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

Part III. Changes in the Lysine Availability

by

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재래식 간장 및 된장 제조가 대두단백질의 영양가에 미치는 영향

제 3 보 Lysine 가용도의 변화

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Abstract

The changes in lysine availability during soybean fermentation were determined by the chemical analysis method as well as the biological methods with rat. The FDNB-reactive lysine determined by the difference (TLMI) method indicated that cooking and Meju fermentation reduced the lysine availability of soybean, but the subsequent ripening restored the availability to the same level of the raw soybean.

On the other hand, the Biological Value, NPU, NER and the Relative lysine availability of the rat experiments showed a general decrease in the lysine availability of soybean during the ripening process as well as Meju fermentation.

Introduction

Evaluation of protein quality on the basis of the total amino acid pattern can result in misleading figures for some cases, particularly for the processed foods. The chemical score is based on the assumption that the amino acid in a protein is completely digested and 100% utilized for the maintenance and growth of the animal. However, this assumption is not valid for most of the foods. The digestibility of the protein and individual amino acids varies with the type of food. Food processing can reduce the availability of

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amino acid drastically without any changes in the amount of the amino acid. The concept of available, as distinct from total amino acid present in a protein is used to differentiate between amino acids modified or damaged in some way during denaturation with the consequent loss of nutritive value and those which remained nutritionally available to the metabolic processes.

Lysine has been known to be one of the most susceptible essential amino acids in food processing, especially to heat treatment. The effect of foodprocessing on the lysine availability has been widely studied and was recently reviewed by Carpenter [1].

The effect of soybean processing on the lysine value is important to protein nutrition of Koreans, since soybean is considered to be an important lysine supplementing food to the Korean diet, of which lysine appears to be the most limiting amino acid[2]. Studies on the effect of soybean processing on the lysine availability are largely limited by the lack of proper analytical methods. Since soybean contains an abundant amount of lysine and the most limiting amino acid is methionine, the animal feeding experiments with soybean as the only protein source are not able to detect the changes in the lysine value unless the damage in lysine is so severe that lysine becomes the most limiting amino acid of the diet. If the lysine in soybean protein is diluted by mixing it with a lysine poor-protein or with other limiting amino acids resulting in lysine becoming the most limiting amino acid in the diet, the Biological value and the weight gain response of the animal to the diet can be used as the parameters of the lysine value. However, this method has a limitation in that the results are not due to soybean only but to the mixture of different sources of protein used.

Lysine is the only amino acid for which the unavailable form is know chemically and, therefore, the chemical method of measuring the available from appears to be possible. It is assumed that animals cannot utilize lysine if the ε-amino group is bound and this lysine is then referred to as unavailable lysine. Most of the chemical methods measuring the lysine availability are based on this assumption and determine the ε-free lysine. Carpenter[8] established the FDNB(1-fluoro-2,4-dinitrobenzene)-reactive lysine method. This method gives reliable results with the animal proteins but appears to be unsatisfactory for the vegetable protein foods, particularly those rich in carbohydrates. In addition, this method is not applicable to the foods which contain a significant amount of free lysine. The method has been modified by many investigators[4,5,6]

In the present rat feeding experiments, diets were made by mixing the fermented soybean products with gluten providing 80% of the dietary protein in order to make the lysine the most limiting amino acid of the diets. The protein digestibility (TD), biological value(BV) and net protein utilization(NPU) of the diets were determined by using the young rats. The weight gain response to a unit lysine intake was calculated on the basis of the 5 day balance period. In addition, the FDNB-reactive lysine of the fermented soybean products was determined by using the TLMI (total lysine minus inaccessible) method of Silcock. From the result obtained, the effect of the traditional Korean soybean fermentation on the lysine value and the differences in the results of the methods used will be discussed.

Materials and methods

1. Sample preparation

The samples used in this experiment were identical to those used in Part I. and II.

(2) Determination of the FDNB-reactive lysine

The TLMI includes N-terminal lysine and free lysine, but excludes all other amino acids. It requires total lysine, inaccessible lysine and standard to be run. As total lysine in acid hydrolysate and the standard were determined in Part I & II, it was only necessary to measure the inacessible lysine. The inaccessible lysine was determined by measuring the lysine remaining in solution after a separate hydrolysis of the protein treated with 1-fluoro-2,4-dinitrobenzene according to Roach et al.[5], as described below : 1.0 g of the sample was put in a 200ml flask and to it was added 8 ml of a solution of 8% sodiumcarbonate and then it was left for 10 minutes. A freshly prepared 1-fluoro-2,4-dinitrobenzene solution (0.3ml 1-fluoro-2,4-dinitrobenzene disolved in 12ml ethanol per test) was added to the flask and the contents were left overnight.

The ethanol was then removed by using a water bath, after which 25ml of 8 N-HCl and two glass beads were added and then refluxed at 137°C for 16 hours. The condenser was washed with 50ml of water and allowed to stand for more than one hour. The hydrolysate was filtrated into a 200ml volumetric flask and then water was added up to 200ml. One ml of this hydrolysate was put in a column of 10 cm length for the separation, after which the procedure was identical to that of total lysine determination as described in Part I. and II. Available lysine was simply calculated as follows:

Available lysine=Total lysine-Inaccesible lysine

3. Biological experiments with rats

1) Experimental diet preparation

The samples were mixed with gluten providing 80% of the nitrogen of the mixtures. Using these mixtures as the protein source, diets containing 9.375% protein of the drymatter were made. The nitrogen content was adjusted by adding a nitrogenfree mixture, which had the following composition:

Sucrose……………………………9.00%
Cellulose powder……………… 5.20%
Soybean oil………………………5.20%
Potato starch (autoclaved)………80.60%

Mineral and vitamin mixtures were added to the diet at a concentration of 4% and 1.6% of the drymatter, respectively. The diets prepared in portions for groups of 5 rats sufficient for 10 days. Table 1 shows an example of the diet formulation.

Table 1. Example of the experimental diet prepation.

Sample : 1-month Meju
10g drymatter/day/rat……………………………………………………500g daymatter/10 days/5 rats
150mg N/day/rat…………………………………………………… 7.5g N/10 days/5 rats
　　Sample N…………………………………………………………1.5g
　　Gluten N …………………………………………………………6.0g
g gamle(7.02% N, 92.7% drymatter) 1.5×100/7.2=2.37g, 21.37×92.7/100=19.81g
g gluten(13.03% N, 93.01% drymatter) 6.0×100/13.03=46.05g, 46.05×93.01/100=42.83g
Mineral mixture…………………………………………………………500×0.04=20.00g
Vitamin mixture………………………………………………………… 500×0.016=8.00g
　　As drymatter=90.64g

N-free starch mixture (92.53% drymatter)
　　(500−90.64)×100/92.53=442.40g
Total weight of diet for 5 rats for 10 days
　　442.40+21.37+46.05+20+8=537.82g
g diet/rat/day=10.756g

By adding gluten, as much as 80% of the total protein to the diet, the amino acid pattern of the final diets was altered from that of the sample proteins. Table 2 shows the essential amino acid pattern of the final diets. The values were calculated from the amino acid compositions of gluten and of the soybean products determined in Part I and II. The most limiting amino acid of the diets appeared to be lysine supplying 45—49% of the rat[7] and methionine supplying 55—60% of the requirement. Approximately 50% of the lysine of the diets originated from the soybean products.

The salt concentration of the final diet made with the Mejubrine mixtures, which contain salt, as much as 30—40% of the drymatter, was calculated to be about 4% of the drymatter.

2) Raising of rats

Following the balance method of Eggum [8] groups of five Wistar male rats weighing approximately 75g were used in the experiment, in which a preliminary

Table 2. Calculated amino acid composition of the diets for the rat experiments.*
(20% soybean protein+80% gluten) (g/16g N)

Amino acid	Require-ment of rat	Raw soy bean	Cooked soy bean	1-M Meju	3-M Meju	3-M Meju 3-M mix.	Home made Meju	Home made Meju 1-M mix.	Imp-roved Meju	Imp-roved Meju 3-M-mix.
Threonine	4.1	2.8	2.8	2.7	2.7	2.7	2.7	2.7	2.8	2.8
Valine	5.0	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.2
Isoleucine	4.3	3.9	3.9	3.8	3.8	3.9	3.8	3.8	3.9	3.9
Leucine	7.8	7.0	7.0	6.9	6.9	6.9	6.8	6.9	7.0	7.0
Phenylalanine	4.9	4.8	4.8	4.8	4.9	4.8	4.8	4.8	4.8	4.8
Lysine	5.2	2.6	2.5	2.4	2.4	2.3	2.3	2.4	2.4	2.5
Methionine	2.7	1.6	1.6	1.6	1.6	1.5	1.6	1.5	1.6	1.5
Cystine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Histidine	1.8	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Limiting amino acid(%)										
(1) Lysine		49	48	46	46	45	45	47	45	48
(2) Methionine		60	60	59	59	55	58	55	59	55
(3) Threonine		68	68	66	66	66	66	66	69	68

* Tryptophan in sample proteins is assumed to be ca. 70% of the rat requirement

period of 4 days and a balace period of 5 days were employed. Each rat was housed in a separate cage and water was supplied ad libitum. Each rat received 150mg nitrogen and 10g drymatter daily throughout the preliminary and balance periods. The rats were weighed at the begining of the experiment and divided into groups of 5, so that the average weights of the groups differed by no more than±0.5g. The rats were also weighed at the end of the preliminary and balance periods. By using the specially devised cage,[8] the urine and faeces of each rat were collected separately, and the amounts of the diet consumed for the periods were accurately measured.

3) Calculation

Nitrogens in the diet, in the urine and in the feces were analyzed by Kjeldahl method. From these results of the analysis, the TD, BV and NPU were calculated by the following formulas:

$$TD=\frac{\text{N intake}-(\text{Faecal N}-\text{Metabolic N})}{\text{N intake}}\times 100$$

$$BV=\frac{\text{N intake}-(\text{Faecal N}-\text{Met.N})-(\text{Urin.N}-\text{Endo.N})}{\text{N intake}-(\text{Faecal N}-\text{Met.N})}\times 100$$

$$NPU=\frac{TD\times BV}{100}$$

The metabolic nitrogen of the rat consuming 150 mg of N per day for the 5 days of the balance period was assumed to be 102.00 mg and this was corrected when the diet was not completely consumed. The endogenic nitrogen of the same rat was assumed to be 76.00 mg per 5 days.

The weight response of the rats to the protein diets was measured during the 5 days of the balance period in order to observe any toxic effect of the samples. It was calculated as g weight gain per g nitrogen intake and designated as Nitrogen Efficency Ration (NER), although the measurement did not fulfill the requirements of the standard method of the PER determination[9].

$$NER=\frac{\text{g weight gain for5 days of balance period}}{\text{g N intake for the same period}}$$

In order to determine the lysine availability of the sample protein quantitatively, the weight gain of the rats per g lysine eaten was calculated. The relative lysine availability of the proteins was expressed as the percentage of the weight gain achieved by the lysine of cooked soybean. The raw soybean was not a suitable standard for this purpose, since it contained growth inhibiting factors.

$$\text{Relative lysine availability}(\%)=\frac{\text{g weight gain/g lysine of fermented food}}{\text{g weight gain/g lysine of cooked soybean}}\times 100$$

Results

1. Changes in the FDNB-reactive lysine content

As shown in Table 3, raw soybean had 95% of its lysine as FDNB-reactive. Cooking reduced this to 89% and it was further reduced to 85% during the first month of the Meju fermentation. Prolonged Meju fermentation did not reduce it further, but it showed an increasing tendency.

During the subsequent ripening of the Meju-brine mixture, the FDNB-reactivity of lysine increased from 87—90% in the Meju to 96—98% and most of the Meju-brine mixtures ripened for 8 months had as high a lysine availability as that of raw soybean. After the separation of the mixture, lysine showed less availability in the paste than that in the soysauce, with about 97% of lysine in soysauce and 90% in soypaste being FDNB-reactive.

Table 3. Changes in FDNB-reactive lysine of soybean during the making of Korean soysauce and soypaste

Proteins	Total lysine g/16 g N	FDNB-in-accessible lysine g/16 g N	FDNB-reactive lysine g/16 g N	AL/TL×100 (%)
Raw soybean	6.74	0.35	6.39	95
Cooked soybean	6.54	0.70	5.84	89
1-month Meju	5.82	0.86	4.96	85
2-month Meju	5.84	0.79	5.05	87
1- month mixture	5.45	0.62	4.83	89
3- month mixture	8.36	0.57	7.79	93
8- month mixture	5.43	0.40	5.03	93
-paste	5.03	0.53	4.50	90
-sauce	5.97	0.21	5.76	97
3-month Meju	5.97	0.62	5.35	90
1- month mixture	5.90	0.68	5.22	89
3- month mixture	5.60	0.53	5.07	91
8- month mixture	5.71	0.46	5.25	92
-paste	5.29	0.35	4.94	93
-sauce	6.37	0.21	6.16	97
Home-made Meju	5.61	0.74	4.87	87
1- month mixture	6.07	0.52	5.55	91
3- month mixture	6.10	0.47	5.63	92
8- month mixture	4.86	0.31	4.55	94
-paste	4.64	0.52	4.12	89
-sauce	5.19	0.20	4.99	96
Improved Meju	5.71	0.71	5.00	88
1- month mixture	5.89	0.44	5.45	93
3- month mixture	6.48	0.43	6.05	93
8- month mixture	5.50	0.35	5.15	94
-paste	4.93	0.40	4.53	92
-sauce	6.25	0.13	6.12	98

2. The protein digestibility, BV, NPU and NER of the exprimental diets

Table-4 shows the protein digestibility, biological value, net protein utilization and nitrogen efficiency ratio of the diets prepared with the soybean products and gluten providing 80% of the dietary protein in the rat feeding experments. The protein digestibility of the diets made by the fermented soybean products was slightly higher(94-98%) than that of the diet made by raw soybean(94%).

The BV of the raw soybean diet was 56 and it increased to 65 with the cooked soybean diet. The

Table 4. Protein TD, BV, NPU and NER of the diets made by the soybean products providing 20% of the dietary protein.

Soybean products in diet	TD	BV	NPU	NER
Raw soybean	94	56	52	5.1
Cooked soybean	95	65	62	10.3
1-month Meju	95	60	57	5.6
3-month Meju	95	62	59	8.8
3- month mixture	98	55	54	8.5
Home-made Meju	95	54	52	7.0
1- month mixture	94	56	53	6.6
Improved Meju	96	60	57	9.3
3- month mixture	98	55	54	8.4

BV of the diets made by the fermented products was lower than that of the cooked soybean diet, ranging from 54 to 62. The NPU of the diets showed the same pattern of the BV. The NER of the cooked soybean diet was remarkably higher(10.3) than that of the raw soybean diet(5.1). The NER of the diets made by the fermented products was lower than that of the cooked soybean diet, ranging from 5.6 to 9.3. The NERs of the diets made of l-month Meju and Home-made Meju products were significantly lower than the others.

3. Changes in the Relative Lysine Availability of soybean

As shown in Table 5, the gram weight gain of the rats per g lysine intake of raw soybean diet was 32 and it increased to 65 in the cooked soybean diet. The values of the diet made of the fermented products were lower than that of the cooked soybean but were higher than that of the raw soybean diet. The relative lysine availability, using that of cooked soybean as 100, decreased to 58—97% in the diets made of the fermented products. The relative lysine availability of the diets made of the l-month Meju and Home-made Meju prodocts were significantly lower than the others, having 58, 74 and 66, respectively. The Improved Meju diet showed the highest value with 97%. Generally, the relative lysine availability of the diets made of the ripened Meju-brine mixtures was lower than that of the diets made of the orignal Mejus.

Table 5. Lysine availability of the soybean products by the weight gain method of rats and by the TLMI-Method.

Soybean products	g weight gain per g lysine intake	Relative lysine availability (%)	% FDNB-reactive lysine
Raw soybean	32	—	95
Cooked soybean	65	100	89
1-month Meju	38	58	85
3-month Meju	58	89	90
3- month mixture	58	89	91
Home-made Meju	48	74	87
1- month mixture	43	66	91
Improved	63	97	88
3- month mixture	54	83	93

Discussion

Since the experimental diets had only one variable in the composition, i.e. the soybean products, the differences in the protein digestibility, BV, NPU and NER of the diets can reflect the differences in the protein quality of these products, even though they provided only 20% of the dietary protein. The difference in the BV of the diets and the weight gain response per unit lysine intake of the rats can also reflect the differences in the lysine availability of the soybean products, since the most limiting amino acid of the diets was lysine and the soybean products provided approximately 50% of the lysine of the diets.

These premises are only justifiable on the assumption that the products do not contain any toxic factors.

Unfortunately, a possible antinutritional effect was observed in l-month Meju. The NER of the l-month Meju was as low as that of the low soybean, although the amino acid pattern of the Meju did not significantly deteriorate during the fermentation, as shown in Part-l. On the other hand, the reductions in the NER of the other fermented proudcts appered to be consistent with the degeneration of the amino acid pattern during the fermentation process.

On the assumption that there was no antinutritional effect with the exception of the l-month Meju, the results of the rat experiments indicated that the protein digestility of soybean was increased by the fermentation process, whereas the lysine availability of soybean decreased during the Meju fermentation and further reduced during the subsequent ripening process.

The chemical method of the lysine availability of the fermented soybean products showed rather contradictory results compared with the biological findings. The FDNB-reactive lysine of the soybean products indicated that the lysine availability was increased by the ripening process of up to 3 months. However, Mauron[10] and Carpenter[1] have pointed out that the TLMI method of Silcock can over-estimate the lysine availability of the foods subjected to the keto llard reaction, since the method includes deoxy-Mailysine in the available lysine. The low lysine ility of similar fermented soybean products availabd etermined by the Carpetner method[3], reported earlier[11], appears to be erroneous, since no consideration was given to the free lysine in the products, resulting in a large underestimation.

The effect of the fermentation of Korean soysauce and soypaste on the amino acid availability of soybean needs further study.

요 약

재래식 간장 및 된장 제조중에 일어나는 대두단백질중의 lysine 가용도의 변화에 관하여 화학적 방법 및 생물학적 방법을 이용하여 측정하였으며 서로 다른 측정방법에 의한 결과의 차이에 대하여 고찰 하였다.

TLMI법에 의한 FDNB-reactive lysine의 동향을 보면 대두의 삶음과 메주 발효과정중 lysine의 가용도는 저하하나, 8개월간의 메주 장숙성과정에서 그 가용도가 다시 증가되어 원료대두 단백질중의 lysine가용도와 거의 같은 수준으로 되었다.

한편 백쥐의 사양시험에 의한 생물가 (BV), NPU, NER 및 상대적 lysine 가용율등에 의하면 메주 제조과정에서 뿐만 아니라 숙성과정중에도 lysine 가용도는 계속 저하되는 것으로 나타났다.

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