}	0	-3.31
Fiber	7.38±0.50^{ns}	6.90±0.09^{ns}	7.24±0.16^{ns}	7.09±0.10^{ns}	-1.90	+2.75
Ash	8.24±0.01^{a}	8.31±0.03^{b}	8.32±0.05^{b}	8.23±0.02^{a}	+0.97	-0.96
		Average % changed			-0.02	+0.44
		Total % changed			-0.12	+2.19
Microbial (cfu/g)						
TPC	7.86×10^{3}±0.54	3.20×10^{3}±0.29	7.484×10^{3}±2.14	3.42×10^{3}±1.06	-4.78	+6.88
Yeast & mold	3.20×10^{2}±0.50	1.74×10^{2}±0.27	3.67×10^{2}±1.41	1.67×10^{2}±0.47	+14.69	-4.02
		Average % changed			+4.96	+1.43
		Total % changed			+9.91	+2.86

Note: Values are expressed as means±standard deviation. Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different (p <0.05).

2.4 FA composition storage stability

The FA compositions of the RB samples are shown in Table 57. The NSRB contained approximately 4.23% of saturated and 9.85% of unsaturated FA, while the SRB contained approximately 4.71% of saturated and 10.72% of unsaturated FA. The SRB samples possessed more saturated, monounsaturated, polyunsaturated and total unsaturated FA content than the NSRB. Similarly, Kim et al. (2014) reported this was because a significant amount of lipids (FA) in the NSRB would be bound to proteins or lipids, and applying thermal energy might generate these linkages and release the lipid compounds. On the other hand, saturated, monounsaturated, polyunsaturated, and total unsaturated FA contents decreased in both the NSRB and SRB after storage. The monounsaturated, polyunsaturated, and total unsaturated FA may have undergone oxidation with the hydronium ion changing the double bond to

be a single bond (Fennema, 1996). The quantities of omega-6 and omega-9 in the SRB samples were higher than those in the NSRB, but all declined during storage by 95.97% and 96.52%, respectively.

The results indicated that the effect of the IR-VC on the RB could insignificantly change the FA composition ($p < 0.05$). The average percentage change in the SRB was lower than the NSRB by 1.26 times. This indicated that the IR-VC treatment method created the non-stabilization of the FA composition. The observed results found arachidic acid (C20:0) and palmitoleic acid (C16:1n7) in the NSRB and SRB, which were significant increases during the storage time; however, the polyunsaturated and unsaturated FA were decreased during the storage time. The trans-fat was increased during storage time. This caused an oxidation reaction during the storage time to break the double bond in the polyunsaturated and unsaturated FA into single bonds of trans fat.

Table 57 Fatty acid profile in NSRB and SRB stabilized by IR-VC during storage

Fatty acid composition	Week (g/100g)				% Changed	
	Week 0		Week 8			
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Myristic acid (C14:0)	0.07	0.08	0.06	0.06	-14.19	-25.00
Palmtic acid (C16:0)	3.36	3.81	ND	ND	-100	-100
Heptadecanoic acid (C17:0)	ND	ND	0.02	0.02	-	-
Stearic acid (C18:0)	0.34	0.35	ND	ND	-100	-100
Arachidic acid (C20:0)	0.13	0.12	5.31	5.64	+3984	+4600
Heptadecanoic acicd (C21:0)	0.15	0.16	0.17	0.19	+13.33	+18.75
Behenic acid (C22:0)	0.07	0.06	ND	ND	-	-
Lignoceric acid (C24:0)	0.11	0.10	0.09	0.09	-18.18	-10.00
Saturated fat	4.23	4.71	5.66	6.02	+33.80	+27.81
Palmitoleic acid (C16:1n7)	0.02	0.03	3.06	3.24	+15200	+10700
Trans-9-Eladic acid (C18:1n9t)	ND	ND	0.31	0.32	-	-
Cis-9-Oleic acid (C18:1n9c)	5.03	5.45	ND	ND	-	-
Monounsaturated fatty acid	5.05	5.49	3.38	3.57	-33.07	-34.97
Trans-Linolelaidic acid (C18:2n6t)	ND	ND	2.66	2.74	-	-
cis-9,12-Linoleic acid (C18:2n6)	4.71	5.15	ND	ND	-	-

Table 57 (cont.)

Fatty acid composition	Week (g/100g)				% Changed	
	Week 0		Week 8			
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Gamma-Linoleic acid (C18:3n6)	0.07	0.08	0.13	0.13	+85.7	+62.5
Alpha-Linoleic acid (C18:3n3)	0.07	0.08	0.08	0.09	+14.2	+12.5
cis-11,14-Eicosadienoic acid (C20:2)	0.01	ND	ND	ND	-	-
cis-8,11,14-Eicosadienoic acid (C20:3n6)	ND	ND	0.05	0.06	-	-
Polyunsaturated Fatty acid	4.80	5.23	2.94	3.02	-38.8	-42.3
Unsaturated fat	9.85	10.72	6.72	6.39	-31.8	-40.4
Tran fat	ND	ND	2.97	3.05	-	-
Omega 3	0.08	0.08	0.09	0.09	+12.5	+12.5
Omega 6	4.71	5.15	0.19	0.18	-96.0	-96.5
Omega 9	5.03	5.46	ND	ND	-	-
Total % changed					+18,911	+14,985
Average % changed					+1,261	+999

Note: ND = not detected

2.5 Tocols and oryzanol storage stability

The results of SRB stabilized by the IR-VC treatment was significantly affected the γ-tocotrienol, α-tocopherol, and γ-oryzanol content in the NSRB and SRB during the 8 weeks of storage (Table 58). The losses of γ-tocotrienol, α-tocopherol, and γ-oryzanol up to approximately 18.8%, 8.7%, and 22.1%, respectively were observed in the SRB that was stabilized at high IR-VC radiation (997 W) for a long period of time (598 seconds) with the vacuum strength (610 mmHg).

γ-Tocotrienol in the NSRB (dry basis) on the initial day of storage was 331.20±6.05 mg/kg, which decreased during the storage time to 263.87±11.02mg/kg, a reduction of 67.33mg/kg. In comparison, γ-tocotrienol in the SRB, which was found on the initial day of storage to be 268.72±8.94 mg/kg, gradually decreased during the storage time to 251.90±7.38mg/kg, a reduction of 16.87mg/kg.

Similarly, α-tocopherol in the NSRB (dry basis) on the initial day was 104.37±0.99 mg/kg and decreased during the storage time to 92.67±0.50mg/kg, which was a reduction of 11.70mg/kg. In comparison, α-tocopherol in the SRB, which was found on the initial day to be 95.33±0.38 mg/kg, gradually decreased during the storage time to 91.73±0.86 mg/kg, a reduction of 3.60mg/kg.

γ-Oryzanol in the NSRB (dry basis) on the initial day was 994.87±11.05mg/kg, then decreased during the storage time (8 weeks) to 716.07±7.53 mg/kg, which was a reduction of 278.8 mg/kg. In comparison, γ-oryzanol in the SRB, which was found on the initial day to be 774.67±16.67 mg/kg, gradually decreased during the storage time to 624.47±5.20 mg/kg, a reduction of 150.2 mg/kg.

Hence, the γ-tocotrienol, α-tocopherol, and γ-oryzanol content in the SRB resulting from the IR-VC heating stabilization in the optimal condition were maintained tocols and oryzanol content higher than the NSRB. Observation of the SRB by IR-VC treated showed preserved γ-tocotrienol, α-tocopherol, and γ-oryzanol. The low temperature and fast mass transfer conferred by the vacuum combined with the rapid energy transfer by IR heating generated very rapid, low temperature drying; thus, it had the potential to improve the energy efficiency and product quality (Giri, &

Prasad, 2007, as cited in Salehi, & ashaninejad, 2018) resulted in preservation of tocols and oryzanol content of the IR-VC stabilization process.

Thanonkaew et al. (2012) reported that the γ-oryzanol content of SRB by HA treated (150°C for 10 minutes), roasting (at 150°C for 10 minutes), steaming (at 130°C for two minutes), and MW (800 W, 2450 MHz at 150°C for 3 minutes) was significantly higher than that of NSRB ($p < 0.05$). Kim et al. (2014) also reported that the γ-oryzanol content in SRB treated by HA (80°C and 100°C for 1hour), MW (1,200 W, 100% power for two and five minutes), and autoclave (121°C for 20 minutes) were relatively higher than that of the NSRB. The average percentage of change in the SRB was 2.02 times lower than the NSRB. This indicated that the IR-VC treatment method effectively maintained the tocols and oryzanol content more than the NSRB.

Table 58 Tocols and oryzanol in NSRB and SRB by IR-VC method during storage

Analysis	Week 0		Week 4		Week 8		% Changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB	NSRB	SRB
Tocols and oryzanol (mg/kg dry basis)								
γ-Tocotrienol	331.20±6.05^{d}	268.77±8.94^{bc}	280.70±1.99^{c}	254.57±5.26^{a}	263.87±11.02^{ab}	251.90±7.30^{a}	-20.33	-6.28
α-Tocopherol	104.37±0.99^{c}	95.33±0.38^{b}	95.07±0.91^{b}	92.93±0.55^{a}	92.67±0.50^{a}	91.73±0.86^{a}	-11.21	-3.78
γ-Oryzanol	994.87±11.05^{f}	774.67±16.67^{d}	857.60±13.08^{e}	676.73±5.59^{b}	716.07±7.53^{c}	624.47±5.20^{a}	-28.02	-19.39
			Total % changed				-59.56	-29.45
			Average %changed				-19.85	-9.82

Note: Values are expressed as means ± standard deviation. Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

2.6 Shelf life of NSRB and SRB by IR-VC method

The shelf life of the NSRB and SRB samples stored at 25°C, 35°C, and 45°C was evaluated using the FFA value as an indicator (table 59). R^2 was created as a linear modeling approach to demonstrate the relationship between the FFA (%) and storage time (seconds) in the RB samples. High R^2 values were observed in the NSRB in which the 25°C, 35°C, and 45°C storage temperatures were 0.9458, 0.9189, and 0.9256, respectively, and the SRB were 0.9611, 0.9750, and 0.9852, respectively. The actual shelf life values of the SRB samples at 25°C, 35°C, and 45°C compared with NSRB were improved 3.41, 4.10, and 2.75 times, respectively. The predicted shelf life of the SRB at 5°C, 25°C, 30°C, 35°C, 40°C, and 45°C were 226.6, 145.0, 129.7, 116.0, 87.3, and 66.3 days, respectively.

The kinetics was determined by the regression relationships of the kinetic data. A zero-order kinetic equation was used to predict the daily rate of the FFA increase in the RB. Table 59 shows that the initial FFA contents (A_0) of the NSRB and SRB were 1.79% and 1.50%, respectively. At all three storage temperatures, the FFA formation rate (k_S) in the SRB was lower than that in the NSRB. Within the three storage temperatures, the lower temperature corresponded to lower k_S. The lowest k_S (0.024%) was found in the SRB stored at 25°C. This could be compared with Wang et al. (2017), who reported the kinetics of the formation of the FFA and estimated the storage time for single and multiple IR/tempering treatments for different MC at ambient conditions of temperature (21±1°C) of simultaneous rough rice drying and RB stabilization using IR radiation heating. They found that by increasing the tempering time from one to five hours, the daily rate of the FFA increased in the RB of the rough rice drying samples before milling by IR heating with the MC of 25.54%, which decreased from 0.2022%/day (RB from rough rice non-stabilized before milling) to 0.0834% per day (IR heating process) for the one-pass drying samples. Moreover, with an increase in the initial MC, the lipase activity was further reduced under the one- and two-pass dryings (IR heating process). At four hours of tempering time, the daily rate of the FFA decreased from 0.149% per day and 20.02% to 0.036%/day and the initial MC of 32.54%. The corresponding values under the two-pass drying were 0.023%/day.

Table 59 Shelf-life and FFA formation rate of NSRB and SRB by IR-VC method

Condition		Linear eq.	R^2	Initial FFA content (A_O)	Final FFA content (A_S)	Actual shelf-life in days, %FFA ≤ 5%	FFA formation rate (k_s)	Predicted shelf-life in days, 30°C
Sample	Storage temperature (°C)							
NSRB	25	Y= 0.4832x+2.0640	0.9458	1.79	5.00	42.5	0.076 %	
	35	Y= 0.5860x+2.6327	0.9189	1.79	5.00	28.3	0.113 %	34.8
	45	Y= 0.6470x+2.7753	0.9256	1.79	5.00	24.1	0.133 %	
SRB	25	Y= 0.1713x+1.4513	0.9611	1.50	5.00	145.0	0.024 %	
	35	Y= 0.2122x+1.4824	0.9750	1.50	5.00	116.0	0.030 %	129.7
	45	Y= 0.3837x+1.3653	0.9852	1.50	5.00	66.3	0.053 %	

3. Storage stability of SRB by the IR-HA stabilized method

3.1 FFA storage stability

The NSRB and SRB by IR-HA method were stored at 35°C for 8 weeks, and the FFA content was monitored every week (Figure 26). The FFA content of the SRB obtained at the optimal conditions by the IR-HA was compared with the NSRB. The FFA of the NSRB increased from initially 1.88% to 11.14% after 8 weeks of storage, while a steady increase in the FFA level was observed in the SRB sample (Figure 25). It is important to note that at the end of week 8 of storage, the FFA of the SRB was approximately 7.87%, which was in the suitable range for oil extraction.

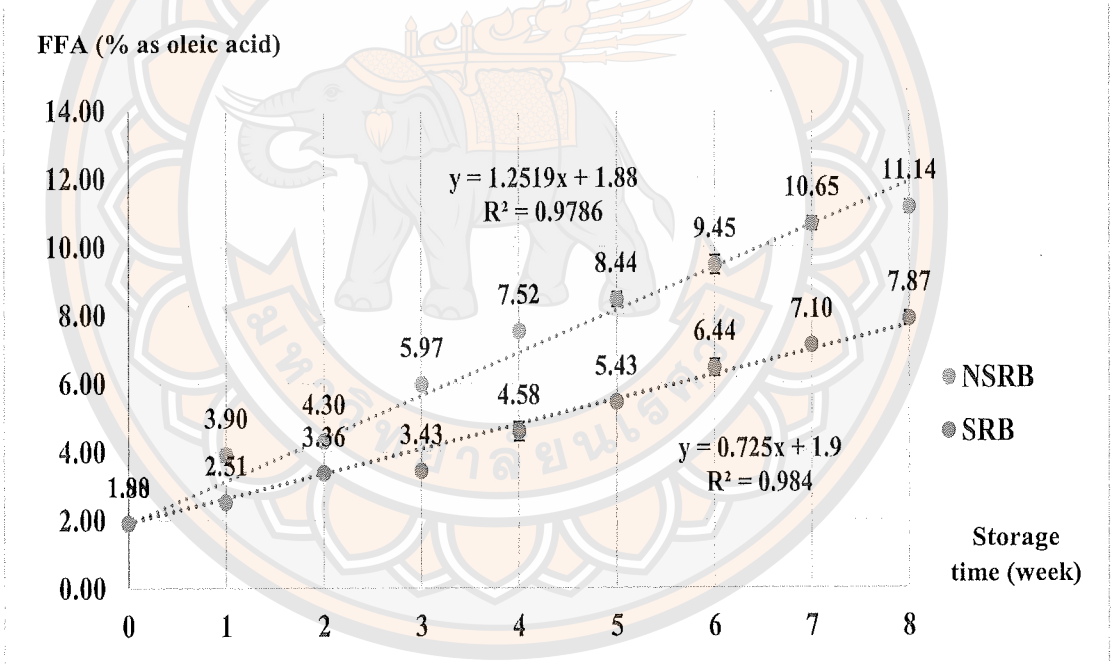


Figure 26 FFA content of NSRB and SRB by IR-HA method during storage

3.2 MC, a_w, and color storage stability

The MC and a_w were also affected by the IR-HA treatment, which was related to the FFA results. The MC and a_w were between 1.57-5.15% and 0.147-0.346, respectively (Table 60). The color results are presented in table 60. The SRB was darker than the NSRB sample with regard to the lower lightness (L*) value. The ΔE value of the SRB (1.18-5.11) was also found to be higher than that of the

NSRB (0-2.45). The effect of hot air temperature on color was similar to Kim et al.'s study (2014) that reported the brightness (L*) of RB after the dry heating (hot air heating) treatment decreased, but the redness (a*) and yellowness (b*) increased as the dry heating temperature increased. The ΔE value expressed as a single value in the color difference of L*, a*, and b* also significantly increased with the increase in the dry heating temperature. Similarly, the decrease in brightness (L*) of the RB with the increase in the heating temperature was reported (Aliva, & Silva, 1999). This result could be due to the formation of some products from the Maillard reaction induced by the heat treatment. The SRB showed an intense yellow color, which could be due to the intensity of the intermediate stages of the Maillard reaction and could be partly due to the thermal oxidation of the RBO (Irakli et al., 2018).

Table 60 MC, a_w, color and ΔE changed in NSRB and SRB stabilized by IR-HA stabilization method during storage

Sample	Storage week	MC (%)	a_w	L*	a*	b*	ΔE
NSRB	0	5.84 ± 0.15^{h}	0.410 ± 0.003^{j}	67.45 ± 0.03^{bcde}	3.68 ± 0.01^{abc}	19.84 ± 0.06^{ab}	0.00 ± 0.00^{a}
	1	6.05 ± 0.11^{i}	0.424 ± 0.001^{k}	68.38 ± 0.27^{fgh}	4.59 ± 0.22^{d}	20.36 ± 0.98^{bc}	1.71 ± 0.43^{g}
	2	7.02 ± 0.15^{m}	0.430 ± 0.001^{kl}	68.52 ± 0.06^{fgh}	3.59 ± 0.12^{abc}	20.14 ± 0.17^{b}	0.65 ± 0.12^{cdef}
	3	6.66 ± 0.05^{l}	0.445 ± 0.005^{m}	69.55 ± 0.07^{j}	3.42 ± 0.01^{ab}	19.18 ± 0.20^{a}	2.45 ± 0.23^{h}
	4	6.21 ± 0.18^{ij}	0.446 ± 0.002^{m}	68.63 ± 0.06^{fghi}	3.41 ± 0.09^{ab}	19.60 ± 0.35^{ab}	0.79 ± 0.06^{ef}
	5	6.06 ± 0.06^{i}	0.429 ± 0.002^{kl}	69.00 ± 0.01^{hij}	3.22 ± 0.74^{a}	19.95 ± 0.52^{ab}	1.59 ± 0.28^{g}
	6	6.35 ± 0.11^{jk}	0.432 ± 0.003^{l}	68.76 ± 0.04^{ghi}	3.36 ± 0.08^{ab}	20.29 ± 0.15^{bc}	1.03 ± 0.14^{f}
	7	6.47 ± 0.06^{kl}	0.444 ± 0.004^{m}	69.24 ± 0.15^{ij}	3.40 ± 0.16^{ab}	19.79 ± 0.16^{ab}	1.67 ± 0.06^{g}
	8	5.83 ± 0.08^{h}	0.441 ± 0.002^{m}	69.28 ± 0.06^{ij}	3.19 ± 0.09^{a}	20.09 ± 0.22^{ab}	1.86 ± 0.01^{g}
SRB	0	1.57 ± 0.06^{a}	0.147 ± 0.002^{a}	65.77 ± 0.08^{a}	3.87 ± 0.11^{bc}	20.53 ± 0.15^{bc}	1.71 ± 0.18^{g}
	1	2.39 ± 0.09^{b}	0.211 ± 0.001^{b}	66.80 ± 0.04^{b}	3.69 ± 0.21^{abc}	20.07 ± 0.92^{ab}	0.54 ± 0.07^{bcde}
	2	3.64 ± 0.16^{d}	0.240 ± 0.001^{c}	66.87 ± 0.09^{b}	3.82 ± 0.08^{bc}	20.35 ± 0.08^{bc}	0.33 ± 0.03^{abcd}
	3	3.66 ± 0.22^{d}	0.246 ± 0.002^{d}	67.19 ± 0.02^{bc}	4.03 ± 0.07^{c}	20.22 ± 0.16^{bc}	0.19 ± 0.05^{bc}
	4	2.62 ± 0.16^{c}	0.270 ± 0.003^{e}	67.41 ± 0.06^{bcd}	3.35 ± 0.84^{ab}	19.67 ± 1.05^{ab}	0.68 ± 0.28^{def}
	5	5.15 ± 0.04^{g}	0.293 ± 0.002^{f}	67.65 ± 1.50^{cde}	3.54 ± 0.16^{abc}	21.07 ± 0.68^{c}	1.75 ± 0.58^{g}
	6	4.53 ± 0.10^{f}	0.337 ± 0.009^{h}	67.61 ± 0.02^{cde}	3.63 ± 0.15^{abc}	20.53 ± 0.15^{bc}	0.29 ± 0.13^{abcd}
	7	4.30 ± 0.15^{e}	0.325 ± 0.003^{g}	68.11 ± 0.05^{efg}	3.09 ± 0.18^{abc}	19.96 ± 0.21^{ab}	0.26 ± 0.04^{abc}
	8	3.67 ± 0.08^{d}	0.346 ± 0.004^{i}	68.07 ± 0.05^{def}	3.43 ± 0.12^{ab}	20.35 ± 0.06^{bc}	0.38 ± 0.08^{abcd}

Note: Values are expressed as means ± standard deviation. Values for the same rice bran fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

3.3 Proximate and microbial analysis

The NSRB and SRB samples stored for 0 and 8 weeks were analyzed for their proximate composition and microbial analysis in order to understand their basic differences (Table 61). The ash, protein, fat, fiber, and carbohydrate content (dry basis) increased as a result of the IR-HA stabilization. Only the MC decreased because of the evaporation of water. Additionally, it was observed that the fiber content of the NSRB and SRB at the initial storage was significantly higher than that of week 8. The average percentage of change during storage time of the proximate content of the NSRB and SRB decreased by 2.61% and 0.89%, respectively. This indicated that the percentage change of the proximate in the NSRB was higher than the SRB, and the IR-HA effectively preserved the proximate content in the RB.

The TPC of the NSRB and SRB are displayed in table 61. The TPC in the SRB showed significant growth during storage. The IR-HA treatment effectively reduced the TPC, and yeast& mold. The TPC in the NSRB and SRB were increased during storage, which the TPC in the SRB had a lower survival rate than the NSRB (+37.0 % change in the NSRB and +4.49 % change in the SRB). These TPC of the SRB samples had high stability against the microbial growth during the storage period. The yeast and mold in the SRB was decreased during the storage period, which was lower than the initial time after 8 weeks, but the yeast and mold in the NSRB was higher than the initial time after 8 weeks. The conclusion of this study indicated that the combination of the IR wattage, treatment duration, and hot air heating inhibited the growth of microbes.

Table 61 RB proximate and microbial analysis in NSRB and SRB by IR-HA method during storage

Analysis	Week 0		Week 8		% Changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Proximate (g/100g dry basis)						
Carbohydrate	50.88±0.50^{a}	50.87±0.24^{a}	52.89±0.99^{b}	51.09±0.34^{a}	+3.95	+0.43
Fat	16.54±0.24^{b}	16.62±0.16^{b}	15.61±0.57^{a}	17.03±0.10^{b}	-5.62	+2.47
Protein	14.58±0.06^{a}	14.52±0.06^{a}	14.67±0.15^{ab}	14.80±0.09^{b}	+0.62	+1.93
Fiber	9.47±0.49^{b}	9.46±0.20^{b}	8.32±0.22^{a}	8.48±0.12^{a}	-12.14	-10.36
Ash	8.52±0.24^{ns}	8.51±0.12^{ns}	8.53±0.07^{ns}	8.60±0.03^{ns}	+0.12	+1.06
		Average % changed			-2.61	-0.89
		Total % changed			-13.07	-4.47
Microbial (CFU/g)						
TPC	5.00×10^{4}±0.14	4.45×10^{3}±0.06	6.85×10^{4}±0.13	4.65×10^{3}±0.05	+37.0	+4.49
Yeast & mold	4.60×10^{2}±0.07	3.40×10^{2}±0.03	4.25×10^{2}±0.03	3.30×10^{2}±0.03	+7.61	-2.94
		Average % changed			+22.3	+0.78
		Total % changed			+44.6	+1.55

Note: Values are expressed as means ± standard deviation.
Values for the same rice bran fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different (p <0.05)

3.4 FA composition storage stability

The FA compositions of the rice bran samples shows in Table 62. The NSRB contained approximately 4.16% of saturated and 12.42% of unsaturated FA, while the SRB contained approximately 4.25% of saturated and 12.71% of unsaturated FA. The SRB samples possessed more saturated, monounsaturated, polyunsaturated, and total unsaturated FA content than the NSRB. The monounsaturated, polyunsaturated and total unsaturated FA in the NSRB that decreased during the storage time (6.12-5.91%, 6.30-5.79*, and 12.42-11.70%, respectively) may have undergone oxidation with the hydronium ion changing the double bond to be a single bond (Fennema, 1996). However, the SRB compounds were stable (6.39-6.40%, 6.47-6.39%, and 12.71-12.79%, respectively), which the IR-HA method had the efficiency

to maintain the health beneficial FA compounds in the RB. The quantities of omega-6 and omega-9 in the SRB samples were higher than those in the NSRB, and all had stable contents during storage. The omega-3 content did not change during the IR-HA processing and maintained stable content during storage.

The average percentage of change during storage of FA composition in the NSRB was higher than the FA composition in the SRB by IR-HA. These results indicated that the effect of the IR-HA on the RB could significantly maintained the FA composition, especially the polyunsaturated and unsaturated FA in the SRB that were increased, while the NSRB decreased. The results in this study were not similar to Li et al. (2016), who reported no significant change of the main FA in wheat germ that had been stabilized with IR heating. The reason for the differences between Li et al. (2016) and this study was the combination of hot air with the IR used in this study was to improve the efficiency to stabilize the FA composition.

Table 62 FA composition in NSRB and SRB by IR-HA method during storage

Fatty acid composition	Week (g/100g)				%Changed	
	Week 0		Week 8			
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Myristic acid (C14:0)	0.08	0.08	0.07	0.08	-12.5	0
Palmtic acid (C16:0)	3.40	3.47	3.18	3.48	-6.47	+0.29
Heptadecanoic acid (C17:0)	ND	ND	ND	ND	-	-
Stearic acid (C18:0)	0.36	0.36	0.34	0.37	-5.56	+2.78
Arachidic acid (C20:0)	0.15	0.15	0.15	0.16	0	+6.67
Heneicosanoic acicd (C21:0)	ND	ND	ND	ND	-	-
Behenic acid (C22:0)	0.06	0.06	0.06	0.07	0	+16.7
Lignoceric acid (C24:0)	0.10	0.10	0.10	0.11	0	+10.0
Saturated fat	4.16	4.25	3.92	4.29	-5.77	+0.94
Palmitoleic acid (C16:1n7)	0.03	0.03	0.03	0.03	0	0
Trans-9-Eladic acid (C18:1n9t)	ND	ND	ND	ND	-	-
Cis-9-Oleic acid (C18:1n9c)	5.98	6.10	5.77	6.25	-3.51	+2.46
Cis-11-Ecosenoic acid (C20:1n11)	0.09	0.09	0.09	0.10	0	+11.1
Monounsaturated fatty acid	6.12	6.39	5.91	6.40	-3.43	+0.16
Trans-Linolelaidic acid (C18:2n6t)	ND	ND	ND	ND	-	-
cis-9,12-Linoleic acid (C18:2n6)	6.06	6.24	5.58	6.16	-7.92	-1.28

Table 62 (cont.)

Fatty acid composition	Week (g/100g)				%Changed	
	Week 0		Week 8			
Gamma-Linoleic acid (C18:3n6)	ND	ND	ND	ND	-	-
Alpha-Linoleic acid (C18:3n3)	0.22	0.22	0.19	0.22	-13.64	0
cis-11,14-Eicosadienoic acid (C20:2)	ND	ND	ND	ND	-	-
cis-8,11,14-Eicosadienoic acid (C20:3n6)	ND	ND	ND	ND	-	-
Polyunsaturated Fatty acid	6.30	6.47	5.79	6.39	-8.10	+1.24
Unsaturated fat	12.42	12.71	11.70	12.79	-5.80	+0.63
Trans fat	ND	ND	ND	ND	-	-
Omega 3	0.22	0.22	0.19	0.22	-13.64	0
Omega 6	6.07	6.24	5.59	6.17	-7.91	-1.12
Omega 9	5.99	6.11	5.78	6.26	-3.51	+2.46
Average % changed					-5.54	+2.97
Total % changed					-97.76	+53.03

Note: ND = not detected

3.5 Tocols and oryzanol storage stability

Table 63 shows the γ-tocotrienol content of up to approximately 9.71%, which was observed in the SRB that was stabilized at high IR radiation (999 W) for a long period of time (596 seconds) and in 72°C of a combination of hot air. The NSRB contained 298.97 mg/kg dry basis, and its loss was approximately 17.6% and 25.8% at four and 8 weeks of storage time, respectively, while the SRB contained 328.03 mg/kg dry basis that was a loss of approximately 1.14% and 4.29% at four and 8 weeks of storage time, respectively. The reason for the tocopherol content in the NSRB was because of the faster decrease than the SRB, which was a reaction from oxidation. The high moisture in the NSRB was the main cause of the increasing oxidation reaction, and the γ-tocotrienol changed to be another substance; such as, FFA, saturated FA, flavor, etc.

The α-tocopherol content in the NSRB lost approximately 4.53% that was observed in the SRB. The NSRB lost approximately 2.60% and 7.73% at four and 8 weeks of the storage time, respectively, while the SRB lost approximately 0.42%

and 0.77% at four and 8 weeks of the storage time, respectively. This inferred that the IR-HA stabilized RB processing had the efficiency to maintain γ-tocotrienol.

The oryzanol in the NSRB on the initial day was 1476.67 mg/kg dry basis, then decreased during the storage to 1082.90 mg/kg dry basis, which was a reduction of 26.67%, in comparison with oryzanol in the SRB, which was found on the initial day to be 1692.50 mg/kg dry basis and gradually decreased during the storage time to 1,518.33 mg/kg dry basis, a reduction of 10.29%. Yilmaz et al. (2016) reported that stabilization parameters; such as, IR power and process time (500-700 W and 3.0-7.0 minutes) did not significantly affect the oryzanol content of the processed bran samples ($p > 0.05$). Observation of the RB's IR-HA heating stabilization significantly preserved γ-tocotrienol, α-tocopherol, and γ-oryzanol.

Table 63 Tocols and oryzanol in NSRB and SRB by IR-HA method during storage

Analysis	Week 0		Week 4		Week 8		% Changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB	NSRB	SRB
Tocols and Oryzanol (mg/kg dry basis)								
γ-tocotrienol	298.97±28.71^{b}	328.03±19.95^{b}	246.37±18.83^{a}	324.30±4.83^{b}	221.87±68.21^{a}	313.97±18.81^{b}	-25.79	-4.29
α-tocopherol	50.07±0.45^{c}	47.80±0.10^{b}	48.77±0.42^{bc}	47.60±0.35^{b}	46.20±1.64^{a}	47.43±0.15^{ab}	-7.73	-0.77
γ-oryzanol	1,476.67±77.41^{c}	1,692.50±38.39^{d}	1,258.13±30.38^{b}	1,555.40±49.29^{c}	1,082.90±73.14^{a}	1,518.33±50.47^{c}	-26.67	-10.29
			Average % changed				-20.06	-5.12
			Total % changed				-60.19	-15.35

Note: Values are expressed as means ± standard deviation.

Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

3.6 Shelf life FFA formation rate of NSRB and SRB by IR-HA method

High R^2 values were observed in the NSRB (0.9757, 0.9859, and 0.9919 at the storage temperatures of 25°C, 35°C, and 45°C, respectively) and the SRB were 0.9619, 0.9875, and 0.9803 at the storage temperatures of 25°C, 35°C, and 45°C, respectively. These high ranges of linear regression enabled the storage time to be calculated with a high degree of accuracy.

The actual shelf life values of the SRB samples at 25°C, 35°C, and 45°C were 2.98, 1.92, and 1.35 times higher, respectively than those of the NSRB samples when compared to the same storage temperatures. The predicted shelf-life of the SRB at 30°C was 2.40 times of the NSRB. To further describe the effect of the IR-HA heating on the inactivation of the lipase in the RB and to estimate the storage time in which the FFA concentration was maintained below 5%, the kinetics of the formation of the FFA were investigated with different storage temperatures (25°C, 35°C, and 45°C, respectively). The kinetics were determined by the regression relationships of the kinetic data.

A zero-order kinetic equation was used to predict the FFA formation rate (k_S) in the RB. Table 64 shows that the initial FFA contents (A_0) of the NSRB and SRB were 1.88% and 1.90%, respectively. At all three storage temperatures, the k_S in the SRB was lower than that in the NSRB. Within the three storage temperatures, the lower temperature corresponded to the lower k_S. The lowest k_S (0.063%) was found in the SRB stored at 25°C. This could be compared to Wang et al. (2017), who reported the kinetics of the formation of the FFA was 0.083 % per day (IR heating process) for the one-pass drying samples.

Table 64 Shelf-life and FFA formation rate in NSRB and SRB by IR-HA method

Condition		Linear equation	R^2	Initial FFA content (A_O)	Final FFA content (A_S)	Actual shelf-life in days, %FFA ≤ 5%	FFA formation rate (k_s)	Predicted shelf-life in days, 30°C
Sample	Storage temperature (°C)							
NSRB	25	Y= 1.0118x+2.6293	0.9757	1.88	5.00	16.4	0.190 %	
	35	Y= 1.1677x+2.3571	0.9859	1.88	5.00	15.8	0.197 %	16.1
	45	Y= 1.4105x+2.1936	0.9919	1.88	5.00	13.9	0.224 %	
SRB	25	Y= 0.5030x+1.4858	0.9619	1.90	5.00	48.9	0.063 %	
	35	Y= 0.7635x+1.6816	0.9875	1.90	5.00	30.4	0.102 %	38.6
	45	Y= 0.9258x+2.5144	0.9803	1.90	5.00	18.8	0.165 %	

4. Storage stability of SRB by the RF method

4.1 FFA storage stability

The NSRB and SRB were stored at 35°C for 8 weeks, and the FFA content was monitored every week (Figure 27). As the RB sample was exposed to a relatively high RF treatment temperature for a longer time, a decrease in the FFA content was noticed. The SRB proved to be effective even with 8 weeks of storage, as the FFA content of the SRB did not exceed 5% (4.51%) (Figure 27) that was considered acceptable for human consumption.

The FFA content of the NSRB was always higher than that of the SRB throughout the studied storage period. The FFA of the NSRB increased from initially 1.88% to 11.14% after 8 weeks of storage, while the SRB sample increased from initially 1.55% to 4.51%. The reason for this was the RF stabilization effectively inhibited the lipase activity and delayed the hydrolytic rancidity resulting in a much lower rate of increase in the FFA value compared to that of the NSRB sample. The optimized conditions of the RF stabilization effectively inactivated the lipase activity and inhibited the FFA occurring during storage.

Suchada et al. (2015) reported the RF treatment for stabilizing extracted *Perilla frutescent* (L) Britt oil at a low temperature of 50°C for any treatment period, which increased the recovery of the oil. The lipase activity significantly affected the quality of the oil. In contrast to this result of a high treatment temperature over 80°C and duration of 10 minutes for the RF treatment, the low activation of lipase occurred. The result showed a low lipase activity in a high RF treatment temperature and RF treatment duration, while a low MC of the sample (115, 117, and 92 U g-1 dry matter at 90°C of RF treatment temperature, 600 seconds of RF treatment duration, and 10% of the MC of *Perilla frutescens* L.).

Ling et al. (2018) also reported the effects of hot air-assisted radio frequency (HAARF) heating on enzyme inactivation, lipid stability, and product quality of the RB. As shown, the initial FFA content between the control and HAARF treated samples did not show any significant difference ($p > 0.05$) regardless of the time-temperature combination applied. As expected, the FFA content of the control increased sharply beyond the acceptable limit within the first 15 days of storage and reached 50.67 g/100 g at the end of storage. However, the FFA accumulations of the

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

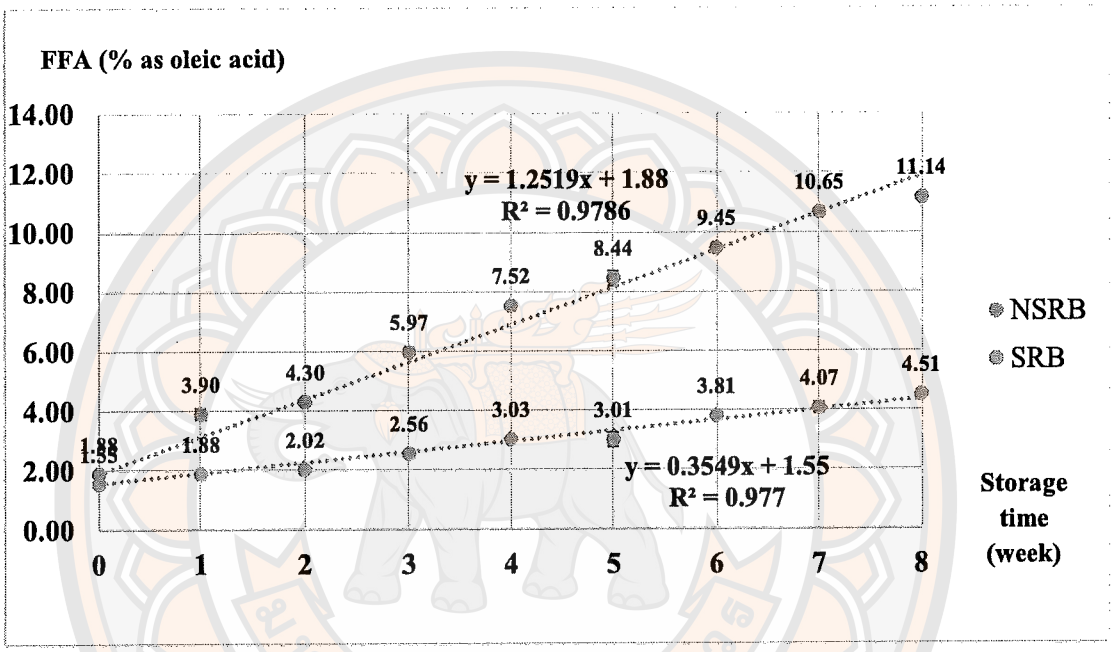


Figure 27 FFA content of NSRB and SRB by RF method during storage

4.2 MC, a_w, and color storage stability

The MC and a_w were an important factor influencing the storage stability of the RB. Table 65 shows that the initial MC and a_w of the NSRB was 5.84 g/100 g dry basis and 0.410, respectively. The strength of the RF treatment affected the MC and a_w. The MC of the RF treatment samples significantly decreased (p<0.05) with the increasing RF treatment temperature and RF treatment duration, and reached their minimum values of 0.14 g/100 g dry basis. Similarly, Ling et al. (2018) reported the effects of the HAARF on the MC and a_w and found that the initial MC and a_w of the NSRB were 11.16 g/100 g wet basis and 0.671, respectively. The MC and a_w of the HAARF treatment samples significantly decreased (p<0.05) with the increasing RF treatment temperature and RF treatment duration, and reached their minimum values

of 3.07 g/100 g wet basis and 0.141 in samples treated at 90°C for 45 minutes, respectively.

Both the MC and a_w of the SRB changed during the storage time, but the NSRB did not significantly change ($p>0.05$). The a_w of the SRB was initially 0.126 during the storage and significantly increased from 0.126 to 0.412 (Table 65). The MC of the SRB was significantly increased due to the permeability of the PE bag that absorbed water to the SRB sample until a new moisture equilibrium was achieved.

Table 65 shows the color properties of the NSRB and SRB samples. The RF treatments altered the color of the SRB among which the effect was more pronounced in the NSRB, which had a larger ΔE value. The SRB sample as a result of the RF treatment significantly differed to the NSRB, and it was darker than the NSRB. The L^* values of the SRB significantly decreased ($p<0.05$) from 65.77 on the initial day to 63.68 at the end of the storage period. This indicated that the RF stabilized samples became darker than the control. The a* and b* values of the SRB were higher than the NSRB and increased ($p<0.05$) during the storage period. The b* value was significantly increased ($p<0.05$) during the storage period. In contrast, Ling et al. (2019) reported the color properties of wheat germ. Both RF treatments altered the color of the wheat germ among which the effect was more pronounced in steam-treated samples, which had a larger ΔE value. The L□ values of the wheat germ increased significantly ($p<0.05$) from 77.9 to 81.5 and 83.4 with the RF heating at 100°C that was withheld for 15 minutes and 110°C, which was withheld for 5 minutes, respectively.

This indicated that the RF stabilized samples became brighter than the control, which could be due to the decrease of the MC and degradation of the heat-labile carotenoids during dry heating (Gili et al., 2017). In this study, the Maillard reaction had an effect that occurred during the thermal treatment. The L* value significantly decreased from 67.45 to 65.77, and the non-enzymatic browning during wet heating should be responsible for this phenomenon (Ling et al., 2019).

Table 65 MC, a_w, color and ΔE in NSRB and SRB stabilized by IR-HA method during storage

Sample	Storage week	MC (%)	a_w	L*	a*	b*	ΔE
NSRB	0	5.84 ± 0.15^{h}	0.410 ± 0.003^{i}	67.45 ± 0.03^{f}	3.68 ± 0.01^{cd}	19.84 ± 0.06^{bc}	0.00 ± 0.00^{a}
	1	6.05 ± 0.11^{i}	0.424 ± 0.001^{j}	68.38 ± 0.27^{g}	4.59 ± 0.22^{e}	20.69 ± 0.41^{f}	1.72 ± 0.43^{cd}
	2	7.02 ± 0.15^{m}	0.430 ± 0.001^{k}	68.52 ± 0.06^{gh}	3.59 ± 0.12^{bcd}	20.14 ± 0.17^{cde}	0.65 ± 0.12^{ab}
	3	6.66 ± 0.05^{l}	0.445 ± 0.005^{m}	69.55 ± 0.07^{l}	3.42 ± 0.01^{abc}	19.18 ± 0.20^{a}	2.45 ± 0.23^{d}
	4	6.21 ± 0.18^{ij}	0.446 ± 0.002^{m}	68.63 ± 0.06^{hi}	3.41 ± 0.09^{abc}	19.60 ± 0.35^{b}	0.80 ± 0.06^{b}
	5	6.06 ± 0.06^{i}	0.429 ± 0.002^{k}	69.00 ± 0.01^{j}	3.22 ± 0.74^{ab}	19.95 ± 0.52^{bcd}	1.59 ± 0.28^{c}
	6	6.35 ± 0.11^{jk}	0.432 ± 0.003^{lk}	68.76 ± 0.04^{i}	3.36 ± 0.08^{abc}	20.29 ± 0.15^{de}	1.03 ± 0.14^{bc}
	7	6.47 ± 0.06^{k}	0.444 ± 0.004^{lm}	69.24 ± 0.02^{k}	3.40 ± 0.16^{abc}	19.79 ± 0.16^{bc}	1.67 ± 0.06^{cd}
	8	5.83 ± 0.08^{h}	0.441 ± 0.001^{l}	69.28 ± 0.06^{k}	3.19 ± 0.09^{a}	20.09 ± 0.22^{cd}	1.86 ± 0.19^{cd}
SRB	0	0.14 ± 0.03^{a}	0.126 ± 0.001^{a}	65.77 ± 0.08^{e}	3.87 ± 0.11^{d}	20.53 ± 0.15^{ef}	1.71 ± 0.18^{cd}
	1	1.52 ± 0.11^{b}	0.192 ± 0.001^{b}	62.91 ± 0.4^{6a}	6.77 ± 0.09^{h}	21.29 ± 0.03^{g}	16.91 ± 0.75^{f}
	2	2.30 ± 0.17^{cd}	0.267 ± 0.002^{c}	63.52 ± 0.06^{bc}	5.42 ± 0.07^{fg}	24.32 ± 0.04^{k}	19.47 ± 0.28^{g}
	3	2.37 ± 0.17^{d}	0.301 ± 0.003^{d}	63.70 ± 0.03^{cd}	5.45 ± 0.04^{fg}	23.52 ± 0.04^{h}	15.55 ± 0.30^{e}
	4	2.13 ± 0.14^{c}	0.330 ± 0.001^{e}	63.91 ± 0.02^{d}	5.13 ± 0.11^{f}	23.81 ± 0.08^{hij}	15.35 ± 0.18^{e}
	5	3.57 ± 0.08^{fg}	0.353 ± 0.003^{f}	63.11 ± 0.06^{a}	5.64 ± 0.08^{g}	24.17 ± 0.28^{jk}	20.91 ± 1.34^{h}
	6	3.65 ± 0.11^{g}	0.404 ± 0.002^{h}	63.43 ± 0.04^{b}	6.54 ± 0.08^{h}	23.98 ± 0.09^{ijk}	20.94 ± 0.22^{h}
	7	3.03 ± 0.10^{e}	0.395 ± 0.004^{g}	63.37 ± 0.08^{b}	5.37 ± 0.09^{fg}	23.65 ± 0.24^{hi}	17.20 ± 0.69^{f}
	8	3.40 ± 0.03^{f}	0.412 ± 0.00^{i}	63.68 ± 0.08^{c}	5.14 ± 0.01^{f}	24.00 ± 0.04^{ijk}	17.02 ± 0.13^{f}

Note: Values are expressed as means ± standard deviation. Values for the same rice bran fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

4.3 Proximate and microbial analysis

The NSRB and SRB samples were analyzed for their proximate composition and microbial analysis (Table 66). The ash, protein, fiber, and carbohydrate content (dry basis) were stable as a result of the RF stabilization. Only the fat increased because the RF process was deteriorating the cell walls, thereby releasing the fat of the cell walls. RF heating was used to heat the dielectric materials with electromagnetic waves at frequencies between 1-300 MHz (Huang et al., 2015). As RF generates heat within the commodity by molecular friction, it can rapidly raise the temperature in a uniform, volumetric fashion. RF heating also has greater penetration depths compared to IR or MW heating, while also having simpler and more uniform field patterns compared to MW heating (Awuah et al., 2015).

The TPC of the NSRB and SRB are displayed in Table 66. The TPC, yeast, & mold in the SRB showed an increasing trend as the storage period increased. The SRB samples showed a high stability against the microbial growth during the 8 weeks of storage. The RF stabilization could inhibit the growth of microbes. The main reason for this was that the low MC and a_w values in the SRB could retard the microbial growth and made the bran more stable during the storage duration.

Table 66 RB proximate and microbial analysis in NSRB and SRB by RF method during storage

Analysis	Week 0		Week 8		%changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Proximate (g/100g dry basis)						
Carbohydrate	50.88±0.50^{a}	51.34±0.50^{a}	52.89±0.99^{b}	50.49±0.63^{a}	+3.95	-1.66
Fat	16.54±0.24^{b}	17.33±0.29^{c}	15.61±0.57^{a}	17.91±0.53^{c}	-5.62	+3.35
Protein^{ns}	14.58±0.06	14.63±0.12	14.67±0.15	14.70±0.13	+0.62	+0.48
Fiber	9.47±0.49^{b}	8.24±0.34^{a}	8.32±0.22^{a}	8.38±0.23^{a}	-12.14	+1.70
Ash^{ns}	8.52±0.24	8.47±0.08	8.51±0.12	8.52±0.03	-0.12	+0.59
	Average % changed				-2.66	+0.89
	Total % changed				-13.31	+4.46
Microbial (cfu/g)						
TPC	3.25×10^{4}±0.04	4.45×10^{3}±0.06	8.15×10^{4}±0.08	6.45×10^{3}±0.05	+150	+44.9
Yeast & mold	2.80×10^{3}±0.06	3.40×10^{2}±0.03	3.15×10^{3}±0.03	2.25×10^{2}±0.03	+12.5	-33.8
	Average % changed during storage time				+81.2	+5.55
	Total % changed during storage time				+162	+11.1

Note: Values are expressed as means ± standard deviation. Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different (p <0.05).

4.4 FA compositions of the storage stability

The FA compositions of the NSRB and SRB by the RF stabilization method are shown in Table 67. The NSRB contained approximately 4.16% of saturated and 12.42% of unsaturated FA, while the SRB contained approximately 4.47% of saturated and 13.31% of unsaturated FA. The SRB samples possessed more saturated, monounsaturated, polyunsaturated, and total unsaturated FA content than the NSRB. The monounsaturated, polyunsaturated and total unsaturated FA in the NSRB that were decreased during storage may have undergone oxidation with the hydronium ion changing the double bond to a single bond (Fennema, 1996). Nevertheless, for the SRB by the RF stabilization method, these compounds were stable, which the RF stabilized method had the efficiency to maintain the health beneficial FA compounds (unsaturated FA) in the RB.

The quantities of omega-6 and omega-9 in the SRB samples were higher than those in the NSRB and had a stable content throughout storage. The omega-3 content did not change during the RF processing, and its content was stable during the storage period. The results presenting the effects of the RF on the RB could insignificantly change the FA profile ($p > 0.05$) as already observed by Ling et at. (2018), who reported that the HAARF treatment at 100°C for 15 minutes did not significantly influence the FA composition of the RB ($p > 0.05$).

The possible explanation for the decrease of the FAs contents during the storage of the NSRB could be due to the abundance of FFA hydrolyzed by the lipase activity in the NSRB. FA decreases (or FFA increases) might be due to the water absorption in the RB during storage agreeing with the increases of the MC and a_w (Table 65). Based on the previous studies of Labuza, & Dugan (1971), the enzyme activity in foodstuff had a significantly positive correlation on its MC. This study further proved that it would be difficult to irreversibly inactivate the lipase activity in the NSRB with high MC conditions, and the activity of the endogenous enzyme in the NSRB by dry heating could be partly inhibited only if the bran moisture increased to atmospheric equilibrium during storage.

Table 67 FA composition in NSRB and SRB by RF method during storage

Fatty acid composition	Week (g/100g)				%Changed	
	Week 0		Week 8			
	NSRB	SRB	NSRB	SRB	NSRB	SRB
Myristic acid (C14:0)	0.08	0.08	0.07	0.08	-12.5	0
Palmtic acid (C16:0)	3.40	3.66	3.18	3.64	-6.47	-0.55
Heptadecanoic acid (C17:0)	ND	ND	ND	0.01	-	-
Stearic acid (C18:0)	0.36	0.38	0.34	0.38	-5.55	0
Arachidic acid (C20:0)	0.15	0.15	0.15	0.16	0	+6.67
Heneicosanoic acicd (C21:0)	ND	ND	ND	ND	-	-
Behenic acid (C22:0)	0.06	0.07	0.06	0.07	0	0
Lignoceric acid (C24:0)	0.10	0.11	0.10	0.11	0	0
Saturated fat	4.16	4.47	3.92	4.46	-5.77	-0.22
Palmitoleic acid (C16:1n7)	0.03	0.03	0.03	0.03	0	0
Trans-9-Eladic acid (C18:1n9t)	ND	ND	ND	ND	-	-
Cis-9-Oleic acid (C18:1n9c)	5.98	6.38	5.77	6.33	-3.51	-0.78
Cis-11-Ecosenoic acid (C20:1n11)	0.09	0.10	0.09	0.10	0	0
Monounsaturated fatty acid	6.12	6.53	5.91	6.49	-3.43	-0.61
Trans-Linolelaidic acid (C18:2n6t)	ND	ND	ND	ND	-	-
cis-9,12-Linoleic acid (C18:2n6)	6.06	6.53	5.58	6.41	-7.92	-1.83
Gamma-Linoleic acid (C18:3n6)	ND	ND	ND	ND	-	-
Alpha-Linoleic acid (C18:3n3)	0.22	0.24	0.19	0.23	-13.63	-4.17
cis-11,14-Eicosadienoic acid (C20:2)	ND	ND	ND	ND	-	-
cis-8,11,14-Eicosadienoic acid (C20:3n6)	ND	ND	ND	ND	-	-
Polyunsaturated Fatty acid	6.30	6.78	5.79	6.60	-8.09	-2.65
Unsaturated fat	12.42	13.31	11.70	13.13	-5.80	-1.35
Trans fat	ND	ND	ND	ND	-	-
Omega 3	0.22	0.24	0.19	0.23	-13.64	-4.17
Omega 6	6.07	6.54	5.59	6.42	-7.91	-1.83
Omega 9	5.99	6.39	5.78	6.35	-3.51	-0.63
Average % changed					-5.43	-0.67
Total % changed					-97.73	-12.12

Note: ND = not detected

4.5 Tocols and oryzanol storage stability

In this study, approximately up to 10.0 % of γ-tocotrienol was observed in the SRB that was stabilized by the RF stabilization method. The NSRB contained 298.97 mg/kg dry basis, and lost approximately 17.59% and 25.79% at weeks 4 and 8 of the storage period, respectively, while the SRB contained 326.93 mg/kg dry basis, which was an approximate loss of 4.67% and 7.08% at weeks 4 and 8 of the storage period, respectively. This inferred that the RF stabilized RB processing had the efficiency to maintain γ-tocotrienol (Table 68).

The α-tocopherol content did not have a significant change in the SRB ($p > 0.05$). The NSRB lost approximately 2.60% and 7.73% at weeks 4 and 8 of the storage period, respectively, while the SRB approximately lost 0.20% and 1.25% at four and 8 weeks of storage time, respectively. This inferred that the RF stabilized RB processing had the efficiency to maintain α -tocopherol than the control (NSRB). In comparison, Kim et al. (2014) reported that most of the tocopherol was bound to proteins or phospholipids, and the thermal process could break these linkages, which after 60 days of storage, the total tocopherol content of the control and RF treatment sample decreased from 48.06 mg/kg to 30.01 mg/kg and 55.05 mg/kg to 45.74 mg/kg, decreasing by 38% and 17%, respectively. Similar decreases were also observed in α and β+γ-tocopherol. This implied that the tocopherols in raw RB were less stable than the HAARF treated RB during storage.

γ-Oryzanol in the NSRB (dry basis) on the initial day was 1,476.67 mg/kg dry basis, then decreased during the storage period to 1,082.90 mg/kg dry basis, which was a reduction of 26.67%, in comparison with oryzanol in the SRB, which was found on the initial day to be 1,538.87 mg/kg dry basis and gradually decreased during the storage period 1,407.47 mg/kg dry basis, a reduction of 8.54%. Similarly, Chun, et al. (2005) reported that tocopherol had a significant antioxidant effect during lipid oxidation in stored peanuts, and its losses were highly correlated with an increase in the PV and control sample (NSRB) that contained 2.97 g/100 g oil of γ- oryzanol. Statistically, the effect of HAARF heating and storage on the γ-oryzanol content of RBO was not significant ($p > 0.05$). Similarly, Rodchuajeen et al. (2016) also reported that the storage time did not significantly affect the γ-oryzanol content of the RB stabilization by different

moving bed drying methods. Nonetheless, overall, γ-oryzanol in the RB was stable during the HAARF stabilization and storage (Ling et al., 2018).

Table 68 Tocols and oryzanol in NSRB and SRB by RF method during storage

Analysis	Week 0		Week 4		Week 8		%Changed	
	NSRB	SRB	NSRB	SRB	NSRB	SRB	NSRB	SRB
Tocols and Oryzanol (mg/kg dry basis)								
γ-tocotrienol	298.97±28.71^{b}	326.93±10.23^{b}	246.37±18.83^{a}	311.77±2.71^{b}	221.87±68.21^{a}	303.77±2.22^{b}	-25.79	-7.08
α-tocopherol	50.07±0.45^{b}	50.20±0.46^{b}	48.77±0.42^{b}	50.10±0.36^{b}	46.20±1.64^{a}	49.57±0.84^{b}	-7.73	-1.25
γ-oryzanol	1476.67±77.41^{cd}	1538.87±45.79^{d}	1258.13±30.38^{b}	1442.57±35.66^{cd}	1082.90±73.14^{a}	1407.47±22.18^{c}	-26.67	-8.54
			Average % changed				-20.06	-5.62
			Total % changed				-60.19	-16.87

Note: Values are expressed as means ± standard deviation. Values for the same RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

4.6 Shelf life and FFA formation rate of the NSRB and SRB by RF method

The shelf life of the NSRB and SRB samples stored at 25°C, 35°C, and 45°C were evaluated using the FFA content as an indicator (Table 69). High R^2 values were observed in both the NSRB (25°C, 35°C, and 45°C were 0.9757, 0.9859, and 0.9919, respectively) and SRB (25°C, 35°C and 45°C were 0.9631, 0.9807, and 0.9785, respectively) at all storage temperatures. These high ranges of linear regression enabled the storage time to be calculated with a high degree of accuracy.

The actual shelf life values of the SRB samples at 25°C, 35°C, and 45°C were 7.35, 4.21, and 4.00 times higher, respectively than those of the NSRB samples when compared to the same storage temperatures. The predicted shelf life of the SRB at 30°C was 89.6 days and was 5.57 times compared to the NSRB.

A zero-order kinetic equation was used to predict the FFA formation rate in the RB. Table 69 shows that the initial FFA contents (A_0) of the NSRB and SRB were 1.88% and 1.55%, respectively. At all three storage temperatures, the FFA formation rate (k_S) in the SRB was lower than that in the NSRB. Within the three storage temperatures, the lower temperature corresponded to lower k_S. The lowest k_s (0.024%) was found in the SRB stored at 25°C.

Table 69 Shelf-life and FFA formation rate of NSRB and SRB by RF method

Condition		Linear eq.	R^2	Initial FFA content (A_O)	Final FFA content (A_S)	Actual shelf-life in days, %FFA ≤ 5%	FFA formation rate (k_s)	Predicted shelf-life in days, 30°C
Sample	Storage temperature (°C)							
NSRB	25	Y= 1.0118x+2.6293	0.9757	1.88	5.00	16.4	0.190 %	
	35	Y= 1.1677x+2.3571	0.9859	1.88	5.00	15.8	0.197 %	16.1
	45	Y= 1.4105x+2.1936	0.9919	1.88	5.00	13.9	0.224 %	
SRB	25	Y= 0.2113x+1.3558	0.9631	1.55	5.00	120.7	0.024 %	
	35	Y= 0.3740x+1.4418	0.9807	1.55	5.00	66.6	0.030 %	89.6
	45	Y= 0.4185x+1.6771	0.9785	1.55	5.00	55.6	0.053 %	

Conclusion of Section 3

Due to the different qualities (physic-chemical properties) of the RB's raw material (different seasons), it could be concluded that,

1. The NSRB and SRB by the stabilized method were stored at 35°C for 8 weeks and the FFA content was monitored every week. This IR and RF that the effectiveness of the stabilized RB methods sequencing compare with NSRB from high to low was RF, IR-VC, IR, and IR-HA, respectively.

2. The MC, a_w and color of the RB were investigated storage stability. All the SRB's MC and a_w stabilization treatments were lower than the NSRB. The MC and a_w of the NSRB sample gradually decreased during the storage time, while both values of the SRB were found to increase. The color results presented in the SRB were darker than the NSRB sample with regard to the lower lightness (L*) value. The* and b* values of the NSRB were higher than the SRB. The ΔE value of the SRB was also found to be higher than that of the NSRB. This IR and RF that the effectiveness of the maintained color of SRB sequencing compare with NSRB from high to low was IR-VC, IR-HA, IR and RF, respectively.

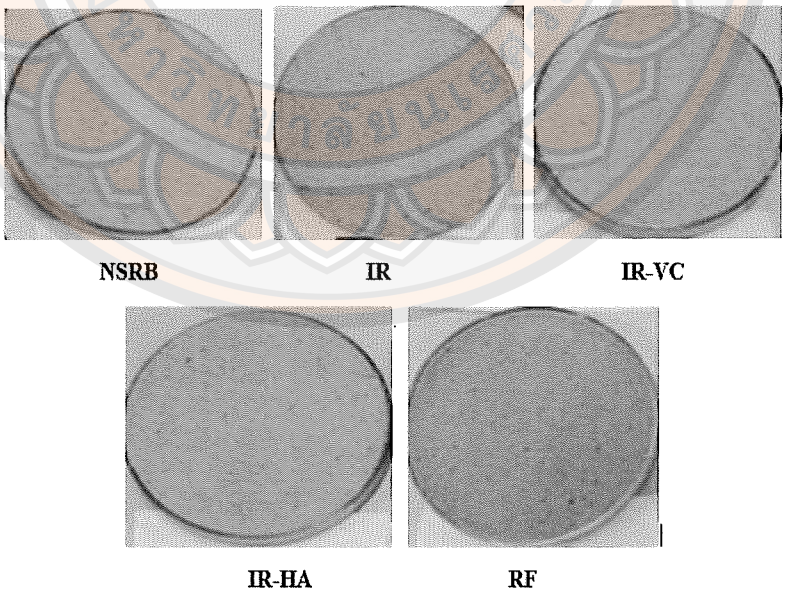


Figure 28 Appearance of NSRB and SRB's IR, IR-VC, IR-HA and RF methods

3. The ash, protein, fat, fiber and carbohydrate content (dry basis) in the SRB significantly increased as a result of the IR, IR-VC, and IR-HA methods, but the RF method was unstated. The IR method was observed for the protein content of the SRB that was significantly higher than the fresh counterpart, but the fiber content was significantly lower than the fresh counterpart. Additionally, the fat in the SRB's RF method was higher than the NSRB. The IR-VC method noted for the observed fiber content of the SRB was significantly higher than that of the fresh counterpart. The TPC of all treatments had non-significant growth and remained at the standard level during storage. Yeast & mold were detected in the SRB samples. The SRB sample of all treatments showed high stability against the microbial growth during storage. This IR-VC that the effectiveness of maintained and improved proximate composition, while stability microbial during storage.

4. FA composition of the RB was investigated storage stability. This included the important FA composition of the RB samples as saturated, unsaturated FA (monounsaturated and polyunsaturated). All SRB samples possessed more saturated, monounsaturated, polyunsaturated, and total unsaturated FA content than the NSRB. The quantities of omega-3, omega-6, and omega-9 in the SRB samples were higher than those in the NSRB, which had a stable content during storage. The results indicated that the effect of the IR, IR-VC, IR-HA, and RF stabilized method on the RB could insignificantly change the FA profile ($p > 0.05$). This inferred the efficiency to maintain the health beneficial FA compounds in RB.

5. Tocols and oryzanol of the RB were investigated storage stability. In the current study, up to approximately 9.71%, 18.8%, 9.71%, and 10.0%, respectively of γ-tocotrienol were observed in the SRB by the IR, IR-VC, IR-HA, and RF methods, respectively. The NSRB had a greater loss in the γ-tocotrienol content than the SRB during the 8 week storage period. All the stabilized RB processing methods had the efficiency to maintain γ-tocotrienol, especially the IR-VC method. The α-tocopherol content in the NSRB showed a non-significant change in the SRB by the IR and RF methods, while up to 8.7% was found in the SRB by the IR-VC method but there was a loss of 4.53% in the SRB resulting from the IR-HA method. The IR-VC method had the efficiency to maintain α-tocopherol, while the IR-HA could not maintain α-tocopherol. The α-tocopherol content in the NSRB had a greater loss than the SRB

during storage. Up to approximately 8.90%, 22.1%, 14.6%, and 4.25%, respectively of γ-oryzanol were observed in the SRB by the IR, IR-VC, IR-HA, and RF methods respectively. The NSRB had a greater loss in the γ-Oryzanol content than the SRB during storage. All the stabilized RB processing methods had the efficiency to maintain γ-oryzanol.

6. The shelf life of SRB by IR, IR-VC, IR-HA, and RF methods compared to NSRB were improved for 2.77, 3.73, 2.40, and 5.57 times, respectively. It was 44.7, 129.7, 38.6 and 89.6 days, respectively. This inferred that the IR-VC and RF stabilization methods had a long shelf life, it recommend to using for stabilization rice bran.

The optimized stabilization RB conditions by the IR, IR-VC, IR-HA, and RF heat treatments were selected to be used in the next section. The NSRB and SRB from section 2 were extracted by screw press and investigated the percentage yield of the crude RBO. The NSRBO and SRBO samples from the IR, IR-VC, IR-HA and RF heat treatments were stored at 35°C for 8 weeks. The samples were collected each week during that period for the determination of the FFA content of each sample. The shelf life and formation of the FFA in the RBO were also investigated. The NSRBO and SRBO samples were collected at Weeks 0, 4, and 8 for the determination of the AV, PV, FA composition, tocotrienol, tocopherol, and oryzanol contents.

Section 4 Crude RBO yield, qualities and stability

1. Crude RBO yield

The extraction of RBO has been performed using conventional techniques like solvent extraction and mechanical pressing, but solvent extraction (hexane) is more commonly used in the RBO industry. These techniques usually use organic solvents, which are expensive and sometimes toxic. Solvent oil extraction is the most efficient method; however, its application presents some industrial disadvantages; such as, plant security problems, emissions of volatile organic compounds into the atmosphere, high operation costs, and poor quality products caused by high processing temperatures (Uquiche et al., 2008).

Therefore, the cold-pressed extraction process gave a better quality of crude oil and contained a higher vitamin, phytochemical, and mineral content than that of solvent extraction (Thanonkaew et al., 2012). In the current study, the RB sample was stabilized by IR and RF heating for the cold-pressed RBO process. Experiments were carried out by using IR, IR-VC, IR-HA and RF, and NSRB, which had the oil extraction yield of stabilized RBO of 7.10, 8.87, 6.97, 5.63, and 5.37 (g/100g bran dry basis), respectively (Table 70). The IR-VC stabilization method had the highest extraction yield of oil as a result of the cold-pressing method, and the RF stabilization method showed a non-significant difference in the control. The IR-HA stabilization method had higher non-significant extraction efficiency than the IR, but had a greater extraction yield of oil than the NSRB. This inferred that hot air did not improve the IR heating extraction yield of the oil. Thanonkaewa et al. (2012) reported that the oil extraction yield from RB increased according to the application of hot air, roasting, and microwave heating (MH) ($p<0.05$).

Thanonkaew et al. (2012) reported the oil extraction yield of the NSRB, SRB by HA, MH, roasting, and steaming were 3.29 g/100 g, 5.53 g/100 g bran, 4.81 g/100 g bran, 4.77 g/100 g bran, and 3.41 g/100 g bran, respectively. The SRB by steaming displayed no significant difference in the oil extraction yield from the NSRB ($p<0.05$). Uquiche et al. (2008) reported that an optimal MC for domestic heating existed, and the provision of a higher MC had a significant impact on the extraction yield. The low MC in SRB could make it be more brittle, therefore resulting in a

greater rupture of tissue and increase in the extraction of oil during the mechanical pressing.

There are two types of lipids in oilseeds: storage lipids, which are mainly triacylglycerols and which are high in quantity and localized in the oil bodies of the tissues, and membrane lipids, which are mainly phospholipids (Liu, & Brown, 1996). Azadmard-Damirchi et al. (2010) reported that pretreatment of rapeseed with MH could enhance the oil extraction yield of the cold pressing and solvent extraction methods. The oil extraction yield from hazelnut seed increased according to the application of MW (Uquiche et al., 2008). The heating may modify the cellular wall, which would result in greater porosity. It could also vaporize the water of the vegetable substrate microstructure by increasing the pressure in its interior; its release would cause the disintegration of the material (Aguilera, & Stanley, 1999; Starmans & Nijhuis, 1996), rupture the cell membrane, and improve the efficiency of the pressing extraction of the oil from the oilseeds, thus enabling the passage of oil from the cell membrane (Uquiche et al., 2008).

These results were in agreement with other forms of plant oil extraction, especially the IR-VC, IR, and IR-HA methods that were most efficient for modifying the cellular wall, which resulted in greater porosity in SRB. It could also vaporize the water of SRB microstructure by increasing the pressure in its interior; its release would cause the disintegration of the material, rupture the cell membrane, and improve the efficiency of the pressing extraction of the oil. Nevertheless, the RF method was not a significant stabilization method to improve the extracted RBO (p>0.05), as it had a different mechanism for the IR method. The RF heating was heating by a dielectric property to heat low energy (high wavelength, low frequency and low penetration). The SRB by RF method had no strength to increase the pressure in its interior and was not released, which caused the disintegration of the material, ruptured the cell membrane, and did not improve the efficiency of the pressing extraction of the oil.

Table 70 RBO yield of crude NSRBO and SRBO obtained from IR, IR-VC, IR-HA, and RF methods

No.	RB stabilized method	RBO yield by cold press method (g/100g bran dry basis)
1	Non-stabilized	5.37±0.38[a]
2	IR	7.10±0.50[b]
3	IR-VC	8.87±0.42[c]
4	IR-HA	6.97±0.38[b]
5	RF	5.63±0.15[a]

Note: Values are expressed as means ± standard deviation.
Values for the same stabilized RB fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different ($p<0.05$)

2. Appearance of NSRBO and SRBO obtained by IR, IR-VC, IR-HA and RF methods

Color is another important characteristic for determining the visual acceptance of RBO. The influence of the IR, IR-VC, IR-HA and RF methods on the color of cold-pressed RBO was observed. The color of the crude NSRBO and SRBO by cold pressing is shown in Figure 29. The SRBO differed in its colors, as all of the heating methods could reduce the lightness, while the SRBO obtained from RF method mostly reduced the lightness of the RBO. The NSRBO sample mostly maintained lightness, followed by the IR-VC, IR, IR-HA, and RF stabilization methods, respectively. However, there were no color standards for cold-pressed RBO, and the L*, a*, and b* measurements could thus be used for the color classification (Thanonkaew et al., 2012).

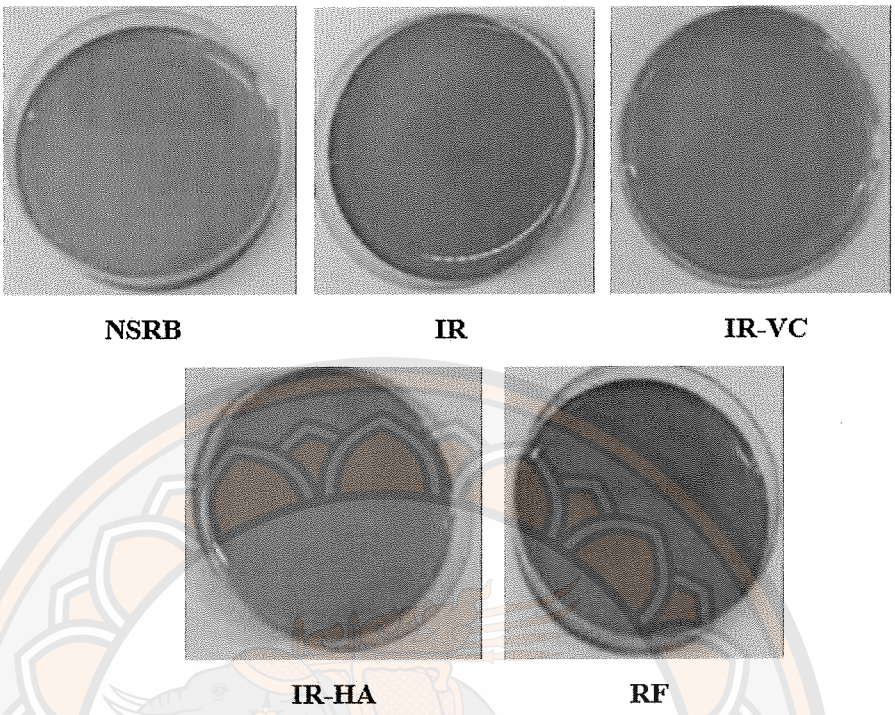
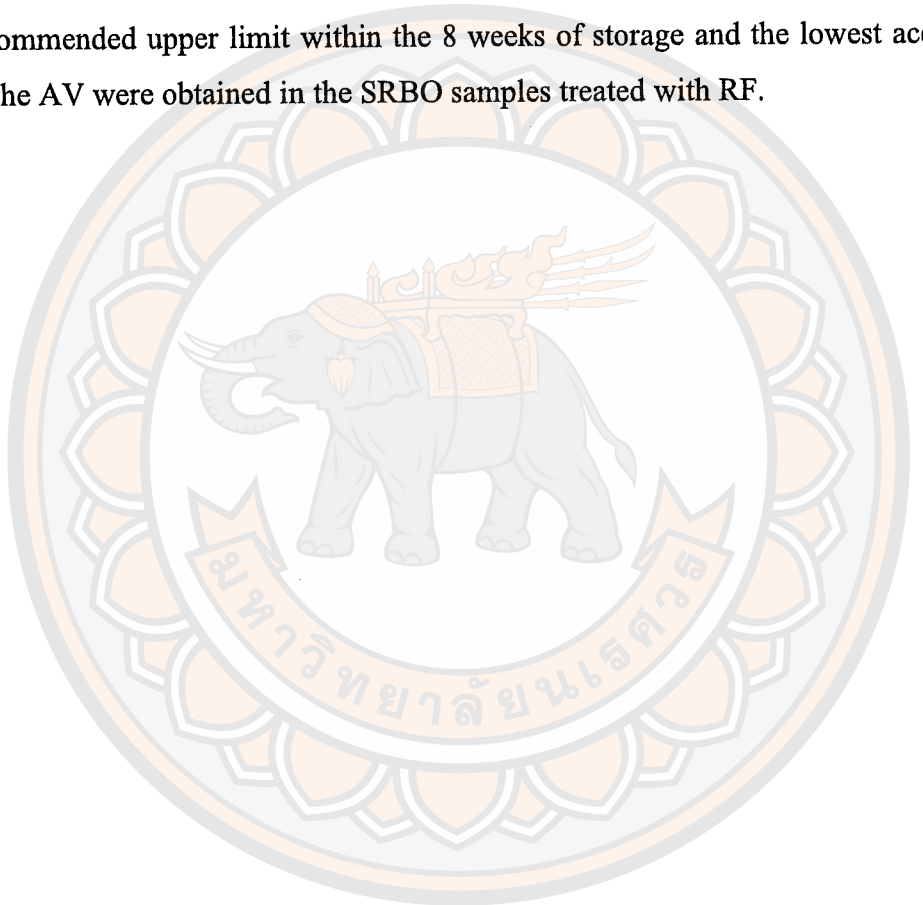


Figure 29 Appearance of crude NSRBO and SRBO obtained by IR, IR-VC, IR-HA and RF methods

3. AV storage stability

The initial AV between the NSRBO, SRBO obtained from the IR, IR-VC, IR-HA and RF stabilization methods had a different value (Fig. 30). The AV of the NSRBO and SRBO obtained from the IR, IR-VC, IR-HA, and RF stabilization methods were 7.78, 5.64, 5.08, 6.76, and 5.64 mg KOH/g RB oil, respectively, regardless of the different conditions of the stabilization methods applied. Thanonkaew et al. (2012) reported that the initial AV of the NSRBO and SRBO obtained from hot air, roasting, steaming, and MH were 11.11, 6.98, 7.56, 9.01 and 6.30 mg KOH/g RB oil, respectively.

Within the 8 weeks of the storage period, the AV of the NSRBO increased sharply beyond the acceptable limit and reached 30.96 mg KOH/g RB oil at the end of the storage period. However, the accumulation of AV of the RF method samples was lower than other stabilization methods (14.86 mg KOH/g RB oil) and the NSRBO at each storage interval. The AV of SRBO obtained from IR-HA, IR, IR-VC, and RF were lower than the NSRBO. The ranking of AV from high to low was NSRBO, SRBO obtained from IR-HA, IR, IR-VC, and RF methods, respectively. The recommended upper limit within the 8 weeks of storage and the lowest accumulation of the AV were obtained in the SRBO samples treated with RF.



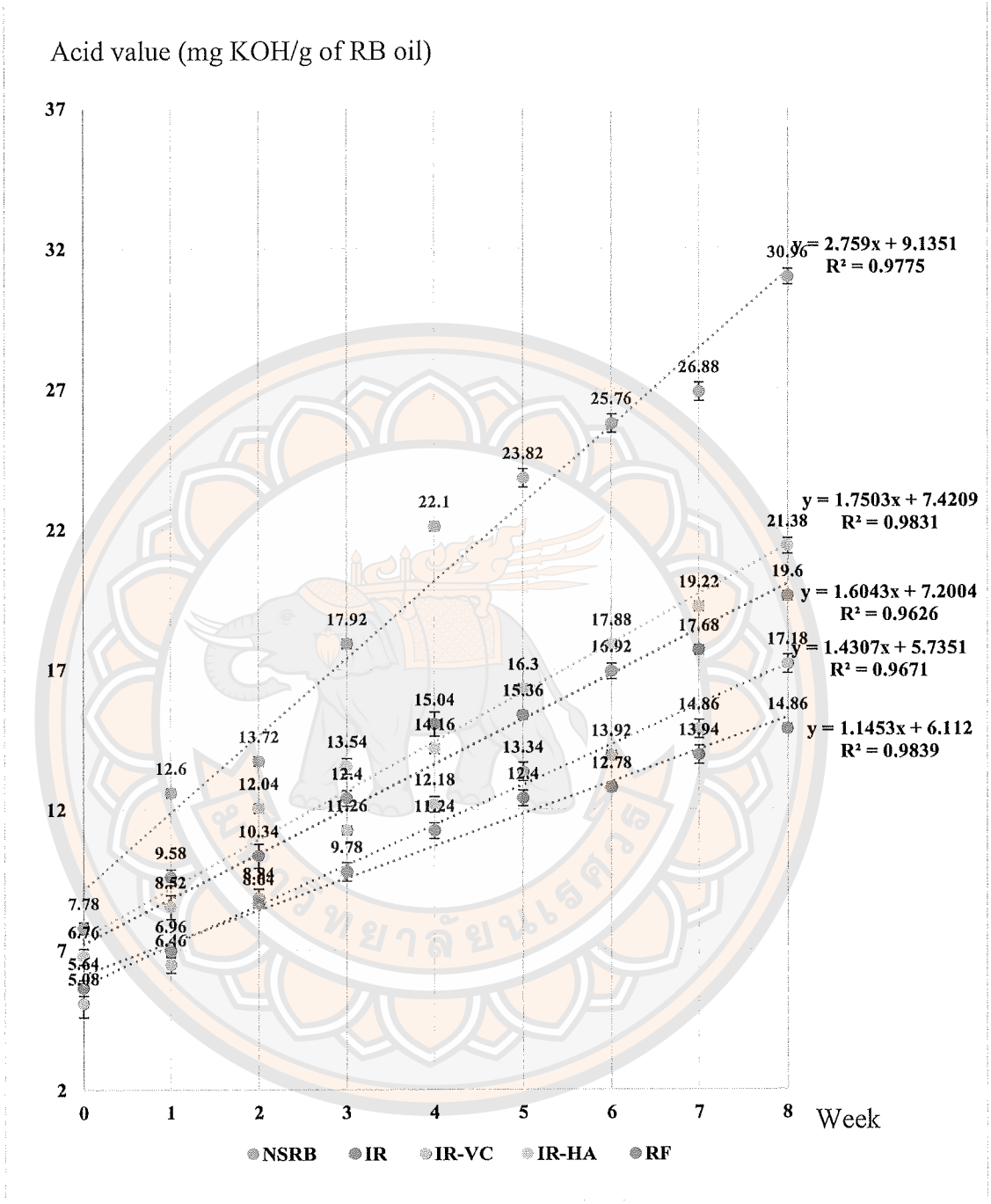


Figure 30 Acid value of crude NSRBO and SRBO obtained by IR, IR-VC, IR-HA, and RF methods during storage

4. PV storage stability

In general, PV is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of the lipid oxidation, which indirectly indicate the freshness of the oil. The detection of PV gives the initial evidence of rancidity in unsaturated fats and oils. Other methods are available, but the PV is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation, and the extent of secondary oxidation may be determined from a p-anisidine test. The double bonds found in fats and oils play a role in autoxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. The best test for autoxidation (oxidative rancidity) is the determination of the PV. PV is intermediates in the autoxidation reaction. Autoxidation is a free radical reaction involving oxygen that leads to the deterioration of fats and oils, which form off-flavors and off-odors. The PV, concentration of peroxide in an oil or fat, is useful for assessing the extent to which spoilage has advanced. According to the Codex Alimentarius Commission, the acceptable limit of the PV for RBO is <15 meq/kg (by cold-pressed method) (Patil et al., 2016).

Figure 31 shows the changes PV on the RBO samples during storage. In the initial storage period, stabilization caused a significant increase in the PV compared to the raw RBO. The PV in the RBO obtained from IR, IR-VC, IR-HA, and RF stabilization methods were 3.33 meq.O_2/kg, 4.08 meq.O_2/kg, 3.64 meq.O_2/kg, 3.80 meq.O_2/kg, and 3.72 meq.O_2/kg /kg, respectively. Oils with a high degree of unsaturation are most susceptible to autoxidation. Therefore the reason for this case was the stabilization of the heating to make the oils have a role in the autoxidation without any enzymes.

At weeks 4, the PV of NSRBO and SRBO obtained from IR, IR-VC, IR-HA, and RF were 8.50 meq.O_2/kg, 6.93 meq.O_2/kg, 5.88 meq.O_2/kg, 7.94 meq.O_2/kg, and 4.91 meq.O_2/kg, respectively. All treated remained well within the acceptable limit of 10 meq/kg. At the end of during storage, the PV of SRBO obtained from IR-VC and RF stabilization methods were remained well within the acceptable limit of 15 meq.O_2/kg (8.65 meq.O_2/kg and 6.16 meq.O_2/kg of the RBO).

The results demonstrated the efficacy of both the IR-VC and RF stabilization methods in controlling the rise in the PV until the end of the during storage. Lipase

and lipoxygenase enzyme were mainly responsible for the formation of the hydroperoxides. Furthermore, heating treatments might suppress the activity of the respective enzymes resulting in the reduced levels of peroxide of the RB. The findings could be substantiated based on the fact that an energy-efficient heating process could induce the decomposition of some of the formed hydroperoxide resulting in volatile degradation of the products (Shaker et al., 2013). A similar reduction in the PV of RBO after stabilization was reported by Dhingra & Chopra (2014) and Shaker et al. (2013).

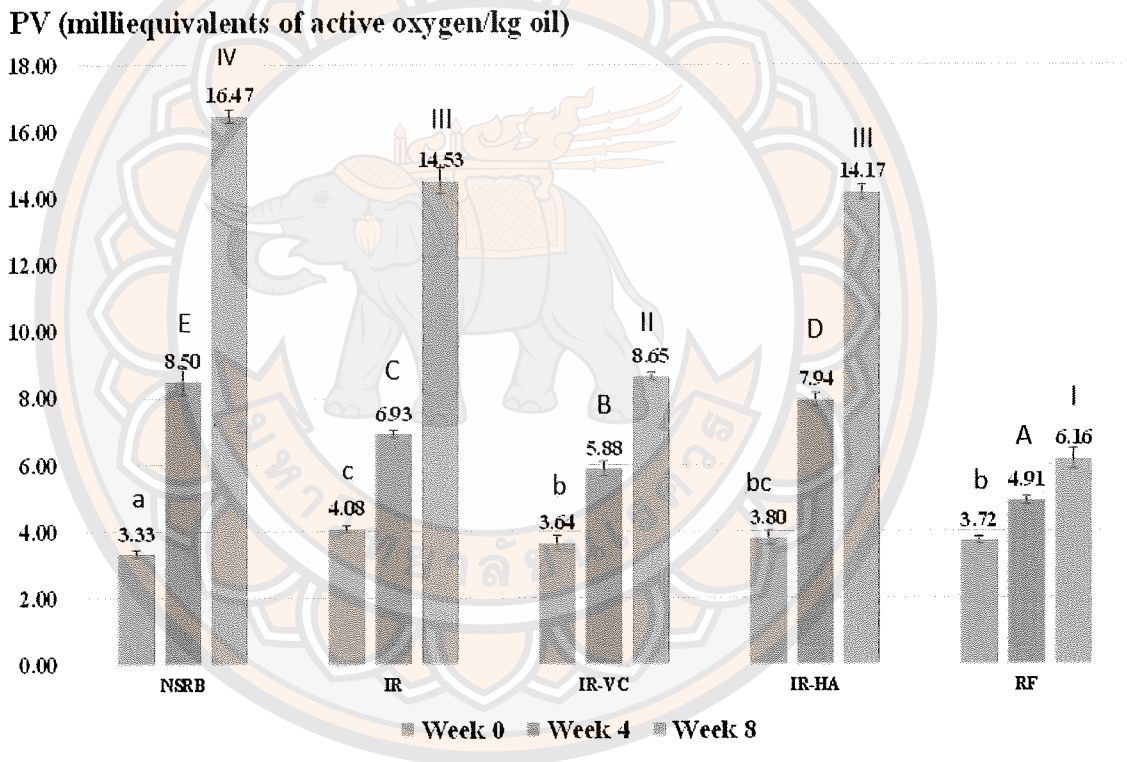


Figure 31 PV of crude NSRBO and SRBO obtained from IR, IR-VC, IR-HA and RF stabilization methods on during storage

Note: Values are expressed as means ± standard deviation. Values for the same stabilized RB storage duration week: the small, capital, and roman numerals labeled with the same week are not significantly different, but values labeled with different letters are significantly different ($p < 0.05$)

4. FA composition storage stability

The FA composition of the NSRBO and SRBO samples shows in table 71. The NSRBO contained approximately 24.89% of saturated and 75.11% of unsaturated FA (43.03% of monounsaturated and 32.08% of polyunsaturated FA) and contained 0.05% of trans fat, 1.26% of omega-3, 30.79% of omega-6, and 42.25% of omega-9. The SRB of the IR, IR-VC, IR-HA, and RF at the initial storage period contained approximately 24.89-25.08% of saturated and 74.92-75.11% of unsaturated FA (43.00-43.26% of monounsaturated and 30.50-30.77% of polyunsaturated FA) and also 0.02-0.06 of trans fat, 1.26-1.26% of omega-3, 30.54-30.80% of omega-6, and 42.22-42.49% of omega-9. There was no differences between the NSRBO and SRBO from all stabilization treatments in all FA compositions.

Similarly, no significant reduction of the main FA in wheat germ stabilized with IR heating was found by Li et al. (2016), and similar results observed by Ramezanzadeh et al. (2000) and Yilmaz et al. (2014) also mentioned that the MW of RB could be stored at 4-5°C for up to 16 weeks without any adverse effects on the quality of the proximate and FA composition. Because the RBO sample treated by the hot air assisted RF (HAARF) heating at 100°C for 15 minutes had the longest shelf life, only this sample was chosen for quality evaluation. It was observed that the HAARF treatment at 100°C for 15 minutes had no significant influence on the FA composition of the RB ($p > 0.05$).

Similar results were also observed in RB stabilized by MW (Ramezanzadeh et al., 2000). After 60 days of storage, the FA composition and content in the HAARF treated sample did not change significantly ($p>0.05$), while the content of the linoleic acid and linolenic acid in the control showed a significant decrease ($p<0.05$). The possible explanation for the decrease of the FA content during storage could be due to the abundant FFA hydrolyzed by the lipase activity in the control. FFAs; such as, linolenic acid, are an excellent substrate for liquid oxygen (LOX) and auto-oxidation leading to the generation of primary oxidation products.

Table 71 Fatty acid composition of NSRBO and SRBO by IR, IR-VC, IR-HA and RF method during storage

Fatty acid composition	Week 0					Week 8				
	NSRBO	IR	IR-VC	IR-HA	RF	NSRBO	IR	IR-VC	IR-HA	RF
Myristic acid (C14:0)	0.36	0.36	0.35	0.37	0.37	0.36	0.36	0.36	0.38	0.36
Pentadecaric acid	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.04	0.03
Palmtic acid (C16:0)	20.64	20.69	20.51	20.60	20.52	20.67	20.66	20.65	20.68	20.53
Heptadecanoic acid (C17:0)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stearic acid (C18:0)	2.21	2.22	2.23	2.19	2.30	2.21	2.21	2.22	2.44	2.22
Arachidic acid (C20:0)	0.85	0.87	0.88	0.85	0.87	0.86	0.86	0.86	0.86	0.87
Heneicosanoic acicd (C21:0)	0.04	0.04	0.02	0.04	0.02	0.03	0.03	0.03	0.03	0.03
Behenic acid (C22:0)	0.27	0.31	0.31	0.29	0.30	0.27	0.30	0.30	0.28	0.30
Tricosanoic acid (C23:0)	0.02	0.03	0.03	0.02	0.03	0.02	0.03	0.02	0.02	0.03
Lignoceric acid (C24:0)	0.42	0.48	0.49	0.44	0.47	0.43	0.47	0.47	0.44	0.47
Saturated Fatty acid	24.89	25.08	24.89	24.90	24.96	24.92	25.00	24.97	25.22	24.87
Palmitoleic acid (C16:1n7)	0.24	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Trans-9-Eladic acid (C18:1n9t)	0.05	0.02	0.05	0.05	0.06	0.05	0.06	0.02	0.08	0.05
Cis-9-Oleic acid (C18:1n9c)	42.22	42.23	42.46	42.20	42.40	42.37	42.21	42.47	42.32	42.52
Cis-11-Ecosenoic acid (C20:1n11)	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
Erucic acid (C22:1n9c)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Monounsaturated fatty acid	43.03	43.00	43.26	43.00	43.22	43.18	43.03	42.35	43.17	43.32
cis-9,12-Linoleic acid (C18:2n6)	30.76	30.61	30.55	30.77	30.50	30.60	30.66	30.48	30.32	30.49
Gamma-Linoleic acid (C18:3n6)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Alpha-Linoleic acid (C18:3n3)	1.26	1.23	1.23	1.26	1.25	1.23	1.24	1.23	1.22	1.25
cis-11,14-Eicosadienoic acid (C20:2)	0.03	0.03	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.03
Arachidonic acid (C20:4n6)	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
Polyunsaturated Fatty acid	32.08	31.92	31.85	32.10	31.83	31.90	31.97	31.78	31.61	31.81
Unsaturated Fatty acid	75.11	74.92	75.11	75.10	75.04	75.08	75.00	75.03	74.78	75.13
Trans fat	0.05	0.02	0.05	0.05	0.06	0.05	0.06	0.02	0.08	0.05
Omega 3	1.26	1.23	1.24	1.26	1.25	1.23	1.24	1.23	1.22	1.25
Omega 6	30.79	30.65	30.58	30.80	30.54	30.63	30.70	30.52	30.36	30.52
Omega 9	42.25	42.26	42.49	42.22	42.43	42.40	42.24	42.50	42.35	42.54

Note: ND = not detected

5. Tocols and oryzanol storage stability

According to Revilla et al. (2009)'s reported, the characterization of minor water-soluble enzymatic extract from rice bran (EERB) functional components were sterols, tocols (tocopherols and tocotrienols) and γ-oryzanol. The most characteristic component of the EEBR was γ-oryzanol. It found a major concentration in RBO (1260 ±50 mg/Kg), which is 3.4-fold that of the original raw material (RB). Oils from RB or palm were also rich sources of tocols and γ-oryzanol compounds (Xu et al., 2001). The possible regulatory role of tocotrienols in cholesterol dynamics includes hypocholesterolemic action (Qureshi et al., 2002). They found that the major components of vitamin E in the EEBR were δ-tocotrienol, γ-tocotrienol, α-tocopherol, and γ-tocopherol.

In this study, the γ-tocotrienol, α-tocopherol, and γ-oryzanol content in the NSRBO and SRBO stabilized by IR, IR-VC, IR-HA, and RF stabilized treatment methods shows in table 72. The highest contents of γ-tocotrienol, α-tocopherol, and γ-oryzanol at the initial storage period were found in the NSRBO, SRBO stabilized by the IR, and SRBO stabilized by the IR-VC; the values of which were, 1,279.73±38.56 mg/kg RBO, 479.43±15.82 mg/kg RBO and 8,079.17±86.09 mg/kg RBO, respectively.

In the initial storage periods, the γ-tocotrienol content in the SRBO obtained by RF method and IR-HA treated had a loss of NSRBO approximately 2 times. The IR-VC mostly maintained γ-tocotrienol. The γ-tocotrienol content in the SRBO obtained from IR, IR-VC, IR-HA, and RF methods were significantly decreased compared to the NSRBO. In clarifying the γ-tocotrienol loss, these were destroyed by all heat treatments, especially the RF and IR-HA stabilization treatments, which were strongly decreased. At the end of the storage period, the IR-VC significantly maintained γ-tocotrienol, while the RF stabilization treatment significantly lost γ-tocotrienol.

The α-tocopherol contents in the SRB obtained from IR, IR-VC, and IR-HA methods were significantly higher than the NSRBO and RF method. The α-tocopherol content in the RF method lost α-tocopherol content in the NSRBO. In contrast, Ling et al. (2018) reported that the total content of the tocopherols in the control increased after the HAARF treatment with the α-tocopherol and total contents of the tocopherol increasing from 30.12 to 48.06 mg/kg and 34.64 to 55.05 mg/kg,

respectively. This could be due to the excessive heating of the RF stabilization of the RBO that might decompose some of the heat-sensitive tocols and reduce the total content of the tocols. After 8 weeks of storage, the α-tocopherol content of the NSRBO and SRBO obtained from the IR, IR-VC, IR-HA, and RF methods samples decreased from 332.30 to 231.67mg/kg, 479.43 to 359.00mg/kg, 466.37 to 360.37mg/kg, 345.67 to 293.87mg/kg, and 310.57 to 281.40 mg/kg, respectively. This implied that the α-tocopherol in the NSRBO was significantly less stable than all the stabilization methods.

The IR and IR-VC stabilization methods during storage were significantly stable than the IR-HA, and RF stabilization methods, and the RF methods was less stable than all the stabilization method. Yilmaz et al. (2016) reported that the stabilization parameters; such as, IR power and process time (500-700 W and 3.0-7.0 minutes) did not significantly affect the oryzanol content of the processed bran samples ($p>0.05$). In the current study, the IR and IR-VC methods significantly increased the γ-oryzanol content up to approximately 18.92% and 21.16%, respectively, but the IR-HA and RF methods significantly decreased the γ-oryzanol content by approximately 7.92% and 11.65%, respectively.

Therefore, SRBO obtained from IR-HA and RF methods excessive heating could destroy the heat-sensitive γ-oryzanol and reduce the total γ-oryzanol content. After 8 weeks of storage, the γ-oryzanol content of the NSRBO and SRBO obtained from IR, IR-VC, IR-HA, and RF methods decreased from 6668.63 to 5863.63 mg/kg, 7930.23 to 5940.60 mg/kg, 8079.17 to 6920.40 mg/kg, 6140.80 to 5768.57 mg/kg, and 5891.90 to 5571.43 mg/kg, respectively. This implied that the γ-oryzanol content in the SRBO obtained from the IR-HA and RF methods on during storage was significantly less stable than the NSRBO, IR and IR-VC methods, respectively. The IR and IR-VC stabilization methods during storage were significantly more stable than the IR-HA, and RF stabilization methods. The SRBO obtained from RF stabilized method had tocols and oryzanol content less than other methods.

Table 72 Tocols and oryzanol content of NSRBO and SRBO obtained from IR, IR-VC, IR-HA and RF methods during storage

SRB sample method	γ-Tocotrienol (mg/kg)			α-Tocopherol (mg/kg)			γ-Oryzanol (mg/kg)		
	Week 0	Week 4	Week 8	Week 0	Week 4	Week 8	Week 0	Week 4	Week 8
NSRB	1279.73±38.56^{d}	1032.53±68.11^{b}	920.33±25.21^{c}	332.30±20.36^{ab}	247.30±3.05^{a}	231.67±3.10^{a}	6668.63±15315^{c}	6260.17±34.61^{b}	5863.63±146.27^{b}
IR	988.60±12.72^{b}	993.03±23.99^{b}	844.57±18.40^{b}	479.43±15.82^{c}	418.40±10.76^{c}	359.00±4.42^{c}	7930.23±149.87^{d}	6351.37±10.19^{b}	5940.60±92.46^{b}
IR-VC	1207.50±20.26^{c}	1167.87±20.60^{c}	1033.07±3.44^{d}	466.37±8.25^{c}	404.00±11.38^{c}	360.37±11.32^{c}	8079.17±86.09^{d}	7415.40±254.40^{c}	6920.40±221.96^{c}
IR-HA	639.77±18.49^{a}	619.93±17.61^{a}	612.63±6.71^{a}	345.67±6.22^{b}	307.87±4.17^{b}	293.87±8.26^{b}	6140.80±143.51^{b}	5953.30±94.32^{a}	5768.57±59.01^{ab}
RF	624.11±4.64^{a}	616.17±3.47^{a}	590.50±22.09^{a}	310.57±15.11^{a}	302.10±14.68^{b}	281.40±8.07^{b}	5891.90±127.55^{a}	5894.63±37.98^{a}	5571.43±73.46^{a}

Note: Values are expressed as means ± standard deviation. Values for the same stabilized rice bran fraction labeled with the same letters are not significantly different, but values labeled with different letters are significantly different (p<0.05)

6. Shelf life and FFA formation rate of NSRBO and SRBO obtained by IR, IR-VC, IR-HA and RF methods

The application of an accurate kinetic model to understand and predict the formation of the FFA could be useful to determine the rancidity rate of the RBO and feasibility of the full utilization of RBO (Wang et al., 2017). The shelf life of the NSRBO and SRBO stabilized by the IR, IR-VC, IR-HA, and RF samples stored at various temperatures of 25°C and 35°C was evaluated using the FFA value as an indicator (Table 73).

Linear regression (R^2) was created as a linear modeling approach to demonstrate the relationship between the FFA (%) and storage time (days) in the RBO samples. High R^2 values were observed in the NSRBO with storage at 25°C, and 45°C being 0.9382 and 0.9341. The SRBO obtained from IR, IR-VC, IR-HA and RF stabilization treatments stored at 25°C were 0.9604, 0.9154, 0.9763, and 0.9799, respectively, and at 35°C were 0.9500, 0.9341, 0.9683, and 0.9535, respectively. These showed the high ranges of the linear regression relationship between the FFA (%) and storage time (days) in the RB samples that enabled the storage time to be calculated with a high degree of accuracy. The actual shelf life values of the NSRBO at 25°C and 35°Cwere 2.39 days and 1.19 days, while the predicted shelf life of the SRBO from the IR, IR-VC, IR-HA, and RF stabilization treatment samples at 30°C were 16.8 days, 27.4 days, 13.4 days , and 39.2 days, respectively.

To further describe the effect of the IR, IR-VC, IR-HA, and RF stabilization methods on the inactivation of lipase in the RB and estimate the storage time, which the FFA concentration was maintained below 5%, the kinetics of the formation of the FFA were investigated with different storage temperatures (25°C and 35°C). The regression relationships of the kinetic data determined the kinetics of the formation of the FFA. A zero-order kinetic equation was used to predict the daily rate of the FFA increase in the RB. Table 73 shows that the initial FFA contents (A_0) of the NSRBO and SRBO as a result of the IR, IR-VC, IR-HA, and RF stabilization treatments were 3.89%, 2.82%, 2.54%, 3.38%, and 2.82% (as oleic acid), respectively. At 25°C and 35°C during storage, the daily rate of the FFA increase (k_s) in all the SRBO was lower than that in the NSRBO. Within the two storage temperatures, lower temperatures corresponded to lower k_s. The lowest k_s (0.033%) was found in the SRBO from the RF stabilization treatment at a storage temperature of 25°C.

Table 73 Shelf-life and FFA formation rate of NSRBO and SRBO obtained by IR, IR-VC, IR-HA and RF methods

Condition		Linear equation	R^2	Initial FFA content (A_0)	Final FFA content (A_S)	Actual shelf-life in days, %FFA ≤ 5%	FFA formation rate (k_s)	Predicted shelf-life in days, 30°C
Sample	Storage temperature (°C)							
NSRBO	25	Y= 0.8139x+4.7255	0.9382	3.89	5.00	2.39	0.464 %	1.70
	35	Y= 1.4035x+4.7617	0.9341	3.89	5.00	1.19	0.933 %	
SRBO-IR	25	Y= 0.5008x+3.2983	0.9604	2.82	5.00	23.78	0.092 %	16.8
	35	Y= 0.8395x+3.5458	0.9500	2.82	5.00	12.12	0.180 %	
SRBO-	25	Y= 0.3911x+2.7250	0.9154	2.54	5.00	40.72	0.060 %	27.4
IR-VC	35	Y= 0.6582x+3.2675	0.9341	2.54	5.00	18.42	0.134 %	
SRBO-	25	Y= 0.4955x+3.4580	0.9763	3.38	5.00	21.63	0.075 %	13.4
IR-HA	35	Y= 0.8924x+3.9367	0.9683	3.38	5.00	8.34	0.194 %	
SRBO-	25	Y= 0.2405x+2.7433	0.9799	2.82	5.00	65.68	0.033 %	39.2
RF	35	Y= 0.5735x+3.0792	0.9535	2.82	5.00	23.44	0.093 %	

Conclusion of Section 4

The effect of the stabilization of the RB by domestic heating on the mechanical extraction yield, quality and biochemical content of cold-pressed RBO was investigated.

1. The highest extraction oil yield (%) by the cold press was found in SRB from IR-VC treatment (8.87±0.38g/100g bran), followed by those obtained from IR (7.10±0.50 g/100g bran), IR-HA (6.97±0.38g/100g bran), RF (5.63±0.15g/100g bran), and NSRBO (5.37±0.38g/100g bran).

2. The NSRBO and SRBO obtained from IR, IR-VC, IR-HA and RF methods were differed in color. The heating methods could reduce lightness, while the RF method could reduce the most lightness of the RBO. The most lightness RBO sample was the NSRBO and the stabilization method was the IR-VC, followed by the IR, IR-HA, and RF method, respectively.

3. The rancidity of RBO storage stability, the AV of the NSRBO increased sharply beyond the acceptable limit within the 8 weeks of storage and achieved 30.96 mg KOH/g RB oil at the end of storage. The RF stabilization methods were the most effective for inhibiting rancidity in SRBO, which provided a low AV and PV on during storage.

4. The FA composition of NSRBO and SRBO obtained from IR, IR-VC, IR-HA stabilization methods did not differ in all FA compositions. Moreover, these did not change while in during storage.

5. The γ-tocotrienol content of NSRBO was higher than SRBO obtained from IR, IR-VC, IR-HA, and RF stabilization methods. The γ-tocotrienol content of the SRBO obtained from RF and IR-HA methods lost from the NSRBO approximately two times. In the during storage, the IR-VC method mostly maintained γ-tocotrienol, while the RF method also significantly lost γ-tocotrienol. The α-tocopherol content in SRBO obtained from IR, IR-VC, and IR-HA methods had a significant higher content than the NSRBO, but the stabilization treatment from the RF method had a lower content than the NSRBO. After 8 weeks of storage, the α-tocopherol content of the NSRBO and SRBO obtained from IR, IR-VC, IR-HA, and RF methods significantly decreased. The α-tocopherol content in the NSRBO was significantly less stable than all the stabilization methods, while the IR and IR-VC stabilization methods were

significantly more stable than IR-HA and RF methods. The RF stabilization method was stable than all the stabilization methods. The RBO obtained from IR and IR-VC stabilization methods significantly increased the γ-oryzanol content from the NSRBO, but the SRBO obtained from IR-HA and RF stabilization treatments significantly decreased the γ-oryzanol content from the NSRBO. After 8 weeks of during storage, the γ-oryzanol content in the SRBO obtained from IR-HA and RF stabilization methods was significantly stable than the NSRBO, but the IR and IR-VC stabilization methods were significantly more stable than the NSRBO, and the RF was less stable than all the stabilization treatments of the RBO. The IR-VC stabilization method could improve and maintain the tocols and oryzanol content in RBO.

6. The actual shelf life values of the NSRBO at 25°C and 35°C was 2.39 days and 1.19 days, while the predicted shelf life of the SRBO obtained from the IR, IR-VC, IR-HA, and RF stabilization methods at 30°C were 16.8 days, 27.4 days, 13.4 days, and 39.2 days, respectively. At the storage temperatures of 25°C and 35°C, the FFA formation rate (k_s) in SRBO was lower than that in the NSRBO. Within the 2 storage temperatures, lower temperature corresponded to lower k_s. The lowest k_s (0.033% as oleic acid/ day) was found in the SRBO as a result of the RF stabilization method at a storage temperature of 25°C.

In conclusion, the mostly effective stabilization of the RB by the IR-VC method could be applied to RBO extraction prior to pressing to improve the oil extraction yield, qualities, and useful biochemical such as tocols and oryzanol of cold-pressed RBO.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The optimization of RB stabilization conditions by IR, IR-VC, IR-HA and RF methods using RSM and CCD was studied with key factors, including FFA, MC, and a_w in SRB. It was found that the optimal condition of the IR treatment was 993 W IR wattage and 598 seconds of the treatment duration. Additionally, the optimal condition of the IR-VC was 997 W IR wattage, 598 seconds of treatment duration, and 610 mmHg of vacuum strength, while the 999 W IR wattage, 598 seconds of treatment duration and 72°C of hot air temperature were an optimal condition of the IR-HA method. The RF method at 130°C of the RF treatment temperature, 10.22 % of the RB moisture content, and 322 seconds of the treatment duration were optimal condition. The optimal conditions of the IR, IR-VC, IR-HA, and RF stabilization methods found the FFA contents of 3.59%, 0.33%, 4.22%, and 0.85%, respectively. The MC of 4.58%, 1.00%, 4.68%, and 1.10%, respectively were also found. In addition, these had an a_w of 0.432, 0.117, 0.286, and 0.164, respectively.

2. The effects of the IR, IR-VC, IR-HA and RF stabilization methods on the physico-chemical properties and the bioactive compounds of the RB during storage and shelf life were investigated. The qualities of the SRB in comparison with those of NSRB were investigated in terms of the FFA, MC, a_w, color, proximate analysis, FA composition, and bioactive compounds content. The stability of the RB samples was monitored and evaluated for 8 weeks.

2.1 The comparison of the occurring rate of the FFA content during storage found an increase in the sequencing from high to low as the NSRB, IR-HA, IR, IR-VC, and RF, respectively. This IR that the efficiency of the SRB treated with the FFA key factor of sequencing from high to low was RF, IR-VC, IR, and IR-HA, respectively.

2.2 All the SRB's MC and a_w stabilization methods were lower than the NSRB. The MC and a_w of the NSRB sample gradually decreased during storage, while

the MC and a_w of the SRB were found to increase. This could be a result of the moisture transfer phenomenon between the NSRB and SRB during storage even though it was stored in sealed PE bags. Once the equilibrium stage was achieved, the constant MC and a_w had similar stable contents in the NSRB and SRB samples.

2.3 The color results presented that the SRB from the IR-HA, IR, IR-VC, and RF stabilization methods were darker than the NSRB sample with regard to the lower lightness (L*) value. The a* and b* values of the SRB were higher than the NSRB. The ΔE value of the SRB was also found to be higher than that of the NSRB. This inferred that the efficiency of the SRB treated with the color key factor was IR-VC method.

2.4 The proximate and microbial analysis of the RB storage stability had an average percentage change in the proximate of the SRB, which was significantly increased as a result of the IR, IR-VC, IR-VC and RF stabilization methods compared to the NSRB. The noted IR stabilization treatment was observed for the protein content of the SRB that was significantly higher than the NSRB, but the fiber content was significantly lower than the NSRB. The IR-VC stabilization method noted for the observed fiber content of the SRB was significantly higher than the NSRB. Additionally, the fat in the SRB's RF stabilization treatment was higher than the NSRB.

2.5 The TPC, yeast and mold detected for the SRB samples for all the stabilized methods showed a decrease and stability against the microbial growth during storage.

2.6 The SRB samples possessed more saturated, monounsaturated, polyunsaturated, and total unsaturated FA content than the NSRB. The quantities of omega-3, omega-6, and omega-9 in the SRB samples were higher than those in the NSRB, and all the NSRB and SRB did not change during storage. The results indicated that the effects of the IR, IR-VC, IR-HA, and RF stabilized methods on the RB could insignificantly change the FA composition.

2.7 The increased γ-tocotrienol contents were observed in the SRB by the IR, IR-VC, IR-HA, and RF stabilized methods compared to the NSRB. The α-tocopherol content in the NSRB had a non-significant change that was observed in the SRB by the IR and RF treatments, while up to 8.7% in the SRB was a result of the IR-

VC treatment, but there was a loss of 4.53% in the SRB from the IR-HA method. This inferred that the IR-VC method had the efficiency to maintain α-tocopherol, while IR-HA could not maintain α-tocopherol. Up to approximately 8.90%, 22.1%, 14.6%, and 4.25%, respectively of γ-oryzanol were observed in the SRB by the IR, IR-VC, IR-HA, and RF methods, respectively. In terms of storage duration, the NSRB lost more γ-tocotrienol, α-tocopherol, and γ-oryzanol content than the SRB during storage. Observation of the RB's IR-VC stabilization methods showed preserved tocotrienol, tocopherol, and oryzanol.

2.8 The shelf life of IR, IR-VC, IR-HA, and RF stabilization methods compared to NSRB were improved for 2.77, 3.73, 2.40, and 5.57 times, respectively. It was 44.7, 129.7, 38.6 and 89.6 days, respectively. This IR-VC and RF stabilization methods had a long shelf life, it recommend to using for stabilization rice bran.

3. The crude oil yield, qualities and stability of crude RBO from the experiment were found as follows,

3.1 The highest extraction oil yield (%) by the cold press was found in SRB from IR-VC treatment (8.87±0.38g/100g bran), followed by those obtained from IR (7.10±0.50 g/100g bran), IR-HA (6.97±0.38g/100g bran), RF (5.63±0.15g/100g bran), and NSRBO (5.37±0.38g/100g bran).

3.2 The RBO differed in its appearance with the most lightness being the NSRBO, it followed by SRBO obtained from IR-VC, followed by IR, IR-HA, and RF stabilization methods, respectively.

3.3 The AV of the NSRBO increased sharply beyond the acceptable limit within the 8 weeks of storage and reached 30.96 mg KOH/ g RBO at the end of storage. The SRBO obtained from RF methods were the most effective for the inhibition of rancidity, which provided low AV (14.86 mg KOH/ g RBO) and PV (8.65 meq.O_2/kg RBO) in the 8 weeks of during storage.

3.4 The FA composition of the NSRBO samples and SRBO obtained by the IR, IR-VC, IR-HA, and RF methods did not differ in FA compositions. Moreover, these did not change while in during storage.

3.5 The γ-tocotrienol content in the NSRBO was higher than SRBO, which the γ-tocotrienol content in the SRBO obtained RF and IR-HA methods had a loss from NSRBO approximately 2 times. At 8 weeks of during storage, the IR-VC

method mostly maintained γ-tocotrienol in SRBO, while the RF method also significantly lost γ-tocotrienol. The α-tocopherol content in SRBO obtained from IR, IR-VC, and IR-HA methods had a significantly higher content than the NSRBO, while the RF method had a lower γ-tocotrienol content than the NSRBO. After 8 weeks during storage, the α-tocopherol content of the NSRBO and SRBO obtained from IR, IR-VC, IR-HA, and RF methods were significantly decreased. The α-tocopherol content in SRBO by RF stabilization method was mostly decreased during storage, while SRBO obtained from IR-VC method was mostly maintained the α-tocopherol content in RBO. The IR and IR-VC stabilization methods were mostly maintained γ-oryzanol content in SRBO, while the IR-HA and RF stabilization methods did not maintained γ-oryzanol content in SRBO.

3.6 The actual shelf life values of the NSRBO at 25°C and 35°C were 2.39 days and 1.19 days, while the predicted shelf life of the SRBO obtained from IR, IR-VC, IR-HA, and RF methods samples at 30°C were 16.8 days, 27.4 days, 13.4 days, and 39.2 days, respectively. The improved shelf life compare with NSRBO by SRBO obtained from the IR, IR-VC, IR-HA, and RF treatments was improved for 9.88, 16.1, 7.88 and 23.0 times, respectively. At the storage temperatures of 25°C and 35°C, the k_s in SRBO, which was lower than that of the NSRBO. Within the 2 storage temperatures, the lower temperatures corresponded to lower k_s. The lowest k_s (0.033%) was found in the SRBO obtained from RF method at 25°C. In conclusion, the most effective stabilization of the RB by the IR-VC stabilization method could be applied to the RBO extraction prior to pressing to improve the oil extraction yield, quality, and maintain biochemicals; such as, tocols and oryzanol of cold-pressed RBO.

Recommendations

1. The composition, qualities, and regularity of the RB raw material were important factors that could influence the a_w, MC, and FFA content of RB.

2. Fresh rice bran should be stabilized as soon as possible after milling process since the rancidity cause by lipase occurs rapidly.

3. The initial FFA of the fresh should not be over 2% in order to obtain stabilized rice bran with less than 5% FFA, which would be suitable for human consumption and useful for RBO production.

4. The final MC of the SRB sample should be appropriate for conducting the cold-press extraction of the RBO to avoid high heat during extraction process. The high temperature of the cold-press extraction may alter the color, quality, and composition of the RBO.

5. RBO obtained by cold-press extraction should have the acid value (AV) lower than 4.0 mg KOH/g oil according to CODEX standard (Codex Standards for Fats and Oils from Vegetable Sources).

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APPENDIX

APPENDIX A ANALYSIS METHODS

1. The FFA content

The FFA content of each sample is determined using a standard titration method (Kim, et al., 2014, AOCS., 2004). Each rice bran sample (2 g) collected as described is dissolved into 100 mL of a mixture of ethanol and diethyl ether (1:1, v/v) and titrated with a KOH/ethanol solution (0.01 N). The FFA content is calculated as oleic acid equivalent.

2. Fatty acid profile

The Fatty Acid Composition of the sample is determined by GC-FID using a Supelco (USA) 100m × 0.25mm × 0.2μm column. The temperature of injection is 250°C, and the detector is 250°C. The oven temperature is initially 140°C for 5 min, and then increased 3°C/min to 250°C, followed by a 17 min hold. The He gas flow rate is 1.1mL/min. The flow rates of the FID's H_2, air, and make up gas are 40 ml/min, 450 ml/min, and 45 ml/min, respectively. The split ratio is 100:1 and the sample injection volume is 1 μL (AOAC, 2012).

3. Tocols and oryzanol

The α-Tocopheral, γ-Tocotrienol (Tocols) and γ-Oryzanol content of sample are determined using the methods of Pestana-Bauer, V.R. *et al.* (2012) with some modifications. The HPLC system (Alliance e2695, Waters, UK) consisted of a Column Heater (M15SMH049G, Waters, UK), Detector 2998 (M15998529A, Waters, UK) and Detector 2475 (K15475783G, Waters, UK). A Luna 5 μ PEP (2) 100A column (250 mm,4.6 mm) (Phenomenex, USA) is also used. Sample portions of 250 mg are weighed out and dissolved into 5 ml of isopropanol. After centrifugation at 9000 rpm (7.245g) for 6 min (Universal 32R, Hettich Zentrifugen, Germany), the supernatant is transferred to a 1.5 ml vial. Aliquots of 10 μl are injected into the HPLC. Separations are performed at 25°C with a flow-rate of 1 ml/min. The UV-Vis spectrophotometer detector used for analysis is set at 298 nm. The mobile phases are 50:40:10 (A) and 30:65:5 (B) acetonitrile–methanol–isopropanol mixtures (v/v/v). Separation of α-Tocopheral, γ-Tocotrienol and γ-Oryzanol, is carried out by isocratic elution with phase A for 5 min, followed by a 10 min linear gradient from phase A to 100% phase B, followed by a final 5 min isocratic elution with phase B (adapted from

Chen, & Bergman, 2005). The data is acquired and processed using Waters Empower 3 (build 3471) software. Standard solutions of α-Tocopheral, γ-Tocotrienol, and γ-Oryzanol are used to construct the calibration curves.

4. Acid value

Weigh, to the nearest mg, 5 g RBO well-mixed sample into 250 ml Erlenmeyer. Add 50 ml alcohol-ether mixture (Equal volumes alcohol and ether) and 0.1 mL phenolphthalein solution (1% in alcohol or alcohol denatured with methanol). Titrate with 0.1 alcoholic KOH until permanent faint pink appears and persists for ≥10s.

Acid value = ml alcoholic KOH solution x normality alcoholic KOH solution x 56.1 / g RB oil. Difference between duplicate determination should be ≤ 0.1 KOH/ g RB oil.

5. Peroxide value

The RBO samples has to be protected from the air, stored in a cool place and should not be opened before the onset of the determination. Other tests have to be carried out afterwards. Solid fats may not be melted before the determination. - Transfer approx. 3 g of the sample, accurately weighed, taken from the center of the sample (attention must be paid to the fact that no sample is taken from the surface) into a 250 mL Erlenmeyer flask closed immediately with glass stopper. - Add 50 mL of the appropriate solvent mixture (Solvent mixture might be prepared by measuring volume of glacial acetic acid and: chloroform: 3:2) - Add 1 mL of saturated potassium iodide solution, freshly prepared, and allow reacting for 60 seconds ± 1 second while agitating manually but vigorously the solution at least twice. - Add 100mL of water and shake. - Titrate with the appropriate sodium thiosulfate solution (0.01 or 0.1 mol/L, part 3.2.3), using 1 mL starch solution or 0.1 g of Thyodene indicator from a purple to a slight yellow or colourless endpoint The indicator should be added towards the end of the titration but while the pale straw colour is still present. During titration shake until the blue colour disappears. Carry out a blank titration under the same conditions. No more than 0.5 mL of sodium thiosulfate solution should be consumed for this purpose. If this volume is exceeded, it is necessary to re-examine the quality of the reagents (AOCS, 2000)

APPENDIX B FIGTURE

Figure 32 RB raw material

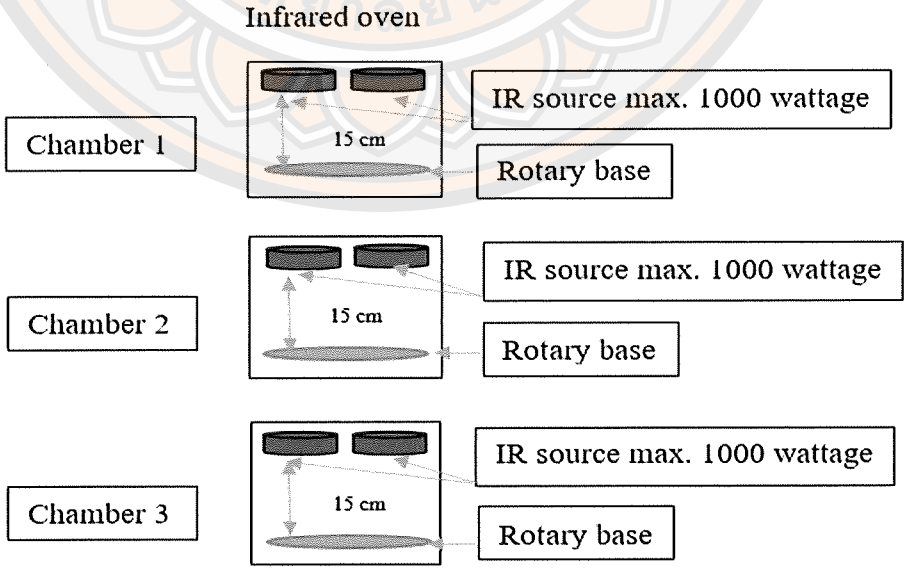

Figure 33 Schematic diagram of IR stabilization process of RB

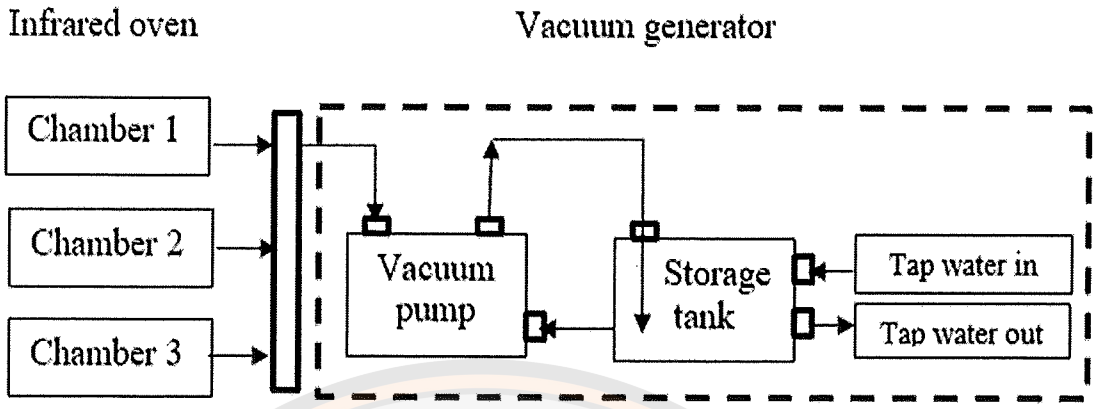

Figure 34 Schematic diagram of IR-VC stabilization process of RB

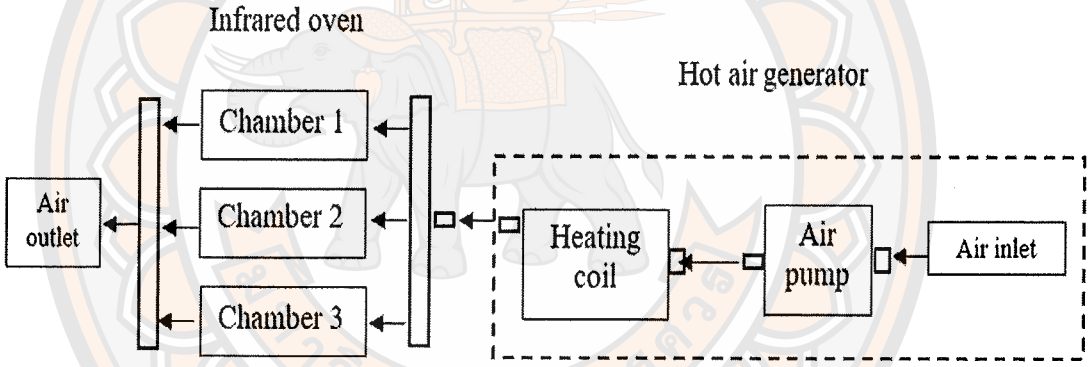

Figure 35 Schematic diagram of IR-HA stabilization process of RB

Figure 36 The IR, IR-VC and IR-HA for stabilization process of RB

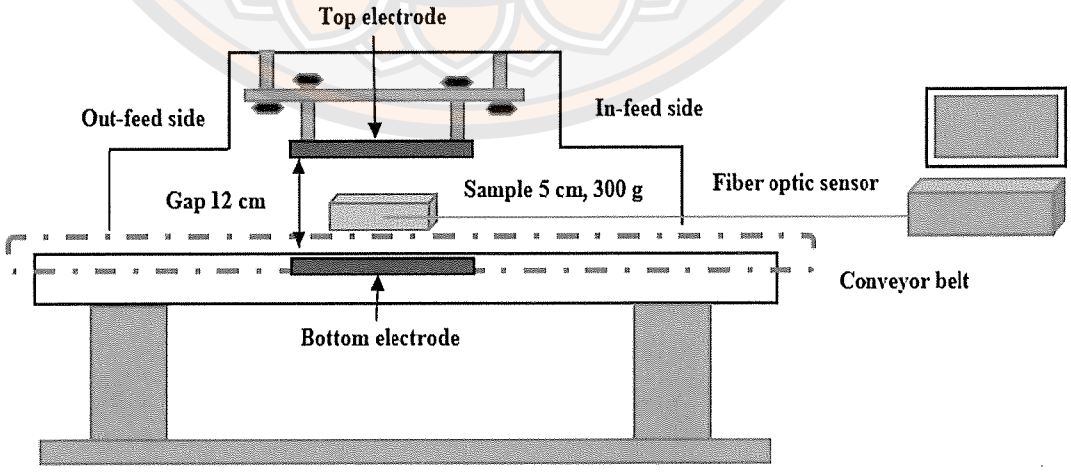

Figure 37 Schematic diagram of RF stabilization process of RB

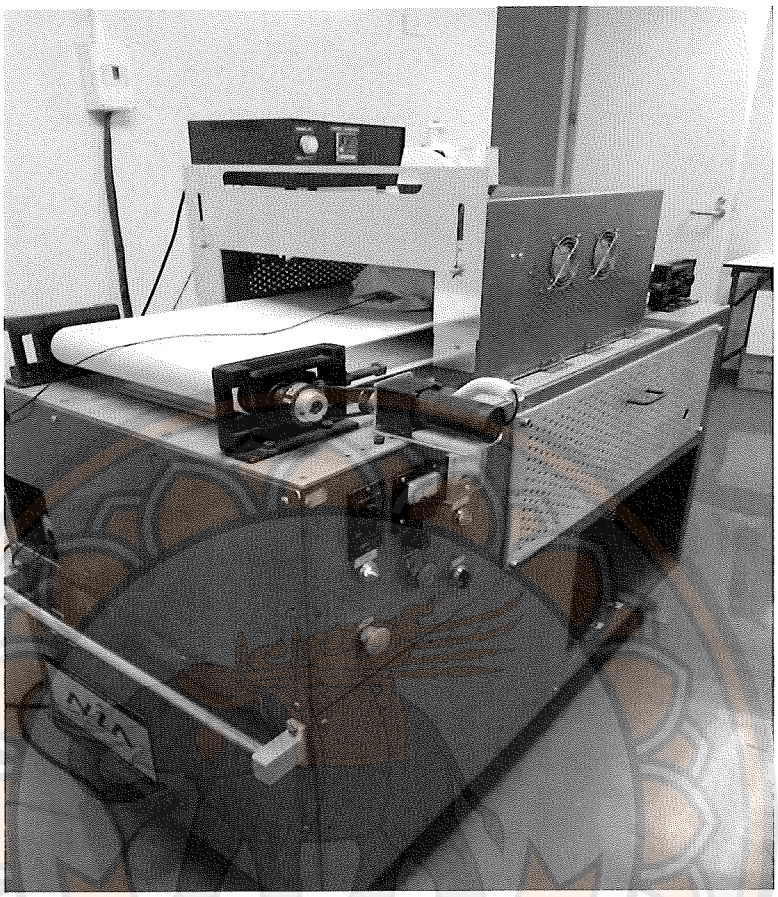

Figure 38 The radio frequency for stabilization process of RB

Figure 39 The incubators for during storage of RB sample and crude RBO sample

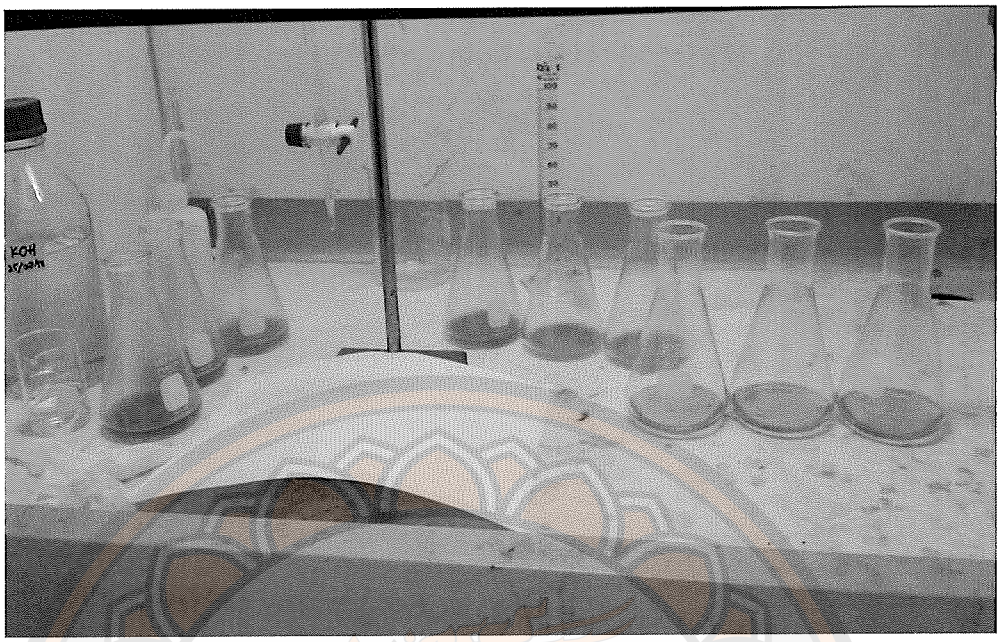

Figure 40 FFA analysis

Figure 41 RB color measurements

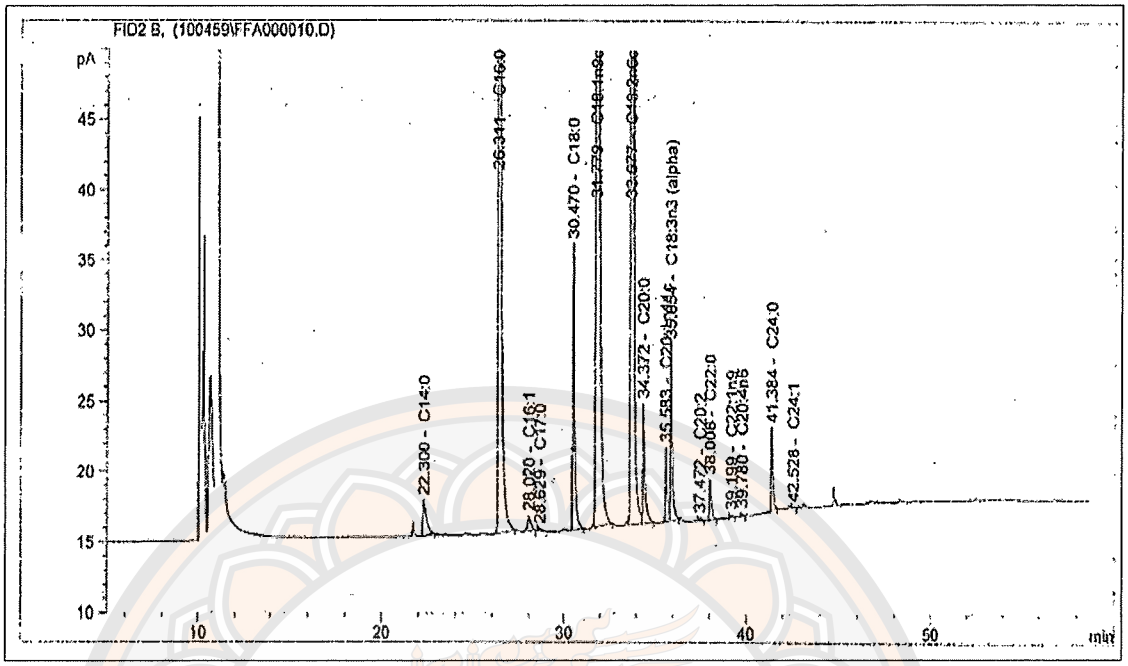

Figure 42 FA composition chromatogram using GC-MS

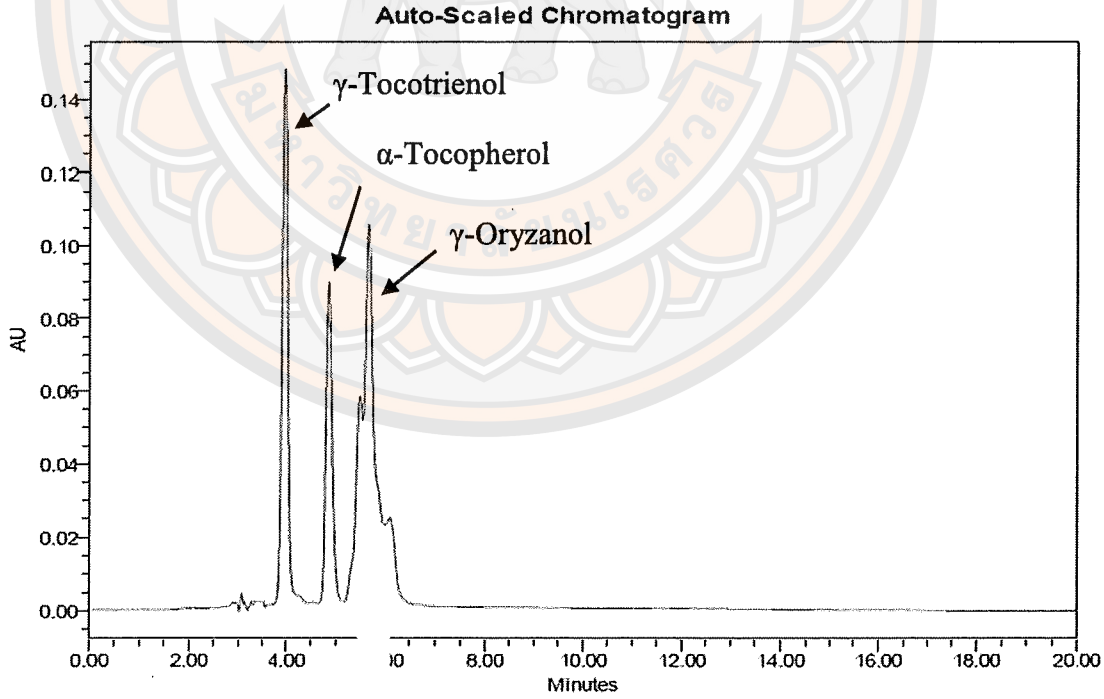

Figure 43 γ-Tocotrienol, α-tocopherol and γ-oryzanol chromatogram using HPLC

Figure 44 Cold-press of RB extraction

Figure 45 Triplicate of extraction of RBO yield determination

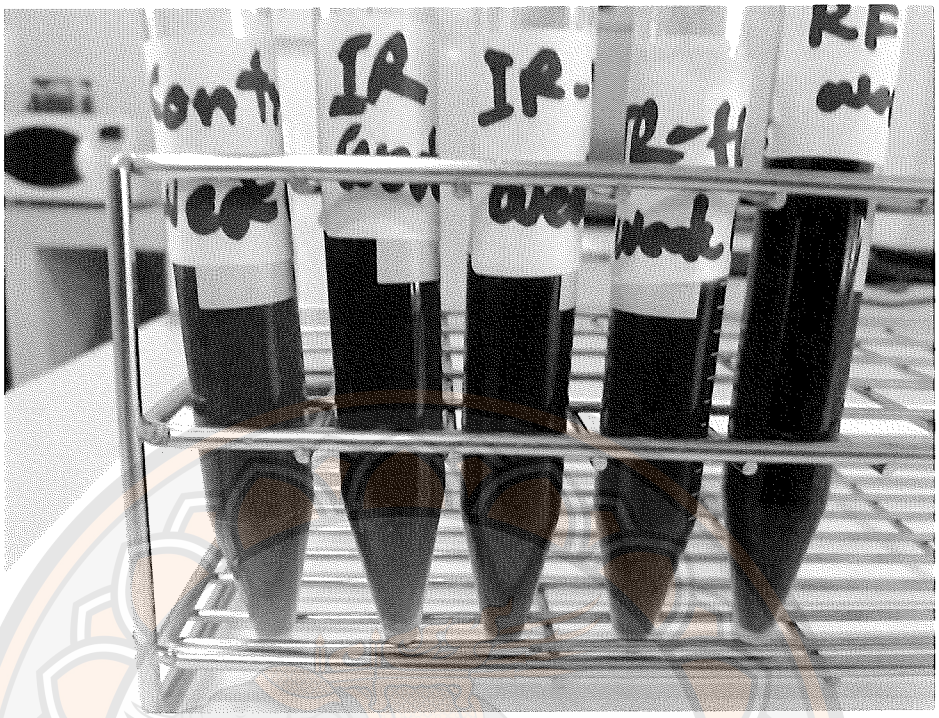

Figure 46 Crude NSRBO and SRBO samples obtained from IR, IR-VC, IR-HA and RF methods