CHAPTER 4

Rice bran stabilization by using radio frequency heat treatments

Abstract

The use of rice bran as a food ingredient was greatest restricted by its instability during storage. The study was to investigate suitable temperature of radio frequency (RF) heat treatments for alternative stabilization technique in rice bran. The experiment was designed in split-split-plot in CRD with 4 replications. Main plot was 3 temperatures from radio frequency application and sub-plot was 2 different kinds of package as well as three months storage were sub-sub-plot. The non-glutinous rice cv. Khaw Dawk Mali 105 (KDML 105) and glutinous rice cv. San-pah-tawng 1 (SPT1) were each exposed to radio frequency RF (27.12 MHz) application at the temperature of 80, 100 and 120ºC for 5 minutes. Each treated sample 200 g were packed in laminate plastic and closed with 2 different pressure levels of vacuum sealer with gas modifier (70 and -100 kPa). Before and after treating, then storage for 1, 2 and 3 months, the moisture content, oil, protein and fiber contents, rancidity by thiobarbituric acid number (TBA) as well as the activity of lipase enzyme were determined. After treating, the moisture of both cultivars significantly decreased. The oil and protein content was unchanged while the fiber content significantly increased from 17.84 to 18.52% when treated with 120ºC. After storage for 3 months, the moisture of all bran in both packing increased due to equilibrium moisture activity. Treating temperatures had no effect on oil and protein content during storage. However, both type of packaging and storing for 1 month significantly increased oil, protein and fiber content after that it decreased along 2-3 months. Storage of 1 month also resulted in TBA value of bran packed in 70 kPa sealer especially the sample treated at 80ºC showed the highest value (18mg MDA/kg rice bran) whereas the value of bran in vacuum was unchanged. The stabilized rice bran using RF application were influenced the activity and stability of lipase by using temperature of 100 and 120ºC could also retained 17-26% of the maximal activity.

4.1 Introduction

Thai rice has been produced not only milled white rice but also a by-product also obtain from milling process. Rice bran known as a potential source of human food due to containing of protein, carbohydrates, ash, vitamins, and minerals i.e. vitamin B, E, beta-carotene, gamma-oryzanol and etc. (Qureshi *et al*., 2000; Saunders, 1985; Mori, 1999) which can be used a source of nutritious human food complement. Presently, huge amount of rice bran was brought to industrial processes, unfortunately lipid content of rice bran approximately 18–22% involved in failure of using rice bran for food purposes. Juliano (1993) noted that rice bran contains 15.0-19.7% oil which include 20% saturated fatty acids and approximately equal amounts of oleic (40-50%) and linoleic (30-35%) fatty acids (Rukmini and Raghuram, 1991). Rancidity can be happened with hydrolysis and any oxidation reaction. Saunders (1986) reported that the rate of free fatty acid formation by lipase promotion is also depended on environmental condition which the formation is as high as 5–7% free fatty acids per day and up to 70% in a month. Therefore, the reduction of rice bran quality must be stabilized immediately after production. Numerous researches have been done to stabilize rice bran by various thermal methods. Barber and Bendito de Barber, 1980 showed that inactivation of lipase enzyme containing in rice bran were depended on temperature and duration of heat treatment including the moisture content of the material. Fernando and Hewavitharana (1993) developed fluidized bed drying and the rice bran was stabilized at temperature of 90-130ºC. On the other hand, the fluidized bed method was required high air velocity which was less uses as uneconomical. As well as drum drying at temperature of 156ºC was also developed for rice bran stabilization (Delahaye *et al*., 2005).In recent year, ohmic heating is a promising method of stabilizing rice bran (Lakkakula *et al*., 2004). The principle of the method regarding to the route of alternating current was passed through a food sample which results in ohmic or electrical heating by virtue of the sample's electrical resistance (Halden, *et al*., 1990). In addition, microwave energy is use an inexpensive source of thermal processing the method has been considered to be one of the most energy-efficient types and a rapid method for heating food items (Yoshida *et al*. 1991; Yoshida *et al*. 2003). Ramezanzadeh *et al*. (2000) also revealed that stabilizing rice bran by the microwave heating process did not result in any deleterious effect on major nutrients in the bran.

To achieve proper stabilization, the objective of this experiment was to study the effect of radio frequency heat treatment on Thai rice bran stabilization, extension of its storage life when packed in different conditions and stabilization of bran nutrients.

4.2 Materials and methods

4.2.1 Rice bran preparation

White rice (*Oryza sativa* L.) 2 cultivars (San-pah-tawng 1; SPT1 and Kaw Dawk Mali 105; KDML105) was used in the experiment. The rice was cultivated in paddy field of Plant Science and Natural Resources. After harvest, the rice seed each cultivar was milled by milling factory. Freshly milled rice bran was immediately passed through a 100 mesh to remove broken rice and husks. The bran sample was determined for moisture content before subject to stabilization by using radio frequency applicator.

4.2.2 Experiment

The split-split plot was design in this experiment by using in each cultivar. The main plot was storage time for 3 month and the sub-plot was package conditions (70 and -100kPa) as well as sub-sub plot was treatment temperature. The rice bran sample (200g) was subjected to radio-frequency heat treatments at 27.12 MHz under the temperatures of 80, 100, 120ºC for 5 minutes. After treating, the bran was packed in 2 conditions and then storage at 25ºC for 3 month. In each month, the bran was sampled and determined as shown in sample analysis below.

4.2.3 Sample Determination

1) Moisture content (AOAC, 2005)
2) Oil content (AOAC, 2005)
3) Protein content (AOAC, 2005)
4) Fiber content (AOAC, 2005)
5) Thiobarbituric acid number (TBA number) (AOAC, 2005)
6) Lipase activity (Prabhu *et al*., 1999)

4.2.4 Data analysis

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

4.3 Results and Discussion

4.3.1 Effect of radio frequency heat treatment on quality of rice bran 2 cultivars

Rice bran sample cv. SPT1 and KDML105 with different levels of moisture content were subjected to radio frequency heating at temperature of 80, 100 and 120ºC. The result showed that different temperature significantly reduced moisture content of rice bran (Table 4.1). This demonstrate that energy transfer is the main characteristic of radio frequency heat treatment by dipole rotation and ionic conduction through reversals of dipoles and displacement of charged ions present in the solute and the solvent, then energy is delivered directly to materials via different conversions of electromagnetic energy into thermal energy (Routray and Orsat, 2011). Although the lipid content was different in cultivar, it was unchanged when treated with different temperature. Result showed the protein content range from 10.54-10.93% and it slightly increased with increasing of treated temperature. The fiber content of between cultivars were different and it varied among treated temperature.

Table 4.1 Moisture content and chemical compositions of rice bran after radio frequency heat treatment

Rice bran variety	RF temp(°C)	Quality of rice bran			
		Moisture (%)	**Lipid Content**	**Crude Protein**	**Crude Fiber**
SPT1	control	9.50	13.93	10.93	15.54
	80	7.60	14.25	11.22	15.35
	100	5.39	15.01	11.52	15.68
	120	4.76	14.16	11.77	15.37
KDML105	control	10.0	13.41	10.54	17.84
	80	6.87	12.77	10.84	18.36
	100	4.40	13.09	11.25	17.51
	120	3.25	13.75	11.23	18.52
LSD $_{0.05}$variety		0.13	0.37	ns	0.17
LSD $_{0.05}$temperature		0.27	ns	0.09	0.18
LSD $_{0.05}$varietyx temperature		0.38	0.80	ns	0.25

The extent of lipid peroxidation of rice bran after exposure to radio frequency was monitored by the formation of TBA value as compared to the raw rice bran (control) are displayed in Table 4.2. The TBA value was determined. The result found that RF heat at several temperatures showed no effect on TBA value which was 7.7-10.26mg MDA/kg-rice bran cv. SPT1 and 9.79-11.53 mg MDA/kg-rice bran in cv.KDML105.

Lipase was showed the activity in endogenous of rice bran and influenced directly on the further shelf life. The treated temperature expressed significantly result on the lipase activity in both rice bran cultivars (Table 4.2). This paralleled with reported of Castro *et al*. (2004) who found that the electric field had a significant effect on the lipoxygenase and it was higher inactivation with conventional heating (water bath) approximately 5 times.

Table 4.2 Rancidity and lipase activity of rice bran after radio frequency heat treatment

Rice cultivar	**RF temperature(ºC)**	**TBA value mg MDA/kg-rice bran**	**Lipase activity (µmol p-NPB/min)**
SPT1	control	7.7	5.70
	80	9.89	5.09
	100	10.26	3.40
	120	7.89	2.51
KDML105	control	11.23	8.04
	80	12.09	3.49
	100	11.53	1.38
	120	9.79	2.06
LSD $_{0.05}$variety		ns	ns
LSD $_{0.05}$temperature		ns	1.39
LSD $_{0.05}$variety x temperature		ns	ns

4.3.2 Effect of radio frequency heat treatment on the proximate composition of rice bran 2 cultivars after storage

The effect of radio frequency heat on moisture content of rice bran 2 cultivars after storage was presented in Table 4.3. The initial moisture content differences between the bran samples must be due to the different radio frequency thermal processing treatments. The result found that treated temperature and storage time showed significantly effect on change of moisture content of both cultivar as well as the interaction between temperature and storage time significantly affected only in rice bran cv. KDML105. With different moisture content at initial time resulted from RF heat, the moisture content sharply increased with storing time and their moisture at 3 month storage were also different. It can be observed that lower moisture at initial time of storage contained lower moisture content at the end of storage. This is occurred because of equilibrium moisture content. As well as plastic type of package also impacted on gas exchange.

The fat content in rice bran is the main effect which contributed to the bran instability. Present experimental result of several temperatures on lipid content of rice bran after storing for 3 months is shown in Table 4.4. The treatment temperatures, packing and interaction between them showed no effect on lipid content of rice bran cv. SPT1, however the storage time significantly affected on change of the bran which reduced with storage time. On the other hand, the lipid content of the bran cv. KDML105 was significant difference between vacuum packs and the average of lipid of bran packed in 70 and -100 kPa were 13.33 and 13.04%.

The effect of radio frequency heat at several temperatures on crude protein of rice bran stored for 3 months is expressed in Table 4.5. The treated temperature was significant change in protein content only in rice cv. SPT1 while the storage time significantly affected on protein content of both cultivars. After storage for 1 month, the lipid significantly increased, after that it decreased in 2^{nd} month and slightly decreased at the end of storage time.

The change of ash content of treated rice bran during storage was illustrated in Table 4.6. The RF treated temperature, storage time and interaction between them significantly influenced on ash content. The average of ash content of rice bran cv. KDML105 (18.13%), it increased in 1 month storage (average of 18.77%), after that it sharply decreased with storage time for 2 and 3 month to 18.15 and 17.16%.

Table 4.3 Moisture content of RF treated rice bran 2 cultivars packed in different vacuum packs and stored for 3 months

Package air pressure	RF temperature (ºC)	San-pah-tawng 1 (SPT1)				Kaw Dawk Mali 105 (KDML 105)			
		Storage time (month)				Storage time (month)			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
		Moisture content (%)				**Moisture content (%)**			
70 kPa	80	4.76	5.79	7.42	8.78	4.11	6.07	8.00	8.28
	100	2.61	4.53	6.56	8.02	2.71	5.86	6.87	8.43
	120	1.71	2.45	5.83	7.70	1.03	4.87	6.12	7.32
-100 kPa	80	4.76	6.24	7.90	8.08	4.11	7.04	7.89	6.93
	100	2.61	4.43	5.66	6.84	2.71	5.79	6.96	6.49
	120	1.71	4.06	5.64	6.57	1.03	4.25	5.58	6.57
Average		3.03	4.59	6.50	7.67	2.62	5.65	6.96	7.33
Temperature (T)		0.39				0.74			
Package (P)		ns				0.36			
T x P		ns				ns			
Storage month(M)		0.63				0.34			
T x M		ns				0.69			
P x M		ns				ns			
T x P x M		ns				ns			

Table 4.4 Lipid content of RF treated rice bran 2 cultivars packed in different vacuum packs and stored for 3 months

Package air pressure	**RF-temperature (ºC)**	San-pah-tawng 1 (SPT1)				Kaw Dawk Mali 105 (KDML 105)			
		Storage time (month)				Storage time (month)			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
		Lipid content (%)				**Lipid content (%)**			
70 kPa	80	14.25	13.98	13.82	14.30	12.77	13.73	13.20	13.86
	100	15.01	14.15	14.24	13.57	13.09	13.94	13.35	13.51
	120	14.16	14.84	14.47	13.57	13.75	14.22	12.91	11.60
-100 kPa	80	14.25	13.40	14.16	13.49	12.77	13.43	13.39	11.49
	100	15.01	14.46	14.00	14.50	13.09	13.87	13.50	11.69
	120	14.16	15.18	13.98	14.71	13.75	14.05	13.20	12.30
Average		14.48	14.33	14.11	14.02	13.20	13.87	13.23	12.41
Temperature (T)		ns				ns			
Package (P)		ns				0.29			
T x P		ns				ns			
Storage month(M)		0.3				0.53			
T x M		ns				ns			
P x M		ns				0.60			
T x P x M		ns				ns			

Table 4.5 Protein content of RF treated rice bran 2 cultivars packed in different vacuum packs and stored for 3 months

Package air pressure	RF-temperature (ºC)	San-pah-tawng 1 (SPT1)				Kaw Dawk Mali 105 (KDML 105)			
		Storage time (month)				Storage time (month)			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
		Protein content (%)				**Protein content (%)**			
70 kPa	80	11.22	12.67	11.27	11.16	10.84	12.23	11.12	10.87
	100	11.52	13.01	12.84	11.22	11.25	12.00	11.62	10.78
	120	11.77	12.82	12.12	11.21	11.23	12.26	11.71	10.79
-100 kPa	80	11.22	12.42	11.31	11.34	10.84	11.94	10.67	10.96
	100	11.52	12.78	12.23	11.56	11.25	12.29	10.77	11.01
	120	11.77	13.29	11.41	11.48	11.23	11.64	10.90	11.00
Average		11.50	12.83	11.86	11.33	11.11	12.06	11.13	10.90
Temperature (T)		0.30				ns			
Package (P)		ns				ns			
T x P		ns				ns			
Storage month(M)		0.32				0.24			
T x M		ns				ns			
P x M		ns				ns			
T x P x M		ns				ns			

Table 4.6 Fiber content of RF treated rice bran 2 cultivars packed in different vacuum packs and stored for 3 months

Package air pressure	RF-temperature (ºC)	San-pah-tawng 1 (SPT1)				Kaw Dawk Mali 105 (KDML 105)			
		Storage time (month)				Storage time (month)			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
		Fiber content (%)				**Fiber content (%)**			
70 kPa	80	15.35	15.51	15.31	16.04	18.36	18.70	18.27	17.23
	100	15.68	16.02	15.39	16.41	17.51	18.66	17.76	17.20
	120	15.37	15.48	15.78	15.63	18.52	19.03	18.48	17.42
-100 kPa	80	15.35	15.90	15.37	15.42	18.36	19.00	18.15	16.96
	100	15.68	16.34	16.22	15.17	17.51	19.27	18.00	17.16
	120	15.37	16.28	16.05	15.28	18.52	17.97	18.26	16.99
Average		15.47	15.92	15.69	15.66	18.13	18.77	18.15	17.16
Temperature (T)		0.09				0.18			
Package (P)		ns				ns			
T x P		0.13				0.25			
Storage month(M)		ns				0.32			
T x M		0.18				0.26			
P x M		ns				ns			
T x P x M		0.26				0.51			

Table 4.7 Rancidity of RF treated rice bran 2 cultivars packed in different vacuum packs and stored for 3 months

Package air pressure	RF-temperature (ºC)	San-pah-tawng 1 (SPT1)				Kaw Dawk Mali 105 (KDML 105)			
		Storage time (month)				Storage time (month)			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
		mg MDA/kg rice bran				mg MDA/kg rice bran			
70 kPa	80	9.89	10.79	13.00	12.11	12.09	18.52	13.06	12.72
	100	10.26	10.01	9.89	21.52	11.53	15.52	12.32	13.65
	120	7.89	10.77	8.15	15.29	9.79	16.92	10.68	9.91
-100 kPa	80	9.89	13.63	11.25	15.19	12.09	10.76	15.56	9.19
	100	10.26	12.21	9.69	12.69	11.53	14.58	11.47	15.07
	120	7.89	16.48	8.61	6.56	9.79	16.59	15.85	16.61
Average		9.35	12.32	10.10	13.89	11.14	15.48	13.16	12.86
Temperature (T)		ns				ns			
Package (P)		ns				0.29			
T x P		ns				ns			
Storage month(M)		0.3				0.53			
T x M		ns				ns			
P x M		ns				0.60			
T x P x M		ns				ns			

Table 4.8 Lipase activity of RF treated rice bran 2 cultivars packed in different vacuum packs and stored for 3 months

Package air pressure	**RF-temperature (ºC)**	San-pah-tawng 1 (SPT1)				Kaw Dawk Mali 105 (KDML 105)			
		Storage time (month)				Storage time (month)			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
		µmol p- NPB/min				µmol p- NPB/min			
70 kPa	80	5.09	4.21	5.33	5.47	3.49	4.55	3.11	4.51
	100	3.40	5.61	5.44	4.93	1.38	1.94	1.07	4.07
	120	2.51	1.27	2.43	3.82	2.06	1.22	1.61	3.56
-100 kPa	80	5.09	4.12	5.37	3.28	3.49	5.79	4.14	2.10
	100	3.40	5.19	4.67	1.92	1.38	2.52	3.06	3.19
	120	2.51	1.42	1.08	1.96	2.06	1.57	2.11	2.54
Average		3.67	3.64	4.05	3.56	2.31	2.93	2.52	3.33
Temperature (T)		0.42				0.28			
Package (P)		0.12				ns			
T x P		ns				ns			
Storage month(M)		ns				0.53			
T x M		0.84				0.57			
P x M		0.24				1.21			
T x P x M		ns				0.81			

4.3.3 Effect of radio frequency heat treatment on quality of rice bran 2 cultivar

The rancidity of stabilized bran as measured by thiobarbituric acid (TBA) as reactive substances value, an indicator of stability is presented in Table 4.7. The results expressed as a milligram malondialdehyde per kilogram of rice bran by according to the principle that the 3-carbon compound malonaldehyde was a major carbonyl decomposition products of auto-oxidized, polyunsaturated lipid materials (Tamura *et al*. 1991; Crawford *et al*. 1966). The experiment found that heat temperatures, package and interaction between them showed no effect on the TBA value of rice bran cv. SPT1. However, storage time significantly affected on TBA value and it increased in 1 month at room temperature (25ºC) and then decreased after 2 month and steady at the end of storage time. As can be seen in Table 4.7, the TBA values in the bran increased with increasing lipid content as shown in Table 4.4, which generally agrees with the fact that the higher lipid content gives the higher TBA values in rice bran sample. For rice bran cv. KDML105, treated temperatures had also no influence on TBA value; however package condition, storage time and interaction between them significantly changed the value. The previous study by Ramezanzadeh *et al*. (1999) reported the effect of storage time and temperature on free fatty acid levels in microwave-heated rice bran packed in zipper-top bags and vacuum packs and stored at 25 and 4-5ºC for 16 week storage. There was a small but steady increased in the free fatty acid value for both methods of packaging over the 16-week storage period when stored at 25ºC. The change of free fatty acid content microwave-heating bran in both packaging types when stored at 4-5ºC for 16 week were unchanged.

The rice bran lipase activity was measured by using the specified substrate as p-Nitrophenyl-Buryrate (p-NPB) and p-Nitrophenyl-Laulate (p-NPL) were used and the result are shown in Table 4.8. It was found that treated temperatures affected on lipase activity of both cultivars. Lipase activity of rice bran treated at temperature of 120ºC significantly decreased the enzyme activity to be the lowest in both cultivars. A previous study (Castro, 2004) showed that the presence of an electric field can influence biochemical reactions by changing molecular spacing and increasing inter chain reactions and it may remove the metallic prosthetic groups present in the enzymes Lipoxygenase and Phospholipase, therefore the heat energy caused the enhancement of activity loss. Moreover, the storage time and interaction between time and temperature significantly involved in lipase activity of rice bran.

4.4 Conclusion

The results of this investigation have shown that Thai rice bran 2 different cultivars can be stabilized by heating bran with radio frequency heat treatment energy to inactivate lipase enzyme. Application of radio frequency heating process at all temperatures decreased moisture content. Rice bran stabilized by radio frequency heating and stored for up to 3 months affected on proximate content. The results of this study suggested that radio frequency heat treatment is an effective stabilization method especially inactivation lipase enzyme activity. However, the vacuum packing (-100 kPa) did not show any significantly benefit over 70kPa package. The adverse effect was seen by any increase in the moisture content of rice bran. Therefore, it can be concluded that storage condition for prevention of hydrolytic rancidity in radio frequency heated rice bran at 25°C is a main factor affecting the further lipid oxidation.