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Changes in Free Amino Acids by Lipid Deterioration in the Biological System of Rice Bran

Yeong-Ok Song, Hong-Sik Cheigh† and Jae-Hyeong Pyeun*

Dept. of Food Science and Nutrition, Pusan National University, Pusan 609-735, Korea
**Dept. of Nutrition and Food Science, National Fisheries University of Pusan, Pusan 608-737, Korea*

Abstract

The damaging effect of peroxidized lipid on amino acid was studied in rice bran by determining the free amino acid content in a biological system. The content of free amino acid in the rice bran stored in the controlled atomsphere of 35 ℃ with relative humidity of 65% for 180 days, increased during the first 60 days of storage, and then decreased as the lipid peroxidation proceeded. The content of free amino acid in the sample exposed to the air of 25-30 ℃ with relative humidity of 70-90% for 100 days decreased rapidly in the beginning period of the storage. The lipid oxidation developed much faster in the rice bran exposed to the air than in the rice bran stored in the controlled atmosphere. The correlation coefficients between the total content of free amino acid and degree of peroxidation for the samples of both conditions were above −0.8, which is significant($p<0.05$). The changes in the concentration of serine, glutamic acid, proline, methionine, lysine, histidine, and arginine were significantly correlated with the degree of lipid oxidation($p<0.05$) for the samples stored in the controlled atmosphere and the open air. It was observed that peroxidized lipid has damaging effects on protein in the biological system of rice bran.

Key words : rice bran, lipid oxidation, free amino acid, degradation and interaction, storage

INTRODUCTION

It is well known that amino acid and protein can interact with peroxidized products of lipid oxidation[1]. Lipid and protein interact mainly through the free radical interaction between peroxidized lipid and reactive residue of the amino acid or protein. Production of protein or amino acid radical by the peroxidized lipid have been confirmed by Electron Spin Resonance experiment[2, 3]. The proposed mechanisms of these reactions are proteinprotein polymerization, protein-lipid adduct formation, protein scission, and amino acid destruction. Lipidamino acid interactions have been manifested through phenomena such as the antioxidant effect of amino acids[4, 5], the production of browning pigment by Maillard reactions[6-8], and the decrease of amino acid in enzyme or protein[9-11]. The antioxidant effects of amino acid may be accounted for by the covalent linkage of the hyd-roperoxide radical of lipid with the electrons from nitrogen in the amino acid during lipid-amino acid adduct formation[4, 6]. Yong and Karel[8] reported that hexanal, an important product of lipid oxidation, seemed to react with histidine to form a Schiff's base which leads to the browning. Lysine, histidine, methionine, tryptophan, glycine, proline and serine are the major amino acids known to be involved in the browning reaction[12, 13]. The amino radicals liable to the peroxidized lipids are -SH (cystein), -OH(serine, threonine, tyrosine), =NH (histidine), and $-SCH_3$(methionine)[14].

† To whom all correspondence should be addressed

Most of the studies of the effects of peroxidized lipid on the deterioration of protein have been performed in the model systems[4-11]. Recently, the damaging effects of peroxidized lipid on the protein in the rice bran have been studied in the biological[15] and model system[16]. The purpose of this research is to study the lipid-protein interaction in the biological system by determining the free amino acid contents in the rice bran at various stages of lipid oxidation.

MATERIALS AND METHODS

Sample preparation

Fresh rice bran of the Dong-jin variety grown on the Kim-hae plain was obtained from a local miller at two different times. Two groups of sample were prepared in the same manner reported in reference[15]. One group of sample was stored in an atmosphere controlled to have a limited amount of oxygen at 35℃ in order to enhance the lipid hydrolysis, and the other group was exposed to the air to enhance peroxidation as well as the lipid hydrolysis.

Lipid analysis

Total lipid was extracted with 10 volume of n-hexane for 15 hours. Peroxide and carbonyl value were assayed by using the methods of AOCS Cd 8-53[17] and Henick[18], respectively.

Determination of free amino acid

Meal portion of the rice bran extracted with n-hexane was used for free amino acid determination. Defatted rice bran was homogenized for 5 minutes with 10 volumes of 70% ethanol. Sample was refluxed at 80℃ for 15 minutes and then centrifuged at 14,000×g for 20 minutes. Residue portion of the sample was washed twice with 5 volumes of 70% ethanol before being centrifuged. The water layer was measured up to 100ml of solution with distilled water after ethanol was evaporated by vacuum evaporator. Sulfsalicilic acid (5%, w/v) was added to the sample solution to remove non-amino acid materials. Sample was kept in cold room(4℃) for an hour and then centrifuged(14,000×g). The supernatant was evaporated completely, and then was measured up to 10ml with lithium citrate buffer of pH 2.2. Free amino acid was analyzed by amino acid autoanalyzer (L.K.B. 4150, England).

Statistical analysis

Correlation coefficients between the concentration of free amino acid and the degree of lipid oxidation at various stage of storage were analyzed by Pearson's R test[19] and the significance was determined at the level of 0.05.

RESULTS AND DISCUSSION

Lipid deterioration of rice bran during storage

The peroxide and carbonyl values of the rice bran stored in the controlled atmosphere of RH 65% changed slowly, while corresponding values of the rice bran exposed to air changed drastically (Figs. 1 and 2). This suggests that production of the primary and secondary products of oxidized lipid depends on the amount of oxygen in the system, since oxygen reduces the activation energy for the formation of hydroperoxide[20].

Changes in the contents of free amino acid in rice bran stored in the controlled atmosphere

The contents of most of the amino acids increased during the first 60 days of storage and began to decrease afterwards. The content of free amino acid increased up to 135% during 60 days (Table 1). This may indicate the presence of protease in the rice bran. The decrease in the contents of free amino acid was significant when lipid peroxidation reached to a considerable degree during the storage (Fig. 1). The amount of histidine and glutamic acid decreased most significantly during storage. Their contents decreased to 92% and 89% of their initial values, respectively, in 180 days of storage. Changes of methionine, proline and lysine were also significant, losing as much as 50% of their initial contents. The decrease in the content of free amino acid during storage may be due to the transformation of the structure of native protein into an insoluble form by the lipid protein interaction. The destruction of amino acid by reactive free radicals of oxidized lipid, such as hydroperoxide and carbonyl compounds, can also contribute to the reduction of free amino acid. Song

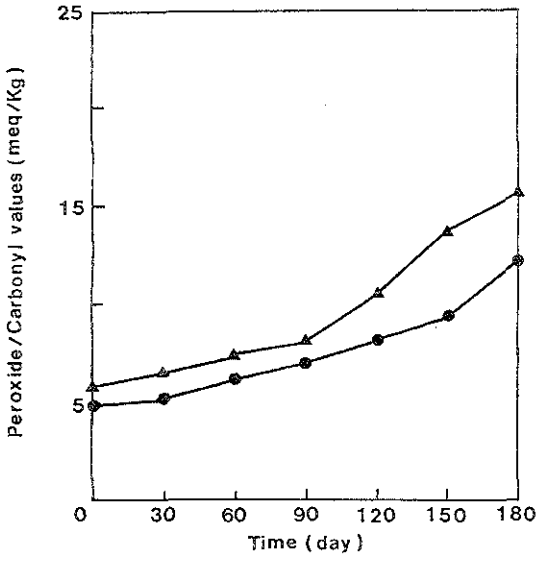

Fig. 1. Lipid oxidation pattern of rice bran stored in controlled atmosphere for 180 days.
Rice bran was stored at 35℃ with limited amount of oxygen where relative humidity was controlled with sat. lithium acetate solution to be 65%.
▲–▲ Peroxide value ●–● Carbonyl value

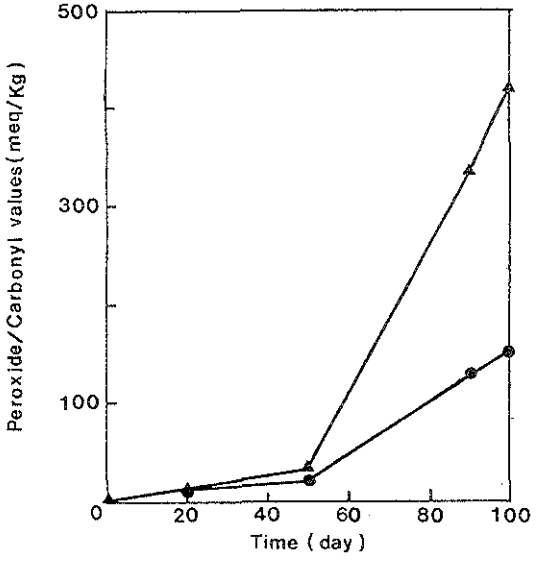

Fig. 2. Lipid oxidation pattern of rice bran exposed to air for 100 days.
Rice bran was left open in air where the temperature was 25-30℃ and the relative humidity was 70-90%.
▲–▲ Peroxide value, ●–● Carbonyl value

Table 1. Changes in the concentration of major free amino acid of rice bran stored in controlled atmosphere for 180 days†

(mg/100g sample)

Amino acid	Storage time, days						
	0	30	60	90	120	150	180
Threonine	42.04	54.14	100.64	60.21	40.29	29.64	34.29
Serine	12.82	15.29	17.71	12.10	10.81	9.17	9.27
Glutamic acid	175.58	133.80	152.14	60.48	50.12	43.57	37.84
Proline	8.32	13.49	13.07	13.10	8.00	5.36	4.90
Glycine	3.78	10.89	13.65	10.46	7.30	5.60	5.13
Alanine	20.41	28.27	47.22	30.21	18.09	13.01	15.71
Valine	3.72	6.42	8.41	5.60	4.50	3.86	5.06
Methionine	2.11	3.22	2.77	1.80	1.69	1.56	1.10
Isoleucine	1.65	2.91	3.59	2.36	2.22	2.01	1.89
Leucine	2.73	4.78	9.30	5.20	2.54	2.93	2.44
Tyrosine	4.46	7.24	8.26	6.17	5.20	4.02	4.06
Phenylalanine	3.34	4.67	6.99	4.00	3.89	2.62	2.69
Lysine	5.10	6.94	6.08	4.44	3.00	2.78	2.20
Histidine	5.38	5.98	3.94	1.46	0.81	0.71	0.43
Arginine	17.30	24.64	22.10	20.08	15.82	12.17	13.49
Total	308.74	322.68	415.87	237.67	174.28	139.01	138.50

†See the footnote of Fig. 1.

and Cheigh[16] analyzed the amino acids in the protein isolate of rice bran using the method of enzyme hydrolysis, and found that the content of most of amino acids in the protein isolate of rice bran decreased by more than 90% during interaction with the oxidized rice bran oil of peroxide value 1200 meq/kg. Decrease in the proteolytic activity of rice bran protease by the primary and secondary products of peroxidized rice bran oil was also observed.

Table 2. Correlation coefficient between the content of free amino acid and degree of lipid oxidation of rice bran stored in controlled atmosphere for 180 days†

Amino acid	Peroxide value	Carbonyl value
Threonine	-0.516	-0.480
Serine	-0.756*	-0.746
Glutamic acid	-0.818*	-0.838*
Proline	-0.792*	-0.741
Glycine	-0.432	-0.403
Alanine	-0.578	-0.537
Valine	-0.563	-0.562
Methionine	-0.780*	-0.817*
Isoleucine	-0.377	-0.383
Leucine	-0.439	-0.428
Tyrosine	-0.612	-0.586
Phenylalanine	-0.556	-0.541
Lysine	-0.868*	-0.873*
Histidine	-0.831*	-0.853*
Arginine	-0.801*	-0.756*
Total amino acids	-0.815*	-0.805*

†See the footnote of Fig. 1.
*Changes in the concentration of free amino acid were significantly correlated with the degree of lipid peroxidation ($p<0.05$)

The correlation coeffecients between the degree of lipid oxidation and the content of free amino acid are presented in Table 2. Changes in the content of serine, glutamic acid, proline, methion-ine, lysine, histidine, arginine and the degree of peroxidation during storage were found signifi-cantly correlated ($p<0.05$). This suggests that $-\varepsilon$, $-OH$, and $-NH$ groups of amino acid are very reactive to the lipid oxidation[14]. It was observed that the damaging effect of peroxidized lipid was serious even in cases where the degree of lipid oxidation was not so significant.

Changes in free amino acid in the rice bran exposed to air

The initial content of free amino acids in the rice bran used in this study was 263.60 mg%, which was slightly less than the value observed in the sample stored in the controlled atmosphere of 35℃. The concentration of amino acid began to decrease gradually as soon as the rice bran was exposed to air (Table 3). Losses in free amino acid became more significant as the lipid oxidation progressed

Table 3. Changes in the content of major free amino acid in rice bran exposed to air for days†

(mg/100g sample)

Amino acid	Storage time, days				
	0	20	50	90	100
Threonine	22.00	23.48	12.39	10.48	-*
Serine	10.10	12.00	12.00	-	-
Glutamic acid	157.50	156.36	37.18	0.09	-
Proline	7.80	9.23	8.17	5.12	4.04
Glycine	3.20	-	-	-	-
Alanine	20.30	34.45	12.27	-	-
Valine	3.90	3.09	1.66	0.80	-
Methionine	0.90	0.93	0.42	-	-
Isoleucine	2.30	2.36	1.53	1.30	0.80
Leucine	1.30	0.70	0.21	-	-
Tyrosine	3.10	4.48	3.55	2.21	-
Phenylalanine	2.20	2.22	1.68	1.41	1.02
Lysine	15.50	2.86	2.85	-	-
Histidine	4.50	6.16	1.78	-	-
Arginine	9.00	8.97	6.35	-	-
Total	263.60	267.29	102.04	21.41	6.04

†See the footnote of Fig. 2.
*Not detected

Table 4. Correlation coefficient between the content of free amino acid and degree of lipid oxidation of rice bran exposed to air for 100 days†

Amino acid	Peroxide value	Carbonyl value
Threonine	-0.928*	-0.875*
Serine	-0.977*	-0.977*
Glutamic acid	-0.814	-0.829
Proline	-0.943*	-0.951*
Glycine	-0.343	-0.364
Alanine	-0.846	-0.853
Valine	-0.776	-0.790
Methionine	-0.901*	-0.918*
Isoleucine	-0.901*	-0.918*
Leucine	-0.741	-0.762
Tyrosine	-0.884*	-0.874
Phenylalanine	-0.904*	-0.914*
Lysine	-0.890*	-0.900*
Histidine	-0.836	-0.848
Arginine	-0.969*	-0.979*
Total amino acids	-0.880*	-0.893*

†See the footnote of Fig. 2.
*Changes in the concentration of free amino acid were significantly correlated with the degree of lipid peroxidation ($p<0.05$)

(Fig. 2). Total amount of free amino acid decreased drastically in 100 days. For the first 50 days the content of free amino acid of rice bran exposed to air decreased in contrast to the sample stored in the controlled atmosphere where the content of free amino acids increased. The diff-erence in the degree of lipid oxidation of two samples may account for the degree of destruction of amino acid.

The correlation coefficients between the contents of free amino acids and the degree of peroxidation are shown in the Table 4. It can be seen that lipid peroxidation is strongly related to the decrease of contents of free amino acid. The amount of decrease in contents of the amino acids—threonine, serine, proline, methionine, isoleucine, tyrosine, lysine, and arginine—were found significantly correlated to the peroxide and carbonyl values ($p<0.05$). All of the major amino acids in the rice bran suffered a great loss during the lipid oxidation. It can be inferred that lipid—amino acid interaction might have developed to a considerable degree, which is enough to change the native structure of protein or destroy the amino acid residue so that the free amino acid was not extracted in the buffer solution.

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미강지방질의 산패에 따라 생성된 산화 지방질이 유리아미노산의 변화에 미치는 영향

송영옥 · 최홍식 · 변재형*

부산대학교 식품영양학과
*부산수산대학교 식품영양학과

요 약

상대습도 65%, 온도 35℃의 저장 조건하에서 지방질의 가수분해를 촉진 시킨 미강과 상대습도70-90% 온도 25-30℃의 공기중에 방치하여 가수분해와 산화를 동시에 촉진시킨 미강을 시료로 지방질의 산화에 따라 생성된 산화 생성물이 단백질의 변패에 미치는 영향을 유리아미노산 함량의 경시적인 변화로써 살펴 보았다. 산소농도를 제한한 system에서는 저장초기에 유리아미노산의 함량이 증가하다가 지방질의 산화가 진행되면서 감소하기 시작하였고, 이러한 감소현상은 공기중에 방치한 실험군에서는 저장 초기부터 일어나기 시작하여 산화가 급격하게 진행된 저장 말기에는 그 감소 현상이 현저하였다. 이들 아미노산의 감소와 지방질의 산패정도를 통계처리한 결과, 유리아미노산 중 serine, glutamic acid, proline, methionine, lysine, histidine, 그리고 arginine 등의 함량 감소는 지방질의 산패에 의해 현저한 영향을 받았다($p<0.05$).