KOREAN J. FOOD SCI. TECHNOL.
Vol. 22, No. 5, pp. 596~601 (1990)

Changes in Functional Groups of Protein by Lipid Deterioration in the Biological System of Rice Bran

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Abstract

The effects of peroxidized lipid on the protein in the biological system of rice bran was studied by determining the changes in the content of functional groups under two different storage conditions. One stored at controlled atmosphere of 35 °C with relative humidity 65% and the other one was exposed to the air of 25 °-30 °C with relative humidity 70-90%. The lipid peroxidation started after the lipolysis was almost completed. The autoxidation occurred much faster in the bran exposed to the air than that stored in the controlled atmosphere. Substantial changes in the physiochemical characteristics were observed in all of the major functional groups in both of the samples. The content of sulfhydryl and available lysine decreased as lipid peroxidation progressed. Protease activity was lost almost completely. Protein solubility and *in vitro* digestibility also decreased during storage. The lipid peroxidation and contents of major protein functional groups were significantly correlated ($p<0.05$) and the correlation coefficients were higher than −0.8, for the both of the sample. peroxidized lipid was found to deteriorate protein in the biological system as well.

Key words: peroxidized lipid, biological system, functional groups, protein deterioration, rice bran

Introduction

It has been known that lipid causes various kinds of damages in the biological system during its peroxidation[1]. During autoxidation process, lipids produce hydroperoxides and their secondary products such as carbonyl compounds and low molecule acids. These peroxidized products, having highly reactive free radicals, can interact with protein, amino acid, enzyme, or biologically active compounds and damage the biochemicals in the biological system[2]. The toxicity thus developed can lead to the protein deterioration or other kind of undesirable quality deterioration in foods[3]. The peroxidized lipid interact with the protein mainly through the free radical reactions[2,4-6]. The types of radical reactions that can occur in the lipid-protein interaction are protein-protein cross-links, protein scissions, protein-lipid adduct formations and amino acid destructions[1,2,6,7]. The protein-lipid reactions in the biological system can alter the functional groups of protein. The disulfide produced by the oxidation of reactive sulfhydryl residue in amino acid can cause intermolecular linkage, and this in turn may result in the aggregation of protein[7]. The protein aggregation reduces solubility and digestibility of protein, hence the bioavailability of amino acid in food decreases during interactions.

Rice bran, the outer layers of the rice caryopsis, is an ideal biological model system for the study of lipid-protein interaction, since it is composed of lipase, unsaturated lipid, protein body, and protease. Various kinds of peroxidized lipid-protein interactions can occur in the rice bran during storage. Thus, by studying the rice bran, we can learn how the physiochemical changes of protein affect the nutritive characteristics of proteins and enzyme activities.

The effect of peroxidized lipid on protein degradation has been studied only through the model systems. The purpose of this research is to study the lipid deteriorations and the protein degradations during lipid peroxidation in the biological system of rice bran. Changes in the contents of SH, S-S, available lysine, proteae activity, solubilities and *in vitro* digestibilities of protein in each samples were determined at various stages of lipid oxidation.

Materials and Methods

Sample preparation

Fresh rice bran of the Dong-jin variety, obtained from a local miller located near the Kimhae plain,

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was prepared for the sample within 2 hours after the milling. To prepare the lipid hydrolysis enhanced sample, rice bran was sieved and then put in cheese cloth bag, each weighing 100g for the sample store. Samples were placed on the top of the glass rod bridge of polyethylene bottle where relative humidity of 65% was maintained using saturated lithium acetate solution. The bottle was then sealed air tight with the cellophane tape and stored at the atmosphere of limited amount of oxygen controlled to have temperature of 35 °C. For the lipid hydrolysis and peroxidation enhanced sample, the sieved rice bran was put in polyvinylene plastic boxes and left to the open air. Samples were stirred several times a day in order to enhance the peroxidation.

Lipid analysis

Total lipid was extracted with 10 volume of chloroform: methanol (3:2) for 15 hours. Acid, peroxide, and carbonyl value were assayed by the methods of AOCS Cd 3a-63[8], AOCS Cd 8-53[8], and Henick[9], respectively.

Functional properties of protein determination

Rice bran was defatted with 10 volume of n-hexane for 15 hours. Meal portion obtained after extraction was dried in the air and used as the sample. All experiments were carried out in the cold room (4 °C). Sulfhydryl and disulfide groups of protein were determined spectrophotometically using 5,5'-dithiobis[10]. The content of available lysine in rice bran was determined by the method of Booth[11], the modified version of Carpenter's. For the determination of protein solubility, protein was first extracted with 20 volume of 10 mM phosphate buffer containing 10% NaCl and then fractionationed into the water and salt soluble protein after dialysing against water and 10% NaCl solution, respectively. The protein concentration was determined by the method of Lowry[12]. Proteolytic activity of protease using the casein as the substrate was determined using the method of Kim[13] with the crude enzyme solution obtained from the fractionation of ammonium sulfate. Proteolytic activity was expressed in U(Tyrosine equiv./mg prot./min). The factor used for converting the absorbance of the assay solution to the concentration of tyrosine was 0.41. The modified multienzyme method of Satterlee *et al.*[14] was used to determine *in vitro* protein digestibility. ANRC sodium casein was used as the references protein.

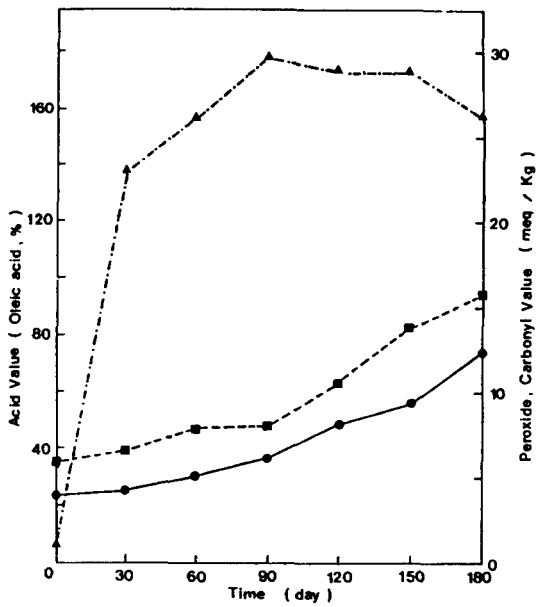

Fig. 1. Lipid oxidation pattern of rice bran stored in controlled atmosphere for 180 days

Rice bran was stored at 35 °C with limited amount of oxygen where relative humidity was controlled with sat. lithium acetate solution to be 65%.

●—● : Carbonyl value, ▲···▲ : Acid value

■···■ : Peroxide value

Statistical analysis

Correlation coefficient between data obtained from the functional group analysis and those from lipid analysis at various stage of storage were analyzed be Pearson's R test and the significance was determined at level of 0.05.

Results and Discussion

Lipid deterioration of rice bran during storage

The acid value of the rice bran which was stored at the controlled atmosphere of 65% relative humidity changed rapidly during the storage, while peroxide and carbonyl value changed slowly (Fig. 1). However, the rate of fatty acid hydrolysis of lipid in rice bran exposed to the air increased at a constant rate, as the lipid peroxidation took place slowly up until the 50th day and then changed drastically afterward (Fig. 1). Lipase seem to play the major

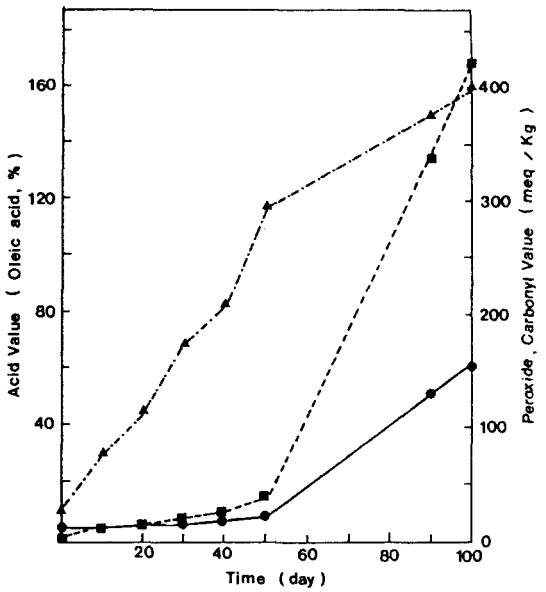

Fig. 2. Lipid oxidation pattern of rice bran exposed to the air for 100 days

Rice bran was left open in the air where temperature was 25-30°C and relative humidity was 70-90%.
▲···▲ : Acid value, ●—● : Carbonyl value
■···■ : Peroxide value

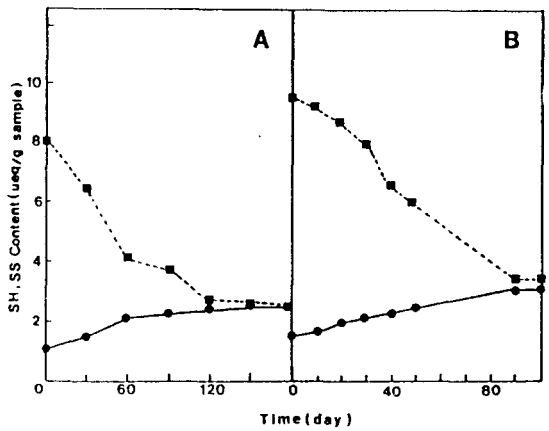

Fig. 3. Changes in sulfhydryl and disulfide content of rice bran

A: Rice bran stored in controlled atmosphere for 180 days. B: Rice bran exposed to the air for 100 days
■ ■ : SH Content, ●—● : SS Content

role in hydrolyzing the free fatty acids in both samples. When sample was exposed to the air, sufficiently enough amount of hydroperoxide might have been produced for the lipid peroxidation contrast to the case where rice bran was stored at the controlled atmosphere where the oxygen was deficient. Oxygen might have reduced the activation energy for the formation of hydroperoxide[15].

Changes in functional groups of protein during storage

The concentration of free sulfhydryl and disulfide in the fresh rice bran were 8.37 ± 0.25 and1.20 ± 0.17u eq per gram sample. The amount of sulfhydryl group decreased while disulfide increased during storage. The highly reactive sulfhydryl group seem to have participated in the reactions. The reaction reached to the equilibrium in 120 days of storage for the sample stored in the controlled atmosphere while the sulfhydryl group started to decrease noticeably at the 40th day of exposure to the air and reached to their equilibria after 90 days (Fig. 3). Higher rate of decrease of sulfhydryl in the sample exposed to the air, compared to the one stored in the controlled atmosphere, is probably due to the sufficient amount of oxygen in the air and the large amount of peroxidized lipid produced. In fact, the decrease of sulfhydryl was significantly correlated to the degree of lipid peroxidation ($p<0.05$).

The changes in the concentration of available lysine during storage are shown in Fig. 4 which shows that it decreases continuously. The rate of decrease in available lysine content was more significant in the sample exposed to the air than in the one stored in the controlled atmosphere. About 22% and 38% losses in concentration were observed respectively in the sample exposed to the air at the 50th and 100th day of storage, in contrast to the loss of 27% in 180 days of storage of rice bran stored in the controlled atmosphere. This high rate of decrease in available lysine might be due to the lipid oxidation occured during storage. ε-Lysine, known as the most liable side chain in amino acids is very reactive with other component. The liability of ε-lysine has been investigated intensively by studying the maillard reaction which is initiated by the sugar-amino condensation. But recent studies found that lipid-amino condensation also can initiate the browning by maillard reaction[16]. It has been reported that aldehydes, especially the malonaldehyde, which are the secondary products of lipid hydroperoxide, can initiate the maillard reaction by forming the Schiff's base with amino group[16,17].

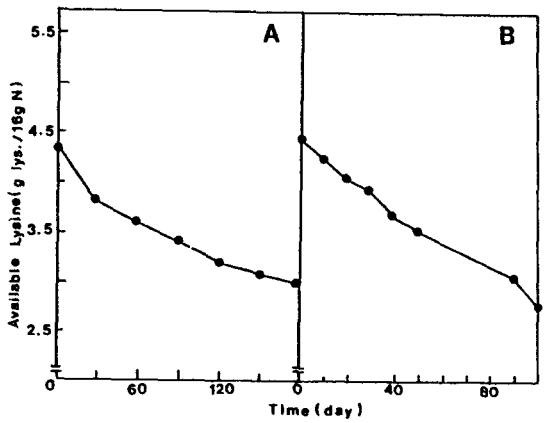

Fig. 4. Changes in available lysine content of rice bran
A: Rice bran stored in controlled atmosphere for 180 days, B: Rice bran exposed to the air for 100 days

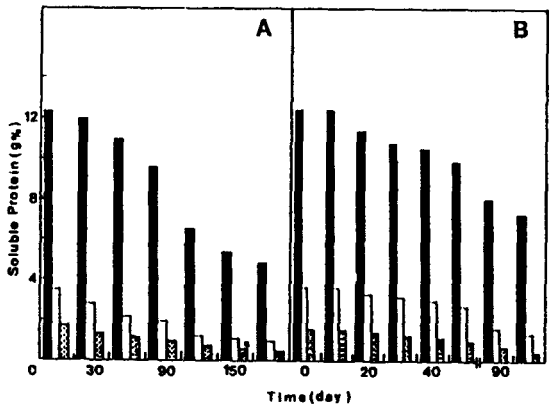

Fig. 5. Changes in protein solubility of rice bran
A: Rice bran stored in controlled atmosphere for 180 days, B: Rice bran exposed to the air for 100 days
■: Total Extract, □: Water Soluble, ▨: Salt Soluble

Brown discorloration observed in the present experiment seemed to be initiated by both of the sugar-amino and lipid-amino condensation.

The concentration of soluble protein of fresh rice bran extracted with buffer solution was 12.6 ± 0.2 g per 16 g nitrogen in which the fraction of water and salt soluble protein occupied 29% and 13% of total soluble protein, respectively. After 180 days of storage in the controlled atmosphere, the concentration of total, water, and salt soluble protein decreased down to 46%, 30%, and 36% of their initial concentrations (Fig. 5). Water soluble protein fractions, losing about 70% of its contents, showed the biggest changes among the various protein fractions. The losses observed in the sample exposed to the air were about 50-60% of their initial contents in 100 days of storage (Fig. 5). This findings agree with other authors who observed that decreases of protein solubilities were greater in water and salt soluble protein fractions than in other fractions during storage[18]. The decrease of protein solubility might have been caused by changes in functional groups of protein, for such changes can either transform the structure of protein or promote the polymerization of protein that can change the native protein into an insoluble form[7]. The changes in protein solubilities observed in this experiment was similar to the change of sulfhydryl during storage. It seems that the increase of disulfide group by the oxidation of sulfhydryl is closely related to the decrease of protein solubility[19].

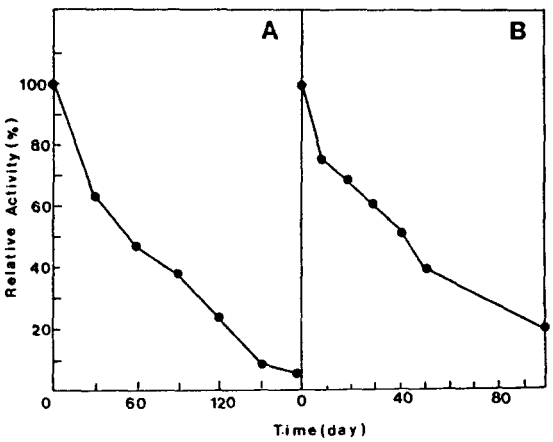

Fig. 6. Changes in protease activity of rice bran
A: Rice bran stored in controlled atmosphere for 180 days, B: Rice bran exposed to the air for 100 days

The changes of protease activity of rice bran are shown in Fig. 6. Proteolytic activity of rice bran protease stored in the controlled atmosphere decreased very rapidly during storage. The loss of protease activity was 37% in 30 days, 50% in 60 days, and was more than 95% in 180 days. This rapid loss of enzyme activity might be attributed to the low water activity and low relative humidity of the experimental environment, since natural enzymes can not exist in such a condition. The reaction of free radicals of oxidized lipid with amino acids at the active site of enzyme might have also contributed to the loss of enzyme activity. When the rice bran was exposed to the air, about 38% and

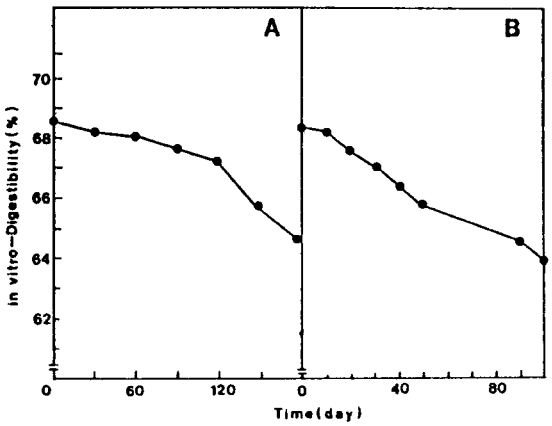

Fig. 7. Changes in *in vitro*-digestibility of rice bran
A: Rice bran stored in controlled atmosphere for 180 days, B: Rice bran exposed to the air for 100 days

Table 1. Correlation coefficients between protein functional groups and degree of lipid oxidation of rice bran stored in controlled atmosphere for 180 days[a)]

Functional group	Peroxide value	Carbonyl value
SH group	-0.835*	-0.960*
S-S group	0.835*	0.959*
Avail. lysine	-0.827*	-0.954*
Total sol.	-0.941*	-0.943*
Water sol.	-0.882*	-0.981*
Salt sol.	-0.854*	-0.972*
Protease Act.	-0.887*	-0.963*
Digestibility	-0.948*	-0.751

a)* Changes in functional groups during storage were significantly correlated with the degree of lipid peroxidation ($p<0.05$)

65% losses in enzyme activity was observed after 10 and 100 days of storage, respectively.

The *in vitro* digestibility of fresh rice bran was $68.43 \pm 0.11\%$, lower than that of rice which was about 85%. The digestibility decreased by 10% in 100 days for the sample exposed to the air, while 7% decrease was observed in the sample stored in the controlled atmosphere in 180 days (Fig. 7). This substantial decrease in the digestibility could be attributed to the lipid peroxidation. It was reported that lipid-protein interaction was one of the factor contributing most significantly for the reduction of the digestibility of fish products[20]. The protein-protein aggregation or protein-lipid polymerization may lead to the transformation of native protein into a form that is structurally inadequate for the enzyme hydrolysis[5].

Table 2. Correlation coefficients between protein functional groups and degree of oxidation of rice bran exposed to the air[a)]

Functional group	Peroxide value	Carbonyl value
SH group	-0.866*	-0.887*
S-S group	0.866*	0.887*
Avail. lysine	-0.839*	-0.854*
Total sol.	-0.833*	-0.855*
Water sol.	-0.961*	-0.972*
Salt sol.	-0.976*	-0.985*
Protease Act.	-0.668	-0.649
Digestibility	-0.886*	-0.904*

a)* Changes in functional groups during storage were significantly correlated with the degree of lipid peroxidation ($p<0.05$)

Correlation between lipid oxidation and functional properties

The correlation coefficients between the degree of lipid peroxidation and concentration of each functional group of protein in the rice bran are shown in Table 1 and 2. For the case of rice bran stored in the controlled atmosphere, despite the peroxidation degree was low, peroxide and carbonyl value were significantly correlated ($p<0.05$) to the concentration of each functional group. The correlation coefficients were close to -0.9 for all of functional groups (Table 1). Significant correlation coefficient ($p<0.05$) were also found for the case of rice bran exposed to the air (Table 2). This is consistent with the observation of the rapid lipid oxidation and significant changes in the functional group in a relatively short time compared to that of rice bran stored in the controlled atmosphere. From the correlation analysis, it can be inferred that the lipid peroxidation is closely related to the functional groups of protein which deteriorate the protein in rice bran.

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(Received Aug. 13, 1990)

미강 저장 중 지방의 산패에 따라 생성된 산화 지질이 단백질의 기능기에 미치는 영향에 관한 연구

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미강의 산소 농도를 제한하고 상대습도 65%, 온도 35℃의 저장 조건하에서 함유 지방질의 가수분해를 최대한 유도한 시료와 온도 25-30℃, 상대습도 70-80%인 공기 중에 방치하여 함유 지방질의 가수분해와 지방질의 산화를 동시에 유도한 미강을 시료로 하여 지방의 산화 양상에 따른 단백질의 물리 화학적인 변화 즉, sulfhydryl과 disulfide group, 단백질의 용해도, 유효성 lysine, protease의 활성변화 등을 실험하였다. 산소 농도를 제한한 system에서는 지방질의 가수분해는 급격히 일어난 반면 산화는 서서히 진행되었고, 공기 중에 방치한 system에서는 지방질의 가수분해와 자동산화가 현저하게 진행되었다. 이 때 각종 단백질 기능기의 함량의 감소 현상은 저장기간보다는 지방의 산패 정도와 더 깊은 상관성을 보였다. 지방질의 산패와 주요 단백질 기능기 함량과의 상관계수는 두 system 모두에서 −0.8 이상으로서, 지방질의 산패는 단백질의 이화학적인 변화에 중요한 영향을 미침이 관찰되었다 ($p<0.05$).