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# The Effect of Korean Soysauce and Soypaste Making on Soybean Protein Quality

## Part II. Chemical Changes During Meju-brine Ripening

by

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# 재래식 간장 및 된장 제조가 대두 단백질의 영양가에 미치는 영향

## 제 2 보 메주장의 숙성중에 일어나는 성분 변화

이 철 호
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## Abstract

The laboratory Mejus as well as home-made Meju and improved Meju received from Korea were ripened in the brine for up to 8 months and the changes in the chemical composition during the process were determined and the differences between the types of Meju were compared. On the basis of the amino acid pattern, the changes in the protein quality of soybean during the process was evaluated.

No significant changes in the general chemical composition of Meju were noticed during the ripening for 8 months. However, the nitrogen solubility of Meju increased for 13~29% to 66~78% during 8 month ripening of the Meju-brine mixture. The concentration of free amino-N to the total-N increased from 4~7% in Meju to 29~35% in the 8month ripened mixture. The concentration of amino-N to the total-N increased from 1~4% in Meju to 5~14% in the 8month ripened mixture and the changes varied with the type of Meju used.

Remarkable changes in the amino acid pattern of soybean were occured during the ripening

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process. The concentration of methionine decreased to the half of original Meju during the first month of ripening. Arginine and histidine were destroyed rapidly by the ripening longer than 1 month. A considerable amount of ornithine was synthesized during the ripening. The amino acid pattern of Meju did change drastically during the ripening longer than 3 months and the changes varied with the type of Meju.

The retention of the nutrients in soybean during 8 month ripening of the laboratory 3 month Meju in the brine was 49% for carbohydrates, 107% for crude fat, 93% for crude protein and 74% for the total amino acid. Histidine, arginine and methionine and 74% for the total amino acid. Histidine, arginine and methionine were the most damaged during the process, retaining only 25%, 27% and 49% of the contents in raw soybean, respectively, whereas lysine retained 79%.

By the separation of the 8 month ripened mixture, approximately 60% of crude protein, all of crude fat and 80% of carbohydrates in the mixture were retained in soypaste. Soypaste contained higher concentrations of amino acids per 16gN compared to soysauce, except for lysine

The most limiting amino acid of the protein was the S-containing amino acids in all cases studied, whereas the second limiting amino acid varied from valine in soybean to threonine in most of Mejus and the brine mixtures, lysine in most of soypastes and tryptophan in some of soysauces.

According to the protein quality evaluation made by the reference of the FAO provisional pattern of amino acid, the chemical score of raw soybean was 82, which was reduced to 77 by cooking and further reduced to 71∼74 by Meju fermentation. At the eighth month of ripening the chemical score of the Meju-brine mixtures were reduced to 51∼66. After the separation, the chemical score of soypaste ranged from 60 to 71, whereas that of soysauce varied from 45 to 57. Generally, the products made from improved Meju recorded the highest score, whereas those made from homemade Meju showed the poorest protein quality.

The essential amino acid index(EAAI) of the samples was similar to the chemical score, but it appeared to fit the overall changes in the amino acid pattern during the process better than the chemical score.

## Introduction

In part. 1, the changes in the chemical composition of soybean during Meju making were studied. In the present experiment the laboratory Mejus as well as home-made Meju and improved Meju received from Korea were ripened in the brine for up to 8 months and the changes in the chemical composition during the process were determined and the differences between the types of Meju were compared. The retention of the nutrients during the whole process, from soybean to the 8 month ripened Meju-brine mixture, was estimated. The distribution of the nutrients into soysauce and soypaste by the separation of the ripened mixture was also estimated. On the basis of the amino acid pattern, the changes in the protein quality of soybean during the process were evaluated.

## Materials and methods

### 1. Sample preparation

The Meju-brine mixtures were prepared from homemade Meju and improved Meju as well as the laboratory Mejus, which were fermented for one month or more, by adding 1 part salt to 4 parts water to 1 part Meju. The Meju-brine mixtures were ripened at room temperature for varying periods of 1 month, 3 months and 8 months for each Meju.

Soypaste and soysauce were obtained by the filtrarion of the Meju-brine mixtures ripened for 8 months.

The samples were freeze dried, when necessary, and were ground to powder for the analyses. Soysauce were analysed without further preparation.

### 2. Analytical methods

All the analytical methods used in the present experiment were identical to those described in part. 1, except for the determination of lysine content in soypaste and soysauce.

The occurence of ornithine in hydrolysates of proteins of microbial origin has been widely recognized[1] and the Moor and Stein system of amino acid analysis of protein hydrolysates includes ornithine, if it exists, in the lysine content. Furthermore, the unusually large amount of ammonia in soysauce disturbed the measurement of the basic amino acids by the automatic amino acid analyzer. The lysine content of these products was determined by the Peterson and Bernlohr method [2]. This method is a modification of the ordinary Moor and Stein method separating, among others ornithine from lysine by changing the pH of the elusion buffer and other elusion conditions with the same column used for the ordinary method. The difference between Peterson and Bernlohr method and the ordinary Moor and Stein method are as follows:

| | Peterson & Bernlohr | Moor & Stein |
|---|---|---|
| pH of elusion buffer | 4.60 | 5.28 |
| Elusion rate | 60 ml/hr | 68 ml/hr |
| Ninhidrin flow rate | 30 ml/hr | 34 ml/hr |
| Column temperature | 42°C. | 55°C. |
| Resin | Bio-Rad Aminex A-5 | Amberlite CG-120 |

## Results

### 1. Changes in the general chemical composition

Table 1. shows that the concentration of crude protein, crude fat, carbohydrates and ash of the Meju-brine mixtures did not change significantly during ripening periods of up to 8 months. The high concen tration of ash in the mixtures was due to the addition of salt which amounts to 16~17% of total weight.

### 2. Nitrogenous components

Table 2. shows the changes in the nitrogenous components during the 8 month ripening of Meju-brine mixtures made from the different types of Meju. The soluble nitrogen of the mixtures increases rapidly during the first month of ripening, moderately from the second to the third month and then rather slowly for the remaining 5 months. All types of Meju studied showed the same tendency, although the ratio of soluble nitrogen to total nitrogen in the mixture of improved Meju was lower (65%) than for the others (75%).

The liberation of free amino acid in the mixtures showed the same tendency as for that of soluble nitrogen. The ratio of free amino-nitrogen to the total nitrogen increased from 4~7% in the Meju to 29~35% in the Mejubrine mixtures after 8 months of ripening.

**Table 1. Changes in the general chemical composition of Meju-brine mixtures for the different periods of ripening.** (%)

| Meju | Period of ripening | Moisture | Ash | Crude Protein | Crude fat | Carbohydrates |
|---|---|---|---|---|---|---|
| 2-month Meju | 1 month | 68.06 | 17.17 | 7.43 | 3.98 | 3.36 |
| | 3 months | 68.10 | 17.30 | 7.59 | 4.31 | 2.70 |
| | 8 months | 68.53 | 17.49 | 7.00 | 4.44 | 2.54 |
| 3-month Meju | 1 month | 67.25 | 16.71 | 7.42 | 4.30 | 3.32 |
| | 3 months | 67.20 | 17.55 | 7.65 | 4.27 | 3.33 |
| | 8 months | 67.87 | 16.68 | 7.73 | 4.69 | 3.03 |
| Home-made Meju | 1 month | 67.95 | 16.78 | 8.60 | 3.47 | 3.20 |
| | 3 months | 68.43 | 16.13 | 8.40 | 2.94 | 4.10 |
| | 8 months | 68.45 | 17.72 | 8.10 | 3.45 | 2.28 |
| Improved Meju | 1 month | 69.77 | 16.89 | 7.14 | 3.08 | 3.12 |
| | 3 months | 71.35 | 14.93 | 7.13 | 3.28 | 3.31 |
| | 8 months | 68.86 | 17.01 | 7.27 | 3.59 | 3.27 |

**Table 2. Changes in the nitrogenous components during the ripening of Meju-brine mixture.**

| Meju | Period of Ripening | % soluble-N SN/TN×100 | % free amino-N FAN/TN×100 | % ammonia-N $NH_3$-N/TH×100 |
|---|---|---|---|---|
| 2-monthMeju | Meju | 29.0 | 7.1 | 2.0 |
| | 1 month | 54.8 | 16.7 | 2.4 |
| | 3 months | 70.1 | 30.4 | 6.3 |
| | 8 months | 77.5 | 34.7 | 8.2 |
| 3-month Meju | Meju | 27.8 | 6.4 | 1.4 |
| | 1 month | 50.7 | 17.4 | 1.3 |
| | 3 months | 63.5 | 21.6 | 4.1 |
| | 8 months | 74.6 | 29.0 | 8.7 |
| Home-made Meju | Meju | 25.5 | 5.8 | 4.4 |
| | 1 month | 52.6 | 21.3 | 6.4 |
| | 3 months | 61.8 | 29.0 | 7.6 |
| | 8 months | 74.9 | 31.5 | 13.6 |
| Improved Meju | Meju | 13.9 | 3.7 | 0.7 |
| | 1 month | 45.4 | 19.7 | 3.3 |
| | 3 months | 58.1 | 28.9 | 5.3 |
| | 8 months | 65.5 | 34.9 | 5.0 |

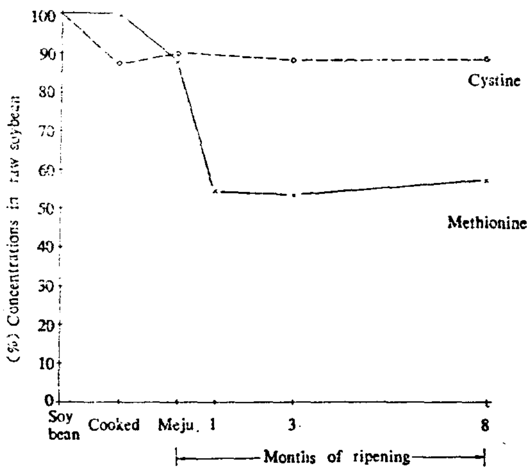
In this case, improved Meju showed the highest liberation of free amino acids from the protein compared with other Mejus.

On the other hand, the concentration of amino nitrogen increased slightly during the first month of ripening but developed rapidly for the remaining 7 months of ripening. The ratio of amino nitrogen to total nitrogen increased from 0.7~4.4% in the Meju to 5.0~13.6 in the Meju-brine mixtures after 8 months of ripening. There was observed a large variation between the different types of Meju. Home-made Meju produced a suprisingly large amount of ammonia between the third and the eighth month of ripening, whereas, in the case of improved Meju, the concentration decreased for the same period and was only 1/3 of that of the home-made Meju-brine mixture.

### 3. Amino acid pattern

The amino acid composition of Mejus and Meju-brine mixture for different ages of ripening are shown in Tables 3, 4, 5 and 6. The concentration of aspartic acid, glycine, alanine, tyrosine and phenylalanine in the different types of Meju did not change significantly for the entire ripening period of 8 months.

Generally, the amino acid pattern of Meju did not change significantly during the first month of ripening in the brine mixture except for methionine, which was reduced by 40% of the original concentration in the Meju during the same period and remained at the same level for the remainder of the ripening period. On the other hand, as shown in Fig. 1, the cystine which was reduced by 12% of the concentration in




Fig' 1. Changes in the concentrations of methionine and cystine during cooking, 3-month Meju fermentation and ripening.

**Table 3. Changes in amino acid composition of Meju-brine mixture during the ripening of 2 month Meju.** (g/16g N)

| Amino acid | 2 month Meju | Ripening period | | |
|---|---|---|---|---|
| | | 1 month | 3 months | 8 months |
| Aspartic acid | 11.33 | 11.23 | 11.55 | 11.12 |
| Threonine | 3.66 | 3.46 | 3.51 | 2.88 |
| Serine | 4.27 | 4.26 | 3.02 | 2.23 |
| Glutamic acid | 19.56 | 19.45 | 16.62 | 14.05 |
| Proline | 4.49 | 4.87 | 4.79 | 4.66 |
| Glycine | 4.05 | 4.12 | 4.05 | 3.84 |
| Alanine | 4.08 | 4.12 | 4.37 | 4.39 |
| Valine | 4.71 | 4.75 | 6.54 | 5.44 |
| Isoleucine | 4.52 | 4.47 | 4.35 | 4.14 |
| Leucine | 7.29 | 7.18 | 6.92 | 6.69 |
| Tyrosine | 4.08 | 3.87 | 4.47 | 3.69 |
| Phenylalanine | 4.94 | 5.29 | 5.27 | 4.96 |
| Lysine** | 5.84 | 5.45 | 8.36 | 8.30 |
| Histidine | 2.38 | 2.27 | 1.74 | 0.98 |
| Arginine | 6.38 | 5.93 | 3.72 | 2.95 |
| Methionine | 1.30 | 0.79 | 0.78 | 0.98 |
| Cystine | 1.19 | 1.13 | 1.27 | 1.33 |
| Tryptophan | — | — | — | 1.14 |
| (Ammonia) | (1.99) | (1.81) | (2.07) | (1.76) |
| Total amino acid | 94.07* | 92.64* | 91.33* | 83.77 |
| EA/TA×100 | 39.9* | 39.3* | 45.4* | 41.2 |

* Tryptophan is not included ** Ornithine included

**Table 4. Changes in amino acid composition of Meju-brine mixture during the ripening of 3 month Meju.** (g/16g N)

| Amino acid | 3 month Meju | Ripening period | | |
|---|---|---|---|---|
| | | 1 month | 3 months | 8 months |
| Aspartic acid | 10.79 | 11.05 | 10.47 | 10.55 |
| Threonine | 3.54 | 3.51 | 3.54 | 2.87 |
| Serine | 4.27 | 3.99 | 4.00 | 2.27 |
| Glutamic acid | 20.29 | 19.75 | 18.43 | 14.66 |
| Proline | 4.67 | 4.78 | 3.78 | 4.40 |
| Glycine | 4.16 | 4.15 | 3.84 | 3.39 |
| Alanine | 4.10 | 4.27 | 4.08 | 3.29 |
| Valine | 4.78 | 4.89 | 4.98 | 5.02 |
| Isoleucine | 4.50 | 4.53 | 4.63 | 4.14 |
| Leucine | 7.22 | 7.01 | 7.34 | 6.69 |
| Tyrosine | 4.19 | 4.11 | 4.17 | 3.95 |
| Phenylalanine | 5.30 | 5.22 | 5.21 | 4.99 |

| | | | | |
|---|---|---|---|---|
| Lysine** | 5.97 | 5.90 | 5.60 | 7.15 |
| Histidine | 2.39 | 2.36 | 2.31 | 0.76 |
| Arginine | 6.23 | 6.30 | 5.01 | 2.15 |
| Methionine | 1.29 | 0.93 | 0.87 | 0.78 |
| Cystine | 1.31 | 1.35 | 1.27 | 1.32 |
| Tryptophan | — | — | — | 0.72 |
| (Ammonia) | (2.00) | (2.31) | (1.36) | (1.45) |
| Total amino acid | 95.00* | 94.10* | 89.53* | 79.10 |
| EA/TA×100 | 40.1* | 39.8* | 42.0* | 40.9* |

* Tryptophan is not included　　　　** Ornithine included

**Table 5. Changes in amino acid composition of Meju-brine mixture during the ripening of home-made Meju.** (g/16g N)

| Amino acid | Home-made Meju | Ripening period | | |
|---|---|---|---|---|
| | | 1 month | 3 months | 8 months |
| Aspartic acid | 10.26 | 10.25 | 10.08 | 9.18 |
| Threonine | 3.46 | 3.41 | 3.69 | 2.75 |
| Serine | 3.98 | 3.90 | 4.15 | 2.30 |
| Glutamic acid | 16.12 | 16.11 | 14.95 | 11.50 |
| Proline | 4.15 | 4.43 | 4.20 | 2.91 |
| Glycine | 4.07 | 4.10 | 3.85 | 3.78 |
| Alanine | 4.15 | 4.29 | 4.06 | 3.95 |
| Valine | 4.80 | 4.92 | 4.79 | 4.48 |
| Isoleucine | 4.25 | 4.45 | 4.37 | 2.89 |
| Leucine | 6.84 | 7.05 | 6.87 | 4.95 |
| Tyrosine | 3.57 | 3.86 | 3.62 | 3.98 |
| Phenylalanine | 4.99 | 5.05 | 5.19 | 4.94 |
| Lysine** | 5.61 | 6.07 | 6.10 | 7.25 |
| Histidine | 2.23 | 2.20 | 2.06 | 1.01 |
| Arginine | 5.97 | 3.94 | 2.30 | 1.11 |
| Methionine | 1.25 | 0.79 | 0.78 | 0.71 |
| Cystine | 1.17 | 1.29 | 1.11 | 1.08 |
| Tryptophan | — | — | — | 1.13 |
| (Ammonia) | (2.71) | (3.08) | (2.15) | (2.15) |
| Total amino acid | 86.87* | 86.11* | 82.17* | 69.90 |
| EA/TA×100 | 41.4* | 42.8* | 44.4* | 41.6 |

* Tryptophan is not included　　　　** Ornithine included

raw soybean by cooking, was quite stable during the ripening for 8 months.

The amino acid pattern of Meju was moderately changed by 3 months of ripening and seriously changed after 8 months of ripening. Arginine and histidine were affected more seriously than the others. As shown in Fig. 2, the concentrations were reduced to half of the original Meju after 8 months of ripening. The concentration of arginine was reduced rapidly for the first three months of ripening, whereas that of histidine was reduced more rapidly between the fourth and eighth month of ripening than in the first three

**Table 6. Changes in amino acid composition of Meju-brine mixture during the ripening of im proved Meju.** (g/16g N)

| Amino acid | Improved Meju | Ripening period | | |
|---|---|---|---|---|
| | | 1 month | 3 months | 8 months |
| Aspartic acid | 11.3 | 11.46 | 10.18 | 10.41 |
| Threonine | 3.90 | 3.80 | 3.84 | 3.69 |
| Serine | 4.74 | 4.52 | 4.46 | 4.05 |
| Glutamic acid | 18.85 | 18.47 | 18.44 | 16.24 |
| Proline | 4.93 | 5.01 | 4.27 | 4.78 |
| Glycine | 4.23 | 4.13 | 4.26 | 3.62 |
| Alanine | 4.30 | 4.13 | 5.02 | 4.16 |
| Valine | 5.01 | 4.98 | 5.19 | 4.93 |
| Isoleucine | 4.72 | 4.65 | 4.68 | 4.52 |
| Leucine | 7.66 | 7.29 | 7.47 | 7.07 |
| Tyrosine | 4.41 | 4.16 | 4.48 | 3.85 |
| Phenylalanine | 5.06 | 4.69 | 5.09 | 4.83 |
| Lysine** | 5.71 | 5.89 | 6.48 | 5.65 |
| Histidine | 2.43 | 2.44 | 2.12 | 1.31 |
| Arginine | 6.86 | 4.84 | 3.88 | 3.04 |
| Methionine | 1.41 | 0.72 | 0.75 | 0.88 |
| Cystine | 1.37 | 1.16 | 1.29 | 1.27 |
| Tryptophan | — | — | — | 1.20 |
| (Ammonia) | (1.86) | (2.03) | (1.48) | (1.27) |
| Total amino acid | 96.92* | 92.34* | 91.90* | 85.50 |
| EA/TA×100 | 40.5* | 40.4* | 42.7* | 38.3 |

* Tryptophan is not included ** Ornithine includded

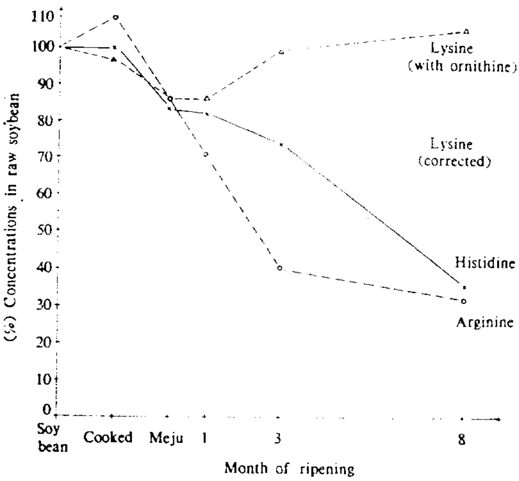



**Fig. 2. Changes in the concentrations of the basic aminoacids during cooking, 3 month Meju fermentation and ripening.**

months.

On the other hand, the concentration of lysine in the mixtures appeared to increase during the ripening period. It is probably caused by the analytical error, i.e., as mentioned earlier, the mixtures were expected to contain a considerable amount of ornithine which was measured as lysine. As shown in Table. 7, the concentration of ornithine in the separated soysauce and soypaste was extraordinarily high ranging from 1.2g to 5.2g per 16g nitrogen. The corrected concentration of lysine in the Meju-brine mixtures aged for 8 months indicated that the lysine content was not affected significantly by the ripening process.

The changes in the pattern of amino acid during the ripening varied with the type of Meju. In the case of homemade Meju, considerable changes in the pattern were observed as early as the first 3 month

**Table 7. Ornithine concentration in the fermented soybean products.(g/16 gN)**

| Products | Ornithine |
|---|---|
| 1-month Meju | 0.18 |
| -brine mixture ripened for 1 month | 0.36 |
| 2-month Meju | |
| -brine mixture ripened for 8 months* | 1.44 |
| -soypaste | 1.17 |
| -soysauce | 1.80 |
| 3-month Meju | |
| -brine mixture ripened for 8 months* | 1.93 |
| -soypaste | 1.59 |
| -soysauce | 2.46 |
| Home-made Meju | |
| -brine mixture ripened for 8 months* | 2.47 |
| -soypaste | 1.75 |
| -soysauce | 3.56 |
| Improved Meju | |
| -brine mixture ripened for 8 months* | 3.36 |
| -soypaste | 1.99 |
| -soysauce | 5.18 |

* Calculated from values in soypaste and soysauce

of ripening, whereas, in the case of improved Meju, the overall changes were first observed after the eighth month ripening. The concentrations of proline, isoleucine and leucine in home-made Meju decreased significantly from the third to the eighth month of ripening, whereas they were not changed significantly in the other types of Meju. The concentrations of threonine and serine decreased considerably from the third to the eighth month of ripening for home-made Meju and laboratory Mejus but not for improved Meju. The content of total amino acid in the crude protein of Meju and of its brine mixture varied with the type of Meju. The gram total amino acid per 100g crude protein tended to decrease during the ripening process, as shown in Fig. 3. Home-made Meju contained 86 g amino acid per 100g crude protein. This was reduced to 70g after 8 months of ripening. Improved Meju had the highest value with 97g amino acid per 100g crude protein and this decreased to 86g after the 8 month ripening.

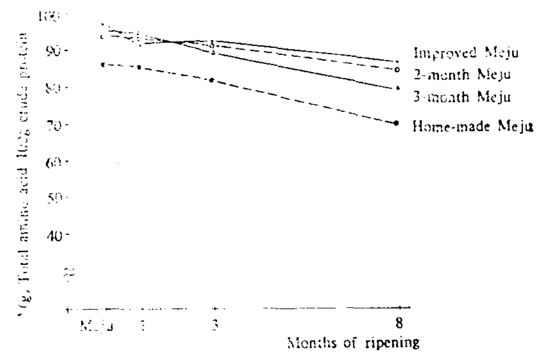



**Fig. 3. Changes in the content of total amino acid in the crude protein of soybean during the ripening of different types of Meju.**

**4. Retention of nutrients**

An estimation was made of the yield of nutrients by the ripening for 8 months of the Meju-brine mixtures made from the different types of Meju. As shown in Table. 8, the crude protein of the original Mejus decreased within the range of 0~9% according to the type of Meju used, whereas crude fat increased by 10~22% of the original amount for the 8 months of ripening. The largest loss observed was of carbohydrates, as in the case of Meju fermentation. The

**Table 8. Yield of nutrients in different types of Meju by 8 months ripening of Meju-brine mixture. (%)**

| Meju | Crude protein | Crude fat | Carbohydrates |
|---|---|---|---|
| 2-months Meju | 94 | 111 | — |
| 3-months Meju | 100 | 115 | 83 |
| Home-made Meju | 94 | 122 | 63 |
| Improved Meju | 91 | 110 | 71 |

amount of carbohydrates in the original Meju was reduced by 17~37% for the ripening period of 8 months. In the case of home-made Meju brine mixture the loss of carbohydrates was larger than for the mixtures made from other Mejus, whereas the gain in crude fat was the highest.

Table. 9 shows the percentage of nutrients in raw soybean retained at each stage of the process using 3 month laboratory Meju. The Meju brine mixture retain-

**Table 9. Retention of nutrients in soybean during the processing of Meju products. (%)**

| Nutrients | Soaking | Cooking | 3-month Meju fermentation | 8 month Meju-brine ripening |
|---|---|---|---|---|
| Carbohydrates | 86 | 81 | 59 | 49 |
| Crude fat | 101 | 96 | 93 | 107 |
| Crude protein | 101 | 96 | 93 | 93 |
| Total amino acid | 99 | 96 | 90 | 74 |
| Lysine | 97 | 93 | 82 | 79 |
| Methionine | 98 | 96 | 81 | 49 |
| Histidine | 97 | 97 | 79 | 25 |
| Arginine | 104 | 104 | 78 | 27 |

ed only one half of the carbohydrates contained in the raw soybean at the completion of the ripening for 8 months. On the other hand, crude fat in the raw soybean decreased by 7% during the soaking, cooking and 3-month Meju fermentation stages but was produced during the subsequent 8 month ripening with the final content in the ripened mixture being 7% larger than in the raw soybean. Although the content of crude protein decreased by only 7% during the entire process, approximately 1/4 of the total amino acid in the raw soybean was lost during the same process. Methionine, histidine and arginine were most seriously damaged retaining only 49%, 25% and 27%, respectively, of those contained in the raw soybean. The largest portion of the loss occured during the ripening period, particularly from the third to eighth month of ripening. On the other hand, lysine was one of the stable amino acids during the process retaining about 80% of the original content in the raw soybean.

5. **Distribution of the nutrients in Meju-brine mixture after the separation into sysauce and soypaste**

Table. 10 shows the general chemical composition of soysuce and soypaste obtained by the separation of the Meju-brine mixture ripened for 8 months. A quantitative estimation was made of the distribution of the nutrients after the separation. As shown in Table. 11, the soypaste contained about 60% of the crude protein and nearly all of the crude fat of the original mixture with no significant difference between the types of Meju being observed.On the other hand, the distribution of carbohydrates varied with the type of Meju with 70~90% contained in the pastes.

**Table 10. General chemical Composition of soypaste and soysauceobtained from the separation of Meju-Brine mixtures ripened for 8 months. (%)**

| Meju | Product | Moisture | Ash | Crude protein | Crude fat | Carbo-hydrate |
|---|---|---|---|---|---|---|
| 2-month Meju | Paste | 60.38 | 17.90 | 8.49 | 8.78 | 4.45 |
| | Sauce | 72.76 | 20.33 | 6.38 | 0.07 | 0.46 |
| 3-month Meju | Paste | 60.93 | 18.42 | 8.47 | 8.30 | 3.88 |
| | Sauce | 72.75 | 20.05 | 6.56 | 0.06 | 0.58 |
| Home-made Meju | Paste | 61.42 | 17.66 | 10.05 | 6.39 | 4.48 |
| | Sauce | 72.17 | 19.59 | 6.69 | 0.06 | 1.49 |
| Improved Meju | Paste | 61.76 | 16.33 | 9.32 | 7.72 | 4.87 |
| | Sauce | 73.96 | 18.92 | 5.47 | 0.00 | 1.65 |

The distribution of crude protein depened on the period of ripening with 30% contained in soysauce from the Meju-brine mixture ripened for 1 month increasing to 40% from the mixture ripened for 8

Table 11. Distribution of the nutrients after the separation of the Meju-brine mixture ripened for 8 months. (%)

| Meju | Crude protein | | Crude fat | | Carbohydrates | |
|---|---|---|---|---|---|---|
| | Paste | Sauce | Paste | Sauce | Paste | Sauce |
| 2-month Meju | 58 | 42 | 99 | 1 | 91 | 9 |
| 3-month Meju | 61 | 39 | 99 | 1 | 89 | 11 |
| Home-made Meju | 60 | 40 | 99 | 1 | 75 | 25 |
| Improved Meju | 57 | 43 | 100 | 0 | 70 | 30 |

Table 12. Essential amino acid concentration in soypaste and soysauce separated from the Meju-brine mixture after 8 month ripening (g/16gN)

| Amino acid | 2-month Meju | | 3-month Meju | | Home-made Meju | | Improved Meju | |
|---|---|---|---|---|---|---|---|---|
| | Paste | Sauce | Paste | Sauce | Paste | Sauce | Paste | Sauce |
| Threonine | 3.87 | 2.71 | 3.43 | 2.50 | 2.53 | 3.50 | 4.11 | 3.82 |
| Valine | 5.10 | 3.95 | 5.09 | 4.58 | 4 97 | 3.96 | 5.26 | 4.20 |
| Isoleucine | 4.63 | 3.37 | 4.33 | 3.20 | 4.62 | 4.30 | 4.90 | 5.62 |
| Leucine | 7.38 | 5.24 | 7.19 | 5.22 | 7.40 | 6.10 | 7.70 | 6.98 |
| Tyrosine | 5.00 | 1.40 | 5.18 | 1.55 | 5.39 | 1.28 | 4.99 | 1.13 |
| Phenylalanine | 5.27 | 4.02 | 5.53 | 4.17 | 5.45 | 3.30 | 5.11 | 3.81 |
| Lysine | 5.03 | 5.97 | 5.29 | 6.37 | 4.64 | 5.19 | 4.93 | 6.25 |
| Methionine | 0.76 | 0.88 | 0.83 | 0.85 | 0.79 | 0.60 | 0.89 | 0.68 |
| Cystine | 1.43 | 0.98 | 1.27 | 1.14 | 1.37 | 0.99 | 1.58 | 0.95 |
| Tryptophan | 0.94 | 1.16 | 1.33 | 0.89 | 1.27 | 0.70 | 1.38 | 0.74 |

months.

Table. 12 shows the concentration of the essential amino acids in soysauce and soypaste separated from the Meju-brine mixtures ripened for 8 months. The concentration of all essential amino acids except lysine was higher in the paste than in the soysauce. The concentration of tyrosine in soysauce was particularly low in comparision with that in the paste. A quantitative estimation showed that the distribution of the essential amino acids after the separation of 8 month mixtures varied slightly for the different types of Meju. Generally, 30~40% of threonine, valine, isoleucine, leucine,phenylalanine, methionine, cystine and tryptophan in the mixture were contained in the soysauce, whereas only 15% of tyrosine was contained in the sauce. Lysine was quite exceptional with about 50% of it was contained in the soysauce.

## Discussion

Since the preparation of Meju and the subsequent ripening of Meju-brine mixture is ill-controlled, the generalization of the effect of the processing on the nutritive value would appear to be unreasonable. However, the process per se has certain means of control. First of all, the raw material,cooked soybean, is a condition selecting certain microorganisms growing on it from the in numerable species occuring naturally. Cho et al.[3] reported that the fungus flora grew on the dried outer layer of the Meju ball, whereas the bacterial flora grew within the ball. The major fungus flora isolated were the species *Mucor, Penicillium* and *Aspegillus*, and the major bacterial flora were *Bacillus subtilis* and *Bacillus pumilus*. These Bacillus species were the most important organisms characterizing the traditional Korean Meju products from other fermented soybean products.

Lee et al.[4] reported that the concentration of salt and the pH of the Meju-brine mixture were the most important factors deciding the growth of microflora during the ripening. They found that the aerobic bacteria, mainly the Bacillus species, increased for the first two weeks of ripening and then gradually decreased. The lactic acid bacteria, ***Pediococcus halophilus*** and ***Leuconstoc meseteroides***, increased for the first 3 weeks, after which they decreased. The lactic acid fermentation lowered the pH value sufficiently for the growth of yeasts resulting in *Saccharomyces rouxii* and *Torulopsis datila* became the dominating organisms in the mixture. *Saccharomyces rouxii* was reported by Lee et al.[5] to be one of the domination organism in the production of Japanese soysauce being present for the entire period of ripening, whereas *Torulopsis* appeared near the completion of the ripening. From these studies a picture of the rise and fall of the important microflora during the process can be drawn as in Fig. 4.

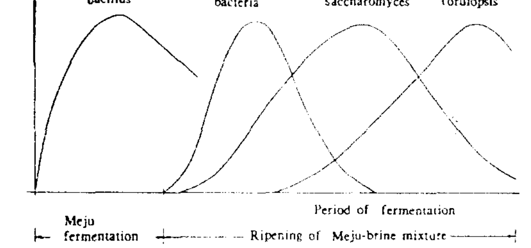



**Fig. 4. The rise and fall of the important microorganisms during the Korean soysauce and soypaste fermentation**

These microbial vicissitudes during the process are closely related to the chemical changes in soybean. The growth of the fungus and bacteria during Meju fermentation results in the hydrolysis of carbohydrates, fat and protein in soybean. These hydrolysates, mainly reducing sugars and free amino acids, are converted to brown pigments through the Maillard reaction. The relatively low water content of the Meju after drying in the sun stimulates this reaction. The Maillard reaction would appear to be the most important chemical reaction during Meju fermentation resulting in the brown colour and meaty flavour of soysauce and soypaste.

The degradation of soybean protein during Meju fermentation did not produce ammonia to any significant extent. This may reflect the fact that the proteins are mainly hydrolyzed to small molecular peptides during Meju fermentation. Kim[6] found active changes in the content of peptides during soybean Koji fermentation and determined 15 different types of oligopeptides in the Koji.

On the other hand, the degradation of the protein during the ripening of the Meju-brine mixture produced a large amount of ammonia. This may imply that a strong deamination process occurs during the ripening. This postulated by the fact that the basic amino acids are damaged more than other amino acids during the ripening. The relatively high content of lysine in the 8 month ripened Meju-brine mixtures did not necessarily mean that the lysine in soybean was stable during the ripening process. The lysine in soybean can be damaged to the same extent as argin ine and histidien during the process, but lysine may be synthesized by the bacteria and yeasts during the ripening resulting in the total amount being unchanged. Many of the yeast proteins are known to have an extraordinarily high concentration of lysine[7].

The high concentation of ammonia in Korean soysauce and soypaste deteriorates the taste character. It can be reduced by modifying the type Meju. Improved Meju produced significantly less ammonia than home-made Meju.

Although the pattern of amino acid in Meju was similar to that of soybean, the pattern of the 8 month ripened brine mixtures was not related to the pattern of soybean. This is perhaps due to the change in the character of the protein in the brine mixture, which after the ripening contains the hydrolysates of soybean protein as well as the proteins of the cells of microorganisms. Therefore, the pattern of amino acid of the final products would appear to be determined by the growth of microorganisms during the ripening.

Even though the reduction of the crude protein in raw soybean was negligible during the process, approximately 1/4 of the total amino acid in raw soybean was lost during the whole process of making Korean soysauce and soypaste.

Cooking destroyed about 12% of the cystine in raw soybean. Methionine was destroyed rapidly during the first month of ripening of the Meju-brine mixtures retaining about one half of that of raw soybean. Since the sulphur-containing amino acids are the most limiting amino acids of soybean protein, these reductions will result in a significant effect on the protein value of soybean.

On the basis fo the amino acid pattern a protein quality evaluation was made of the final products as well as of soybean and the fermented Mejus. The chemical score were calculated using, as the references, the FAO provisional pattern of amino acid and the pattern of whole egg[8]. The essential amino acid index(EAAI) was calculated by the method of Oser[9] using the amino acid pattern of whole egg as the reference. In this calculation arginine was excluded and tyrosine was included with phenylalanine.

As shown in Table. 13, the most limiting amino acid was the sulphur-containing amino acids in all cases studied, whereas the second limiting amino acid varied from valine in soybean to threonine in most of the Mejus and the brine mixtures, lysine in most of the soypastes and tryptophan in some of the soysauces.

**Table 13. Evaluation of protein quality of the products of soybean Meju fermentation by chemical score and EAAI methods.**

| Proteins | Limiting amino acid* | | Chemical score | | EAAI |
|---|---|---|---|---|---|
| | First | 2nd | FAO | Egg | |
| Raw soybean | S-aa. | Val | 82 | 51 | 83** |
| Cooked soybean | S-aa. | Thr | 77 | 48 | 83** |
| 2-month Meju | S-aa. | Thr | 71 | 44 | 79** |
| - 8 mnth mix. | S-aa. | Thr | 66 | 41 | 69 |
| - paste | S-aa. | Lys | 63 | 38 | 73*** |
| - sauce | S-aa. | Thr | 53 | 33 | 60*** |
| 3-month Meju | S-aa. | Thr | 74 | 46 | 80** |
| - 8 month mix. | S-aa. | Thr | 60 | 37 | 61 |
| - paste | S-aa. | Thr | 60 | 37 | 74*** |
| - sauce | S-aa. | Thr | 57 | 35 | 59*** |
| Home-made Meju | S-aa. | Thr | 69 | 42 | 76** |
| - 8 month mix. | S-aa. | Thr | 51 | 31 | 61 |
| - paste | S-aa. | Lys | 62 | 38 | 74*** |
| - sauce | S-aa. | Try | 45 | 28 | 57*** |
| Improved Meju | S-aa. | Thr | 79 | 49 | 83** |
| - 8 month mix. | S-aa. | Trh | 61 | 38 | 72 |
| - paste | S-aa. | Lys | 71 | 43 | 79*** |
| - sauce | S-aa. | Tyr | 47 | 29 | 64*** |

* FAO provisional pattern of amino acid as the reference
** Tryptophan is not included
*** Histidine is not included

The chemical score of a sample protein differed considerably due to the choice of the reference pattern of amino caid, The FAO provisional pattern of amino acid resulted in a higher chemical score of the samples than the egg pattern. However, the two methods rated the protein quality of the samples identically.

According to the evaluation made by the FAO provisional pattern of amino acid as the reference, the chemical score of raw soybean was 82, which was reduced to 77 by cooking and further reduced to 71 ~74 by Meju fermentation according to the type of Meju. home-made Meju showed a lower chemical score than that of laboratory Meju, whereas improved Meju had a higher value.

At the 8 months of ripening, the chemical score of the Meju-brine mixtures were reduced to 51~66 with home-made Meju showing the lowest value. After the separation, soypastes attained a higher chemical score than the mixtures, ranging between 60~71 with improved Meju showing the highest value. The chemical score of soysauce was considerably lower than that of soypaste, varying from 45 in home-made Meju to 57 in Laboratory 3-month Meju.

The EAAI of the samples calculated by using the egg pattern was similar to the chemical score based on the FAO provisional pattern of amino acid. It rated the protein quality of the products essentially in the same way as did the chemical score, and it appeared to fit the changes in the overall amino acid pattern of the products better than did the chemical score. The concentrations of essential amino acids in the protein of soypaste were generally higher than those of the soysauce and the Meju-brine mixture, and therefore, the protein in soypaste was expected to have a higher nutritive value than those in soysauce and in the brine mixture. This fact was emphasized more by the EAAI than by the chemical score. In this respect, the EAAI can be a more suitable method for the determination of the changes in the overall amino acid supplementary potential of the products than the chemical score, since the latter reflects only the changes in the most limiting amino acid.


## 요 약

실험실에서 제조된 메주와 한국에서 보내온 재래식가정 메주와 개량메주를 사용하여 장을 담그고 8개월동안의 숙성(장담금)기간중 일어나는 화학성분변화를 검토하였고 메주 종류에 의한 차이를 비교하였다. 또한 분석된 아미노산 조성에 의거하여 과정에 일어나는 대두단백질의 영양가 변화를 추정하였다.

8개월간의 숙성기간중 일반 화학조성의 변화는 거의 일어나지 않았으나 수용성 질소율은 메주의 13~29%에서 66~78%로 증가되었다. 유리아미노태 질소율은 메주의 4~7%에서 8개월 숙성후 29~35%로 증가되었으며 암모니아태 질소율은 1~4%에서 5~14%로 증가되었다. 메주장 숙성중에 일어나는 이들 질소화합물의 성분변화는 사용된 메주의 종류에 크게 의존하였다.

숙성중 메주의 아미노산 조성도 크게 변화되었으며, 특히 methionine은 숙성 1개월동안 급격히 감소하여 원래 농도의 거의 1/2에 달하게되며 그후 숙성 7개월동안 농도의 변화가 없었다. Arginine과 histidine은 숙성 1개월이 지난 후부터 나머지 7개월 동안 급격히 파괴되었다. 한편 메주제조 과정까지는 거의 존재가 없던 ornithine이 숙성과정중에 상당량이 합성되었다. 대체적으로 숙성 3개월동안은 아미노산 조성의 전반적인 큰 변화가 일어나지 않았으며, 이들 변화는 사용된 메주의 종류에 따라 큰 차이를 보였다.

3개월된 실험실 메주로 8개월 장담금을 하였을 경우 대두중의 영양가 수율은 탄수화물이 99%, 조지방이 107%, 조단백질이 93%, 총 아미노산이 74%였다. Histidine, arginine 및 methionine은 과정중 가장 심한 손상을 보였으며 회수율은 각각 25, 27, 및 49%였다. 한편 lysine회수율은 79%였다.

장 걸름 과정 즉, 간장과 된장의 분리과정에 의하여 메주장중에 함유된 약 60%의 조단백질, 거의 모든 조지방 및 80%의 탄수화물이 된장에 남아 있었다. 또한 된장은 lysine을 제외한 모든 아미노산의 농도(16g 중)가 간장보다 높았다.

대두 및 메주를 포함한 모든 시험장류의 제1한 아미노산은 methionine과 cystine등 유황을 함유하는 아미노산이었으며 제2제한 아미노산은 원료대두에서 valine, 메주와 대부분의 메주장에서 threonine, 된장에서 lysine 그리고 대부분의 간장에서는 tryptophan으로 나타났다

FAO 표준 아미노산 조성을 기준으로 한 원료 대두 단백질의 단백가(chemical score)는 82였으며 이것은 대두의 삶음과정에서 77로 저하되었으며 메주발효과정에서 71~74로 다시 저하되었다. 재래식 가정메주의 단백가는 실험실 메주의 그것보다 낮았으며 개량메주는 실험실 메주보다 높은 단백가를 나타내었다. 메주장의 8개월 숙성후 단백가는 51~66으로 상당히 떨어졌으며 분리에 의하여 된장의 단백가는 60~71로, 간장은45~57의 단백가를 갖게되었다.

일반적으로 개량메주로 만든 장들이 높은 단백가를 가졌으며 가정 메주로 만든 장들은 가장 낮은 단백가를 나타내었다.

계란 단백질 조성을 기준으로한 필수 아미노산지수(EAAI)는 단백가(chemical score)와 유사한 수치를 나타내었다. 그리고 이 필수 아미노산지수는 검체단백질중의 아미노산조성의 전반적인 변화를 단백가보다 더 근사하게 나타내었다.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