Effect of Heat Pretreatment on the Functional Constituents of Rice Germ

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Abstract

Changes in functional constituents of rice germ prepared using three different heat pretreatments: roasting, steaming and microwave heating, were determined and compared with those of non-treated rice germ. The yield of rice germ oil increased generally and then decreased with increasing time for all three heat pretreatments, although the yields of rice germ oil varied among the three heat pretreatments. There were no major differences in fatty acid compositions among the rice germ oils subjected to the three different heat pretreatments. Levels of α -tocopherol in rice germ oil increased up to about 1.5 times at 3 min of roasting and microwave heating, compared to control, and then decreased with increasing treatment time, but α tocopherol concentrations in rice germ oil gradually decreased with increasing steaming time. The contents of three phytosterols (β-sitosterol, stigmasterol and ergosterol) decreased progressively with increases in roasting and steaming time, while concentrations of the three phytosterols increased up to $\sim 15\%$ with 3 min of microwave process as compared to control, and then decreased thereafter. Levels of γ -oryzanol in rice germ oil decreased gradually with increasing time during all three different heat pretreatments. However, levels of γ -aminobutyric acid (GABA) in rice germ decreased gradually with increasing roasting time, while those of GABA increased greatly up to about 2 times after 10 min of steaming process, and then decreased slowly thereafter. During microwave heating, the contents of GABA increased at 3 min of treatment time and then decreased. These results suggest that microwave heating may be the most suitable processing method to preserve functional constituents in rice germ.

Key words: rice germ, functional constituents, roasting, steaming, microwave

INTRODUCTION

Rice germ is obtained as a by-product of the rice milling process, and is also produced by sieving and vibrating the rice bran (1). Rice germ accounts for 20% or more of the weight of rice bran which represents only about 8% of the paddy weight (2).

Rice germ is rich in unsaturated fatty acids such as linoleic acid and linolenic acid, as well as in several functional constituents including γ -aminobutyric acid (GABA), tocol derivatives (tocopherols+tocotrienols), γ -oryzanol and phytosterols (3-6). Therefore, rice germ is receiving a renewed interest as a source of dietary supplement ingredients. However, it is somewhat difficult to produce high quality of rice germ because it contains relatively high levels of a very active lipase which, once the germ is removed from the kernel during milling, promotes rapid hydrolysis of the oil to free fatty acids (7). Therefore, economical and effective heat treatments such as roasting, steaming, and microwave,

and extrusion have been developed for inactivating lipase in rice bran immediately after milling (8).

Heat pretreatments are known to alter the chemical composition and concentrations of bioactive components in several grain seeds (9-17). Shin et al. (10) reported that levels of tocol derivatives and oryzanol in rice bran oil produced by extrusion decreased progressively with increased temperature. Lane et al. (11) reported that heat pretreatment of rice bran, such as roasting and microwave heating, increased the levels of tocol derivatives and oryzanols. In contrast, Kim et al. (14) and Ko et al. (15) reported that levels of α -tocopherol in rice germ oil gradually increased with an increase in roasting temperature and time, while there was no significant differences in γ -oryzanol levels of rice germ oils prepared at different roasting temperatures and times. Additionally, some researchers have reported that roasting of grain seeds caused a temperature-dependent increase in the levels of some functional constituents but a decrease in other phytochemicals (12-15). Thus, the heat pretreatment has inconsistent effects on chemical composition of grains and varies according to the kinds of grains, pretreatment methods, and processing temperature and time. Study on the effects of heat pretreatment in the functional constituents of rice germ is still very limited.

The purpose of present study was to investigate changes in fatty acid compositions and other functional constituents, such as tocopherols, phytosterols, γ -oryzanol, and γ -aminobutyric acid (GABA) of rice germ prepared with three different heat pretreatments: roasting, steaming and microwave heating.

MATERIALS AND METHODS

Materials

Rice (*Oryzae sativa* L., cultivar: Junam) germ used in this study was obtained directly from Haenong Food, Pohang, Gyeongbuk, Korea. Free fatty acids, α -tocopherol, three phytosterols (campesterol, stigmasterol, β -itosterol), and γ -oryzanol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). HPLC solvents were obtained from Merck (Darmstadt, Germany). All other reagents used for this study were analytical grade.

Sample preparation

Rice germ (50 g) was roasted in an electric roaster (Dongkwang, Korea) with constant stirring at 200°C for 1, 3, 5 and 8 min. Meanwhile, the sample (50 g) was steamed at 90~100°C in a domestic stainless steel steamer [dimensions 260 (W)×200 mm (H)] for 10, 20, 30, 40, and 60 min. Finally, the sample (50 g) was placed in a rotating glass container (dimensions 290 mm i.d.) in the center of domestic microwave (MW) oven (Samsung RE-C200T, at a frequency of 2450 MHz, pulsed variable MW power output from 90 to 700 W which was controlled by a timer, inner volume 21.8 L) and heated for 1, 3 and 5 min. The pretreated rice germs were dried for 2 hr in a drying oven at 50°C before analysis of phytochemical constituents.

Preparation of rice germ oil

The dried rice germ (10 g) was extracted twice with CHCl₃-MeOH (2:1, v/v, 100 mL) for 2 hr in an ultrasonic cleaner (Bransonic 5210R-DTH, USA) at room temperature, filtered and evaporated under a reduced pressure. The concentrated sample was redissolved again in *n*-hexane (10 mL) and filtered through a Whatman GF/A glass fiber filter (Whatman Laboratory Products, Clifton, NL, USA) to remove particles, and evaporated *in vacuo* to yield oil.

Analysis of fatty acid composition

Fatty acid composition of rice germ was determined by gas chromatography, as previously described (18). Oils obtained above were esterified by H₂SO₄ in MeOH, and methyl esters of fatty acids were extracted with hexane. The aliquots (1 µL) of the extracts were injected into a gas chromatograph (GC, Hewlett-Packard 6890 series, USA) equipped with a FID.

Analysis of tocopherols

Quantification of four tocopherol isomers in rice germ oil was performed as previously described (18). Rice germ oil (0.1 g) was solubilized in hexane (10 mL) and passed through a 0.45 μ m syringe filter (25 mm, 0.2 μ m, PTFE, Whatman, Clifton, NJ, USA) and finally injected into a liquid chromatograph (LC, Younglin Acme, Seoul, Korea).

Analysis of phytosterols

Quantification of three phytosterols in rice germ oil was conducted as previously described (18). Rice germ oil (0.1 g) was saponified by 2 N KOH in EtOH, purified and then injected into GC.

Analysis of γ -oryzanol

 γ -Oryzanol in rice germ was quantified by HPLC. Rice germ oil (0.1 g) was solubilized in hexane (10 mL) and passed through a PTFE syringe filter (25 mm 0.2 μ m, Whatman, Clifton, NJ, USA) and evaporated under a reduced pressure. The concentrated sample was solubilized in *n*-hexane and injected into an LC to quantify the γ -oryzanol. The LC system consisted of an HPLC system (Younglin Acme, Seoul, Korea), injector with a 10 μ L sample loop and a UV detector (Younglin Absorbance, Seoul, Korea) set at 290 nm. A LiChrosorb DIOL column (5 μ m, 3×100 mm, Merck Co., Chrompack, Palo Alto, CA, USA) was used. The separation was conducted using a linear gradient from solvent A (*n*-hexane:acetic acid=1000:1, v/v) and solvent B (2-propanol) at 1.0 mL/min for 30 min.

Analysis of γ -aminobutyric acid (GABA)

 γ -Aminobutyric acid of rice germ was quantified by an automated amino acid (AA) analyzer using the method of Saikusa et al. (19). Rice germ (10 g) was extracted twice with 8% trichloroacetic acid (100 mL) for 2 hr in an ultrasonic cleaner (Bransonic 5210R-DTH, USA) at 40°C, filtered and brought up to 100 mL volume. The aliquots (20 mL) of the extracts were evaporated and solubilized in 0.2 M lithium citrate buffer (pH 2.8, 2 mL). The solution was passed through a 0.45 μ m membrane filter (Gelman, Ann Arbor, MI, USA), diluted and then injected into an HPLC for quantification of GABA. AA analysis was performed on an HPLC system (Biochrom 30, Biochrom Ltd, UK) equipped with a photo detector (440 nm & 570 nm), EZ-Chrom Elite software and MIDAS autosampler (Spark Holland BV, Netherland) with a 40

µL loop. HPLC analysis was carried out using a U-1631 column (4.6 I.D.×200 mm, Biochrom, Cambridge, UK) at a flow rate of 0.4 mL/min, and a UV detector set at 280 nm. A mobile phase eluted gradiently from solvent 1 to solvent 6 (solvent 1:0.2 M lithium citrate buffer, pH 2.8, solvent 5:1.65 M lithium citrate buffer, pH 3.55, solvent 6:0.3 M lithium hydroxide) for 50 min.

Statistical analysis

For quantitative analysis of functional constituents in rice germs, each heat pretreatment was repeated twice with duplicate samples, and the data presented are means ±standard deviations.

RESULTS AND DISCUSSION

Yield of oil from heat-pretreated rice germs

The yields (%) of rice germ oils prepared by three different heat pretreatments, such as roasting, steaming and microwave heating, are shown in Table 1. The yield of rice germ oils increased progressively up to total ~ 20%, $\sim 17\%$, and $\sim 26\%$ for 3 min, 40 min, and 3 min of roasting, steaming and microwave heating, respectively, as compared to control, and then decreased thereafter. The microwave pretreatment of rice germ was superior to the other two forms of heat pretreatment for yield of oils. Thus, there were small differences in oil yield between the three heat pretreatments. A pleasant aroma or taste developed in the rice germ during roasting processing for 5 min and microwave treatment for 3 min, but roasting and microwave processing for 10 min and 5 min, respectively, resulted in the production of extensive charring, and very low yields (<5%) of rice germ oil (data not shown). Additionally, the yield of rice

Table 1. Yield of rice germ oil prepared by three different heat pretreatments

Heat treatment	Time (min)	Yield (%, rice germ)
Control (no heat treatment)	0	19.5
	1	$22.8\pm1.2^{1}(116.9)^{2}$
Doggting	3	$23.3\pm1.3\ (119.5)$
Roasting	5	$22.5\pm0.9\ (115.4)$
	8	21.8 ± 0.8 (111.8)
	10	19.0±1.1 (97.4)
	20	22.3 ± 1.3 (114.4)
Steaming	30	22.5 ± 1.2 (115.4)
· ·	40	22.8 ± 1.0 (116.9)
	60	21.3±0.9 (109.2)
	1	21.8±1.0 (111.8)
Microwave	3	$24.5\pm1.2\ (125.6)$
	5	17.0±0.9 (87.2)

¹⁾Values are mean ± SD of duplicate analyses.

germ oil from "Junam" paddy cultivar used in this study was higher to somewhat higher than that of rice germ from "Dongjin" cultivar, indicating that the yield of oil from rice germs could be different among cultivars (20).

FA composition

Changes in level of fatty acid compositions of rice germs resulting from the three different heat pretreatments are shown in Table 2. Rice germ oil (non-treated) consisted of 0.22% myristic acid, 21.70% palmitic acid, 0.02% palmitoleic acid, 1.80% stearic acid, 32.11% oleic acid, 42.48% linoleic acid, and 1.66% linolenic acid. There was no significant effects of the three different heat pretreatments on the fatty acid composition of rice germ oils. A similar trend was observed in the fatty acid

Table 2. Changes in fatty acid composition of rice germ oils prepared by three different heat pretreatments

	Time	Fatty acids (Mol %)						
Treatment	(min)	Myristic acid (C _{14:0})	Palmitic acid (C _{16:0})	Palmitoleic acid (C _{16:1})	Stearic acid (C _{18:0})	Oleic acid (C _{18:1})	Linoleic acid (C _{18:2})	Linolenic acid (C _{18:3})
Control	0	0.22±0.01 ¹⁾	21.70±0.82	0.02 ± 0.00	1.80 ± 0.12	32.11±1.21	42.48±1.32	1.66±0.11
Roasting	1 3 5 8	0.20±0.04 0.20±0.07 0.22±0.02 0.22±0.01	21.85±0.62 21.69±1.01 21.86±0.92 21.68±0.40	0.03 ± 0.01 0.02 ± 0.01 0.02 ± 0.00 0.02 ± 0.01	1.84±0.09 1.80±0.10 1.81±0.13 1.78±0.05	32.32±1.32 32.06±1.03 32.37±1.33 32.06±1.23	42.07±1.43 42.48±1.20 42.04±1.74 42.51±1.64	1.69±0.10 1.75±0.10 1.68±0.10 1.73±0.03
Steaming	10 20 30 40 60	0.20±0.02 0.20±0.01 0.20±0.03 0.20±0.03 0.20±0.02	21.77±0.42 21.61±0.52 21.82±0.41 21.85±0.65 21.57±0.45	0.02±0.00 0.02±0.01 0.02±0.00 0.02±0.01 0.02±0.00	1.79 ± 0.01 1.80 ± 0.03 1.84 ± 0.04 1.90 ± 0.04 1.84 ± 0.05	32.07 ± 1.20 32.08 ± 1.02 32.33 ± 1.03 32.27 ± 1.31 32.13 ± 1.42	42.44±1.34 42.58±1.35 42.14±1.53 42.11±1.03 42.56±1.37	1.71±0.09 1.71±0.10 1.65±0.03 1.65±0.06 1.68±0.10
Microwave	1 3 5	0.16±0.01 0.20±0.03 0.17±0.01	21.80±0.33 21.81±0.52 21.78±0.41	0.01±0.00 0.02±0.01 0.02±0.00	1.86±0.10 1.73±0.04 1.81±0.05	32.15±1.25 31.92±1.27 32.35±1.32	42.44±1.42 42.57±1.52 42.31±1.05	1.57±0.04 1.75±0.09 1.56±0.11

¹⁾Values are mean \pm SD of duplicate analyses.

²⁾% change against control.

composition of corn fiber (13), rice germ (14), and grape seed (18) oils during the heat pretreatments by roasting and microwave processes.

Tocopherol composition

Changes in the concentrations of four tocopherol isomers in rice germ oils prepared by three different heat pretreatmetns are shown in Table 3. Among the four tocopherol isomers, only α -tocopherol was detected in any of the rice germ oils after heat processing. The content of α -tocopherol in rice germ oil gradually increased with increased roasting time, reached a maximum (0.18%) at 3 min, and then decreased slowly. However, the levels of α -tocopherol in rice germ oil gradually decreased with increased steaming time. With microwave heating, the concentration of α -tocopherol in rice germ oil greatly

Table 3. Changes in α -tocopherol concentrations of rice germ oil prepared by three different heat pretreatments

Treatment	Time (min)	α -Tocopherol (%, rice germ oil)
Control	0	$0.12\pm0.02^{1)}$
	1	$0.14 \pm 0.07 \ (116.7)^2$
Roasting	3	$0.18 \pm 0.06 \ (150.0)$
Roasing	5	$0.17 \pm 0.07 \ (141.7)$
	8	$0.16 \pm 0.03 \ (133.3)$
	10	0.09 ± 0.01 (75.0)
	20	0.05 ± 0.01 (41.7)
Steaming	30	0.03 ± 0.00 (25.0)
	40	$0.02 \pm 0.00 \ (16.7)$
	60	0.02 ± 0.01 (16.7)
	1	0.14±0.03 (116.7)
Microwave	3	$0.19 \pm 0.05 \ (158.3)$
	5	$0.13 \pm 0.02 \ (108.3)$

¹⁾Values are mean ± SD of duplicate analyses.

increased at 3 min, and then decreased thereafter. Thus, roasting and microwave heating caused increases in α -tocopherol concentrations, while steaming caused a considerable decrease in α -tocopherol concentrations. This finding supports an earlier report (14) that the content of α - tocopherol in rice germ oil gradually increased with increased roasting temperature and time. Contrary to the previous study, the three other tocopherol isomers except for α -tocopherol in rice germ oils were not detected, possibly because the UV detector used in this study was less sentitive than the fluorescence detector used in that study (14).

Phytosterol composition

Changes in concentrations of three phytosterols in rice germ oil after the three different heat pretreatmetns are shown in Table 4. Three phytosterol derivatives, campesterol, stigmasterol and β -sitosterol, were identified, of which β -sitosterol was the major phytosterol component.

Rice germ oil (non-treated) had 0.223% campesterol, 0.075% stigmasterol, and 0.449% β -sitosterol. During roasting and steaming processes, concentrations of the three phytosterols progressively decreased up to totaling ~24% and ~7%, respectively, as compared to the control. However, during microwave heating, concentrations of all three phytosterols gradually increased up to ~15% at 3 min, and then decreased rapidly. Moreau et al. (13) offered a possible explanation for the heat-induced decrease in the levels of free phytosterols in corn fiber oil, suggesting that free phytosterols evaporate easily under vacuum and high temperature due to their low boiling points. Therefore, the microwave heating is considered to be a good heat pretreatment to retain or enhance the

Table 4. Changes in phytosterols concentrations of rice germ oils prepared by three different heat pretreatments

Treatment	Time	Phytosterols (%, rice germ oil)				
	(min)	Campesterol	Stigmasterol	β-Sitosterol	Total sterol ¹⁾	
Control	0	$0.223\pm0.042^{2)}$	0.075±0.017	0.449±0.084	0.747±0.051	
D	1	0.150±0.050	0.061±0.026	0.332±0.062	$0.673\pm0.049 (90.1)^{3}$	
	3	0.067 ± 0.011	0.051 ± 0.031	0.229 ± 0.034	0.340 ± 0.031 (46.5)	
Roasting	5	0.063 ± 0.010	0.029 ± 0.010	0.174 ± 0.021	0.266 ± 0.016 (35.6)	
	8	0.046 ± 0.012	0.014 ± 0.005	0.118 ± 0.019	0.178 ± 0.012 (23.8)	
	10	0.081±0.038	0.017±0.003	0.125±0.019	0.223±0.026 (29.9)	
	20	0.051 ± 0.007	0.014 ± 0.002	0.123 ± 0.021	0.188 ± 0.017 (25.2)	
Steaming	30	0.044 ± 0.008	0.013 ± 0.002	0.095 ± 0.019	0.152 ± 0.010 (20.3)	
	40	0.033 ± 0.007	$ND^{4)}$	0.036 ± 0.009	0.069 ± 0.010 (9.2)	
	60	0.017 ± 0.005	ND	0.034 ± 0.010	0.051 ± 0.002 (6.8)	
	1	0.233±0.061	0.085±0.011	0.491±0.057	0.809±0.049 (108.3)	
Microwave	3	0.242 ± 0.072	0.097 ± 0.012	0.523 ± 0.061	0.862±0.056 (115.4)	
	5	0.018 ± 0.002	0.011 ± 0.004	0.030 ± 0.006	0.348 ± 0.012 (46.6)	

¹⁾Campesterol + stigmasterol + β -sitosterol.

²⁾change against control.

²⁾Values are mean ± SD of duplicate analyses.

^{3)%} change against control.

⁴⁾Not detected.

Table 5. Changes in γ -oryzanol and γ -aminobutyric acid concentrations of rice germ oils prepared by three different heat pretreatments

Treatment	Time (min)	γ -Oryzanol (%, rice germ oil)	GABA ¹⁾ (mg%, rice germ)	
Control	0	$0.554 \pm 0.034^{2)}$	398.69 ± 12.73	
Roasting	1	$0.463 \pm 0.029 \ (83.6)^{3)}$	385.53 ± 28.34 (96.7)	
	3	$0.449 \pm 0.021 (81.1)$	$374.56 \pm 21.82 (94.0)$	
	5	$0.418 \pm 0.037 (75.5)$	338.79 ± 19.34 (85.0)	
	8	$0.411 \pm 0.031 (74.2)$	329.10 ± 18.83 (82.5)	
Steaming	10	$0.321 \pm 0.028 \ (57.9)$	892.54±31.52 (223.9)	
	20	$0.310 \pm 0.023 (56.0)$	$789.85 \pm 30.82 \ (198.1)$	
	30	0.282 ± 0.017 (50.9)	$716.03 \pm 29.32 \ (179.6)$	
	40	$0.272 \pm 0.021 \ (49.1)$	$605.71 \pm 18.23 \ (151.9)$	
	60	$0.270 \pm 0.018 \ (48.7)$	$520.26 \pm 20.73 \ (130.5)$	
Microwave	1	0.503 ± 0.041 (90.8)	$423.02 \pm 18.73 \ (106.1)$	
	3	$0.473 \pm 0.024 (85.4)$	$446.93 \pm 19.27 \ (112.1)$	
	5	$0.451 \pm 0.027 \ (81.4)$	346.52 ± 10.36 (86.9)	

 $[\]frac{1}{\gamma}$ -Aminobutyric acid.

3)% change against control.

levels of valuable cholesterol-lowering phytosterol components (21).

γ -Oryzanol and γ -aminobutyric acid composition

Changes in the concentrations of γ -oryzanol and γ -aminobutyric acid (GABA) in rice germ following the three heat pretreatmetns are shown in Table 5. Rice germ oil (non-treated) contained 0.554% γ -oryzanol and 398.69 mg% GABA. The γ -oryzanol in rice germ oil gradually decreased with all three heat pretreatments as heating time increased, with a more rapid loss from the steaming process than from roasting and microwave heating.

There are large differences in the change of GABA content among three heat pretreatments. During roasting process, GABA content gradually decreased up to a total of $\sim 82.5\%$ at 8 min of roasting time. In contrast, the steaming rice germ unexpectedly increased the levels of GABA up to 893 mg% at the early stage (10 min) and then it decreased slowly until at 60 min when it retained about 520 mg%. However, during microwave heating, the levels of GABA in rice germ gradually increased with time, reached a peak (447 mg%) at 3 min, and then decreased rapidly. Thus, the roasting process caused a modest decrease in levels of GABA in rice germ, while steaming and microwave heating caused considerable increases in GABA concentrations. This observation supports previous reports that the accumulation of GABA in the rice germ occurred during water soaking (22), and the increase of chemical constituents occurred in germinated seeds (moisture content; 31~50%) during microwave treatment (17). Thus, microwave pretreatment exerts considerable influence on chemical and physicochemical characteristics of rice (23), and is also very

effective for extracting phytochemical constituents which are covalent-bonded with other chemical components such as lipid and protein in rice germ (24).

In conclusion, the three heat pretreatments, such as roasting, steaming and microwave heating, have pronounced effects on yield and chemical compositions of rice germ. In particular, a mild microwave heating effectively increased the levels of valuable functional constituents (fatty acids, α -tocopherol, phytosterols and GABA) of rice germ, except for γ -oryzanol, as compared to the other two heat pretreatments. Therefore, the microwave heating is regarded as an alternative drying step for producing high quality of rice germ oils and products to conventional methods including roasting and steaming processes. Further study is required to investigate the optimum conditions to integrate microwave heating into an industrial process. This study was the first to report the changes in functional constituents in rice germ by three different heat pretreatments: roasting, steaming and microwave processes.

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