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Stabilization of Rice Bran by Microwave Energy

Joon S. Rhee and Heeny H. N. Yoon[1]
Korea Advanced Institute of Science and Technology, Seoul 131, Korea

마이크로파 에너지에 의한 쌀겨의 안정화

이준식 · 윤희남[1]
한국과학기술원

Abstract

Initial moisture content and weight of the rice bran and treatment time were identified as important variables for the inactivation of lipase and peroxidase present in rice bran. Multiple regression analysis was used to obtain a prediction equation to measure the effects of moisture content and weight of the sample and microwave treatment time on the residual lipase activity and loss in weight. It was found that the microwave treatment did not affect acid value and extractability of the rice bran oil.

Introduction

Rice bran is a valuable by-product of rice milling industry which can be used as a source of edible oil of good quality and the defatted rice bran is used as an animal feed. However, due to the presence of various enzymes such as lipase, lipoxygenase and peroxidase, quality of the rice bran is very rapidly deteriorated resulting in the development of off-flavor and high content of free fatty acid. Therefore, it is of utmost importance to inactivate these enzymes after the milling for securing an edible oil of good quality.

Studies on the inactivation of the enzymes in rice bran have been relatively meager. Kopeikovski et al.[1] reported that maximal inactivation of lipase was accomplished by moisturizing rice bran to 19-21% moisture content, followed by heating to 105-108°C. Cheigh et al.[3] developed a extrusion technique to inactivate the peroxidase in the rice bran and they reported that the extruded rice bran was very stable.

In the mean time, no work has been done on the application of microwave energy for inactivation of the enzymes present in rice bran, although some studies related to the application of microwave heating to the inactivation of various enzymes in other foods and/or biological materials[4-7]. Copson[4] reported that pectinmethyl esterase in orange juice concentrate was completely inactivated when exposed to microwave energy at a frequency of 2,450*MHz*. Takashima[5] studied the effect of microwave energy on alcohol dehydrogenase and DNA. He found that the activity of the enzyme and DNA structure were not altered even by the prolonged irradiation at high field intensity. The studies of Eapen et al.[6] with whole rapeseed showed the effectiveness of a shorter microwave treatment in inactivating the enzyme, thioglucoside glucohydrolase. He reported not only inactivating effect of the enzyme, but also its adverse effect on the quality of the oil and the meal. Maheshwari et al.[7] studied the effect of moisture content on the activity of thioglucoside glucohydrolase in dehulled rapeseed exposed to microwave energy at 2,450 *MHz*. They reported that the sample with an initial moisture content of 7% required a longer exposure period for the enzyme inactivation than the samples with the initial moisture content of 10% or 13%.

We have investigated various conditions related to microwave irradiation to inactivate the lipase and perox-

[1] Cheil Sugar Co., Seoul 150-01, Korea

idase of rice bran for stabilization of the rice bran.

Materials and Methods

Rice bran used for this study was a product of milling process for *Japonica* variety rice and obtained from a local mill. Upon arrival at our laboratory, the rice bran was sieved through 36 mesh screen and was double-packaged in polyethylene film bags (thickness 0.08*mm*) and then the double packaged rice bran was stored in a cold room (-18 °C) until used.

Moisture control

Lots of original rice bran (10.4% initial moisture content) were adjusted to moisture content of 12.3, 15.1, 17.8 and 19.7. The moisture content was adjusted by spraying with distilled water. After addition of the water, the samples were equilibrated at 0-4 °C for 24 hrs.

Microwave treatment

The samples in Pyrex glass beakers were exposed to irradiation in a microwave oven (Toshiba ER-1017, 1*KW*, 2,450*MHz*) for a predetermined period. Before the microwave treatment, the equilibrated samples were brought to 21°C and magnetron was prewarmed for 2 hr. Samples were placed exactly in the center of the microwave oven for the irradration. The power (watt) was determined by heating 1*l* of distilled water in a 1,000*ml* Pyrex glass beaker for 2 *min* and multiplying the increase in temperature (°C) by 34.5 or by actual calculation. The power input with high output button ranged from 965 to 975*W*, and the power input with middle output button ranged from 480 to 485*W*.

Peroxidase activity assay[8]

15*g* of rice bran was suspended in 100*ml* of 0.1*M* phosphate buffer (pH 7.0). After vigorous mechanical stirring with a magnetic bar for 3 hr, the suspension was filtered through cheese cloth and the turbid solution was centrifuged for 30 *min* at 7,000 *rpm*. The supernatant obtained from the centrifugation was used for peroxidase activity assay. The peroxidase activity was assayed by continuous reading of absorbancy of reaction mixture consisted of 2*ml* of 0.1*M* phosphate buffer (pH 7.0), 0.3*ml* of 0.5*mM* guaiacol solution, 0.3*ml* of the enzyme solution and 0.3*ml* of 0.5*mM* H_2O_2 solution at 470*nm* by using Beckman Spectrophotometer Model 35.

The peroxidase activity was calculated as follows;

$$A_p = \frac{\Delta E}{\Delta t}$$

where A_p is the peroxidase activity, E is the change of the absorbancy at 470*nm* and t is the time interval (sec).

Lipase activity assay[9,10]

15*g* of rice bran was defatted three times with petroleum ether. The defatted rice bran was then suspended in 50*ml* of 0.01*M* $CaCl_2$ solution. After vigorous mechanical stirring with a magnetic bar for 3 hr, the suspension was filtered through cheese cloth and the turbid solution was centrifuged for 30 *min* at 7,000 *rpm*. The supernatant obtained from the centrifugation was used for lipase activity assay. The lipase activity was assayed by adding 5*ml* of the substrate mixture consisted or 20*ml* tributyrin, 1*ml* of Tween 60 and 90*ml* of distilled water to enzyme solution consisted of 15*ml* of 0.05*M* KCl, 5*ml* of 0.005*M* $CaCl_2$ and 5*ml* of the enzyme solution (the supernatant). The final volume of the reaction mixture was made up to 50*ml* with deionized water.

The enzyme reaction was carried out at pH 7.5 and at 35°C with constant stirring. The increase of free fatty acid (FFA) produced from the enzymatic hydrolysis was monitored by titrating with 0.005 *N* NaOH with a pH stat (Radiometer Type TTT-IC). Unit of lipase activity was defined as the number of micro-equivalents of alkali required per minute per gram of dry rice bran to titrate FFA released at pH 7.5 and at 35°C.

Table 1. Definition and levels of independent variables

Independent variables	Symbols	Levels				
		-1.682	-1	0	1	1.682
Moisture content (%)	X_1	10.4	12.3	15.1	17.8	19.7
Sample weight (g)	X_2	50	90.5	150	209.5	250
Treatment time (sec)	X_3	20	28	40	52	60

Experimental design

Combinations of three independent variables (moisture content (X_1), sample weight (X_2), and treatment time (X_3), each at five different levels, were studied as listed in Table 1. A centrol composite experimental design, as described by Cochran and Cox[11], was used to estimate regression coefficients (B_i, and B_{ii}, B_{ij}) according to the model given as follows;

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2$$

where Y is dependent variables (residual lipase activity (Y_1) and loss in weight (Y_2) and B_i, B_{ii} and B_{ij} are coded regression coefficients for corresponding independent variables and their interaction terms. Twenty trials of microwave treatment were performed for residual lipase activity and loss in weight, consisting of a complete 2 factorials (8 trials), 6 points outside of the cubic surface to measure response curvature (6 trials) and 6 replicate center points to estimate experimental errors (6 trials). Statistical significance of the coefficients was determined by the t-test.

$$t = \frac{B}{S.D.}$$

where S.D. is standard deviation and B is regression coefficients (B_i, B_{ii} or B_{ij}).

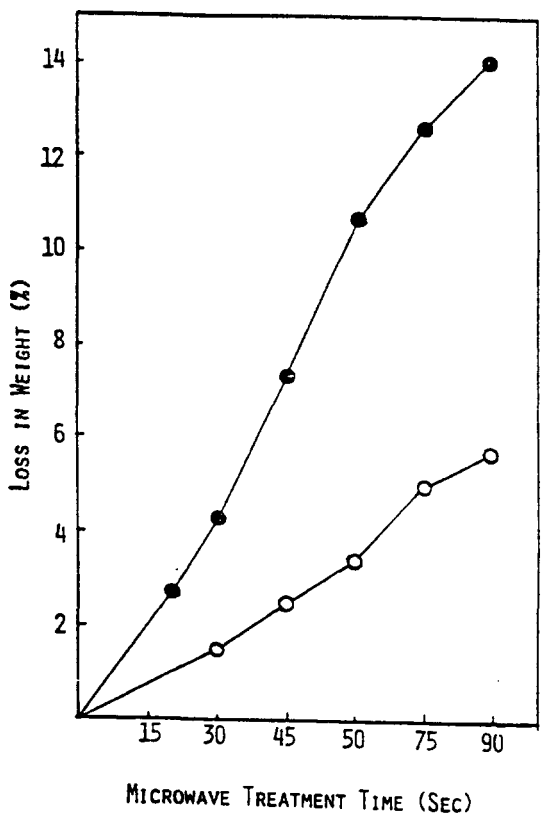

Fig. 1. Effec of initial moisture content on loss in weight at various initial moisture content (50g of sample was irradiated with power input of 970w)
●—● ; 19.3% of moisture content,
○—○ ; 10.4% of moisture content.

Results and Discussion

Exposure of the rice bran to the microwave irradiation resulted in extremely rapid heating and loss of weight from the samples. Experimental results for the loss in weight from the samples with initial moisture contents of 10.4 and 19.7% are presented in Fig. 1. The result shows that the weight of the sample with 19.7% initial moisture content was reduced much faster than the weight of the sample with 10.4% initial moisture content, indicating that the rate of microwave heating is increased with the increase of initial moisture content. This result is in a good agreement with the report of Maheshwari et al.[12] on model soy system and dehulled

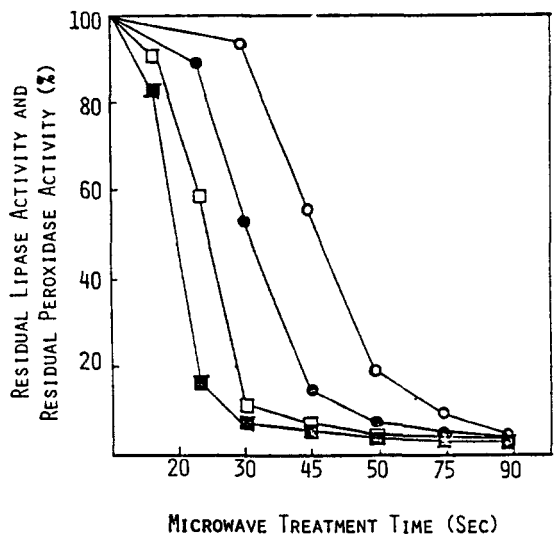

Fig. 2. Effect of microwave treatment time on residual lipase and residual peroxidase activities at various initial moisture contents (50g of sample was irradiated with power input of 970w)
Residual peroxidase activity : □—□ ; 19.3% of moisture content, ○—○ ; 10.4% of moisture content, Residual lipase actity : ■—■ ; 19.3% of moisture content, ●—● ; 10.4% of moisture content.

rapeseed and demonstrates the importance of preconditing for effective adsorption of microwave energy by the samples during such treatment. Effect of the initial moisture content on residual lipase activity and residual peroxidase activity is shown in Fig. 2. Both enzyme in the sample with 10.4% initial moisture content were much more resistant than those in the sample with 19.7% initial moisture content. However, as the treatment time increases, the differences in the residual activities for both enzymes becomes smaller and eventually there were no differences at treatment time of 90 *sec.* The results also indicates that peroxidase is more resistant than lipase when exposed to the microwave energy.

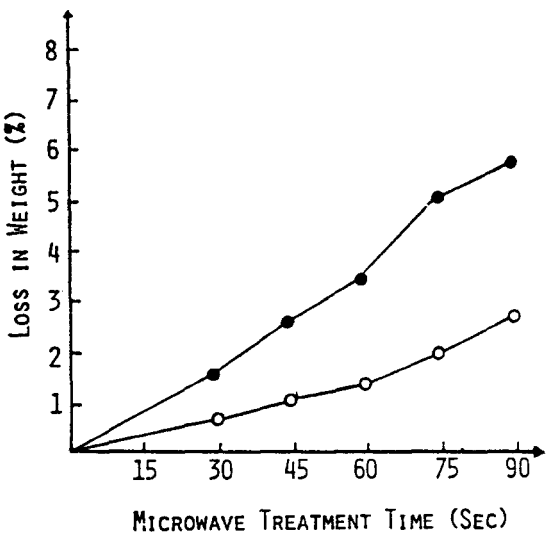

Fig. 3. Effect of microwave treatment time on loss in weight with power input of 480 W and 970 W (50 g of sample (10.4% of initial moisture content was irradiated)
●—● ;power input of 970 W, ○—○ ;power input of 480 W.

The effect of power input on loss in weight and residual activities for both lipase and peroxidase are presented in Fig. 3 and 4, respectively. Fig. 3 shows that the loss is weight with microwave power input of 970 *W* and 480 *W* were almost linearly proportional to the microwave treatment time and the loss in weight with microwave power input of 970 *W* was double that with microwave power input of 480 *W*. Fig. 4 shows that the microwave power input of 970 *W* inactivated both peroxidase and lipase faster than the microwave power input of 480 *W* as expected. The results also confirms the fact that peroxidase is more resistant than the lipase as shown in

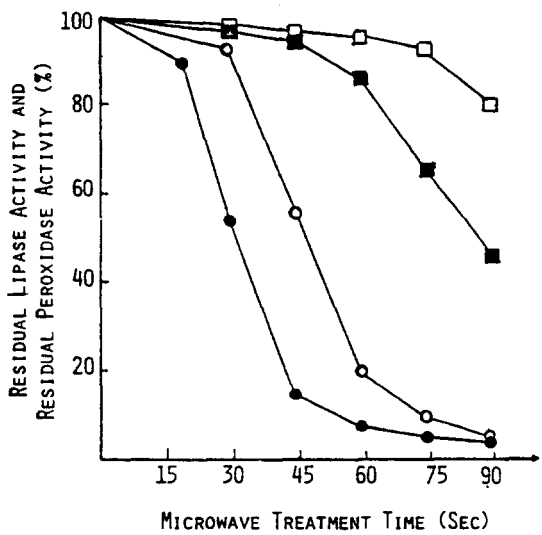

Fig. 4. Effect of microwave treatment time on residual lipase activity and residual peroxidase activity with power input of 480 W and 970 W (50 g of sample (10.4% of initial moisture content) was irradiated)
Residual peroxidase activity : □—□ ; power input of 480 W, ○—○ ; power input of 970 W, Residual lipase activity : ■—■ ; power input of 480 W, ●—● ; power input of 970 W.

Fig. 2. Experimental result for the effect of sample weight on the residual lipase activity is presented in Fig. 5. The results indicates that as the sample

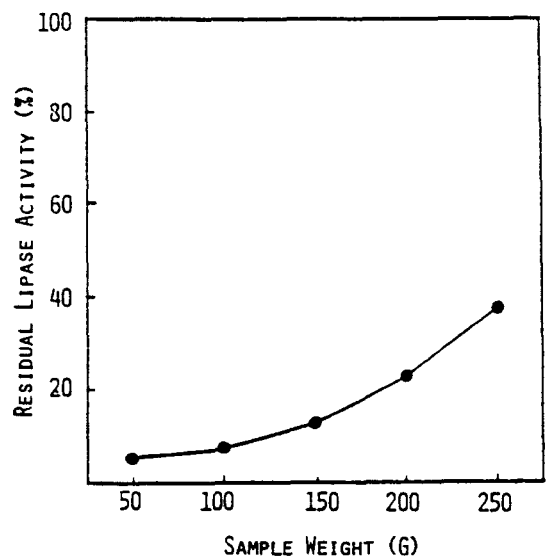

Fig. 5. Effect of sample weight on residual lipase activity (Sample (10.4% of moisture content) was irradiated for 60 sec with power input of 970 W)

Table 2. Effect of independent variable combination upon two dependent variables

Trial No.	Independent variables			Dependent variables	
	X_1	X_2	X_3	Residual lipase activity (%)	Loss in weight (g)
1	-1	-1	-1	59.7	0.65
2	1	-1	-1	22.4	1.41
3	-1	1	-1	80.8	1.17
4	1	1	-1	45.0	2.42
5	-1	-1	1	5.6	2.83
6	1	-1	1	1.0	5.02
7	-1	1	1	27.5	3.44
8	1	1	1	16.5	5.75
9	-1.682	0	0	49.0	0.88
10	1.682	0	0	6.8	3.18
11	0	-1.682	0	7.9	2.38
12	0	1.682	0	45.2	3.20
13	0	0	-1.682	83.8	0.89
14	0	0	1.682	13.3	5.09
15	0	0	0	37.1	3.67
16	0	0	0	38.4	3.37
17	0	0	0	35.8	3.32
18	0	0	0	36.6	3.41
19	0	0	0	37.9	3.50
20	0	0	0	39.4	3.52

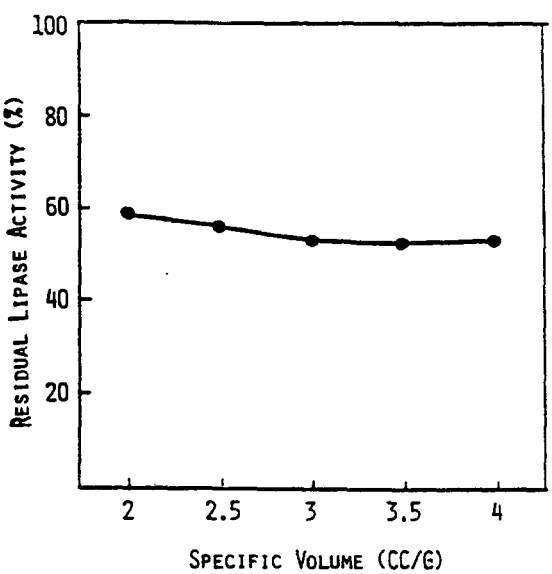

Fig. 6. Effect of specific volume of sample on residual lipase activity (50 g of sample (10.4 % of moisture content) was irradiated for 30 sec with power input of 970 W)

weight increased, the residual lipase activity increased non-linearly, possibly due to the insufficient energy input. Fig. 6 shows that the apparent specific volume of the sample did not affect the residual lipase activity. From all of the results mentioned above, it can be concluded that moisture content and weight of the sample, and microwave treatment time had a great effect on loss in weight and residual activity for both peroxidase and lipase, whereas the effect of specific volume of the sample was almost negligible.

Raw experimental data for two dependent variables (Y) obtained from 20 combinations of independent variables (X) are tabulated in Table 2. Analysis of variance (ANOVA) summaries are given in Table 3 for two dependent variables. Coefficients of determination give the correlation between predicted responses and observed responses. Thus the higher the coefficient of determination, the better fits the mathematical model equation for the variation observed for the dependent variables. Regression coefficients and their statistical

Table 3. Definition and analysis of variance (ANOVA) summaries of two dependent variables

Dependent variable	Description	Coefficient of determination (R^2)	Mean square from regression	Mean square error	$F_{9,10}$	Probaility
Y_1	Residual lipase activity (%)	0.996	1118.80	3.982	280.9	0.01
Y_2	Loss in weight (g)	0.984	4.233	0.061	69.4	0.01

significance for two dependent variables are given in Table 4. Regression coefficients are the least square estimates of the B terms in model equation. It is important to note that the coefficients are in terms of coded levels of independent variables, i.e., -1.682, -1.0, 0, 1.0, 1.682 (see Table 1). Predictions of expected values of dependent variables may be calculated by substituting approximate values of coded independent variables and regression coefficients into the model equaton. The three independent variables—that is, moisture content (X_1), sample weight (X_2) and microwave treatment time (X_3)—significantly affected residual lipase activity (Y_1) and loss of weight (Y_2) of the rice bran samples. The negative regression coefficient of -11.69 for moisture content (X_1) and of -20.2 for microwave treatment time X_3) indicate that adjusting rice bran samples to higher moisture content and exposure for a longer time resulted in a less residual lipase activity. On the other hand, the positive regression coefficient of 10.53 for sample weight (X_2) indicates that increasing sample weight resulted in a more residual lipase activity.

Table 4. Regression analysis of two dependent variables

Model term	Regression coefficient (B_{ii})	
	Y_1 (residual lipase activity)	Y_2 (loss in weight)
Constant	37.60**	3.466**
X_1 (moisture content)	-11.69**	0.760**
X_2 (sample weight)	10.53**	0.311**
X_3 (microwave treatment time)	-20.20**	1.351**
X_1X_2	-0.61	0.076
X_1X_3	7.19**	0.311**
X_2X_3	-0.79	-0.024
X_1^2	-3.79**	-0.448**
X_2^2	-4.28**	-0.179
X_3^2	3.51**	-0.108

** $P < 0.01$

Table 5 shows residual lipase activity, moisture content, acid value and extractability of the initial storage test samples. The results indicate that microwave treatment did not change the acid value and extractability of the rice bran samples.

요 약

쌀겨에 존재하는 지방산 분해효소와 페르옥시다아제를 불활성화 함에 있어서 쌀겨의 초기 수분함량 및 무게와 마이크로파 처리 시간이 중요한 변수들임이 실험결과 알려졌으며, 이들 변수가 지방질 분해효소의 불활성화와 처리중의 쌀겨의 무게 감소에 미치는 영향을 측정하기 위한 추정 방정식을 구하기 위하여 다중 회귀분석법을 사용하였다. 또한 마이크로파 공정을 거친 쌀겨로부터 기름을 추출한 경우 이 기름의 산값과 추출율에는 아무런 변화가 없음을 발견하였다.

Table 5. Residual lipase activity, moisture content, acid value and extractability

Test sample	Residual lipase activity (%)	Moisture content (%)	Lipid content (%)	Acid value	Extractability[c] (%)
Control	100	10.4	22.0	19.2	97.2
Sample #1[a]	45.3	8.5	–	19.1	96.7
Sample #2[b]	6.4	7.5	–	19.3	97.1

a) Microwave irradiated for 55 sec with 970 W.
b) Microwave irradiated for 75 sec with 970 W.
c) Percentage relative to total soxhlet extractable oil.

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