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Radio frequency heating on lipid peroxidation, decreasing oxidative stress and aflatoxin B1 reduction in *Perilla frutescens L.* highland oil seed

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ABSTRACT

The radio frequency (RF) heat treatment had effect to the enzymes activities. On treating temperature over 80 °C for 10 minutes, the low activation of lipase was occurred. The better oil quality which has low level of hydrogen peroxide, peroxide value and TBA value were achieved. RF heating treatments resulted in significantly lower number of *A. flavus* and aflatoxin B1 and the energy consumed than control treatment. Therefore, the RF stabilizing technique provided the high oil quality, less lipid peroxidation and lower energy consumed than convectional drying.

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Keywords: Radio frequency heating, *Perilla frutescens* L. Britton, Lipid peroxidation, seed oil quality, oil seed

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1. Introduction

Perilla frutescens (L.) Britt. (Lamiaceae) is an edible plant frequently used in Asian countries such as China, Korea, Japan and Thailand (Heci, 2001). *P. frutescens* leaf contains the cinnamic, flavonic and anthocyanic derivatives such as Apigenin, Luteolin, Scutellarein, Shisonin, Caffeic acid, and Rosmarinic acid. *P. frutescens* are not only used as food ingredients but also for skin creams, soaps, and medicinal preparations, because of their recognized bioactivities, such as antioxidant (Makino et al. 2003; Takano et al. 2004), anti-allergic (Ueda et al. 2002; Yamasaki et al. 1998), anti-inflammatory (Tao et al. 1993), and anti-HIV-1 activity (Prabhakar et al. 1986). Additionally, perilla seed contains about 35 - 50% of the oil, which is obtained by pressing. Perilla oil is a very rich source of the alpha-linolenic acid (ALA) (omega-3 fatty acid), which contained in about 50 to 60% of the oil. On the other hand, it also contained other poly-unsaturated fatty acid derivatives; linoleic acid (omega-6) (18 - 22**%**) and oleic acid (omega-9) (0.080 - 0.17**%**). However, the oil seed has a high deterioration rate under unsuitable storage condition according to lipid peroxidation, which was activated via lipase and lipoxygenase (LOX) enzyme. Those enzymes activated after perilla seed was mashed which as a catalyst, the triglyceride of oil is quickly hydrolyzed into glycerol and free fatty acids. This mechanism induced the oxidative stress, which was directly affected on the degradation of polyunsaturated fatty acids and oil quality and affected consumer health. The stabilization of raw mashed seed and extraction of oil after mashing process are two effective methods for lipase and LOX enzymes inactivation and prohibition of free fatty acids formation and oxidative stress regeneration. Nowadays, the several stabilization techniques of oil have been studied (Jung et al. 2001; Yamasaki et al. 1998; Tao et al. 1993). Although a number of studies like streaming, dry heating, wet heating, microwave heating, ohmic heating, extrusion, and pH lowering have been conducted for oil stabilization, radio frequency (RF) heating treatment has not been used for this purpose. In fact, it is an environmental friendly technique with a wide range of applications. Widespread use of RF treatment emitting devices increased the exposure to electromagnetic fields (EMFs) from 27.12 MHz to 300 GHz. Various biological effects; such as fungi or insect, their exposure to EMFs have been documented so far, but very little work has been carried out on plants specially affected on the oily material.

2. Materials and methods

The study was aimed to investigate the lipid peroxidation of Perilla frutescens L. oil seed after exposure to radio frequency EMFs, and in particular, to clarify the possible role of Aflatoxin B1 decontamination and oxidative stress in the observation. The experiment was conducted under factorial design with 5 replications. Perilla frutescens L. seed at 10, 14 and 18 g 100g-1 – dry basis moisture content, was exposed to RF heat treatment at 50, 60, 70, 80 and 90 °C for 1, 3, 5, 7 and 10 minutes. The convectional heating techniques were following to Zullaikah et al. (2005), and Amarasinghe and Gangodavilage (20 04) at 110°C for 30 minutes were applied as controlling technique.

The oil yield (AOAC, 1995) free fatty acid composition and concentration (GC/MS technique), peroxide value (AOCS. 1995), *hydrogen peroxide* (H2O2) (O'Kane et al., 1996), enzyme activity; lipase (Prabhu et al., 1999; Hatzinikolaou et al., 1999), lipoxygenase (LOX) (Meshehdani, 1990), superoxide dismutase (SOD) (Oberley and Spitz, 1985), and ascorbate peroxidase (APX) (Nakano and Asada, 1981), Aspergillus flavus (mycelium growth inhibition technique) and aflatoxin B1 (ELISA technique) contamination, total antioxidant activity (DPPH method; Kim et al., 2002) and energy consumed were evaluated.

3. Results and discussion

The RF-heating treatment had the higher efficiency on *A. niger,* and *A. flavus* decontamination. The best condition of RF-heat treatment to control those fungi was 18% wb of initial grain moisture content with 90 °C for 7 minutes. Additionally, the RF-heat treatment also had the high efficiency on Aflatoxin B1 decontamination when applied under conditions; 90 °C for 7 – 10 minutes. (Table 1)

Table 1 *Aspergillus flavus* inhibition and aflatoxin B1 contamination of *Perilla frutescens* (L.) Britt under various radio frequency heating treatment[1]

Treatment	*A. niger* inhibition (%)	*A. flavus* inhibition (%)	Aflatoxin B1 contamination (ppb)
Grain moisture content (g 100g^{-1}- db)			
10	92.31 ± 1.10b	75.10 ± 31.85c	19.40 ± 7.875a
14	99.40 ± 1.51a	87.20 ± 20.17b	15.619 ± 5.874b
18	99.60 ± 1.44a	93.33 ± 15.19a	13.735 ± 5.455c
RF treated temperature (°C)			
50	89.58 ± 5.32b	68.44 ± 27.50d	26.012 ± 6.059a
60	98.24 ± 1.10a	78.67 ± 28.09c	19.086 ± 2.925b
70	98.94 ± 1.58a	90.50 ± 15.14b	14.862 ± 2.329c
80	99.29 ± 1.63a	88.44 ± 26.45b	12.016 ± 1.664d
90	99.45 ± 0.52a	100 ± 0.00a	9.280 ± 2.937e
RF treated duration (min.)			
1	96.52 ± 13.49	61.61 ± 35.37c	18.648 ± 7.966a
3	97.18 ± 9.91	87.11 ± 17.66b	16.574 ± 6.564b
5	97.64 ± 7.05	88.44 ± 20.67b	16.491 ± 7.855b
7	97.04 ± 6.08	92.89 ± 14.24ab	14.938 ± 5.378c
10	97.13 ± 10.03	96.00 ± 10.95a	14.604 ± 6.153c
The convectional heating (110°C for 30 minutes)	56 ± 9.68	61.14 ± 7.719	31.22 ± 3.681

[1]Values are means f standard deviations

The result found that, the initial seed moisture content before treating with RF treatment was the main factor on oil extractability and FFAs content. The oil yield was decreased from 45.44 g 100g^{-1} in low moisture content seed (10 g 100g^{-1} - db) to 40.10 g 100g^{-1} in high moisture content seed (18 g 100g^{-1} - db). Furthermore, the unsaturated FFAs content; α - linolenic acid, linolenic acid, and oleic acid, were gradually decreased in high moisture content seed. On the other hand, the saturated FFAs; palmatic acid and steric acid, remarkably increased in the seed sample. This is probably due to the activation of lipase enzyme. The increase of RF heating temperature significantly decreased the oil extractability of perilla seed. The higher temperature had affect to oil yield that decreased from 48.90 g 100g^{-1} at 50°C to 41.82 g 100g^{-1} at 90 °C. This could be due to some structural alterations in the seed which favor easier extrusion of oil. Maheswari et al. (1981) observed significant disruption of intracellular structure and also coalescence of spherosomes of microwave-treated rapeseed sample. The unsaturated free fatty acids (FFAs) content; α - linolenic acid, were significantly decreased in the high RF heating temperature. The maximum of α - linolenic acid content was found in treated sample for 50 - 60 °C. On the other hand, linoleic acid and oleic acid content was not changed due to the RF heating duration. Otherwise, the saturated FFAs content; palmatic acid and steric acid were significantly increased with the increasing of the RF heating temperature. This could be attributed to the mechanism of lipid peroxidation via the activation of lipoxygenase enzyme, which effected the oil quality. However, the period of RF treatment did not affect the oil extractability and unsaturated and saturated FFAs content. The increase in the period of RF treatment from 1 to 10 minutes did not effect to the oil extractability. Additionally, the increase of RF heating temperature effect not only on oil extractability but also affected on oil quality (Table 2).

The result showed that, initial seed moisture content has the major role in perilla oil quality degradation. At the high seed moisture content, the activation of lipase, and LOX was highest activated in the high moisture seed. Under the high seed moisture condition, the lipolytic enzyme was activated, which is the cause of lipid hydrolysis. Moreover, the activation of LOX may induce the oxidative stress via lipid peroxidation mechanism. Under those conditions, the perilla oil seed quality was degraded while the peroxide and TBA value were significantly increased. The RF heating temperature and duration significantly decreased the activity of lipase enzyme, which has lowest activity at 90 °C for 10 minutes. The lipase enzyme also provided lowest activity in the low seed moisture content sample. The degradation of oil quality may due to the activation of lypolytic enzyme especially on lipase. On the other hand, the RF treatment could decrease the activation of this enzyme that stabilizing the perilla oil. However, the activation of LOX did not affect due to the RF treatment. This indicated greater effect of RF treatment on lipase as compared to LOX. The activities of antioxidative enzymes showed different behavior; SOD and APX decreased after most exposure temperatures. Exceptions were significantly reduced APX activity after longer exposure (10 minutes), while SOD was not changed (Table 3).

Table 2 Lipid content and free fatty acids composition of *Perilla frutescens* (L.) Britt grain under various radio frequency heating treatment

Treatment	Lipid	α - Linolenic acid	Linoleic acid	Oleic acid	Palmatic acid	Steric acid[1]
Grain moisture content (g 100g^{-1}- db)						
10	45.44 ± 4.7554a	6.36 ± 0.665a	0.781 ± 0.0850a	5.90 ± 1.9187a	0.848 ± 0.7342b	36.454 ± 1.4637b
14	40.28 ± 3.2871b	5.63 ± 0.459b	0.478 ± 0.2020b	3.90 ± 1.4614b	0.863 ± 0.7500b	36.167 ± 5.0841b
18	40.10 ± 3.0814b	5.57 ± 0.449b	0.467 ± 0.1892c	3.22 ± 1.3689b	1.562 ± 1.0582a	44.969 ± 7.8464a
RF treated temperature (°C)						
50	48.90 ± 0.653a	10.48 ± 1.345a	0.383 ± 0.054a	3.253 ± 0.054a	0.840 ± 0.085c	31.804 ± 3.714d
60	44.12 ± 0.761bc	9.99 ± 1.764ab	0.366 ± 0.118ab	3.114 ± 1.001ab	0.854 ± 0.077bc	35.397 ± 4.568c
70	46.30 ± 0.452b	10.48 ± 0.931b	0.366 ± 0.046ab	3.108 ± 0.390ab	0.882 ± 0.096b	36.081 ± 0.490b
80	44.25 ± 0.571bc	9.91 ± 1.245bc	0.361 ± 0.058ab	3.070 ± 0.495ab	0.878 ± 0.086b	37.905 ± 3.727b
90	41.82 ± 0.629c	9.41 ± 01.416c	0.345 ± 0.094b	2.937 ± 0.798b	0.914 ± 0.109a	38.404 ± 0.653a
RF treated duration (min.)						
1	45.34 ± 5.234	10.11 ± 1.381	0.357 ± 0.073	3.030 ± 0.622	0.901 ± 0.099a	35.927 ± 5.132
3	44.76 ± 0.766	9.97 ± 1.718	0.356 ± 0.107	3.026 ± 0.909	0.878 ± 0.079ab	35.450 ± 6.105
5	43.96 ± 0.681	9.78 ± 1.322	0.370 ± 0.059	3.144 ± 0.508	0.863 ± 0.099b	35.169 ± 5.444
7	45.88 ± 0.578	10.17 ± 1.261	0.371 ± 0.072	3.153 ± 0.609	0.853 ± 0.093b	36.880 ± 4.942
10	45.42 ± 0.641	10.11 ± 1.327	0.368 ± 0.078	3.128 ± 0.664	0.871 ± 0.096b	36.166 ± 4.948
The convectional heating techniques (110°C for 30 minutes)	42.82 ± 0.751	8.75 ± 1.425	0.273 ± 0.082	2.541 ± 0.432	0.841 ± 0.073	39.178 ± 4.049

Lipid (g 100g^{-1}DM), α - Linolenic acid (mg g^{-1} - DM), Linoleic acid (mg g^{-1} - DM), Oleic acid (mg g^{-1} - DM), Palmatic acid (mg g^{-1} - DM), Steric acid (mg g^{-1} - DM)

[1]Values are means f standard deviations

After exposure the perilla seed to RF treatment, parameters of oxidative stress, such H_2O_2 content as well as activities of antioxidative enzymes were evaluated. The RF treating at 70 °C provided the lowest of H_2O_2 content in perilla seed exposed to RF for 3 - 10 minutes while other exposure treatments did not have an effect. The results showed that RF treatment induced oxidative stress in perilla seed as well as unspecific stress responses, especially of antioxidative enzymes. However, the observed effects markedly depended on the treating temperature as well as exposure duration. Enhanced lipid peroxidation and H_2O_2 content accompanied by diminished antioxidative enzymes activity caused by exposure to investigated RF treatment, especially at high treating temperature and exposure duration, indicate that oxidative stress could partly be due to changed activities of antioxidative enzymes. Additionally, the RF treatment could stabilize perilla oil quality, while peroxide value and TBA value was lowest for 7 minutes RF heating at 80 °C (Table 4).

Interestingly, the RF-heat treatment technique for stabilizing perilla oil quality had the lower energy consumed than those compared to convectional drying technique. While the RF technique consumed less energy about 55 times than convectional drying technique. RF-heat treatment used 68.20 - 88.00 kJ, while convectional drying use 3,780 - 4,860 kJ of energy (Table 5).

Table 3 Total antioxidant activity and enzymatic activity of *Perilla frutescens* (L.) Britt grain under various radio frequency heating treatment

Treatment	Total antioxidant activity (IC_{50})	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 convectional heating techniques (110^0C for 30 minutes)	68.425	118	18.667	18.762	9.987

APX: ascorbate peroxidase, LOX: lipoxygenase, SOD: superoxide dismutase
[1]Values are means f standard deviations

4. Conclusion

RF heating treatment for stabilizing extracted *Perilla frutescens* (L) Britt oil at a low temperature of 50 °C for any treating period 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. This is not surprising because there 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 was occurred. Under this condition, the good oil quality which had low level of hydrogen peroxide, peroxide value and the thiobarbituric acid (TBA) value was achieved. While sufficient moisture may be necessary to generate heat, the content of enzyme-bound water is more important. Interestingly, under this condition, *A. flavus* and aflatoxin B1 could be controlled with least of energy consumed. Therefore, the RF stabilizing technique provided the high oil quality, less lipid peroxidation and lower energy consumed than convectional 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 are responsible for FFA release and the subsequent peroxidation leading to rancidity of oil.

Table 4 Changes in lipids of stabilized *Perilla frutescens* (L) Britt seeds in relation radio frequency heating treatment

Treatment	H_2O_2 concentration (µmole g^{-1} FW)	Peroxide value (meq O_2 kg^{-1} oil)	TBA value (absorbance g^{-1} oil)
Grain moisture content (%-db)			
10	125.32 ± 6.165c	8.40 ± 0.16c	3.36 ± 0.0854b
14	142.45 ± 8.103b	18.80 ± 0.08b	8.36 ± 0.0483a
18	158.29 ± 4.262a	20.00 ± 0.11a	9.09 ± 0.0725a
RF treated temperature (°C)			
50	180.98 ± 7.250a	24.40 ± 0.04a	11.09 ± 2.165a
60	178.99 ± 6.549a	24.00 ± 0.11a	10.67 ± 1.560a
70	153.08 ± 6.797b	23.80 ± 0.12a	10.58 ± 1.614a
80	151.65 ± 8.605b	20.62 ± 0.13b	8.25 ± 1.835b
90	146.44 ± 6.162b	13.40 ± 0.04c	5.36 ± 1.832c
RF treated duration (min)			
1	172.84 ± 7.356a	24.00 ± 0.08a	10.91 ± 2.059a
3	164.00 ± 7.180b	23.58 ± 0.08a	10.48 ± 1.804a
5	157.03 ± 7.151b	23.60 ± 0.04a	10.49 ± 1.480a
7	157.97 ± 6.870b	21.12 ± 0.18b	8.45 ± 1.917a
10	146.74 ± 7.668c	17.83 ± 0.45c	7.13 ± 1.742b
The convectional heating techniques (110 °C for 30 minutes)	168.09	26.45	11.03

Table 5 The comparison of power consumed under various stabilization processes

Treatments	Power consumed (kJ)
T1	68.20 ± 0.503
T2	69.80 ± 0.273
T3	70.20 ± 0.024
T4	80.90 ± 1.491
T5	79.60 ± 0.802
T6	82.20 ± 1.433
T7	85.20 ± 0.829
T8	85.60 ± 0.506
T9	88.00 ± 4.442
T10	4,860 ± 0.000

T1: RF-70 °C 5 min, **T2:** RF-70 °C 7 min, **T3:** RF-70 °C 10 min, **T4:** RF-80°C 5 min, **T5:** RF-80 °C 7 min, **T6:** RF-80 °C 10 min, **T7:** RF-90 °C 5 min, **T8:** RF-90 °C 7 min**, T9:** RF-90 °C 10 min, **T10:** CD-90 °C 30 min
RF: Radio frequency heating treatment**, CD:** Convectional drying

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