CHAPTER 3

Application of radio frequency heat treatment for value-added in purple rice bran

Abstract

A split-split plot in complete randomized design (CRD) with 3 replications was designed in this experiment. The main plot was four cultivars of Thai rice bran which were 2 white rice varieties (SPT1; KDML105) and 2 landrace purple rice (KDSK and KN), sub-plot was temperature of radio-frequency (RF) at 3 levels, and sub-sub-plot was 4 storage periods. The rice bran samples were exposed to RF at frequency of 27.12 MHz at temperatures of 70, 75 and 80ºC for 3 minutes. Then, the treated bran was packed in aluminum foil bag and vacuum sealed at a pressure of 80 kPa and stored at 25°C for 0, 2, 4 and 6 months. In each storage time, the bran was tested for contamination of microorganisms, moisture contents (mc), oil and protein contents and assessment of rancidity by thiobarbituric acid value (TBA) as well as anthocyanin content (in form of cyaniding-3-glucocide: C3G) in purple rice bran was also determined. The results showed that RF heating technique significantly decreased ($p \leq 0.05$) the bran moisture content more than 1 %, however there was no significant difference between all treating temperatures. After 2 months storage, the mc of bran increased significantly due to moisture equilibration in their airtight containers. The RF at temperatures of 70 and 75ºC reduced microbial contamination significantly. Storage for 2-6 months significantly resulted in increasing number of oil content. The interactions between temperature and cultivar also affected oil content. Rice bran treated at 75 and 80ºC showed higher oil content than untreated, especially in *cv.* SPT1, KDML105 and KN. The same happened in protein content. Storage of 2-6 months resulted in TBA value which tended to increase during the first 4 months and decreased in sixth month. The methods also provided higher C3G content extracted from purple

rice bran compared to untreated samples.

3.1 Introduction

Rice bran provides a potential source of human food, especially landrace purple containing high amounts of natural antioxidants and increase immune system so called anthocyanin especially cyanidine 3-glucoside as well as rice bran oil containing gamma oryzanol (Xu *et al*., 2001). Presently, purple rice and their bran have been promoted to be food as medicine or nutraceutical. However, the crude bran is fast to deteriorate during storage because the oil composition is degradation by lypolysis and oxidative rancidity as well as the microorganisms contamination was also detected to be a factor affecting deterioration. Various thermal methods have been made to stabilize rice bran (Prakash, 1996; Lakkakula *et al*., 2004). The application of microwave and RF dielectric heating to agricultural products after post-harvest management is a new technology and has attracted great interest following many researches (Wang *et al*., 2003). In this technology, the radiation energy is dissipated within the sample afterward a great rate of heating can be obtained rapidly. von Hoersten and Luecke (2001) compared five thermal processes for eradicating *Fusarium culmorum* from wheat seed. Hot-air treatment (65–68°C) of seed wrapped in a moisture barrier reduced treatment time less than 2 hr. with no loss moisture content. Microwave-steam treatment at temperatures of 68-75°C was equally effective in less than 10 min. This study was to investigate the effect of radio frequency heat treatment on disinfection and stabilization to extend of their storage time and shelf life of landrace Thai rice bran without adverse effect on the nutrients**.**

3.2 Materials and methods

3.2.1 Rice bran sample preparation

The landrace purple rice (*Oryza sativa* L.) 2 cultivars (Kum Doi Saket and Kum Nan) and 2 white rice cultivars(Khao Dawk Mali 105 and San-pah-tawng 1) were grown to expand seed in experimental fields of Department of Plant Sciences and Natural Resources. After paddy rice harvested, the rice rough rice were cleaned and milled until getting the end product as bran which was used in the experiment. Before exposure rice to radio frequency application, the initial moisture content of bran samples were measured according to ISTA (2001)**.**

3.2.2 Radiofrequency heat treatment

The split-split plot in CRD with 4 replications was designed in the experiment. The main plot was four rice cultivars, sub-plot was three-level temperatures of the radio-frequency (RF) and sub-sub-plot was storage time for 6months. The bran samples will be exposed through RF at wavelength of 27.12 MHz (Figure 3.1) on 3 temperatures of 70, 75 and 80°C for 3 minutes to find the right temperature for decrease the moisture and no adverse effect on the nutritional quality of rice bran.

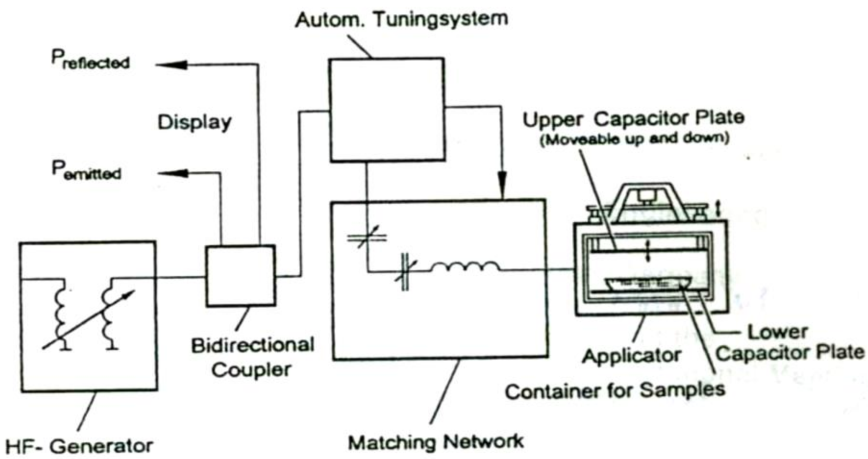


Figure 3.1 Schematic chart of radio frequency instrument

3.2.3 Package and storage of treated rice bran

Each treated rice bran sample 70 g was packed in aluminum foil bag and sealed with sealer bag vacuum (Vacuum sealer with gas modifier; Brand Neo Pak Model NV-002HG) at pressure 80 kPa, then stored at 25°C. In each 2 month, the bran was sampling until 6 months. The samples were determined for contamination of fungi by Total plate count (AOAC, 2005).

3.2.4 Determination of bran sample

1) Moisture content (AOAC, 2005)
2) Protein content (AOAC, 2005)
3) Oil content (AOAC, 2005)
4) Thiobarbituric acid number (TBA number) (AOAC, 2005)

5) Cyanidin-3-glucoside and Peonedin-3-glucoside (Applied from Ryu *et al.*, 1998)

3.2.5 Data analysis

The experiment was arranged in split-split plot in CRD with 4 replications. Data were subject to Statistix 8 (SX 8) analytical software. Mean of treatment effects on rice bran qualities was separated and compared by least significant difference (LSD) at $P \leq 0.05$.

3.3 Result and Discussion

3.3.1 Quality of rice bran after radio frequency heat treatments

The results showed that RF heating technique significantly decreased ($p \leq 0.05$) the moisture content more than 1% (Table 3.1), but there was no significant difference between treating temperatures. The interactions between temperature and cultivar also result oil content. Rice bran treated at 75 and 80ºC showed higher oil content than untreated especially in cv. San pa-tawng 1 (SPT1), Khao Dawk Mali 105 (KDML105) and Kam Nan (KN). The same evident also happened in protein content, but the storage time had no effect in protein.

When the bran sample containing moisture content as 9.21-10.45% was subjected to magnetic field such RF application, the water molecule was rotate as movement back and forth rapidly as the frequency of the electromagnetic wave. In the case of movement in radio-frequency condition, ion motion takes approximately 3 to 300 million times per second, of which the speed of rotation action result on friction and cause to heat up rapidly within 2-3 seconds or about 1 minute after receiving electromagnetic waves. Subsequently, the heat has spread to other cool parts heating migration continuously occurred. Then, the bran’s moisture content significantly decreased which cv. KDML105 decreased for 2.55 to 3.29% after treating under 75ºC while cv. Kam Nan decreased for 0.94, 1.16, 1.66% follow by increasing temperatures at 70, 75 and 80º C, respectively (Table 3.1).However, the interactions between temperature and cultivar significantly affected rancidity of rice bran by the RF heat treatment at temperatures of 75 and 80º C showed the lowest average of TBA value in cv. San-pah-tawng 1.

Table 3.1 Quality of rice bran 4 cultivars before and after treating with various RF treatments

Rice bran variety	RF temp (°C)	mc (%)	Oil content (%)	Protein content (%)	TBA number (mg-malonaldehyde kg^{-1})
SPT1	Control	9.21	28.26	12.54	21.88
	70	8.10	27.51	12.87	20.21
	75	8.21	29.21	12.63	22.93
	80	8.49	26.51	12.83	21.22
KDML105	Control	10.20	26.81	12.00	23.24
	70	7.65	25.51	12.00	20.01
	75	6.91	26.52	12.33	21.84
	80	7.29	27.03	12.41	21.50
KDSK	Control	10.45	9.54	11.31	24.13
	70	8.82	10.33	11.07	19.87
	75	8.99	9.66	10.72	10.19
	80	8.89	9.73	11.23	11.08
KN	Control	9.46	18.47	14.30	23.69
	70	8.52	19.57	14.86	11.63
	75	8.3	19.80	14.43	12.04
	80	7.80	20.11	15.23	14.75
$LSD_{0.05}$variety (V)		0.31	0.22	0.22	3.14
$LSD_{0.05}$ Temperature (T)		0.18	ns	ns	2.58
$LSD_{0.05}$ V x T		0.36	ns	ns	5.16

ns = not significant; mc=moisture content

3.3.2 Effect of radio frequency on control pathogen contaminated in rice bran and after storage

Before and after radio frequency heat treatment at several temperatures, the rice bran sample was determined for total viable plate count and calculated in unit of colony forming unit (x10^4 CFU/ml**)** as shown in Table 3.2. Before exposure to RF, differences of microbial contamination among rice bran cultivars were highly significant. Champagne *et al*. (1992) detailed when rice bran layers were removed from the endosperm during the milling process, the individual cells was damaged and the rice bran lipids disperse into contact with highly reactive lipases. Not only these enzymes containing in the bran but also origin from microorganisms of which initiate hydrolytic deterioration of kernel oil. After exposure to RF application immediately or initial time of storage (month 0), the result found that treated temperature showed significantly effect on control pathogens contamination. The RF at temperatures of 75ºC reduced microbial contamination significantly in rice bran cv. SPT1 and KDML 105 and KN (Figure 3.4, 3.5 and 3.7 resp.) while the total viable plate count number of cv. KSDK was unchanged (Figure 3.6). The results parallel with the report of Ikediala *et al*. (2000) who used to expose food on radio frequency heat treatment at high temperature. In addition Janhang *et al* (2005) also reported that radio frequency at temperature of 75ºC for 3 minutes decreased the percent infection of *Trichoconis padwickii* to 18% fungi. Vassanacharoen *et al*. (2004) also expressed that temperature of 70ºC for 3 minutes reduced percent infection of *Macrophomina phaseolina* in sesame seed.

The temperature showed no significant difference on decreased of the total viable plate count, the average of three temperatures were calculated and expressed in Figure 3.3. After storage for 2 month the disease contamination of all rice bran cultivars were increased. The relationship between fungi contamination and moisture content (Figure 3.8) is according to the study of Abdullah *et al.* (2000) who noted that moisture content and temperature are critical factors providing for fungi growth and production of mycotoxins. Fungi present in rice bran samples were identified as belonging to the genera *Aspergillum, Penicillium* and *Fusarium*. These genera are able to produce mycotoxins, especially aflatoxin, pathogenic to humans and animals; however, in this study, tests to verify the presence of that toxin were not investigated preventing any assertion concerning toxin. After that both of cv. SPT1 and KDSK tended to decrease until 6 month of storage while cv. KN slightly decreased in 4 month, then dropped down till 6 month storage. Whereas the diseases contamination of rice bran cv. KDML105 increased with storage for 2-4 month, after that decreased to 15 (x10^4 CFU/ml**).**

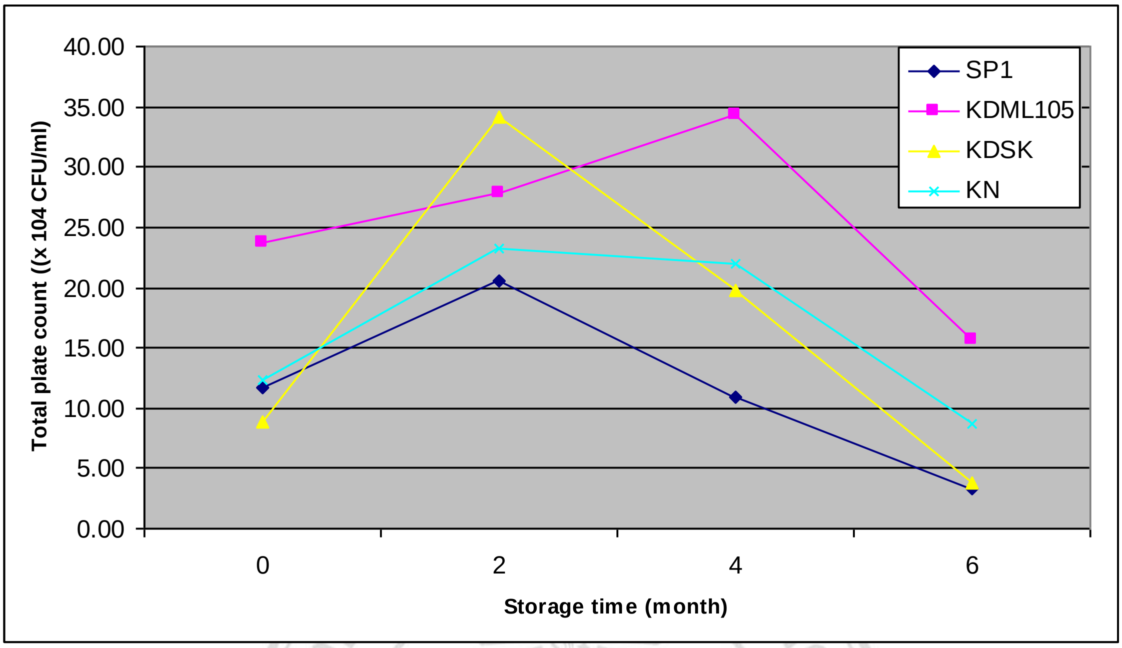


Figure 3.2 Effect of storage time on average of total viable plate count of each 4 treated rice bran cultivars

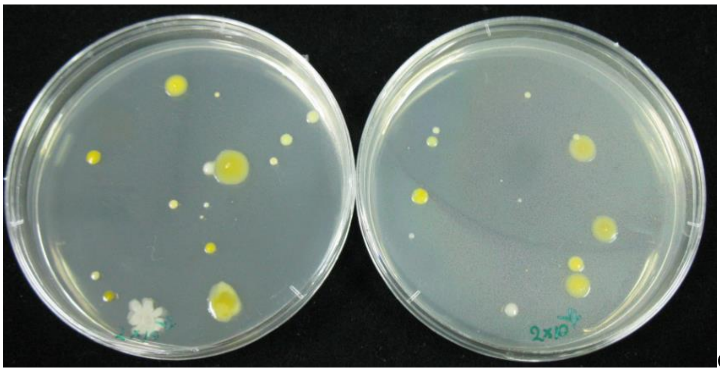
Table 3.2 Total viable plate count of RF treated rice bran after storage for 6 months

Rice bran cultivar	RF temperature (ºC)	Storage time (month))			
		0	**2**	**4**	**6**
		total viable plate count (x 10^4 CFU/ml)			
SPT1	control	18.5	18.5	12.5	1.5
	70	12.5	17.0	9.5	3.0
	75	5.0	21.5	10.5	4.0
	80	11.0	25.5	11.0	4.5
KDML 105	control	41.5	9.0	10.0	2.0
	70	20.5	33.5	37.5	19.0
	75	13.0	30.0	41.5	26.5
	80	20.0	38.5	48.0	15.0
KDSK	control	5.5	92.5	19.5	3.0
	70	14.5	8.5	11.0	3.0
	75	8.0	19.0	37.5	4.0
	80	7.5	16.5	11.0	5.0
KN	control	19.5	31.5	18.0	6.0
	70	15.5	21.5	38.5	11.5
	75	3.5	17.0	15.0	7.0
	80	11.0	23.0	16.5	10.0
$LSD_{0.05}$cultivar(V)			5.7		
$LSD_{0.05}$temperature (T)			ns		
$LSD_{0.05}$ T x V			8.3		
$LSD_{0.05}$storage time(M)			3.6		
$LSD_{0.05}$ V x M			7.14		
$LSD_{0.05}$ T x M			7.14		
$LSD_{0.05}$ T x V x M			14.3		

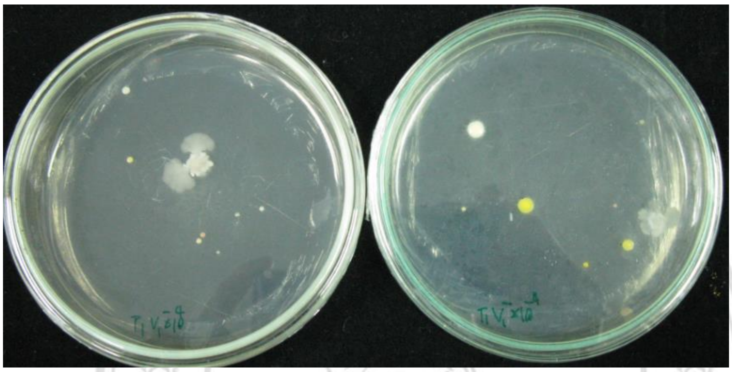
ns = not significant

SPT1=San-pah-tawng 1; KDML105= Kaw Dawk Mali 105

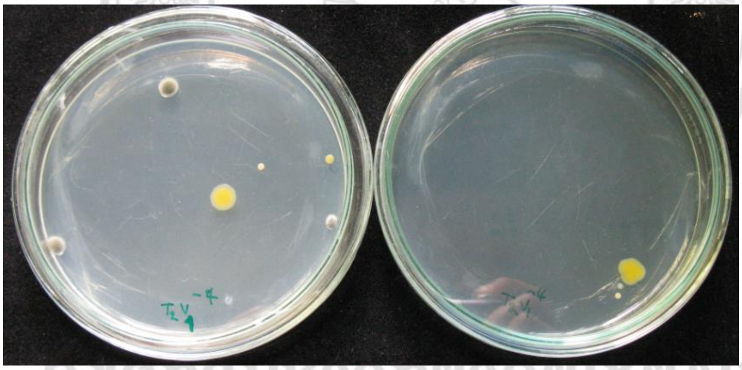
KDSK= Kam Doi Saket; KN=Kam Nan



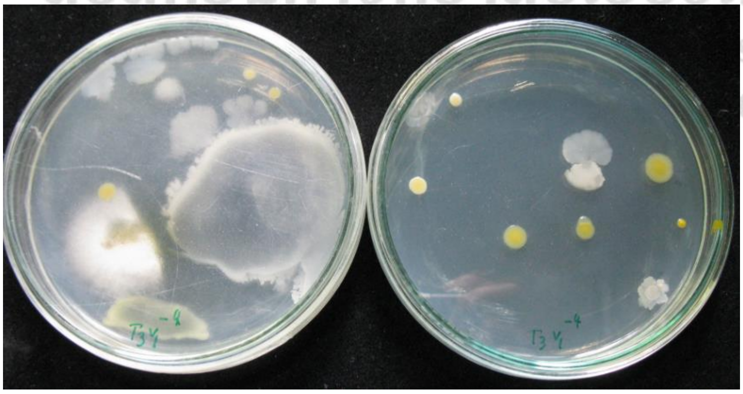
control



70ºC

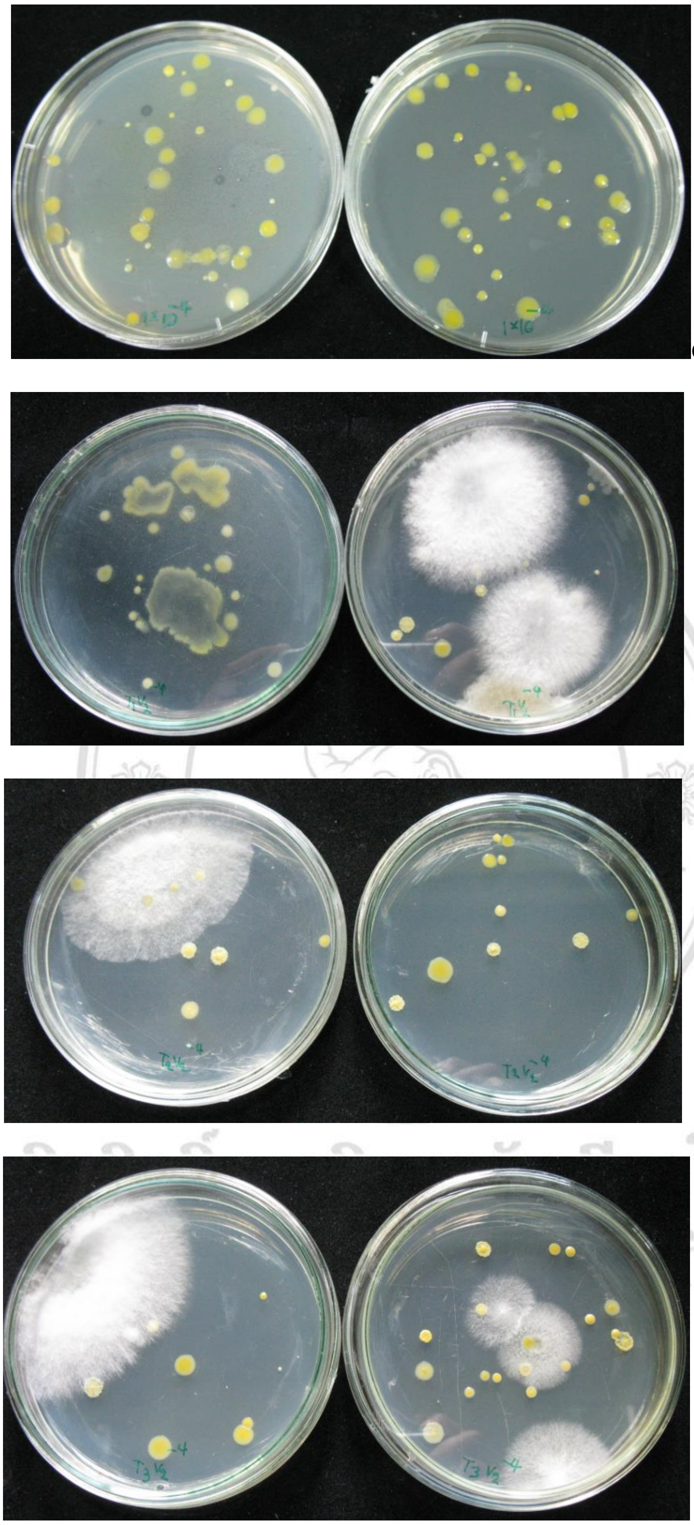


75 ºC



80 ºC

Figure 3.3 Contamination of pathogens by total viable plate count (conc. 10^{-4}) of rice bran cv. San-pah-tawng 1 before and after radio frequency at various temperatures



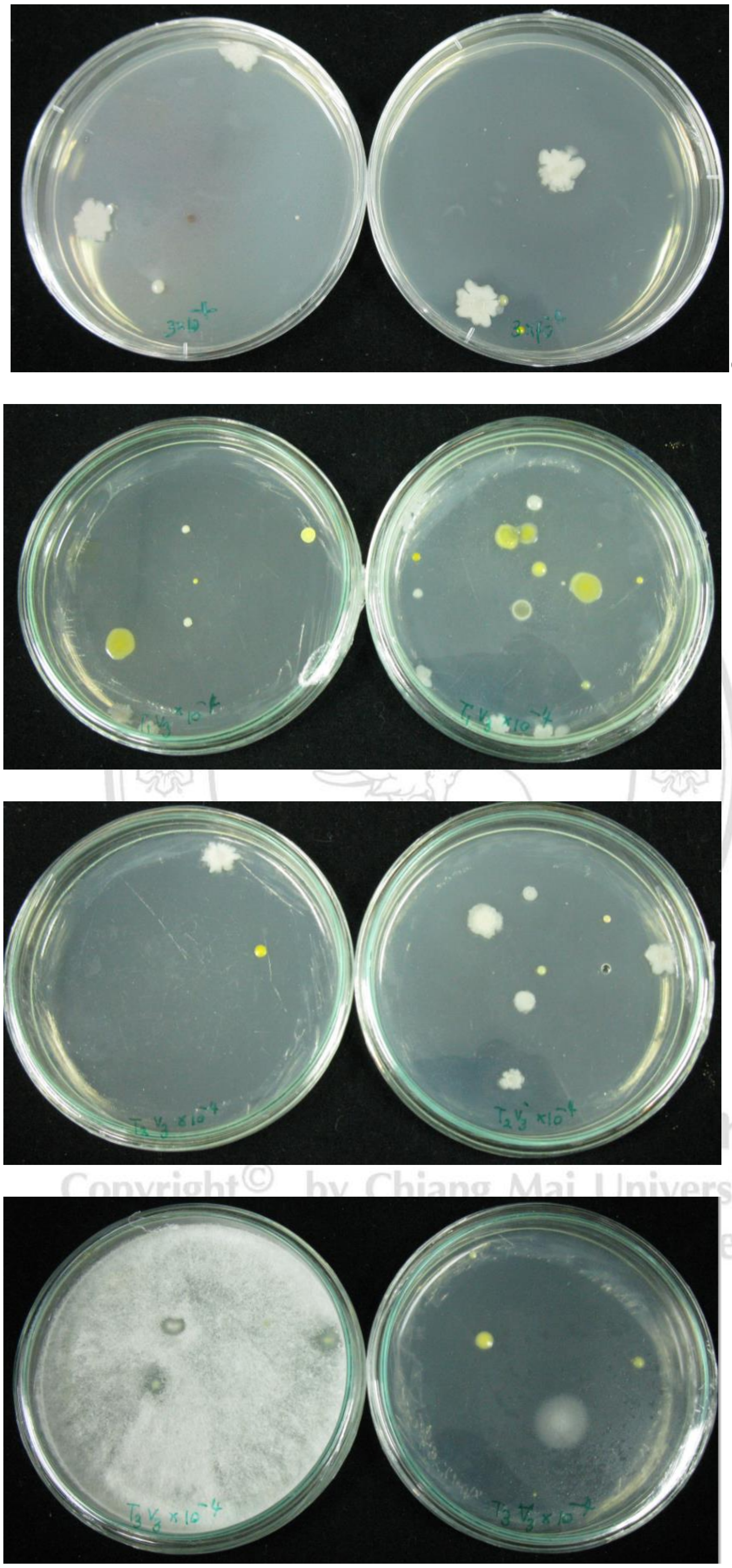
control

70 ºC

75 ºC

80 ºC

Figure 3.4 Contamination of pathogens by total viable plate count (conc. 10^{-4}) of rice bran cv. Kaw Dok Mali 105 before and after radio frequency at various temperatures



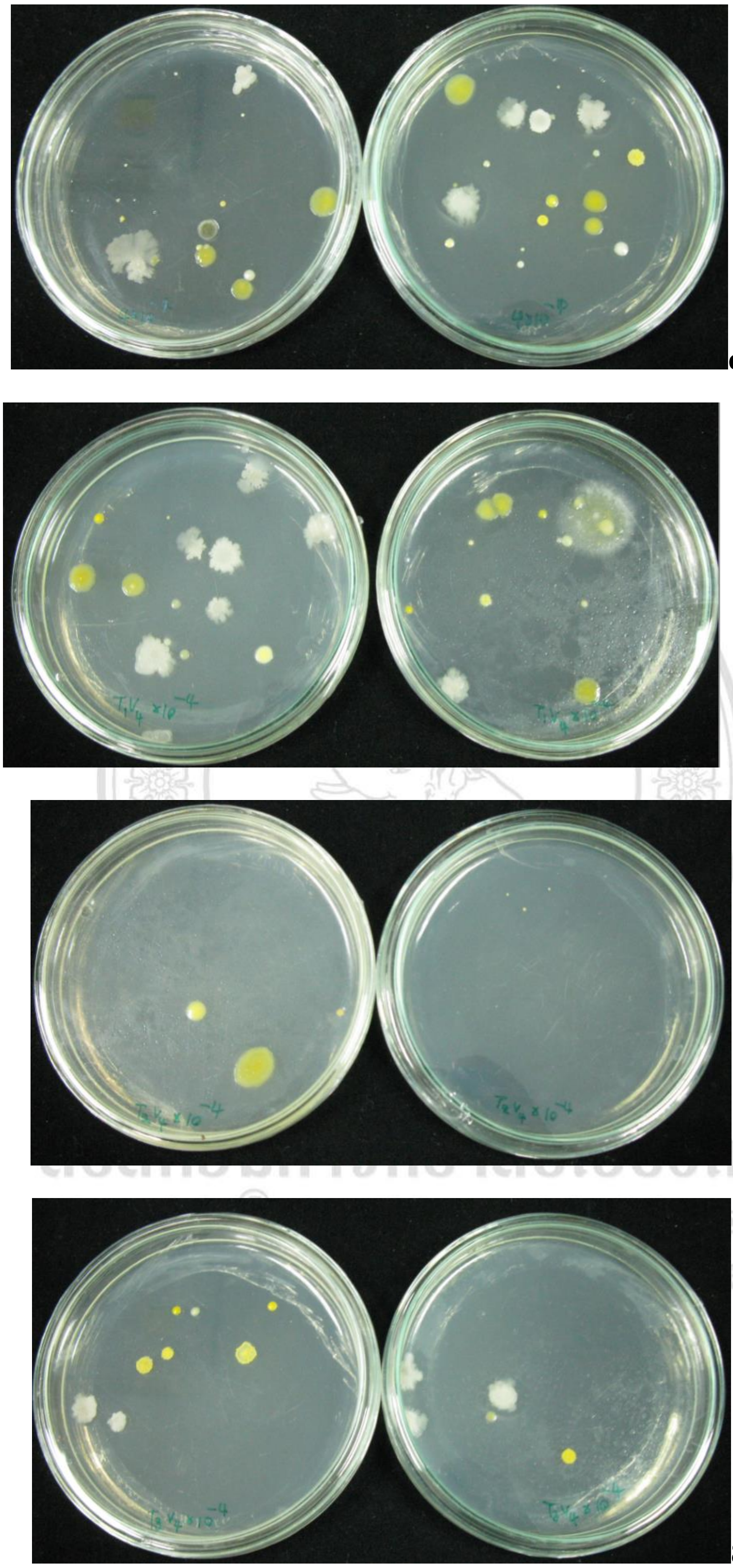
control

70 ºC

75 ºC

80 ºC

Figure 3.5 Contamination of pathogens by total viable plate count (conc. 10^{-4}) of rice bran cv. Kam Doi Saket before and after radio frequency at various temperatures



control

70 ºC

75 ºC

80 ºC

Figure 3.6 Contamination of pathogens by viable total plate count (conc. 10^{-4}) of rice bran cv. Kam Nan before and after radio frequency at various temperatures

3.3.3 Effect of storage time on radio frequency treated rice bran qualities

At initial time of storage, moisture content of rice bran after treating with radio frequency at various temperatures. The moisture of SPT1, KDML 105, KDSK and KN were measured. The average of moisture content amount to 8.27, 7.28, 8.90 and 8.21% and different among the cultivar were highly significant. The treated were closely packed and stored for 6 months at 25ºC. After 2 months storage, the mc of bran increased significantly due to moisture equilibration in their airtight containers. After 4 months, there was no significant between them (Figure 3.2). The ANOVA results show that cultivar, temperature and storage time and interaction between had significant effect on seed moisture content of any rice bran cultivar (Table 3.2).

Table 3.3 Analysis of variance for four rice bran cultivars after treated with various temperatures and stored for 6 months

Source of variation	**Degree of freedom**	***P***				
		Moisture	Total plate count	Lipid content	Protein content	Rancidity
Rice cultivar(V)	3	*	*	*	*	*
RF temperature (T)	3	*	ns	ns	*	ns
T x V	9	*	*	*	*	*
Storage time(M)	3	*	*	*	*	*
V x M	9	*	*	*	*	*
T x M	9	*	*	*	*	*
T x V x M	27	*	*	*	*	*

* = significant at the 0.05 level of probability
ns = not significant

The different treated temperature showed no effect on oil content and thiobarbituric acid number or TBA number of rice bran during storage. Storage for 2-6 months significantly resulted on decreasing number of oil content (Figure 3.9).

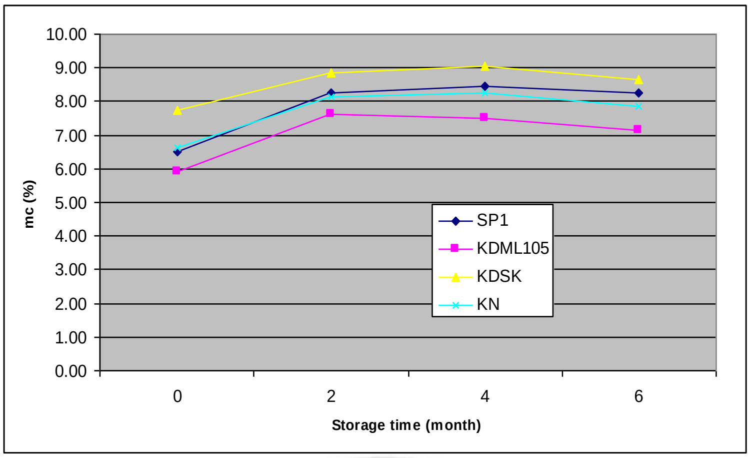


Figure 3.7 Moisture content of RF treated rice bran after storage for 6 months

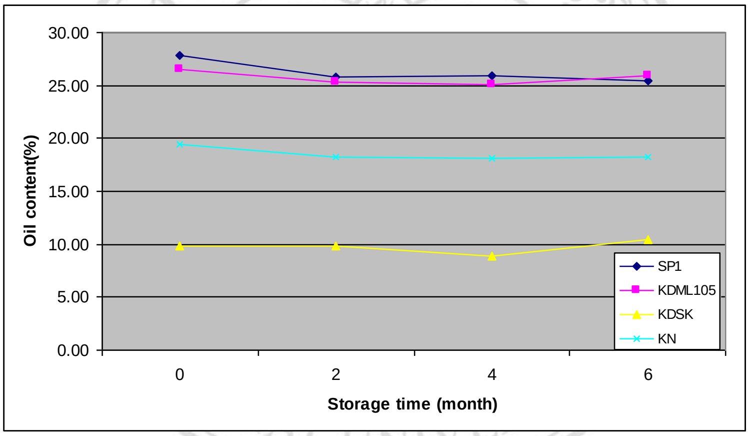


Figure 3.8 Oil content of RF treated rice bran 4 varieties after storage for 6 months

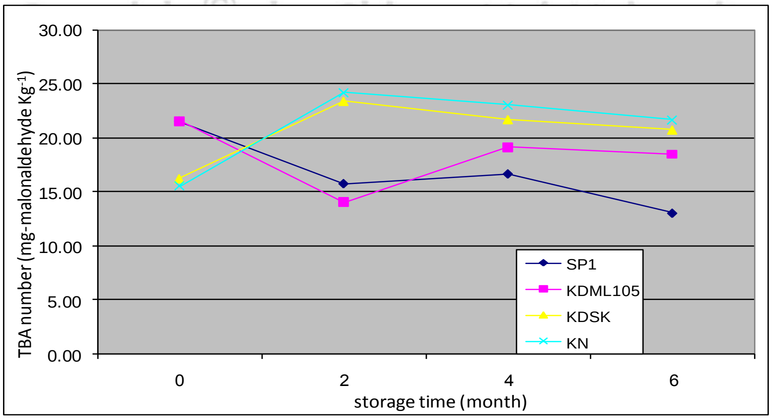


Figure 3.9 TBA acid number of RF treated rice bran 4 varieties after storage for 6 months

The interactions between temperature and cultivar also affected oil content (Table 3.2). Storage of 2-6 months resulted in TBA value of treated purple rice bran which tended to decrease in 2 months after that increased during 4 months and decreased again in sixth month (Figure 3.10). The cause of rancidity form the decomposition of lipids (triacylglycerols) into free fatty acids is typical of the rapid development of hydrolytic rancidity in raw rice bran, of which the products unsuitable for human consumption. Ramezanzadeh *et al.* (1999) also studied the effect of microwave heating (850 Watt for 3 min), packaging and storage temperature on the production of free fatty acid in rice bran. The research found that free fatty acid of raw bran increase rapidly over the 16 weeks of storage when stored at 25ºC increased from 2.5% to 54.9% in vacuum bags while bran packed in zipper-top bags was 48.1%. The result also showed that hydrolytic rancidity of rice bran can be prevented by microwave heating and the recommendation for prolong shelf life of microwave rice bran was storage condition at 4-5ºC in closed tight bag.

3.3.3 Effect of storage time and radio frequency heat treatment on anthocyanin extraction of purple rice bran

After purple rice bran two cultivars 9 (KDSK and KN) exposed to radio frequency at temperature of 70, 75 and 80ºC, the bran were determined anthocyanin content in form of cyanidin 3-glucoside (C3-G) and peonidin 3-glucoside (P3-G) (Table 3.3 and 3.4 resp.). The technique also provided high anthocyanin content extracted from purple rice bran with significantly. All levels of treatment temperature were not significantly different. The treatments increased C3-G extraction of both cultivars.The production of anthocyanins through heating was argued in Yue and Xu (2008) who devoted to change of anthocyanins and anthocyanidins in bilberry extraction during dry heating of which 30% approximately of the degraded anthocyanins were thermally converted to anthocyanidin due to anthocyanin could either be broken down to small molecules or suffer loss of its conjugated sugar to become its corresponding anthocyanidin during heating. When the treated purple rice bran kept at 25ºC, the result found that cultivar, storage time and interaction between them significantly influenced on change of anthocyanin extraction. The storage for 2 month increased anthocyanin extraction and tended to reduce with storage time for 4-6 months.

For P3-G, the result found that cultivar and treatment temperatures showed significantly effect on their amount of extraction. All treated temperatures decreased P3-G extraction of cv. KN whereas P3-G of cv. KDSK was increased after exposure to the waves. However, the storage time and interaction between them played a significant role on P3-G extraction. The P3-G increased when storage for 2 month after that decreased with 4 and 6 months storage. Queiroz *et al*. (2009) also noted that anthocyanins are sensitive to temperature, especially above 70ºC. Kinetic parameters for degradation of anthocyanins were estimated by Mishra *et al*. (2008). The average of P3-G extracted amount of each treated temperature were highly significant after 6 month storage especially cv. KN treated at 70ºC expressed higher P3-G that other temperatures.

Table 3.4 Anthocyanin content in form of Cyanidin 3-glucoside of RF treated purple rice bran 2 cultivars after storage for 6 months

Rice bran cultivar	**RF temp(°C)**	**Storage time (month)**			
		0	**2**	**4**	**6**
		Cyanidin 3-glucoside (µgram/gram-sample)			
KamDoiSaket	control	311.15	837.98	733.66	606.86
	70	709.26	678.49	586.51	615.65
	75	601.50	635.77	606.97	623.90
	80	702.09	741.52	529.37	677.54
Kamnan	control	1279.80	2505.08	2485.78	2207.61
	70	2081.27	2625.47	2483.30	2372.82
	75	1720.74	2395.51	2670.94	2412.60
	80	1684.82	2619.73	2563.19	2416.45
Average		1136.33	1629.94	1582.47	1491.68
$LSD_{0.05}$cultivar(V)		130.23			
$LSD_{0.05}$temperature (T)		ns			
$LSD_{0.05}$ V x T		ns			
$LSD_{0.05}$storage time(M)		63.22			
$LSD_{0.05}$ V x M		89.4			
$LSD_{0.05}$ T x M		126.44			
$LSD_{0.05}$ V x T x M		178.81			

ns = not significant

Table 3.5 Anthocyanin content in form of peonidin 3-glucoside of RF treated purple rice bran 2 cultivars after storage for 6 months

Rice bran cultivar	**RF temp(°C)**	**Storage period (month)**			
		0	**2**	**4**	**6**
		Peonidin 3-glucoside (μ gram/gram-sample)			
KamDoiSaket	control	127.86	435.94	365.58	298.11
	70	313.53	298.99	251.28	260.88
	75	257.90	274.81	260.40	262.15
	80	303.16	322.70	226.35	290.15
Kamnan	control	1709.93	1402.58	1465.63	1143.31
	70	1098.82	2625.47	1355.05	1342.47
	75	1017.65	1335.22	1434.97	1309.11
	80	907.82	1507.97	1448.17	1296.37
$LSD_{0.05}$cultivar(V)		38.59			
$LSD_{0.05}$temperature (T)		60.14			
$LSD_{0.05}$ V x T		ns			
$LSD_{0.05}$storage time(M)		65.84			
$LSD_{0.05}$ V x M		93.11			
$LSD_{0.05}$ T x M		131.68			
$LSD_{0.05}$ V x T x M		186.22			

ns = not significant

3.4 Conclusion

RF heat treatment is an alternative method for decrease moisture content and disease contamination of rice bran especially at the temperature of 75 and 80ºC, subsequently exhibited low rancidity by TBA number without adverse effect on chemical compositions (oil and protein), however storage condition and time showed significant effect on moisture equilibrium as well as auto-oxidation by transportation of oxygen. The radio frequency heating techniques also provided high anthocyanin content extracted from purple rice bran.