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Changes of Protein Pattern of Mungbean Seeds, *Phaseolus aureus* During Germination

D. Y. Park*, S. J. Cho and Y. C. Shin**

Department of Biological Science & Engineering, KAIST, Seoul
**Department of Home Economics Education, Dong Guk University, Kyung-ju*
***Department of Home Economics Education, Cheung Ju College of Education, Chung-Ju*

녹두 발아중 단백질 전기영동 패턴의 변화

박동연*· 조숙자**· 신용철

한국과학기술원 생물공학과

*동국대학교 경주분교 가정교육과 **청주사범대학 가정교육과

Abstract

During the germination of mungbean seeds, the changes of water contents, total and soluble proteins, and electrophoretic pattern of the soluble proteins were examined. The moisture content of a dry mungbean was 12.7%, which was greatly increased after the soaking. Along to the germination period, the moisture content of the mungbean sprouts was gradually increased up to 90.7%. The contents of total and soluble proteins were sharply decreased after the soaking of the mungbean and decreased gradually during the germination. PAGE of the soluble proteins showed two broad bands and three sharp bands. During the germination, two broad bands were weadened but other bands were relatively stable. SDS-PAGE showed 19 discrete bands and during the germination, the most of the bands were thinned or disappeared. But some of the protein bands were stable until the end of germination.

Introduction

Mungbean (*Phascolus aureus*) contains 20-25% protein and sufficient quantities of all amino acids except methionine, cystine and tryptophan.[1] Nevertheless the legume as a protein food source is limited, which can be attributed to three factors: 1) presence of several antinutrients including trypsin inhibitor and hemagglutinating agent, 2) presence of flatulence factors, 3) beany flavors.[2] Inactivation/ removal of these antinutritional factors may, therefore, help improve the legume utilization.

Of the several processing methods used for dry bean processing, germination is a relatively simple method, does not require intensive energy input, and also yields natural products. Germination of legume seeds is accompanied by the metabolism of the reserve proteins stored in pretein bodies in the cotyledons. Although changes in storage proteins of several legume seeds during germination have been investigated,[3-6] no studies on the protein change of mungbean seeds were carried out, except that of the change of oligosaccharides.[7]

In this paper, the changes of protein patterns during the germination of mungbean seeds are investigated by using the technique of polyacrylamide disc gel electrophoresis.

Materials and Methods

Materials

Mungbean sees were purchased from local market, and stored at 4°C until used. Some deformed grains were discarded before use. Unless mentioned otherwise, all chemicals used were reagent grade.

Germination

Each 20*g* of mungbean seeds was soaked in 200*ml* of tap water for 15 hr at 25°C and germinated in the dark at 25°C for 7 days in filter paper-laid flower-pot (20*cm* diameter). During the germination, the tap water was

sprinkled on seeds 3-4 times a day. Unsoaked seeds were ground in a Wiley mill fitted with a 60 *mesh* sieve and served as a control.

Moisture determination

The moisture content of sample was determined by the conventional method at 115°C for 6 hr.

Total protein determination

The protein content of samples was determined by the conventional micro-Kjeldahl method.

Soluble protein determination

The soluble protein of the samples prepared by the precedure described in Fig. 1 was also determined by the same method of micro-Kjeldahl.

Polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Weber *et al.*[8] Briefly, the soluble protein samples were dialyzed against distilled water to remove salts and lyophilized. Samples were dissolved in sample buffer (pH 8.0) containing 0.01*M* Tris base, 0.001*M* EDTA, 1% SDS, and 5% ß-mercaptoethanol and then the equal volume of a tracking dye was added. Samples were heated for 2 min in a boiling water bath and loaded onto the SDS-polyacrylamide gel.

The 7.5% gels were formed from stock solutions in glass gel tubes. Electrophoresis was carried out at 1*mA/gel* in upper gel and at 3*mA/gel* in lower gel. Gels were stained for 5-6 hr in a staining solution containing 0.25% Commassie brilliant blue, 50% methanol, and 10% acetic acid and destained in destaining solution of 5% methanol and 7.5% acetic acid. The polyacrylamide gel electrophoresis without SDS was also conducted by the same method of SDS-PAGE.

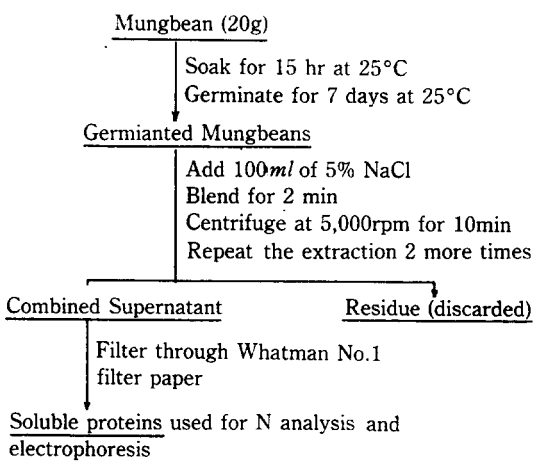

Fig. 1. Procedure for soluble protein extraction

Results and Discussion

Moisture analysis

The change of the moisture content of the mungbean during the germination was studied. As shown in Fig. 2, the moisture content of a dry mungbean was 12.7%, which was greatly increased after soaking the mungbean seeds in water bath at 25°C for 15 hr. Along to the germination period, the moisture content of the mungbean sprouts was gradually increased up to 90.7%. These results are consistent with other results reprted.[6-10]

Total and soluble protein contents

The changes of the total and the soluble protein contents of the mungbean during the germination are shown in Fig. 3. The total protein contents of dry mungbean and mungbean sprouts germinated for 7 days were 24.2% and 2.7%, respectively. The total protein contents of the mungbean sprouts were relatively low, compared to that of dry mungbean, which was mainly due to the higher water content in the mungbean sprouts than in dry mungbean. On dry-weight base, they have similar total protein contents near to 28%.

The total (24.2%) and the soluble (21%) protein con-

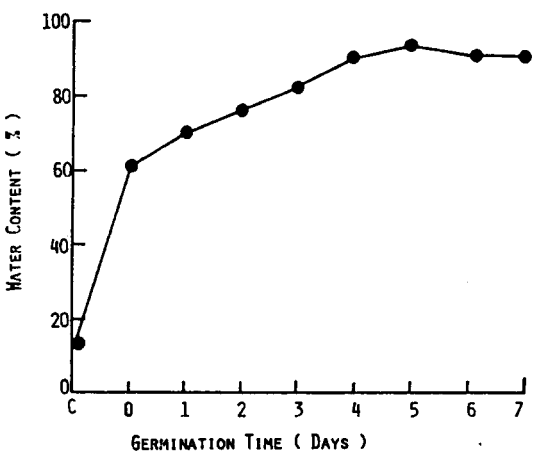

Fig. 2. Change in water content of mungbean during germination

C: dried mungbean, O: soaked mungbean.

tents of the mungbean showed that the most of the mungbean proteins are easily extractable by the treatment of 5% NaCl solution, since mungbean proteins are mostly composed of soluble proteins in 5% NaCl solution such as albumin, globulin, and other non-protein nitrogen (NPN).[11]

As shown in Fig. 3, the total and the soluble protein contents are sharply decreased to 10.8% and 7.3% on soaking of the mungbean and slowly decreased during the germination, which may be explained by considering the water absorption shown in Fig. 2 as well as the metabolic consumption of proteins for sprouting the mungbean seeds. After the 5th day of the germination, the changes of the total and the soluble protein remained constant. This fact suggests that a portion of the mungbean proteins is resistant to the metabolic degradation during the germination, which is evident from the results of the polyacrylamide gel electrophoresis.

Electrophoresis

The changes of the mungbean protein pattern during the germination were examined. The mungbean proteins are progressively degraded during the germination as shown in Fig. 4 and Fig. 5. The soluble fractions of various samples show that the proteins of the mungbean seeds are separated to two main bands, P1 and P5, and three sharp bands, P2, P3, and P4 when they were not treated with SDS (Fig. 4). Kang and Lee[11] obtained 12 bands involving 3 broad bands by the electrophoretic method of Davis.[12] At the present time, the differences are not clearly understood but several reasons are assumed as follows. They extracted the proteins from the defatted mungbean by using the phosphate buffer, pH7.6, containing 0.4*M* *NaCl and 0.01M* ß-mercaptoethanol, while the proteins were extracted from the mungbean powder by using the 0.5% NaCl solution in this studies. Because of the defferent solubility of the proteins in the extraction solution, the different proteins may be obtained. Another reason is the electophoretic methods. Besides, the 0.01*M* ß-mercaptoethanol solution in the extraction solution may reduce the disulfide bonds of the proteins and cleave the proteins to their subunits.

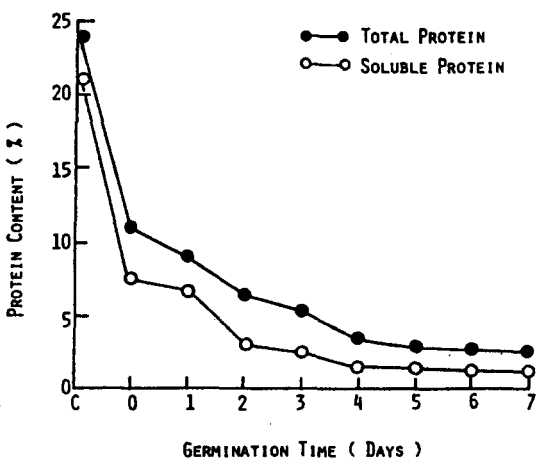

Fig. 3. Changes in total and soluble proteins of mungbean during germination
C: dried mungbean, O: soaked mungbean.

In the early germination periods, the proteins of P1 and P4 were gradually degraded, but the proteins of P2, P3 and P5 were relatively stable in this periods. The proteins of P1 were completely degraded by the 5th day of germination and the proteins of P4, by the 2nd day of germination. The proteins of P5 were slowly degraded by the 4th day of germination, but hereafter further degradation was not occurred. The remained proteins of P5 were intact until the end of germination. In the proteins of P2, the degradation was not occuued during all the days of the germiantion, judged on the basis of band width and intensity. New protein band (NP 1) was appeared on the 6th day of the germination. This protein was considered as the degradation intermediate of the P1 proteins. Apparently, the degradation rate of the mungbean proteins was slower up to 2 days compared to that at the end of the 3rd, 4th, and 5th day of the germination. The reserve protein degradation during the germination has been shown to be slow in other legumes.[13,14] However, the biological roles of the delayed-degradation of the reserve proteins during the germination remain to be elucidated.

As in Fig. 5, SDS-PAGE electrophoretogram of the mungbean proteins shows 19 discrete bands with 6 major bands, S9, S11, S12, S13, S18, and S19. The proteins of S1 and S2 were disappeared at the end of soaking, which indicates that the vital metabolism is in progress in the period of soaking. The most of the proteins were very slowly degraded up to the 2nd day of the germiantion. But the most of the proteins were completely degraded by the 7th day of germination except the proteins of S7, S15, S16, S18, and S19. During the germination, the three new bands (NS1, NS2, and NS3) were observed, which was presumed that they were appeared in the course of degradation of the proteins having higher molecular weight. Relatively, the proteins having higher

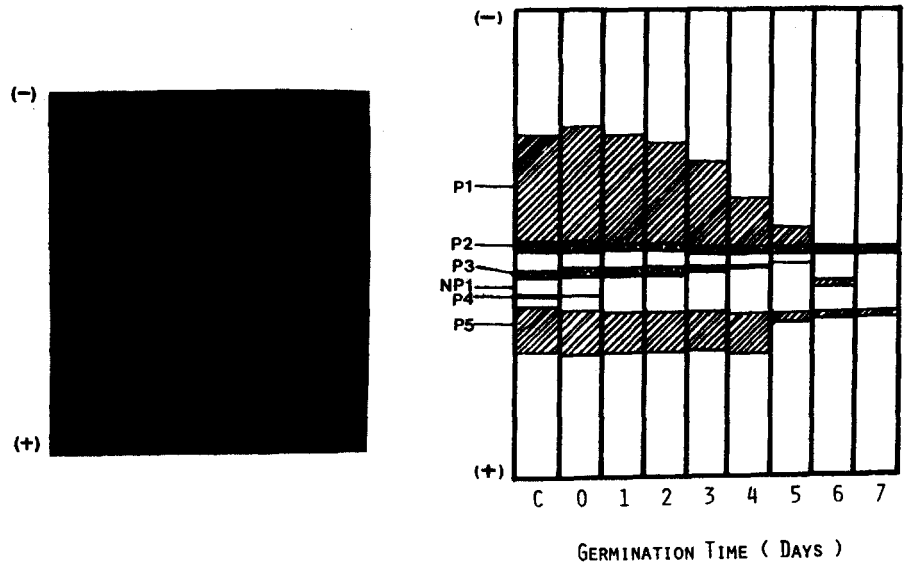

Fig. 4. Polyacrylamide gel electrophoretic patterns of soluble protein of mungbean during germination
C: dried mungbean, O: soaked mungbean.

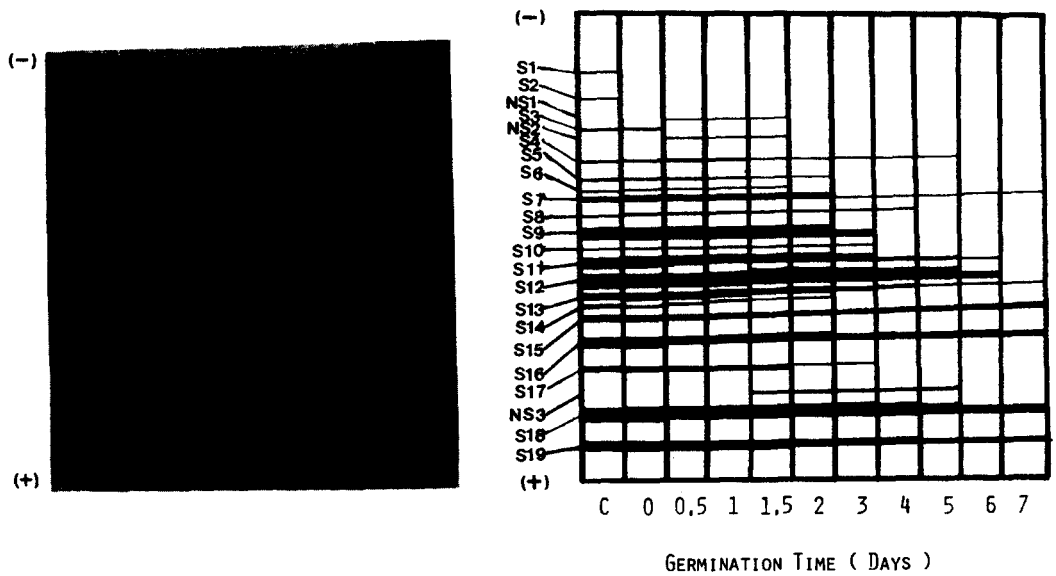

Fig. 5. SDS-polyacrylamide gel electrophoretic pattern of soluble protein of mungbean during germination
C: dried mungbean, O: soaked mungbean.

MW were degraded faster that that having lower MW during the germination of the mungbean.

요 약

녹두의 발아중 수분 함량의 변화, 총 단백질과 가용성 단백질 함량의 변화, 그리고 전기영동에 의한 가용성 단백질의 변화 패턴을 조사하였다. 건조녹두의 수분 함량은 12.7%이고 침지 후에는 60% 였으며 발아함에 따라서 점차 증가하여 90.7%까지 올라갔다. 녹두를 침지하므로써 급격히 총 단백질 함량과 가용성 단백질 함량이 감소하였고 발아 일수가 경과함에 따라서 점진적인 감소를 보였으며, 총 단백질과 가용성 단백질은 비슷한 감소 패턴을 보였다. 녹두의 가용성 단백질을 SDS로 처리하지 않고 전기영동 하였을때 2개의 넓은 band와 3개의 좁은 band를 얻을 수 있었고, 발아함에 따라서 2개의 넓은 band는 점차 희미해져 단백질이 분해되고 있음을 알 수 있었다. 그러나 다른 band의 단백질들

은 비교적 분해되지 않고 남아 있었다. 녹두의 가용성 단백질을 SDS-PAGE로 관찰 하였을때 19개의 분명한 band를 얻을 수 있었고 발아함에 따라서 대부분의 단백질 band는 분해되어 희미해져 갔으나 상당한 부분이 발아 7일째까지 분해되지 않고 남아 있음을 알 수 있었다.

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