KOREAN J. FOOD SCI. TECHNOL.
Vol. 21, No. 4, pp 461~467 (1989)

Effect of CO_2 Concentration in CA Conditions on the Quality of Shiitake Mushroom (*Lentinus edodes*) during Storage

Dong-Man Kim, Hyung-Hee Baek, Hye-Hyun Yoon and Kil-Hwan Kim
Food Science and Technology Lab., Korea Food Research Institute, Seoul

표고버섯(*Lentinus edodes*)의 CA 저장 중 탄산가스농도의 효과

김동만·백형희·윤혜현·김길환
한국식품개발연구원 식품공학연구실

Abstract

The effect of CO_2 concentration on the post-harvest physiology and quality of Shiitake mushroom (*Lentinus edodes*) were investigated during CA storage. The respiratory rates of the mushrooms stored in CA conditions were abruptly increased in proportion to CO_2 concentration after 40 days, and then declined, while that of the mushroom in air was continuously decreased throughout storage period. Large amounts of ethanol and acetaldehyde were produced from the 20 days CA stored mushrooms. The least changes in 5'-GMP content and electrophoresis pattern of protein in the mushroom were observed at CO_2 concentration of 2% during storage. Based on the changes in quality factors of the mushroom during storage, it could be concluded that CO_2 concentration of 2% with fixed O_2 concentration of 2% was more effective in extension of the freshness than any other CO_2 level in this experiment.

Key words: CA storage, Shiitake mushroom (*Lentinus edodes*), CO_2 effect, respiration, N-compounds

Introduction

Shiitake mushroom (*Lentinus edodes*) is one of the important edible fungi in Korea. Most of the mushroom is marketed after dehydration due to its short shelf-life as a fresh produce. But the dehydration results in irreversible and undesirable changes in texture[1] and flavor. Moreover, the nutrients in the mushroom are lost during soaking in water[2] for softening the tissue before cooking. Recently, several attempts have been made to extend the shelf-life of mushrooms as a fresh produce using the methods of cold storage[3], modified atmosphere(MA) storage[4,5] and controlled atmosphere(CA) storage[6].

Little effort has been made, however, to prolong the freshness of Shiitake mushroom by CA storage method at low temperature and to elucidate the effect of gas compositions on the quality of the mushroom during the storage. The freshness of the CA stored produces depends upon a number of factors, particularly the level of CO_2 concentration in the storage atmosphere[7]. The objective of this work was to investigate the effect of CO_2 concentration in CA storage at low temperature on the post-harvest physiology and quality of Shiitake mushroom.

Corresponding author: Dong-Man Kim, Food Sci. and Technol. Lab., Korea Food Research Institute, KAIST, P.O. Box 131 Cheongryang, Seoul, 136-701

Materials and Methods

Materials

Locally grown Shiitake mushroom (*Lentinus edodes*) was used for the storage experiment. The mushroom was commercially harvested at Paju, Oct. 2 and the size graded mushrooms were packed in the field.

The packed cartons were immediately transferred to refrigerated room at 2°C where CA chambers were located. The mushroom were kept

in the room for 3 days before sorting, and then put into the air-tight 100 *l* barrels which were used for CA storage containers. Four kilograms of the mushroom were placed in each barrel and they were separated with plastic baskets in the barrel. The mushrooms were subjected to the 4 storage conditions such as air, 2% CO_2 plus 2% O_2, 4% CO_2 plus 2% O_2 and 6% CO_2 plus 2% O_2.

The desired storage atmosphere were attained within 12 hr after sealing with the cover of the barrel. The concentrations of the gases in the barrel were maintained with appropriate mixture of O_2, CO_2 and N_2 dispensed from pressurized cylinder and were routinely checked with Fyrite gas analyzer during CA storage.

Measurement of respiratory rate

About one hundred grams of the whole mushroom were taken out from the barrels of various atmosphere condition at intervals of 20 days during storage and the respiratory rate was measured by the desiccator method[8]. Weight loss of the mushroom during storage was defined as a difference in wholesome weight between initial and sampling time.

Analysis of ethanol and acetadehyde

Twently grams of the sliced sample were put into a 50 m*l* reaction vial, sealed and then incubated in 55 °C water bath before analysis. After 20 min. for equilibration in each vial, 5 m*l* of headspace gas was injected into stainless steel column (3m × 3.2mm OD) packed with 10% FFAP on Chromosorb AW(80/100 mesh) in GC equipped with a flame ionization detector and a integrator. The other GC conditions for the analysis were substantially the same as the procedures of Hachenberg[9].

Analysis of nucleotides

The nucleotides in the mushroom were extracted from the freeze dried and powdered mushroom with 10% perchloric acid solution by tissuemizer. After pH adjustment of the mixture to 6.5 with KOH solution, it was centrifuged at 4000 × g. The supernatant was passed through 0.45 um membrane filter and C_{18} cartridge, and then injected into C_{18} reverse phase column in HPLC with the conditions proposed by Lee *et al.*[10].

Determination of total and non-protein nitrogen contents

Non-protein nitrogen was collected by using trichloroacetic acid(TCA) precipitation. 250 mg of the freeze dried mushroom powder was combined with 25 m*l* of 10% TCA solution, stirred for 1 hr, then centrifuged. Nitrogen contents of the supernatant and the freeze dried mushroom powder were determined by the Kjeldahl method[11].

SDS-PAGE(Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis)

Electrophoresis was carried out as described by Laemmli's discontinuous system[12] with vertical slab (200 × 200 × 1.5 mm) apparatus. The separating gel was consisted of 10% acrylamide and 2.7% bisacrylamide in 1.5M tris-Cl, pH 8.8 contained 0.4% SDS. The stacking gel was made with 4% acrylamide, 2.7% bisacrylamide in 0.5M tris-Cl, pH 6.8. The run were performed in glycine buffer (pH 8.3) under 25 mA per gel for 4 hr at room temperature. The gel was stained for 24 hr with Coomassie Blue R-250 and destained with several changes of 25% ethanol solution contained 8% acetic acid.

Results and Discussion

Respiratory rate

During CA storage at the various CO_2 concentrations, changes in respiratory rate of the mushroom are presented in Fig. 1. Remarkable decrease in the respiratory activities was observed in the mushroom stored in all conditions used after storage for 20 days. After 40 days the respiratory rate of the mushroom stored in air was gradually decreased. But it was suddenly increased in the CA stored mushrooms in this period, and then

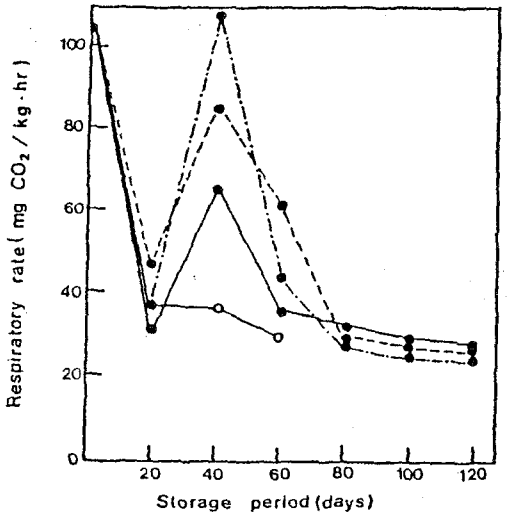

Fig. 1. Changes in repiratory rates of Shiitake mushroom during CA storage.
○—○ : control ●—● : 2%CO_2 + 2%O_2
●---● : 4%CO_2 + 2%O_2 ●-·-● : 6%CO_2 + 2%O_2

continuously declined thereafter.

At the respiratory peaks shown by the CA stored mushrooms, the rate was ranged from 66 to 108 mg CO_2/kg.hr. The highest value was marked by the mushroom stored in the CA condition of 6% CO_2 plus 2% O_2 and this value was mostly same as that by the initial sample at the start of storage. The respiratory rate was comparably proportional to the CO_2 concentration after storage for 60 days, but the reversed trend was shown thereafter. The similar post-harvest rising of respiration was reported by Hammond *et al.*[13] and this rising might be related to stage of development at harvest. From the results of classification in development, the mushroom used in this experiment was considered to be in button or closed cup stage.

Ethanol and acetaldehyde

Fig. 2 shows the changes in the amounts of ethanol and acetaldehyde produced from the mushrooms stored at the different CA storage conditions. At all CA conditions used, the maximum amounts of ethanol and acetaldehyde were produced from the mushrooms after 20 days storage. The levels at the peaks were ranged from 85

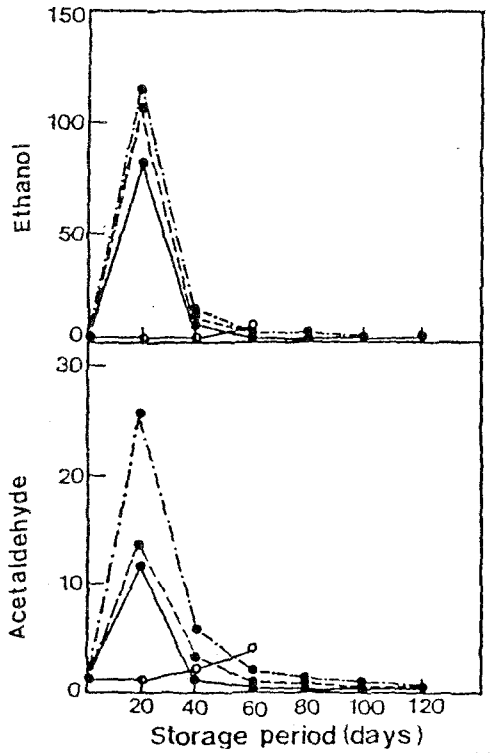

Fig. 2. Changes in amounts of ethanol and acetaldehyde produced by Shiitake mushroom during CA storage.
unit : relative amount against the initial value
○—○ : control ●—● : 2%CO_2 + 2%O_2
●---● : 4%CO_2 + 2%O_2 ●-·-● : 6%CO_2 + 2%O_2

to 115 folds for ethanol, and from 8 to 14 folds for a acetaldehyde in comparison with the amounts reberated by the initial fresh sample, while the levels were negligibly changed in the mushroom stored in air for the same period.

At the peak, the amounts of ethanol and acetaldehyde developed by the mushroom were varied with CO_2 concentration in the CA conditions and the higher values were presented by the mushroom maintained in the higher concentration of the gas. The levels of ethanol and acetaldehyde caused by the CA stored mushrooms were dropped after storage for 40 days. In case of the mushroom held in the air, the amounts were little changed until 40 days after storage, and then increased over the levels exhibited by CA stored mushrooms. It is reported that ethanol and acetal-

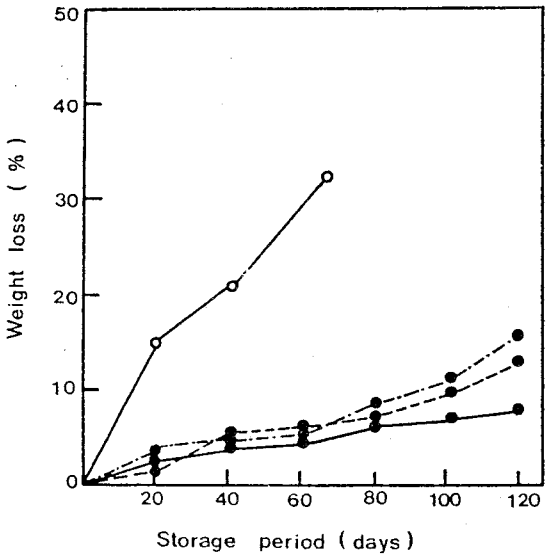

Fig. 3. Weight loss of Shiitake mushroom during CA storage.
○—○ : control ●—● : 2%CO_2 + 2%O_2
●----● : 4%CO_2 + 2%O_2 ●-·-● : 6%CO_2 + 2%O_2

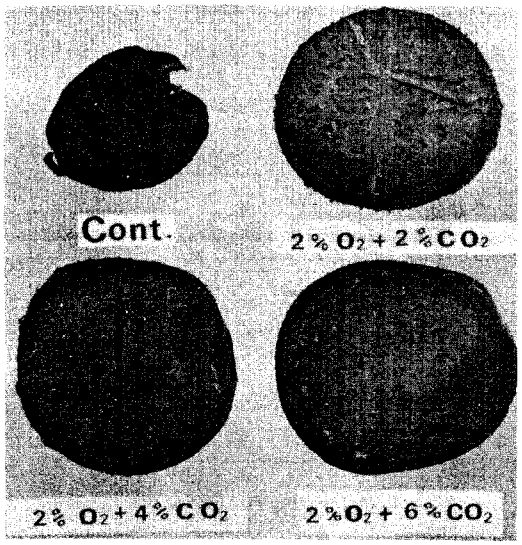

Fig. 4. Photograph of Shiiitake mushrooms stored for 60 days in air and for 120 days at the CA conditions.

dehyde are the major off-flavor compounds developed from any fresh produces stored at MA and CA conditions[4-6]. The accumulated ethanolic odor of the CA stored mushrooms could be eliminated by aeration for a few hours with fresh air as observed by Minamida *et al.*[6].

Weight loss

Weight loss of the mushroom depending on the CA conditions are shown in Fig. 3. The loss was gradually increased in the CA stored mushrooms throughout storage period, whereas it was seriously raised in the mushroom held in air. The difference in weight loss of the mushrooms depended upon CO_2 concentration in CA conditions was unclear until 60 days after storage, but the noticeable reduction of the loss was observed at the CO_2 concentration of 2% during the rest storage period.

Furthermore, at this CO_2 concentration, the mushrooms kept a good appearance for 120 days as shown in Fig. 4. The storable period of the mushroom in this experiment was much longer than than the previously reported results[6] obtained from MA and CA storage test at high temperature.

Nucleotides and its derivatives

Fig. 5 shows the changes in the contents of nucleotides and its derivatives contained in the mushroom during CA storage. The relative amount of 5'-GMP in the mushrooms was continuously decreased in all the storage conditions during storage, except a little increase after 20 days. The decrease seriously occurred in the mushrooms stored in air and the next was in the mushrooms kept in the CA condition of 6% CO_2 plus 2% O_2.

The content of 5'-AMP was reduced without any tendency according to the storage conditions until 60 days after storage, but thereafter the less loss was marked in the mushrooms kept at lower CO_2 concentration. In case of hypoxanthine, the content was gradually increased as storage time increased and the upward tendency in hypoxanthine of the mushroom by storage conditions was more significant than that in xanthine.

Nitrogen

At the start of the storage, total and TCA solu-

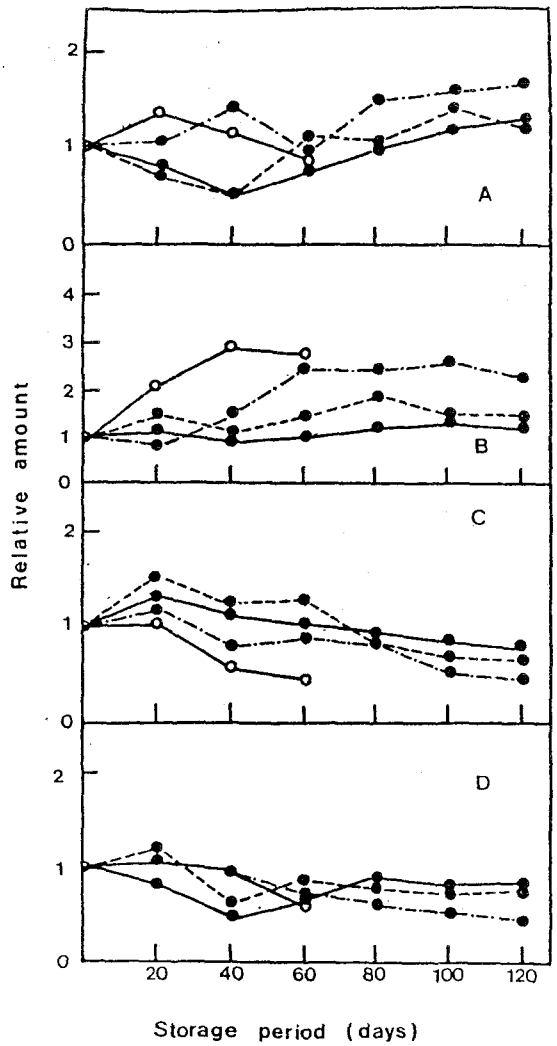

Fig. 5. Changes in the amounts of xanthine(A), hypoxanthine(B), 5'-GMP(C) and 5'AMP(D) of Shiitake mushroom during CA storage.
unit: relative amount against the initial value
○—○ : control
●—● : $2\%CO_2 + 2\%O_2$
●---● : $4\%CO_2 + 2\%O_2$
●-·-● : $6\%CO_2 + 2\%O_2$

ble nitrogen contents of the mushroom were 3.6 and 1.1%, respectively, based on dry weight. They were increased with increase of storage time (Fig. 6). In case of total nitrogen content, more than 41% of the initial nitrogen content was increased in the mushrooms kept at the 3 CA conditions for 120 days. The increase of total nitrogen content was higher than that of TCA soluble nitro-

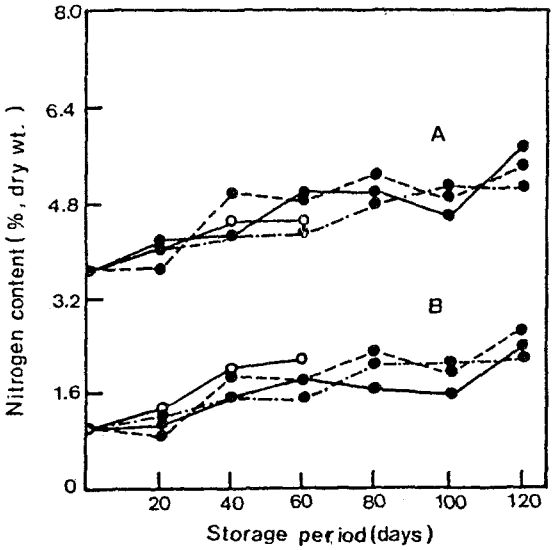

Fig. 6. Changes in the contents of total(A) and TCA soluble(B) nitrogen of Shiitake mushrooms during CA storage.
○—○ : control
●—● : $2\%CO_2 + 2\%O_2$
●---● : $4\%CO_2 + 2\%O_2$
●-·-● : $6\%CO_2 + 2\%O_2$

gen content during storage. To explain the reasons for these unexpected increases, further research on the post-harvest physiology of the mushroom is needed.

The similar result was reported by Minamida *et al.*[3] and it might be related to changes in weight of gills and sporulation during storage. Yamashita *et al.*[5] supposed that changes in the amount of total free amino acid might affect to quality of the stored mushroom. But the contents of total and TCA soluble nitrogen of the mushroom were increased without lucid trends related to quality change of the mushroom during CA storage.

Electrophoresis pattern of protein

The changes in electrophoresis pattern of protein in the mushrooms during storage are presented in Fig. 7. In the mushroom immediately after harvest, several protein bands were observed and the major bands had molecular weight of about 45 kd. The electrophoresis pattern of protein was variously changed by storage conditions. The number of the band was increased in the mush-

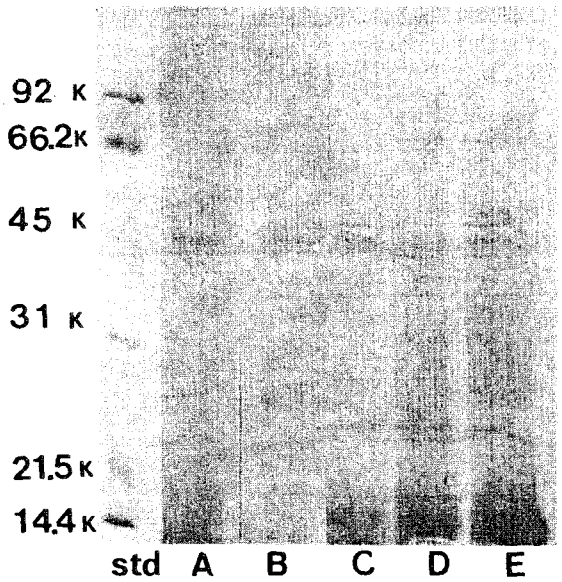

Fig. 7. Protein patterns of Shiitake mushrooms by electrophoresis.
A: Before storage
B: Stored for 60 days at air
C: Stored for 120 days at the CA condition of 2%CO_2 + 2%O_2
D: Stored for 120 days at the CA condition of 4%CO_2 + 2%O_2
E: Stored for 120 days at the CA condition of 6%CO_2 + 2%O_2

rooms stored in the CA condition of 6% CO_2 plus 2% O_2 while it was little changed in the mushrooms stored in the 2% CO_2 plus 2% O_2 in comparison with that shown by the immediately harvested mushroom. This result suggest that postharvest metabolism of protein in the mushroom at low CO_2 concentration slowly progressed during storage under the this CA conditions.

요 약

생표고버섯의 신선도 연장을 위하여 2℃에서 산소의 농도를 2%로 고정하고 탄산가스의 농도를 각각 2%, 4%, 6%로 조절한 CA 저장조건으로 표고버섯을 저장하면서 탄산가스의 농도에 따른 호흡율, off-flavor, 단백질의 전기영동 패턴 등을 비롯한 품질에 관련된 인자들의 변화를 조사하였다. CA 저장한 표고버섯의 호흡율은 대조구와는 달리 저장 40일 후 급격히 증가한 후 다시 저하되었는데 환경가스 조성 중 탄산가스의 농도에 반비례하였으며 off-flavor 성분인 ethanol 및 acetaldehyde의 양은 호흡율이 급속히 증가하기 20일 전에 최대치를 보였다. 한편 탄산가스의 농도에 따라 정미성분인 5′-GMP, 단백질의 전기영동 패턴 등도 차이를 보였는데 환경가스 조성 중 산소의 농도를 2%로 고정시, 탄산가스의 농도를 2%로 유지하는 것이 표고버섯의 품질 보존에 효과적인 것으로 나타났다.

References

1. Willis, C.A. and Teixeira, A.A.: Controlled reduction of water activity in celery, *J. Food Sci.*, **53**, 111 (1988)
2. Aoyaki, Y. and Sugahar, T.: Studies on the rehydration of dried Shiitake mushroom, *J. Food Sci. and Technol. (Jpn.)*, **33**, 244 (1986)
3. Minamida, T., Habu, T. and Ogata, K.: Effect of storage temperature on keeping freshness of mushroom after harvest, *J. Food Sci. and Technol. (Jap.)*, **27**, 281 (1980)
4. Minamida, T., Tsuruta, M. and Ogata, K.: Studie on keeping freshness of Shiitake mushroom (*Lentinus edodes* Sing) after harvest, *J. Food Sci. and Technol. (Jpn.)*, **27**, 26 (1980)
5. Yamashita, I.K., Takahashi, T., Shimoda, T., Kikuchi, K. and Shibata, S.: Changes in some chemical characteristics in Shiitake mushroom during modified atmosphere packaging storage and on exposure to air, *J. Food Sci and Technol. (Jap.)*, **34**, 834 (1987)
6. Minamida, T., Nishikawa, T. and Ogata, K.: Influence of CO_2 and O_2 on the keeping freshness of Shiitake mushroom after harvest, *J. Food Sci. and Technol. (Jap.)*, **25**, 505 (1980).
7. Kader, A.A.: Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables, *Food Technol.*, **5**, 99 (1986)
8. Tarutani, T. and Manebe, M.: Studies on the utilization of persimmon. 3. Effect of storage temperature on persimmons, *J. Jap. Soc. Hort. Sci.*, **29**, 114 (1960)
9. Hachenberg, H. and Schimt, A.P.: Gas Chromato-

graphic Headspace Analysis, Heyden & Son Ltd., London, p. 19 (1979)

10. Lee, E.H., Koo, J.G., Ahn, C.B., Cha, Y.J. and Oh, K.S.: A rapid method for determination of ATP and its related compounds in dried fish and shellfish products using HPLC, *Bull. Korean Fish Soc.*, **17**, 369 (1984)
11. A.O.A.C.: *Official Methods of Analysis*, 14th ed., Association of Official Analytical Chemists, Washington, D.C., p. 16 (1984)
12. Laemmli, U.K.: Cleavage of structural proteins during inserting of the head of bacteriophage T4. *Nature (London)*, **227** 680 (1970)
13. Hammond, B.W. and Nicholas, R.: Changes in respiration and soluble carbohydrate during the postharvest of mushroom (Agaricus bisporus), *J. Sci. Fd. Agri.*, **26**, 835 (1975)
14. Kasmire, R.F., Kader, A.A. and Klaustermeyer, J.A.: Influence of aeration rate and atmosphere composition during simulated transit on visual quality and off-odor production by broccoli, *HortScience*. **9**, 228 (1974)

(1988년 10월 25일 접수)