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Effect of Moist Heat Sterilization on Phytochemical Content, Anti-Oxidant Property and Microbial Sterility of Black Rice Bran

Noppawat Pengkumsri[1], Chaiyavat Chaiyasut[1], Bhagavathi Sundaram Sivamaruthi[1], Sasithorn Sirilun[1], Sartjin Peerajan[2], Prasit Suwannalert[3], Khontaros Chaiyasut[2] and Periyanaina Kesika[1]*

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.
[2]Health Innovation Institute, Chiang Mai 50230, Thailand.
[3]Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

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Rice bran (RB) contains a variety of bioactive components with pharmaceutical values. Black RB (BRB) is rich in phytochemical content. The sterilization process affects the quality of the RB bioactive constituents. The aim of this study was to evaluate content and activity of phytochemicals of BRB after moist heat sterilization. BRB samples were subjected to moist heat at different temperature and time followed by polar and non-polar extractions. The yield of BRB oil was not significantly affected by sterilization. The sterility of treated BRB was evaluated by plate count method. BRB treated at 90 °C for 15 min showed no detectable level of bacterial load. The total phenolic, flavonoid, anthocyanin, and γ-oryzanol content of the BRB extracts were assessed by colorimetric, and HPLC methods. The total phenolic, flavonoid, anthocyanin, and γ-oryzanol content of the treated BRB were decreased with respect to the increased temperature and time. The antioxidant property of sterilized BRB was reduced compared to the control. The present study also suggested that the moist heat at 90 °C for 15 min is the optimal condition for RB sterilization with negligible loss in quality, which can be further subjected to the detailed pharmacological analysis without any microbial contamination.

Keywords: Sterilization, Black rice bran, Phytochemical content, Anti-oxidant property.

Rice bran (RB) is a derivative of the rice milling process, constitutes about 10% of rice grain[1, 2]. RB contains a variety of bioactive components with antioxidant, anti-inflammatory, and chemopreventive activity that includes tocopherols, tocotrienols, γ-oryzanol, phytosterols, ferulic acid, caffeic acid, tricin, coumaric acid, phytic acid, and carotenoids[3]. Most of the above-mentioned phytochemicals are recognized as bioactive compounds, which improves the human health and well-being. Based on the composition of RB, the color of the rice differs and categorized as red, purple, and black rice[4]. Black rice is rich in anthocyanin pigments, phenolic compounds, protein, and vitamins. Thailand is one of the leading black rice cultivating countries.

Although rice and RB are enriched with many biologically active compounds, the activity is affected mostly during different processing like thermal treatments. For example, total sterols, total tocopherols, and γ-oryzanol in RB oil was decreased under high thermal treatments (180 °C), and cause loss of the oxidative stability of RB oil[5]. Additionally, hot air and microwave treated RB oil showed the low acid, and peroxide value. Also, retained the total phenolic compounds of RB oil. The RB oil treated with high temperature for the short period resisting the high content of γ-

* To whom all correspondence should be addressed.
Tel.: +6653944341; Fax: +6653894163;
E-mail: p.kesiaka@gmail.com

oryzanol[6]. The heat and pressure based pretreatments diminish the anthocyanins in the black rice[7]. The subcritical water extraction of defatted RB pre-treated with microwave showed relatively high antioxidant activity than untreated samples[8]. The heat treated various rice cultivars indicated that the temperature effectively preserves the phenolic components and antioxidant activity[9]. But there is no detailed information about the impact of general moist heat sterilization on the stability and sterility of the RB. Thus, the current study was carried out to assess the impact of moist heat sterilization on the stability, and sterility of black RB with respect to the total phenolic compounds, γ-oryzanol content, and antioxidant property.

MATERIALS AND METHODS

Rice bran preparation

Black rice was collected from cultivation farm at Maerim district, Chiang Mai, Thailand and was dried in hot air oven at 60 °C for 48 h. Fresh rice bran was obtained by milling. Then, black rice bran (BRB) was sieved through a 60-mesh strainer and stored at -20 °C until use.

Sterilization process

About 25 g of BRB was subjected to heat by autoclaving at 50 °C (30, 60, 90, and 120 min at 2 psi), 70 °C (30, 60, 90, and 120 min at 4 psi), 90 °C (15, 30, 45, and 60 min at 8 psi), and 121 °C (5, 10, 15, and 20 min at 15 psi). The samples were further evaluated for the microbial load and phytochemical content.

Evaluation of microbial load

The sterilized BRB was assessed for its sterility by total plate count technique. Briefly, 1 g of sterilized BRB was mixed with 10 ml of sterile 0.85% sodium chloride solution (saline). Then sample (1 ml) was plated by pour plate method with plate count agar (9.0 g/l agar, 1.0 g/l dextrose, 5.0 g/l tryptone, 2.5 g/l yeast extract). The plates were incubated at 37 °C for three days. After incubation, plates were examined for the microbial growth.

Phytochemical extraction

Equal quantity of the sterilized BRB samples were subjected to polar and non-polar solvent extraction as detailed in our previous studies[10, 11]. Briefly, 10 g of BRB was extracted by using 100 ml of 0.1 N hydrochloric acid in ethanol for polar extraction, and 10 g of BRB was extracted by 100 ml of hexane and shaked with 150 rpm at 40 °C for 30 min (three times) for non-polar extraction. The extracted solution was filtrated through 0.45 µm membrane and evaporated under reduced pressure at 60 °C.

Phytochemical determination

The total phenolic, total flavonoid, total anthocyanin, γ-oryzanol content of the BRB extracts were assessed as detailed in our previous studies[10, 11] by colorimetric, and HPLC methods.

Determination of Antioxidant Activity

The changes in the antioxidant properties of sterilized BRB was determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH), ABTS, Ferric reducing antioxidant power (FRAP), lipid peroxidation inhibition assay[10, 11].

Statistical analysis

The quantification of total phenolic, total flavonoid, total anthocyanin, and γ-oryzanol content and determination of antioxidant activities were performed in triplicates. All the values are given as mean ± SD. Analysis of variance (ANOVA) was performed using statistical SPSS software version 17 (Chicago, SPSS Inc, U.S.A). The Least Significant Difference (LSD) post hoc test was performed to analyze the significant differences in antioxidant activities and $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The BRB samples were treated at different temperature and period followed by the extraction of the polar and non-polar parts as detailed in the materials and methods section. The yield of BRB oil was not significantly affected by the pre-treatments, whereas, a slight increase in the yield was observed in all the procedures with a low exposure time (Table 1). The sterility of the treated samples were assessed, and the results were tabulated (Table 2). Obviously, high temperature and less time, and low temperature and extended time effectively sterilize the BRB. About 10^6, and 10^5 CFU/ml of sample was observed after 30 min of treatment at 50, and 70 °C, respectively. Whereas, detectable level of bacterial load was not observed in the samples treated at 90 °C for 15 min. Decreased bacterial load (10^4 CFU/ml of sample) was observed

in the samples treated at 120 °C for 5 min compared to the control samples. In all the case, the control samples displayed about 10^8 CFU/ml of sample (Table 2).

The changes in the phytochemical content of BRB after different treatments were represented in Table 3. The results clearly suggested that the extended time and increased temperature affects the phytochemical nature of BRB with respect to total phenolic, total flavonoids, and total anthocyanin contents (represented as mg Gallic acid equivalent (GAE)/g of bran, µg Quercetin equivalent (GE)/g of bran, and mg Cyanidin chloride equivalent (CCE)/g of bran, respectively). The tested phytochemicals in the BRB treated at 50, 70 °C for 15 min, and 90 °C for 15 min was not drastically reduced compared to other sterilization conditions (Table 3). A study reported that microwave and hot air heating were the effective technique for the stabilization of RB, which exhibited high content of total phenolic compounds[6].

The g-oryzanol content of the BRB was not affected significantly by any of the studied treatment procedures (Table 4). Even though after the treatment at 90 °C for 15 min, BRB samples showed relatively high content of γ-oryzanol (2231 ± 111.25 µg/g of bran) compared to other treatments. High content of g-oryzanol was reported to be observed in the hot air heating compared to the microwave and roasting processes[6]. A study reported that microwave (30 s) treated RB showed significantly increased content of α-tocopherol, α-tocotrienol, γ-tocotrienol, and total vitamin E, whereas, microwave treatments with longer heating degrades the vitamin E content of RB[12].

The antioxidant nature of both polar and non-polar extracts of BRB were assesed by free radical scavenging assays. The results indicated that the moist heat sterilization significantly reduced the antioxidant property of the BRB and the severity of the hindrance depends on the temperature and duration of the exposure (Table 5 and 6). Oil extracted from the rapeseeds pretreated with microwaves increased the yield of phytosterols (15%) and tocopherols (55%)[13]. A study by Kwak *et al*. 2013 revealed that the heat treatment improves the antioxidant property of Korean rice cultivars, *Hongjinjubyeo*, and *Heugkwangbyeo*[9]. The cooking modes like the pressure cooker, rice cooker and on the gas usage promotes the thermal degradation of anthocyanins,

Table 1. The yield of the BRB oil after treatment (autoclave)

Condition	Time (min)	Polar (Mean ± SD)	Non-polar (Mean ± SD)
Control	0	20.15 ± 1.21	13.11 ± 0.75
50 °C	30	21.21 ± 1.27	12.93 ± 0.78
	60	22.00 ± 1.30	13.10 ± 0.67
	90	20.05 ± 1.51	13.13 ± 0.55
	120	20.85 ± 1.34	12.89 ± 0. 87
70 °C	30	21.11 ± 1.27	13.00 ± 0.81
	60	21.00 ± 1.03	13.05 ± 0.79
	90	21.60 ± 1.05	12.85 ± 0.70
	120	20.55 ± 1.21	12.95 ± 0. 68
90 °C	15	22.30 ± 1.25	13.00 ± 0.74
	30	22.50 ± 1.35	12.78 ± 0.82
	45	21.60 ± 1.25	13.08 ± 0.59
	60	21.30 ± 1.30	12.87 ± 0.77
121 °C	5	20.21 ± 1.20	13.50 ± 0.81
	10	20.56 ± 1.35	12.86 ± 0.75
	15	21.15 ± 1.25	12.75 ± 0.70
	20	21.05 ± 1.22	13.22 ± 0.80

Table 2. Microbial load in black rice bran after treatment (autoclave)

S. No.	Time (min)	Temperature 50 °C	Temperature 70 °C	Time (min)	Temperature 90 °C	Time (min)	Temperature 121 °C
1	0 *	+++++	+++++	0 *	+++++	0 *	+++++
2	30	+++	++	15	ND	5	+
3	60	ND	ND	30	ND	10	ND
4	90	ND	ND	45	ND	15	ND
5	120	ND	ND	60	ND	20	ND

Note: * = Control; ND = Not detected; +++++ = 10^8 CFU/ml; +++ = 10^6 CFU/ml; ++ = 10^5 CFU/ml; + = 10^4 CFU/ml

especially cyanidin-3-glucoside and associated production of protocatechuic acid[7]. The microwave pre-treatment (80 °C for 10 min) increased the yield of total phenolic compounds and increased the antioxidant activity of defatted rice bran extract[8].

The current study suggest that the yield of the oil and γ-oryzanol content was not significantly influenced by the pre-treatment. Even though the treatment at 50, 70 °C for 15 min, and 90 °C for 15 min have not drastically affected the phytochemical content in the BRB, only at 90 °C for 15 min treatment showed no detectable level of bacterial load. Therefore this study suggest that sterilization at 90 °C for 15 min is optimum for the BRB in respect to stability, microbial sterility and antioxidant property.

Table 3. The phytochemical changes in rice bran after treatments

Condition	Time (min)	Total phenolic content (mg GAE/g bran)	Total flavonoid content (µg QE/g bran)	Total anthocyanin content (mg CCE/g bran)
Control	0	56.05 ± 4.37[a,b]	300.00 ± 6.10[a,b]	186.87 ± 9.34[a,b]
50 °C	30	54.58 ± 2.91[a,b]	292.12 ± 9.68[a,b]	181.96 ± 10.01[a,b]
	60	50.25 ± 4.70[c]	270.65 ± 8.11[c]	165.19 ± 9.09
	90	46.97 ± 1.23	251.42 ± 8.06	156.61 ± 9.61
	120	41.82 ± 2.76	223.85 ± 7.95	139.44 ± 7.07
70 °C	30	52.74 ± 2.75[a,b,c]	282.28 ± 8.29[b,c]	170.83 ± 9.41[b,c]
	60	45.13 ± 1.06	241.58 ± 7.66	150.38 ± 8.07
	90	44.52 ± 1.01	238.29 ± 8.53	148.43 ± 8.16
	120	42.68 ± 2.84	228.45 ± 8.14	142.30 ± 7.83
90 °C	15	53.72 ± 1.83[a,b,c]	287.53 ± 8.50[a,b,c]	179.10 ± 8.85[a,b,c]
	30	44.77 ± 2.03	239.61 ± 8.21	149.25 ± 8.10
	45	41.82 ± 1.66	223.85 ± 7.95	139.44 ± 7.65
	60	38.39 ± 1.22	205.47 ± 6.22	127.99 ± 7.04
121 °C	5	48.45 ± 2.36	259.30 ± 8.37	161.52 ± 8.08
	10	41.58 ± 1.74	222.54 ± 6.90	138.62 ± 7.02
	15	36.92 ± 1.32	197.59 ± 4.95	123.08 ± 5.77
	20	28.58 ± 1.57	152.95 ± 5.12	95.28 ± 6.04

Note: Values are mean ± SD; [a, b, c] statistically differed at 95% confident interval.

Table 4. The changes in the γ-oryzanol concentration of rice bran after treatments

Condition	Time (min)	γ-oryzanol (µg/g bran)
Control	0	2297.07 ± 116.14
50 °C	30	2245.72 ± 112.47
	60	2231.05 ± 113.69
	90	2204.16 ± 103.91
	120	2193.15 ± 99.02
70 °C	30	2229.83 ± 111.25
	60	2223.72 ± 99.02
	90	2145.48 ± 108.80
	120	2129.58 ± 117.36
90 °C	15	2231.05 ± 111.25
	30	2200.49 ± 97.80
	45	2145.48 ± 105.13
	60	2121.03 ± 105.13
121 °C	5	2205.38 ± 121.03
	10	2184.60 ± 108.80
	15	2139.36 ± 107.58
	20	2096.58 ± 106.36

CONCLUSION

In the present study, the yield of the oil was not significantly affected by the pre-treatments. In conclusion, the current study revealed that the moist heat at different temperature and duration relatively stabilizes the γ-oryzanol content and reduces the content of bioactive compounds in both polar and non-polar extracts. The study also suggested that the moist heat at 90 °C for 15 min is the optimal condition for the sterilization of RB, which can be further subjected to the detailed pharmacological analysis without any microbial contamination.

Table 5. The *in vitro* antioxidant activity of polar part of treated black rice bran

Condition	Time (min)	ABTS assay (IC_{50}) (mg of RB)	DPPH assay (IC_{50}) (mg of RB)	FRAP assay mg eq $FeSO_4$/g Bran	Lipid peroxidation assay (IC_{50}) (mg of RB)
Control	0	1.62 ± 0.07[a]	0.38 ± 0.01[a]	8.31 ± 0.32[a]	2.68 ± 0.12[a]
50 °C	30	1.66 ± 0.08[a,b]	0.39 ± 0.01[a,b]	8.10 ± 0.35[a,b]	2.75 ± 0.15[a,b]
	60	1.80 ± 0.06[c]	0.42 ± 0.01[c]	7.45 ± 0.30	2.99 ± 0.13[c]
	90	1.93 ± 0.07	0.45 ± 0.01	6.97 ± 0.33	3.20 ± 0.14
	120	2.17 ± 0.08	0.50 ± 0.02	6.20 ± 0.25	3.59 ± 0.16
70 °C	30	1.72 ± 0.09[a,b]	0.40 ± 0.01[a,b,c]	7.82 ± 0.32[b,c]	2.85 ± 0.12[a,b]
	60	2.01 ± 0.05	0.47 ± 0.01	6.69 ± 0.30	3.33 ± 0.11
	90	2.04 ± 0.08	0.47 ± 0.01	6.60 ± 0.27	3.38 ± 0.13
	120	2.12 ± 0.06	0.49 ± 0.01	6.33 ± 0.28	3.52 ± 0.16
90 °C	15	1.69 ± 0.05[a,b,c]	0.39 ± 0.01[a,b]	7.97 ± 0.33[a,b,c]	2.80 ± 0.11[a,b,c]
	30	2.02 ± 0.06	0.47 ± 0.01	6.64 ± 0.24	3.36 ± 0.13
	45	2.17 ± 0.08	0.50 ± 0.02	6.20 ± 0.26	3.59 ± 0.12
	60	2.36 ± 0.09	0.55 ± 0.02	5.69 ± 0.19	3.92 ± 0.15
121 °C	5	1.87 ± 0.04	0.43 ± 0.01	7.19 ± 0.26	3.10 ± 0.16
	10	2.18 ± 0.07	0.51 ± 0.02	6.17 ± 0.20	3.62 ± 0.14
	15	2.45 ± 0.05	0.57 ± 0.02	5.48 ± 0.25	4.07 ± 0.20
	20	3.17 ± 0.08	0.74 ± 0.02	4.24 ± 0.19	5.26 ± 0.19

Note: Values are mean ± SD; [a, b, c] statistically differed at 95% confident interval.

Table 6. The *in vitro* activity of non-polar part (γ-oryzanol) of treated black rice bran

Condition	Time (min)	ABTS assay (IC_{50}) (µg of RB)	DPPH assay (IC_{50}) (mg of RB)	FRAP assay mg eq $FeSO_4$/g Bran	Lipid peroxidation assay (IC_{50}) (mg of RB)
Control	-	305.28 ± 2.29[a]	8.67 ± 0.41	3.00 ± 0.15	5.48 ± 0.25
50 °C	30	312.26 ± 2.37[b]	8.87 ± 0.42	2.93 ± 0.16	5.61 ± 0.25
	60	314.31 ± 2.34[b,c]	8.93 ± 0.42	2.91 ± 0.15	5.64 ± 0.25
	90	318.15 ± 2.56[c]	9.04 ± 0.46	2.88 ± 0.17	5.71 ± 0.27
	120	319.74 ± 2.69	9.08 ± 0.48	2.86 ± 0.18	5.74 ± 0.29
70 °C	30	314.48 ± 2.39[b]	8.93 ± 0.43	2.91 ± 0.16	5.65 ± 0.26
	60	315.35 ± 2.69[b]	8.96 ± 0.48	2.90 ± 0.18	5.66 ± 0.29
	90	326.85 ± 2.44	9.28 ± 0.44	2.80 ± 0.16	5.87 ± 0.26
	120	329.29 ± 2.27	9.35 ± 0.40	2.78 ± 0.15	5.91 ± 0.24
90 °C	15	314.31 ± 2.39[b,c]	8.93 ± 0.43	2.91 ± 0.16	5.64 ± 0.26
	30	318.68 ± 2.72	9.05 ± 0.49	2.87 ± 0.18	5.72 ± 0.29
	45	326.85 ± 2.53	9.28 ± 0.45	2.80 ± 0.17	5.87 ± 0.27
	60	330.61 ± 2.53	9.39 ± 0.45	2.77 ± 0.17	5.94 ± 0.27
121 °C	5	317.97 ± 2.20[c]	9.03 ± 0.39	2.88 ± 0.14	5.71 ± 0.24
	10	320.99 ± 2.44	9.12 ± 0.44	2.85 ± 0.16	5.76 ± 0.26
	15	327.78 ± 2.47	9.31 ± 0.44	2.79 ± 0.16	5.88 ± 0.26
	20	334.47 ± 2.50	9.50 ± 0.45	2.74 ± 0.16	6.00 ± 0.27

Note: Values are mean ± SD; [a, b, c] statistically differed at 95% confident interval.

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