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Studies on the Fermentation of Lupin Seed (II)
— Preparation of traditional Korean fermented been Sauce and Paste —

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루우핀콩의 발효에 관한연구(Ⅱ)
—한국 재래식 장유 제조시험—

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Lupin seed was used to make Meju, the fermentation starter for Korean soybean sauce and paste in substitution for soybean and the fermentation characteristics were compared with those of soybean. Mejus were prepared by inoculating *Asp. oryzae* on the cooked whole beans. The dried Mejus were used for making fermented bean sauce and paste by mixing with brine and subsequent ripening for 4 weeks.

In general the protease activity and amylase activity-during ripening were higher in lupin seed Meju than those of soybean Meju. The increase in protease activity correlated to the increase in α-amino nitrogen content of the fermented paste and sauce. The development of dark-brown color of the sauce during ripening faster with lupin seed Meju compared to soybean Meju. In sensory evaluation the flavor score of lupin seed sauce and paste was slightly lower than that of soybean products but the overall quality of fermented lupin seed sauce was acceptable.

In the previous paper[1] the growth rate of *Asp. oryzae* on cooked lupin seed was compared to that of cooked soybean. Cooked lupin seed paste grew *Asp. oryzae* faster than soybean paste at the initial growth phase when cooked whole bean was used as the substrate. The growth of *Asp. oryzae* was slightly hindered by the thick hull of lupin seed, but, in general, lupin seed was considered satisfactory as the substrate for the cultivation of *Asp. oryzae*.

Soybean Meju has been traditionally used in making soysauce and soypaste in Korea. Soybean Meju is made from cooked soybean. Cooked soybean is mashed while it is hot and is moulded in to a shape, of which size and shape varies from like a fist to a house brick. The Meju ball is sun-dried for a week. Molds, mainly *Asp. oryzae*, grow on the drying surface and bacteria, mainly *B. subtilis*, inhabits inside of the Meju ball. The fermented Meju ball is immersed in salt solution and ripened for several months to develop dark brown color and meaty flavor. The ripened Meju-brine mixture is filtered to make soysauce and soypaste.[2]

Recently, improved type of Meju made from cooked whole soybean is used widely in Korea. Cooked whole soybean is covered with amount of wheat flour mixed with the spores of *Asp. oryzae* and incubated in a fermentation room. When the surface of soybean is covered with the spore of *Asp. oryzae*, it was dried and packed in a plastic bag and stored until used.

In the present study, the improved Mejus were prepared from lupin seed and soybean. Fermented bean sauce and paste were made from lupin seed Meju. The changes in the chemical composition and enzyme activities during the fermentation were determined. The color and flavor developments and general acceptability of bean sauce and paste made from lupin seed were compared to those of fermented soybean products.

Materials and methods

Materials

Lupin seeds (*Lupinus angustifolius*) harvested in 1981 were obtained from the Grain Pool of West Australia in 100 Kg lots and were stored in plastic container at

room temperature until used. Soybeans (*Glycin max* L) harvested in 1981 were purchased at the market in Seoul and were stored under similar condition as lupin seed.

Microorganism

The microorganism used was *Asp. oryzae* obtained from the Fermentation Laboratory of Korea University. It was grown on Czapeck agar plates at 25°C for 4 days.

Sample preparation

Improved Mejus were prepared as shown in Figure 1. Raw beans were soaked in water at 25°C overnight, drained and divided into 200 g soaked bean per each 1000 ml-erlenmeyer flask. A part of soaked lupin seeds was cut or scratched on the hull in order to facilitate the growth of mold on the surface. This sample was denoted as lupin seed (cutted). It was sterilized in the flask covered with layers of gauze. Excess water remained after cooking was drained by reversing the flask. Wheat flours were heated in a 120°C oven and after cooling mixed with spores of *Asp. oryzae*, 1.5 g of flour inoculum was added to each flask containing cooked beans. It was incubated at 25°C for 3 days and then dried at 60°C for 3 days. Meju-brine mixture was made by adding 1 part of salt and 4 parts of water to eah part of dried Meju. Part of the Meju was ground into powder and used to make powdered Meju-brine mixture. The mixtures were ripened in an incubator at 27°C, and samples were taken periodically. In order to keep the composition of the mixture constant, one part of bean and 5 parts of the liquid of the mixture were taken from the flask at each sampling.

Analytical methods

Total nitrogen was determined by the micro-Kjeldahl method.[3] Crude protein was culculated by multiplying the total nitrogen content by 6.25. Crude fat was measured by the Soxhlet extraction method. Moisture and ash contents were measured by the usual gravimetrical methods. The carbohydrate content was calculated by subtracting the other components from the total. The reducing sugar content of the sauce was measured by the modified Somogyi method.[3] The free amino nitrogen content in the water extracts of the samples was determind. One gram of sample was put into a 15 ml captube and 10 ml of cold distilled water was added, and extracted for 15 minutes. It was vortexed for 3-4 min prior to centrifugation at 1080 x g for 10 minutes. The supernatant was taken for the determination of free amino nitrogen contents by Sörensens method.[3] The pH of sauce was measured by the Beckman model G. pH-meter. Total acid was measured as fallows. 10 ml of sauce was put into a 100 ml beaker and heated in order to remove the CO_2 gas for 3 min and 40 ml of water was added. Using a magnetic stirrer, 0.1N solution of sodium hydroxide was titrated to pH 8.3 and color density was measured by using spectrophotometer. 1 ml of sauce, and 10 ml of distilled water were mixed and the optical density was measured at 500 nm. The color density was expressed by O.D. x 10.

Preparation of enzyme solution

One gram of ground paste was dispersed in 10 ml of water and extracted at 4°C for 4 hours and then centrifuged at 1080 x g for 10 min. The supernatant was used as the enzyme solution.[4]

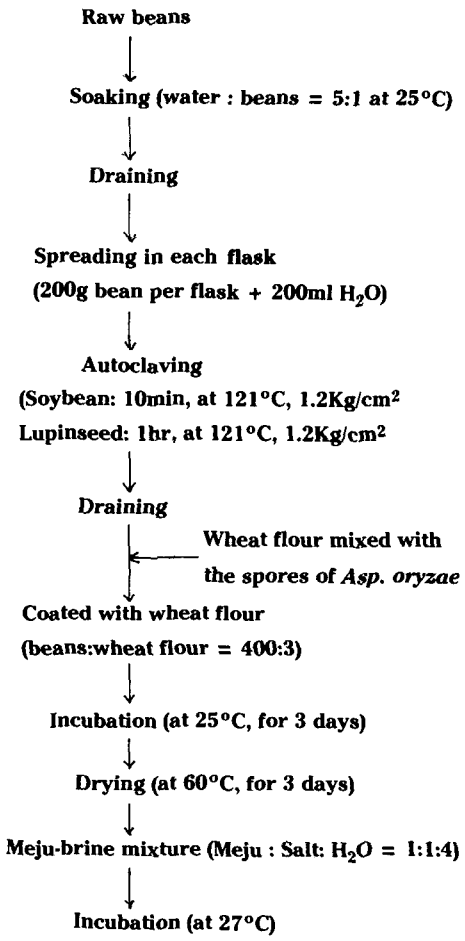


Fig. 1. Scheme of the processing procedure of Improved Meju fermentation and subsequent ripening in brine

Determination of protease activity

A modified Anson's method was used for the determination of protease activities. 5 ml of casein solution (0.6g of casein and 100 ml of 0.04M phosphate buffer at pH 7.0), and 1 ml of enzyme solution were mixed, and the mixture was incubated at 30°C for 10 min. The action of protease was stopped by adding 5 ml of 0.4 M trichloroacetic acid, and the precipitate was removed by filtration with filter paper (Wattman No. 2). Two mililiters of the filtrate, 5 ml of 0.5 M Na_2Co_3 and 1 ml of 0.3 M Folin's reagent were well mixed and after 30 min, the optical density was measured at 660 nm. One unit of protease activity was defined as the amount of enzyme that ceveloped the color equivalent to 1 u mole of tyrosine per min under these conditions.[5]

Amylase activity

The activity of amylase was determined by a modified Wohlgemuth's method which is based on the estimation of the time in which the color of iodine-starch complex decrease to the definite extent.[5] The reaction mixture containing 5 ml of 1% soluble starch, 4.75 ml of 0.1 M acetate buffer (pH 4.8) and 1 ml of the enzyme solution was kept at 40°C for 10 min. The optical density was measured at 660 nm. One unit of enzyme activity was defined as the amount of 1% soluble starch solution hydrolysed by the enzyme for 10 min at 40°C.

Sensory evaluation

Four-week ripended Meju-brine mixture was separated into sauce and paste by filtration. Sensory evaluation on the sauce and paste was made by 20

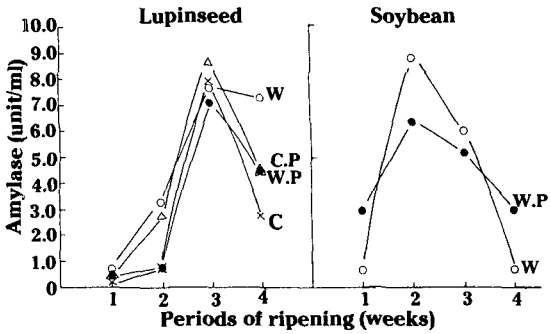


Fig. 2. Changes in Amylase activity of sauce during ripening

W: Whole bean Meju
W.P : Powder of Whole bean Meju
C : Cutted bean Meju
C.P : Powder of Cutted bean Meju

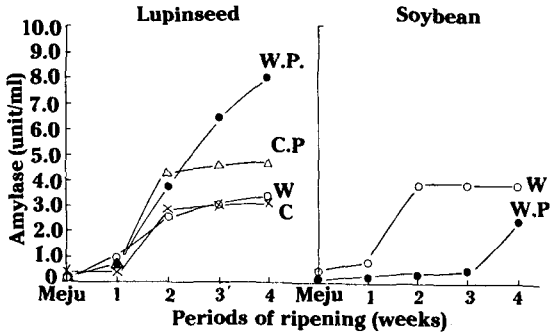


Fig. 3. Changes in Amylase activity of paste during ripening

members of trained panel. The rank-order test was made for flavor, color, taste and bitter taste, using 1 year old soysauce as the reference. For bitter taste, the lupin seed cooked at 121 °C, for 1 hr was used as the reference. The sample which had the nearest character compared to the reference was marked as 1 in the rank order test. In case

Changes during the ripening of mejubrine mixture

Figure 2 shows the changes in the amylase activity of liquid (or sauce) of Meju-brine mixture. The amylase activity in the sauce of lupin seed Meju-brine mixture gradually increased and reached to the maximum value after 3 weeks of ripening, and then decreased sharply. Whereas, in the sauce of soybean Meju-brine mixture, the maximum value was attained after 2 weeks of ripening and then decreased rapidly. The maximum value of the amylase activity was similar between lupin seed and soybean when whole bean Meju was used. The maximum amylase activity was slightly lowered when the powdered Meju was used.

The amylase activity in the paste of Meju-brine mix- of the color of sauce, the most dark brown was ranked as 1.

Results and Discussion

Chemical changes during meju fermentation

Table 1 shows the changes in the dry matter content of the beans during Meju fermentation. From 100 g of raw soybean 72-81 g of fermented Meju was obtained. The dry matter yield was in the range of 73-79%. No significant difference in dry matter retention between lupin seed and soybean was observed. When the hull of

Table 1. Changes in the total weight of beans during fermentation

Sample name	Raw (g)	Soaked (g)	Cooked (g)	Fermented (g)	Dryed fermented (g)	Yield (% of dry matter)
Lupinseed	100	240	238	235	81	79
Lupinseed (Cutted)	100	240	237	233	72	73
Soybean	100	230	226	224	77	75

lupin seed was cut, the loss of dry matter during fermentation became larger.

The contents of curde protein and crude fat were generally increased by the Meju fermentation. This was partly due to the reduction of the content of carbohydrate. It indicates that during fermentation, the energy for growth and propagation of microorganisms was mainly supplied by the carbohydrate in the seed. Lupin seed Meju contained less protein and fat but larger amount of carbohydrate compared to soybean Meju. ture was quite different from that in the sauce. The activity increased gradually for 4 weeks of the ripening. When the powdered lupin seed Meju was used, the amylase activity in the paste was significantly high. In the case of whole bean Meju-brine mixture, the amylase activity in the paste was similar between lupin seed and soybean. In this case, the amylase activity increased for two weeks of ripening and then maintained at the same level for 4 weeks. The carbohydrate content of the paste also

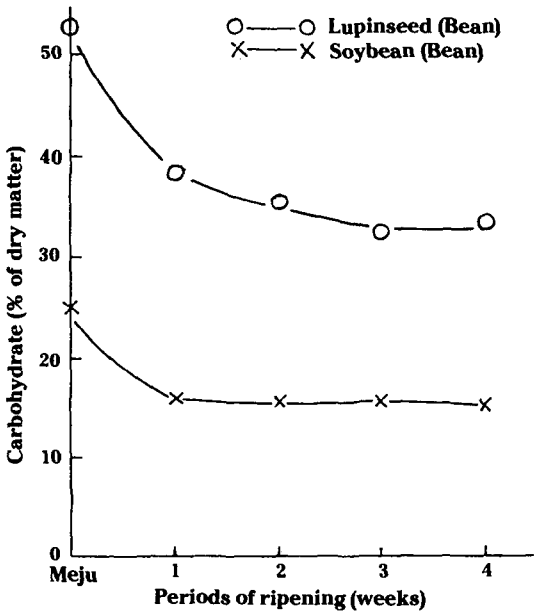


Fig. 4. Changes in the content of carbohydrate of paste during ripening

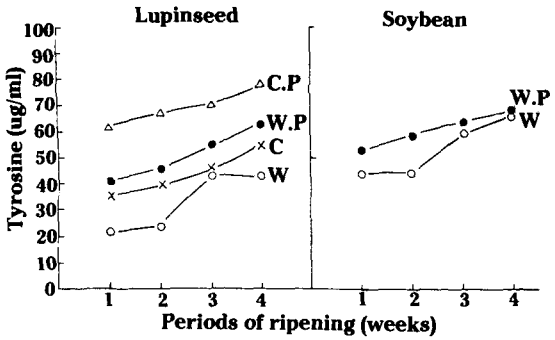


Fig. 5. Changes in protease activity of sauce during ripening

decreased rapidly during the first 2 weeks of ripening and then maintained almost constant level. (Figure 4)

Figure 5 shows the changes in the protease activity in the sauce of Meju-brine mixture. It increased rapidly during the first week of ripening and then gradually increased for 4 weeks of ripening. Significant differences in the activity were found between cutted lupin seed Meju and intact Meju, and also between whole bean Meju-brine mixture and powdered Meju-brine mixture. Cutted lupin seed Meju resulted in higher protease activity in the sauce. Powdered Meju-brine mixture gave higher activity in the sauce than whole bean Meju-brine mixture. No significant diference was noticed between lupin seed and soybean.

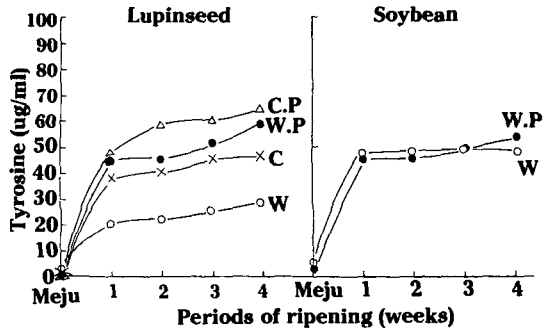


Fig. 6. Changes in protease activity of paste during ripening

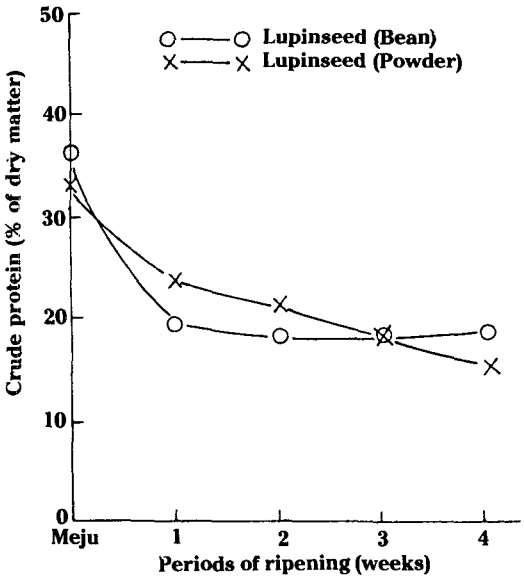


Fig. 7. Changes in the content of crude protein of paste during ripening

Similar results were also found in the paste of Meju-brine mixture; cutted lupin seed Meju and powdered Meju-brine mixture resulted in higher protease activity in the paste, as shown in Figure 6. However, the changes in the crude protein content of the paste during ripening were similar between whole bean Meju-brine mixture and powdered Meju-brine mixture (Figure 7). The content of crude protein decreased rapidly during the first week of ripening and then gradually decreased for 4 weeks of ripening.

The content of free amino nitrogen of the mixture increased during the ripening of Meju-brine mixture (Figure 8). It reached to 40-50% of total nitrogen after two weeks of ripening in the brine. Intact lupin seed Meju gave larger FAN/TN ratio than cutted lupin seed Meju. No significant difference was noticed between lupin seed and soybean. Figure 9 shows the color development of the fermented sauces. In case of lupin seeds, cutted bean Meju and powdered Meju-brine mixture gave higher color density of the sauce. In case of soybean, whole bean Meju-brine mixture gave higher color density in the sauce.

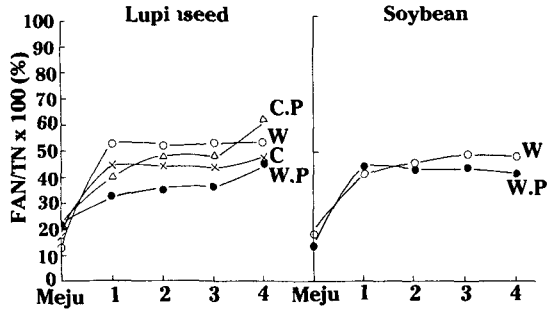


Fig. 8. Changes in % free amino-N (FAN/TN x 100) during the ripening of Meju-brine mixture.

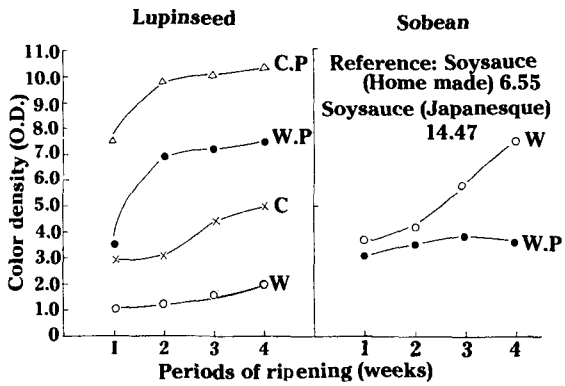


Fig. 9. Changes in the color density of sauce during ripening (O.D., at 500nm)

Quality of fermented lupin seed sauce and paste

Table 3 shows the chemical composition, pH and acidity of the bean sauce ripened for four weeks in Meju-brine mixture. When powdered Meju was ripened in the brine, the crude protein in the sauce increased. Large differences in the chemical composition were found between different fermentation methods. When the seed

Table 2. The general chemical composition of Meju (% of dry weight)

Sample name	Ash	Crude protein	Crude fat	Carbohydrate
Raw Lupinseed	2.71	31.32	7.87	58.10
Lupinseed (Bean)	3.16	36.40	7.74	52.70
Lupinseed (Cutted, Bean)	2.87	34.40	9.11	53.64
Raw Soybean	5.07	40.60	17.33	37.00
Soybean (Bean)	5.58	43.80	25.49	25.13

hull was cutted before Meju making, the resultant sauce had higher crude protein and sugar contents but lower pH and higher acidity compared to the sauce made from intact lupin seed Meju.

The sauce made from whole intact lupin seed Meju had similar pH and acidity to soybean sauce. Table 4 shows the chemical compositon of bean pastes which were separated from the Meju-brine mixture ripened for 4 weeks. The chemical compositon varied widely between different fermentation methods. Fermented lupin seed paste had much less crude protein and crude fat compared to soybean paste.

The results of sensory evaluation of the fermented bean sauces, are shown in Table 5. The sauce made from whole lupin seed Meju marked the weakest color. Powdered Meju-brine mixture gave darker color intensity. The flavor and taste of fermented lupin seed sauce were similar to those of soybean sauce.

In case of fermented paste, the flavor and taste of lupin seed paste were significantly inferior to those of soybean paste. Bitter taste of lupin seed paste was an important factor diminishing the acceptance. It indicates that the bitter taste of lupin seed was not removed by the traditional Korean Chang fermentation method. The bitter taste problem was severe in the paste rather than sauce. Pictures 1 - 2 show the fermented bean products made from soybean and lupin seed.

Table 5. Data of sensory evaluation (Sauce)

Characteristics / Sample name	Color	Flavor	Taste	Bitter taste
Lupinseed (Bean)	5.85*	3.95	3.90	3.75
Lupinseed (Power)	2.25	3.90	3.35	3.70
Lupinseed (Cutted, Bean)	3.75	3.35	3.15	2.90
Lupinseed (Cutted, Powder)	1.25	3.30	3.40	4.25
Soybean (Bean)	2.85	3.65	4.45	3.75
Soybean (Powder)	5.05	2.85	2.75	2.65

* Average of ranking

Lupin seed could be used in place of soybean for making traditional Korean soysauce. It produced acceptable bean sauce of which flavor and taste were similar to soy sauce. The typical dark brown color of fermented bean sauce developed more rapidly with lupin seed Meju compared to soybean Meju. On the other hand, fermented lupin seed paste had inferior quality compared to soybean paste.

Table 3. The general chemical composition of sauce after 4-week ripening (% of dry matter)

Sample name	Moisture content	Ash	Crude protein	Crude fat	Reducing sugar	pH	Acidity (Acetic acid g/100ml)
Lupinseed (Bean)	76.00	67.92	11.88	—	4.50	5.35	0.54
Lupinseed (Powder)	72.00	63.20	22.89	—	4.30	4.20	0.65
Lupinseed (Cutted, Bean)	74.30	63.81	14.79	—	4.80	3.95	0.72
Lupinseed (Cutted, Powder)	70.00	62.00	16.40	—	4.70	4.18	0.70
Soybean (Bean)	75.00	66.40	15.68	—	3.20	5.25	0.56
Soybean (Powder)	72.00	64.64	17.61	—	3.50	5.56	0.54

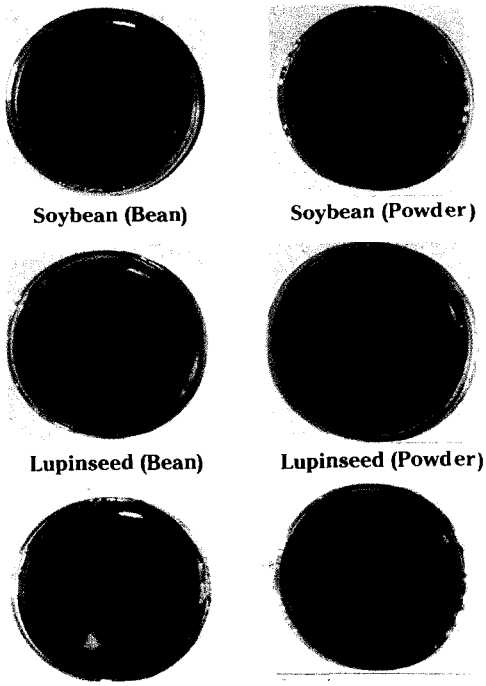
Table 4. The general chemical composition of paste after 4-week ripening (% of dry matter)

Sample name	Ash	Crude protein	Crude fat	Carbohydrate
Lupinseed (Bean)	43.90	18.10	4.52	33.48
Lupinseed (Powder)	43.55	15.44	7.10	33.91
Lupinseed (Cutted, Bean)	39.70	19.15	8.01	33.14
Lupinseed (Cutted, Powder)	49.21	11.51	9.50	29.78
Soybean (Bean)	42.86	21.32.	21.00	15.00
Soybean (Powder)	38.60	24.88	20.44	16.08

Table 6. Data of sensory evaluation (Paste)

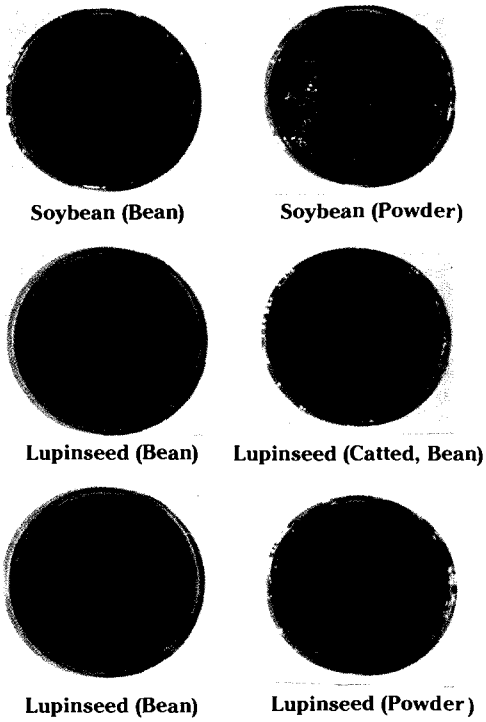
Characteristics / Sample name	Color	Flavor	Taste	Bitter taste
Lupinseed (Bean)	2.30*	3.45	2.85	3.35
Lupinseed (Powder)	4.15	4.15	3.85	4.35
Lupinseed (Cutted, Bean)	4.25	4.10	3.25	3.65
Lupinseed (Cutted, Powder)	4.45	3.75	5.00	4.40
Soybean (Bean)	2.05	2.30	2.50	2.95
Soybean (Powder)	3.80	3.25	3.55	4.25
Lupinseed (Cooked, Salt added)	—	—	—	4.85

* Average of ranking



Picture 1. Bean sauce products made from soybean and lupin seed

The desirable process for making bean sauce from lupin seed was recommended as follows: Raw beans are cleaned and washed in water and soaked in water overnight. Soaked beans are drained and cooked in an autoclave filled with water at 121°C for 1 hr. Cooked beans are drained and cooled to 30°C and innoculated with. *Asp. oryzae* in wheat flour mixture. (bean: flour =400:3)



Picture 2. Bean paste products made from soybean and lupin seed

It is incubated at 25°C for 3 days, and then dried at 60°C for 3 days to make dried lupin seed Meju. Meju-brine mixture is made by adding 4 parts of water and 1 part of salt to 1 part of dried lupin seed Meju. Meju-brine mixture is ripened at 7°C for longer than 4 weeks. At the end of the ripening, sauce is separated from the paste by filtration.

요 약

본 실험은 대두콩 대신 루우핀콩으로 메주를 제조하여 숙성시키면서 숙성기간중에 일어나는성분 변화를 측정함과 동시에 대두콩으로 제조한 메주와 그 발효 특성을 비교 하였다.

완전히 익은 콩에 *Asp. oryzae*를 접종하여 개량식 메주를 제조하였고, 건조된 메주는 소금물에 담궈 4주동안 숙성시켜 간장과 된장을 제조하였다.

일반적으로 숙성기간중, 단백질 분해효소와 전분 분해효소의 활성은 대두콩 메주에서 보다는 루우핀콩 메주에서 더 높았으며, 단백질 분해효소의 활성에서의 증가는 발효된 된장과 간장의 α-아미노태 질소 함량의 증가와 직접적인 상관

관계를 나타내었으며 숙성 중 간장의 색깔이 진한 갈색으로 되는 정도는 대두콩 메주와 비교할 때, 루우핀콩 메주에서 더 빨랐다.

또한 이렇게 발효된 간장과 된장에 대해 관능검사한 결과 루우핀콩 된장은 전반적인 품질면에서 대두콩으로 제조한 된장에 비해 떨어졌으나 루우핀콩 간장은 전반적인 품질면에서 만족할 수 있었다.

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