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

Part I, Chemical Changes During Meju Making

by

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재래식 간장 및 된장 제조가 대두 단백질의 영양가에 미치는 영향

제 1 보 재래식 메주 제조의 성분변화

이 철 호
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(1975년 10월 27일 수리)

Abstract

Fermented soybean Mejus were prepared in the laboratory with varying lengths of fermentation and the changes in the Chemical composition during the Meju making were determined. The moisture of cooked soybean was gradually evaporated during the Meju fermentation, and after 2 months of fermentation the water level reached to the level of the raw soybean. The concentrations of crude fat, crude protein and ash of the dry matter of soybean did not change considerably during soaking, cooking and Meju fermentation of up to 3 months, whereas carbohyrates decreased significantly during soaking and Meju fermentation. The percentage retention of the nutrients were 58 % for carbohydrates and 93% for crude fat and crude protein.

The nitrogen solubility of soybean decreased drastically during cooking, from 79% to 21%, while Meju fermentation increased it to approximately 30% in the first week and this level remained constant for the duration of the fermentation. The concentration of free amino nitrogen in total nitrogen of soybean decreased during cooking, from 7% to 3%, but fermentation of Meju liberated it to the level of raw soybean. The concentration of free amino-nitrogen in the total-N of soybean was increased by cooking and further increased during Meju fermentation.

The amino acid pattern of soybean did not change significantly during soaking, cooking and the

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Meju fermentation up to 3 months. Serine and the basic amino acids, lysine, arginine and histidine, decreased to the range 81~87% of the raw soybean during the first month of Meju fermentation and thereafter remained almost constant. The total amino acid per 16g nitrogen was 99 g incooked soybean and 93 g in 1 month Meju, indicating a 6% reduction.

Introduction

Soybean and its products are important protein supplementary foods providing approximately 8% of the total protein supply in Korea[1]. It is processed in various ways and about 60% of the total supply is used for the making of soysauce and soypastes including red pepper soypaste. More than 2/3 of the fermented soybean foods are processed by the individual households using the traditional methods[2]. The traditional methods are ill-controlled fermentation processes. As a consequence, the composition of the products is not uniform. Many investigations have been carried out to determine the composition of the traditional fermented soybean products. The changes in the chemical composition during the formentation processes have also been studied. However,few studies of the traditional fermentation of soybean have approached the process from a nutritional point of view.

A remarkable increase in the concentrations of soluble nitrogen and free amino acid was recognized in both the short term fementation of Natto[3] and Tempeh[4] as well as in the long term fermentation of the Japanese Miso[5] and of Korean soypaste[6,7]. Kim et al.found that 1/3 of the total amino acid in Korean soypaste was in the form of free amino acid [6].

Standal[8] concluded in her study on the amino acid pattern of the Oriental soybean foods that the Oriental methods of processing soybean did not apparently affect the protein value significantly.This may be true for the non-fermented or the short term fermented foods such as Natto and Tempeh.

However, Sugimura et al.[5]using the microbicilgoal assay technigues reported a 20% decrease in the concentration of total lysine during the 34 days of Japa nese soybean Miso fermentation. Lee [9] found that the amino acid pattern of Korean soysauce made by the traditional method was quite different from that of soybean, and tryptophan was not detected in this sample. In the home-made soybean paste the concentration of lysine increased from 5. 7g/16g N of soybean to 6. 5, whereas the concentrations of methionine and arginine decreased to half of those in soybean. Takeuchi et al. [10] reported a loss of 15% of the total amino acid in soybean during the 9 months of the Japanese soybean Miso fermentation. These investiga tions imply that significant changes in the protein value of soybean can occur during the long term fermention of soybean.

Studies on the changes in the chemical composition of soybean during the making of fermented Meju are scarce, whereas the chemical changes during the subsequent ripening of the Meju-brine mixyture have received some attention by the investigators in Korea [11~15]

In the present experiment the fermented soybean Mejus were prepared in the laboratory with varying lengths of fermentation and the changes in the chemical composition during the process were determined. The retention of nutrients, i.e. carbohydrates, crude fat and crude protein, was also estimated.

Materials and Methcds

1. Sample preparation

Laboratory fermented Meju was made from soybeans received from the Dansk Soyakage Fabrik A/S in Copenhagen, which were imported for oil extraction from the U.S.A.

The method of Meju making followed the traditional Korean method.

Soybeans were washed and soaked in water over night and then drained for 30 minutes and autoclaved at 120°C. for 1 hour. Cooked soybeans were pounded and moulded to a ball weighing about 300g, which is called the Meju ball. Meju balls were dried in the breezy place in day time and during the night they were kept at 30°C with cloth cover. This process

was repeated for a week resulting in microbial growth on the surface and within the Meju ball. Dried Meju balls were stored in a cloth bag for up to 3 months.

A total of 9 Meju balls were prepared and fermented for periods of 1 week, 2 weeks, 3 weeks, 1 month, 2 months and 3 months.

Home-made Meju was received from Korea. It had been made for sale by a small Meju producer in Seoul.

Improved Meju was also received from Korea. It was made by growing *Aspergillus oryzae* on the cooked soybean coated with wheat flour. It was dried and packed in a polyethylene film bag.

No changes in the quality of the Korean Mejus were noticable during the transportation.

The samples were freeze dried, when necessary, and were ground to powder for the analyses.

2. Analytical methods

Total nitrogen was determined by the Kjeldahl method. Crude fat was measured by the Stolt method and moisture and ash contents by the usual gravi metrical methods[16]. The carbohydrate content was calculated by subtracting the other components from the total.

Nitrogenous components were separated and the quantitative analyses were made as follows:

Soluble nitrogen was determined from the water extracts of the samples. The extraction was made from the freeze dried powder of the samples as follows: 4~6g of the sample was put into a 50ml beaker and 40ml of boiling water was added,which was then boiled for one minute in order to inactivate the enzyme activity. As an exception, raw soybean and soaked soybean were not extracted with boiling water but with cold water. After extraction for 15 minutes it was centrifuged for 7~8 minutes at 4,500 rpm. The supernatant was decantated through a glass wool filter into a 200ml volumetric flask. The precipitate was then washed with 40ml of water and centrifuged.This process was repeated once with the supernatants being collected in the same flask. The glass wool was washed with water and the flask was filled to 200ml.

25-50ml of the extract was taken for the Kjeldahl analysis. The nitrogen content of the extract was considered as the water soluble nitrogen.

Free amino acid nitrogen was determined by the Formol-N content of the water soluble extract made above. 25ml of the extract was pipetted to a 100ml beaker and the pH was adjusted to 7.0. 2ml of a 37 % solution of formaldehyde, adjusted to pH 7.0, were added. Using a magnetic stirrer, 0.1 N solution of sodium hydroxide was titrated to pH 9.5[17].

A blank test was made with 25ml of water and 2ml of formaldehyde solution.

Ammonia nitrogen was determined according to the Japanese Standard Method for Sho-yu Analysis[18]. 2g of the freeze dried powder with 5ml of water and poured into a 25 cm-long glass tube with 2-3 drops of amylalcohol as an antifoaming agent, and 10 ml of a saturated solution of potassium carbonate were added. The ammonia formed was introduced into 20ml of 0.1-N solution of sulphuric acid by suction. The sulphuric acid consumed was determined by titrating with 0.1-N solution of sodiumhydroxide. A blank test was made with 10 ml of water.

Determination of the total amino acid concentration:

The total amino acid concentrations were determined by the column chromatographic method based on the Moor and Steinsystem[19,20], using a Beckman Model 120-B, Automatic amino acid analyser.The hydrolysis of protein was made as described by Jakobsen and Weidner[16]. The unoxidative hydrolysis of the protein was applied to all amino acids except methionine, cystine and tryptophan. Samples contain in 9-10 mg nitrogen were boiled in a 6N-HCl solution with stannous chloride for 24 hours.The hydrolyzed amino acid solution was filtered and evaporated in a Büchi-evaporator and then diluted with a buffer solution of pH 2.2. The solution was adjusted to pH 2.2 and filtered and again. Methionine and cystine were oxidized first to methionine sulphone and cysteic acid respectively by a solution of performic acid(0.5ml of 30% hydroperoxide and 4.5ml of conc. formic acid) and then hydrolyzed by the process as mentioned above.

An alkali hydrolysis method was applied for the determination of tryptophan[21]. Samples containing 20mg of nitrogen were hydrolyzed by a 5.5N-$Ba(OH)_2$ solution in a teflontreated flask for 20 hours. After hydrolysis the Ba-ion in the solution was precipitated by a strong sulphuric acid solution and removed by centrifugation. The supernatant was adjusted to pH

2.2.

Lysine, histidine, arginine and ammonia were seperated in a column of 10cm length which was filled with the resin, Amberlite CG-120(Beckman PA 38). The column temperature was adjusted to 55°C. One ml of the hydrolysate was introduced into the column and eluted with a buffer solution of pH 5.28 for 85 minutes at the constant flow rate of 68 ml per hour.

Methionine, cystine, threonine, alanine, serine, valine, proline and glutamic acid were separated in a column of 55cm length with the same resin at the same temperature as above. A buffer solution of pH 3.28 was used for the elution and the flow rate was 68ml per hour. Isoleucine, leucine, tyrosine and phenylalanine were separated by the same method as methionine except that the pH of the buffer solution was 4.25.

In the case of tryptophan a column of 13.5cm length was used. Two ml of the hydrolysate was applied for the separation and eluted with a pH 5.28 buffer solution at a constant flow rate of 30ml per hour.

The colour intensity of the amino acid-ninhidrin complex was measured at wave length 570nm except proline which was measured at 440 nm. The amino acid concentrations were calculated by reference to the standard amino acid chromatogram.

Results

1. Changes in the general chemical composition

Table 1 shows the general chemical composition of soybeans at the different stages of Mejumaking. Homemade and Improved Mejus are comparable in their composition to 8 or 12-week laboratory Mejus. Fig. 1 shows the changes in the dry-matter content of soybeans during Meju making. Water was absorbed by the soybean during the soaking process, about 1.3 times the original weight, which gradually evaporated during Meju fermentation. The dry-matter contents of 8 or 12 week Mejus attained the same level as the raw soybean.

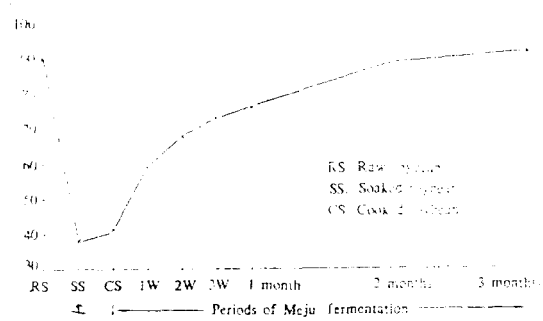

Fig. 1. Changes in the dry matter content of soybean during Meju making.

The contents of ash, crude protein, crude fat and carbohydrates in the dry-matter were calculated and the changes and the changes during the process are shown in Fig 2.

The ash content decreased slightly during soaking and cooking, but was constant during Meju fermenta-

Table 1. Changes in the general chemical composition of soybean during Meju-making.(%)

Sample	Moisture	Ash	Crude protein	Crude fat	Carbohydrates
Raw soybean	8.25	4.89	38.19	19.19	28.76
Soaked soybean	61.55	1.86	16.84	8.95	10.80
Cooked soybean	58.59	1.91	18.22	9.73	11.55
1-week Meju	40.69	2.89	27.32	14.70	14.43
2-week Meju	31.43	3.47	31.75	16.94	16.41
3-week Meju	27.22	3.67	33.86	17.91	17.34
1-month Meju	23.77	3.97	36.07	19.54	16.65
2-month Meju	11.48	—	40.33	21.64	—
3-month Meju	8.40	4.66	43.36	22.96	20.62
Home-made Meju	10.43	5.87	47.86	15.72	20.12
Improved Meju	13.08	4.85	41.57	16.88	23.89

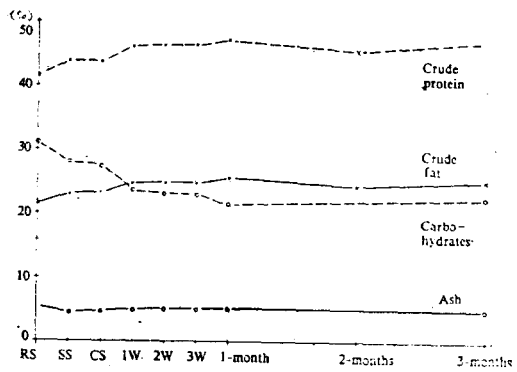

Fig. 2. Changes in the general chemical composition of the drymatter of soybean during Meju making.

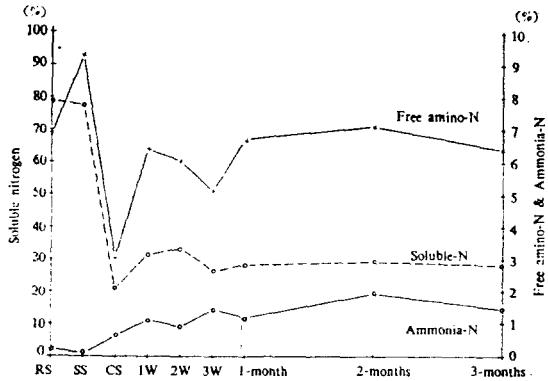

Fig. 3. Changes in the content of soluble. N, free amino-N and amonia-N in the total -N of soybean during Meju making.

tion. A significant reduction of the carbohydrates content was observed during soaking, cooking and the first four weeks of Meju fermentation. For the duration of the fermentation, the level was 70 % of the raw soybean. The contents of fat and protein in the drymatter base did not change to any significant extent. The slight increases shown in the figure were probably caused by the reduction of the carbohydrate content.

2. Nitrogenous components

The nitrogen solubility was calculated from the soluble nitrogen concentration as a percentage of the total nitrogen. The solubility of soybean protein decreased drastically during cooking, from 79% to 21%, as shown in Fig. 3. Fermentation of Meju increased the nitrogen solubility to approximately 30 % in the first week and this level remained constant for the duration of the fermentation.

The concentration of free amino nitrogen in the total nitrogen of soybean decreased as did the nitrogen solubility during the cooking process, from 6.9% in raw soybean to 3.0%, but the fermentation of Meju liberated it to the level of raw soybean as shown in Fig. 3.

The concentation of free amino nitrogen in the total nitrogen of soybean was, on the contrary, increased by cooking and further increased by Meju fermentation. The concentration reached up to 2% of the total nitrogen at the end of the 8-week fermentation, as shown in Fig. 3.

3. Amino acid pattern

Table. 2 shows the total amino acid concentration per 16g nitrogen of soybean at the different stages of Meju making. It was observed that the amino acid concentrations did not change significantly during the process except for serine and the basic amino acids, arginine, lysine and histidine. The concentrations of these amino acids decreased to the range 81~87% of the raw soybean during the first four weeks of fermentation and thereafter remained almost constant.

A reduction in the sum of total amino acids per 16g nitrogen was observed during the fermentation. The magnitute was 99g in cooked soybean and 93g in 4 week Meju, indicating a 6% reduction. This reduction was mainly attributed to the decrease in the basic amino acids and serine. The methionine concentration decreased 87 % of raw soybean during the four weeks of Meju fermentation. Cystine concentration was decreased by cooking slightly but was not affected by the fermention. The ratio of essential amino acid to the total amino acid of raw soybean was about 0.40 and it did not change during the process of Meju making. Tryptophan was not included in the calculation.

4. Retention of nutrients

An estimation was made of the retention of carbohydrates, crude fat and crude protein of soybean during the Meju making. Since it was based on the scale attained in the laboratory, it may not reflect the

Table 2. Changes In the amino acid pattern of soybean duriing Meju making. (g amino acid/16g nitrogen)

Amino acid	Raw soybean	Soaked soybean	Cooked soybean	1-week Meju	1-month Meju	2-month Meju	3-month Meju	Home-made Meju
Aspartic acid	11.35	11.18	11.60	10.91	10.93	11.33	10.79	10.26
Threonine	3.92	3.77	3.80	3.69	3.54	3.66	3.54	3.46
Serine	4.91	4.79	4.72	4.73	4.19	4.27	4.27	3.98
Glutamic acid	19.02	19.02	19.36	19.78	18.87	19.56	20.29	16.12
Proline	4.99	4.65	4.84	4.85	5.20	4.49	4.67	4.15
Glycine	4.25	4.18	4.19	4.17	3.99	4.05	4.16	4.07
Alanine	4.35	4.25	4.30	4.32	4.04	4.08	4.10	4.15
Valine	4.75	4.59	4.91	4.89	4.89	4.71	4.78	4.80
Isoleucine	4.71	4.64	4.82	4.60	4.46	4.52	4.50	4.25
Leucine	7.48	7.49	7.66	7.42	7.02	7.29	7.22	6.84
Tyrosine	4.00	3.73	3.80	4.00	3.82	4.08	4.19	3.57
Phenylalanine	5.12	5.06	4.94	5.59	4.91	4.94	5.30	4.99
Lysine	6.74	6.48	6.54	6.30	5.82	5.84	5.97	5.61
Histidine	2.80	2.65	2.79	2.72	2.28	2.38	2.39	2.23
Arginine	7.45	7.65	8.07	6.82	6.37	6.38	6.23	5.97
Methionine	1.48	1.44	1.48	1.40	1.37	1.30	1.29	1.25
Cystine	1.40	1.52	1.23	1.31	1.39	1.19	1.31	1.17
(Ammonia)	(1.90)	(1.87)	(1.89)	(1.88)	(1.90)	(1.99)	(2.00)	(2.71)
Total amino acid*	98.72	97.09	99.05	97.50	93.09	94.07	95.00	86.87
EA/TA×100*	39.8	39.9	39.6	40.2	40.0	39.9	40.1	41.4

* Tryptophan is not included

Table 3. Retention of nutrients in soybean during Meju making. (%)

Nutrients	Soaking	Cooking	1 month Meju fermentation	3 months Meju fermentation
Carbohydrates	86	81	58	59
Crude fat	101	96	95	93
Crude protein	101	96	95	93

working loss during the process in the home or on the industrial scale, howevr, it can give an approximate pattern of the nutrien tretention during the process.

As shown in Table 3, 14% of the carbohydrates in the raw soybean were lost by soaking, 5% by cooking and more than 20% during the first month of Meju fermentation. No more was lost by the prolonged fermentation of up to 3 months. Crude fat and crude protein were decreased slightly by cooking and by the Meju fermentation of up to 3 months. 93% of these in raw soybean were retained in the Meju fermented for 3 months.

요 약

대두를 이용하여 재래식 메주를 실험실에서 제조하고 발효과정중에 일어나는 경시적 화학조성의 변화를 관찰하였다.

메주중의 수분은 발효기간중 점차적으로 증발되어 발효 2개월 후에는 원료대두의 수분함량과 같은 수준으로 되었다. 대두의 건물중 조단백질 조지방 및 회분 함량은 수침, 삶음과 메주발효중 크게 변화하지 않으나 탄수화물 함량은 수침과 발효중 상당히 감소하였다. 메주

제조과정 중 대두 영양분 회수율은 탄수화물이 58%, 단백과 조지방이 각각 93%이었다.

대두의 총 질소중 수용성 질소율은 대두를 삶는 과정중 79% 에서 21% 로 감소하였으나 메주발효 1주일동안 30% 로 증가되었으며, 이후 3개월동안 거의 같은수준을 유지하였다. 총 질소중 유리 아미노태질소 농도는 원료대두에서 7% 였고 삶음 과정에서 3% 로 감소하였으나 메주발효과정중 다시 원료대두에서와 같은 수준으로 증가되었다. 총 질소 중 암모니아태 질소 농도는 삶음과 발효과정에서 계속 증가하여 발효 종료 후 2%에 도달하였다.

대두단백질의 아미노산 조성은 수침, 삶음 및 3개월간의 메주발효과정 중 크게 변화되지 않았으나, serine과 염기성 아미노산 즉, lysine arginine 및 histidine이 메주발효 1개월 동안 원료대두의 81~87%로 감소되었으며 계속되는 발효 3개월동안 같은 수준을 유지하였다. 100 g 조단백질중 아미노산 함량은 원료대두에서 99 g이 었으나 메주 발효 1개월후 93 g으로 감소되어 메주발효과정중 6%의 아미노산 감소를 나타내었다.

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