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

Part 4. Protein Supplementary Effect of Soybean Products to the Rice Diet

by

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재래식 간장 및 된장제조가 대두단백질의 영양가에 미치는 영향

제 4 보 백미식이에 대한 대두제품의 단백질 보충효과

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Abstract

Cooked soybean, soybean curd, soymilk residue and fermented soybean Meju products were prepared in the laboratory and the protein supplementary effect of these foods to the rice diet was determined by the rat feeding experiments. The soybean products providing 20% of the dietary protein were added to steamed rice and the protein digestibility (TD), Biological Value (BV), NPU, PER and Partial Carcass Nitrogen Value of the diets were measured.

The protein supplementary effect of soybean products indicated that the non-fermented products generally improved the protein quality of the rice diet, whereas the fermented products did not but reduced it in some cases.

There was observed a possible antinutritional effect for the diet supplemented with Home-made Meju. This growth retarding effect of Home-made Meju disappeared during the subsequent ripening in the brine for 6 months.

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Two sorts of soybean were used in the experiment. For the preparation of the low-salt diet American soybean purchased in Copenhagen was used. For the high-salt diet Korean soybean was used. Soybeans were soaked in water overnight. and drained for 30 minutes and then autoclaved at 120°C for 60 minutes. The cooked soybeans were freeze dried and grinded to powder.

Soybean curd and soymilk residue were prepared from American soybean as follows: Soybeans were soaked in water overnight and then grinded for 3 minuts in a mixer. The amount of water in the ground soybean mash was about by weight 10 times that of raw soybean. The soybean mash was boiled for 5-10 minutes and filtrated immediately through a cheese cloth. The solids obtained were used as soymilk residue. When the filtrate was still hot(75°C), the coagulant, $CaCl_2$, in the amount 1/20 of the weight of raw soybean, was added to the soymilk. It was allowed to coagulate for 30 minutes and then the soybean curd was put in a wooden box covered with a cheese cloth and pressed with a weight in order to remove the whey. Both the soybean curd and soymilk residue were freeze dried and grinded to powder.

Meju-brine mixtures were made from Home-made Meju and Improved Meju, which were received from Korea, by the same method in Part 2. Four bottles of brine-mixtures, two for each Meju, were prepared and ripened for 6 months at room temperature. At the end of the ripening one of each Meju-brine mixture was freeze dried without separation into soysauce and soypaste and then grinded to poweder. The remaining mixtures were filtrated through a cheese cloth separating soysauce from the paste. The soypaste obtained was freeze dried and grinded to powder.

The experimental diets were prepared by mixing a soybean product providing 20% of the protein and cooked rice providing 80% of the protein. To each diet a mineral mixture and a vitamin mixture were added in the amount of 4% and 1.6% of drymatter by weight, respectively.

Table 1 shows the compositions of the soybean products and the experimental diets.

2. Experimental method

The protein digestibility, BV and NPU of the diets were determined of Eggum as described in Part-3. Rats were fed restricted to 10g of drymatter per day per rat for a preliminary period of 4 days and a balance period of 5 day. An exception was that the protein content of the diets was not fixed at 9.375% of the drymatter. This was caused by the low protein content of rice which provides 80% of the diet protein. As a result, the protein content of the diets was lower than the standard 9.375% and varied slightly with the diets.

In the calculation the BV of the diets was corrected to the value corresponding to a diet protein level of 9.375%, by using the regression equation established by Eggum providing the relationship between the BV of soybean meal and the diet protein level in the rat experiments as follws:

$$BV = 88.29 - 1.37 \times \% \text{ protein diet } (r = -0.99)$$

The NPU of the diets was calculated from the TD and the corrected BV of the diets.

The weight gain of the rats and the protein efficiency ratio (PER) of the diets were measured for a total of days of the entire feeding trial.

Partial carcass nitrogen was determined by using one of the hind legs of the rats at the end of the experiments. The rats were killed under ether anaesthesia and the legs were cut at the pelvic joint after removal of the skin. The nitrogen content of the whole leg was determined by Kjeldahl method.

Results

1. Protein supplementing effect of non-fermented soybean products on the rice diet

Table 2 shows the effect of non-fermented soybean products on the protein quality of the rice diet in the rat experiment. The diets supplemented with non-fermented soybean products showed somewhat lower protein digestibility than the diet made of cooked rice only.

The BV of cooked rice was calculated to be 72.4 at the protein level of diet 9.375%. Supplementation with cooked soybean providing 20% of the protein increased the BV to 76, with soybean curd to 78 and with soymilk residue to 80. The protein supplementing effect of soymilk residue was equal to that of casein.

The effect of protein supplementation with non-

fermented soybean products on the NPU value of the rice diet was similar to that on the BV. The NPU of the diet supplemented with soymilk residue was lower than that supplemented with casein because of the low protein digestibility of soymilk residue.

The PER value of cooked rice was 1.59 at th diet protein level of 6.7%. It was increased to 1.82 by adding cooked soybean providing 20% of the diet protein. The protein supplementing effect of soybean curd was similar to that of cooked soybean. Soymilk residue providing 20% of the protein increased the PER value of the rice diet to 2.01, and this was higher than those attributed to cooked soybean or soybean curd and slightly lower than of casein. The PER value of the rice diet supplemented with casein was 2.18.

The partial carcass-N content of the rats showed the same effect of the protein supplementation as observed in PER and BV except fot the soymilk residue group which had a suprisingly low value.

2. Protein supplementing effect of fermented soybean products on the rice diet

The protein digestibility of the diets supplemented with Home-made Meju and its products was lower than the diet made of rice only, whereas Improved Meju and its products increased the protein digestibility of the rice diet remarkably (Table 3).

The BV of the diets supplemented soybean products was lower than that of the diet supplemented with cooked soybean, ranging from 67 to 74. Particularly, the BV and NPU of the diets supplemented with Home-made Meju products were lower than those of the diets consisting of rice only.

The effect of the high salt content (4%) of the diet comparing Diet-2 and Diet-6 was not significant.

On the other hand, the PER value of the diets supplemented with fermented soybean products was higher than that of the diet supplemented with cooked soybean except for the diet of Home-made Meju. Home-made Meju reduced the PER of rice diet to 1.56.

In the partial carcass-N determination, the group fed Home-made Meju had a lower value than the group fed rice only. The other groups fed fermented soybean products showed higher partial carcass-N values than the group fed cooked soybean.

Discussion

It appears that the making of soybean curd and soymilk residue does not reduc the protein value of soybean. The proteins of soybean curd and soymilk residue can be superior to those of cooked soybean in supplementing the rice diet. Hackler et al.[4] also reported the high PER value of soymilk residue compared with that of cooked soybean.

While the results of the different methods of protein evaluation used in the present experiments agreed well for the protein supplementary effect of the non-fermented soybean foods, they were rather confusing for the fermented soybean Meju products.

As the present experiment followed the mothod of measuring the BV of the diets, the interpretation of the results should be on the basis of the BV. It is apparent that the fermentation of Meju products deteriorates the protein quality of soybean. The extent of the deterioration appears to be dependent upon the type of fermented Meju. Soypaste made of Home-made Meju appears to have a very low protein quality. It did not improve the BV of the rice diet, on the contrary, it reduced the BV of the rice diet from 72 to 69. On the other hand, the soybean paste made of Improved Meju appears to be better, the BV of the rice diet was increased slightly by the supplementation.

Improved Meju products increased the protein digestibility of the rice diet significantly and, consequently, the NPU of the diets supplemented with these products were comparable to that of the diet supplemented with cooked soybean,

The effect of the dietary protein level on the PER value in the rat experiments have been studied by Morrison and Campbell[11]. They showed that in the casein diet the PER value decreased proportionally with the increasing dietary protein level. On the contrary, in the plant protein PER value increased proportionally with the increasing dietary protein level during the 4 weeks of feeding trials and, in this case, the difference in PER between different levels of dietary protein (7-15%) was relatively small.

In the present expeiment, the protein level of the

diet made of rice only was considerably lower than for the other diets, for which the protein level varied between 7.4-8.3%. It would appear that the PER values obtained from the latter are comparable one another, since the differences in the protein level of the diets is slight.

There was observed a slight reduction in the food intake of the rats in the diets supplemented with 6 month ripened soypaste made from Home-made Meju and with 6 month ripened Improved Meju-brine mixture. Since the diets supplemented with fermented soybean prnducts contain as much as 4% salt of the drymatter, the reference diet supplemented with cooked Korean soybean (Diet-6) was prepared with the same level of salt. No significant effect of the dietary salt content on the protein quality evaluation was noticed.

The PER value of the diet supplemented with Home-made Meju was remarkably low, it was lower than the PER of the diet made of rice only and this was not caused by th redused food intake. This fact may imply that Home-made Meju cotains some ant-inutritional or toxic factors. This was also observed in the experiment with a mixture of 1-month laboratory Muju and gluten as the protein sources, as described in Part 3.

This growth retarding effect of Home-made Meju disappeared with the subsequent ripening process of the Meju in the brine. Since Mejus are not consumed directly but subjeted to a Iong period of Meju-brine ripening, this effect may have not practical importance if the ripening process removes the unfavorable factors completely.

There has been much concern regarding mycotoxins in Meju and soypaste. There has been an article in Time magazine[12] warning that the frequent incidence of stomach cancer of Koreans is probably due to the aflatoxin in Korean soypaste, based on the paper of Dr. David J.Seel presented at the James Ewing Society in New York City. This paper was based upon an epidemiologic investigation, employing statistical analysis of the clinical material available. In his mycologic studies done at Chonbuk National University, the predominant mold organism in Korean soypaste was found to be *Aspergillus flavus* (Personal communication), but no chemical analysis had been carried out.

Lee and Lee[13] could not detect the presence of aflatoxin from 15 samples of Korean Meju and soypaste. Many fluorescencing spots having identical Rf value with aflatoxin Bl were detected from every sample of their experiment, but none of them were identified as aflatoxin in the ultraviolet spectrophotometry.

Table 1. Composition of the experimental diet for the rats

Diet No.	Composition of dried sample			Composition of diet*	
	Samples	% day matter	% N	Soybean product(g)	Rice (g)
1	Steamed rice	94.57	1.09	0	499.10
2	Cooked American soybean	96.35	6.35	23.62	475.00
3	Soybean curd	95.54	7.62	19.69	479.00
4	Soymilk residue	94.21	3.90	38.46	461.00
5	Casein			10.80	486.32
6	Cooked Korean soybean	96.41	7.12	21.07	478.00
7	Home-made Meju	90.53	7.64	19.63	480.00
8	-6 month Mix.	95.85	3.82	39.27	459.00
9	-6 month paste	96.12	4.07	36.86	461.00
10	Improved Meju	91.80	6.93	21.65	478.00
11	-6 month Mix.	95.74	3.58	41.90	457.00
12	-6 month paste	96.91	3.22	46.58	452.00

*20 g mineral mixture and 8 g vitamin mixture were added to each diet.

Table 2. Effect on the protein value by the supplementation of non-fermented soybean products to the rice diet of the rats

	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5
True Digestibilty	94.1	92.9	92.4	91.2	95.8
Corrected BV*	72.4	75.8	78.0	80.1	79.6
Corrected NPU*	68.1	70.4	72.1	73.0	76.2
Diet protein(%)	6.7	8.0	8.1	7.9	8.2
Weight gain for 9 days(g)	9.1	13.0	12.8	14.0	15.8
Food consumption(g)	84.3	88.1	84.3	88.3	88.5
Protein Efficiency Ratio	1.59	1.82	1.84	2.01	2.18
Partial carcass-N(mg/leg)	117.0	120.6	122.0	113.6	130.4

*Corrected for the dietary protein level 9.375%

Table 3. Effect on the protein value by the supplementation of fermented soybean Meju products to the rice diet of the rats

	Diet-6	Home-made Meju products			Improved Meju products		
		Diet-7	Diet-8	Diet-9	Diet-10	Diet-11	Diet-12
True digestibility	92.8	91.9	91.3	91.8	95.2	98.7	92.4
Corrected BV*	78.3	73.9	66.8	68.7	74.1	73.4	73.4
Corrected NPU*	72.6	67.9	60.9	63.0	70.6	72.4	67.5
Diet protein(%)	8.0	8.3	7.8	7.9	7.9	7.6	7.4
Weight gain for 9 days(g)	12.6	11.3	12.9	13.9	15.2	13.1	17.0
Food consumption(g)	87.7	87.4	89.0	83.8	91.9	78.1	86.1
Protein Efficiency Ratio	1.79	1.56	1.83	2.18	2.10	2.12	2.68
Partial carcass-N(mg/leg)	125.6	111.2	131.4	128.6	134.2	129.2	122.6

*Corrected for the diet protein lewel 9.375%

In Japan, according to Ebine[14], no strains producing aflatoxin were detected from 238 strains collected from the factories which prepare Koji. No aflatoxin was detected from 108 industrial Miso samples, 30 Home miso samples and 20 soysauce samples collected throughout Japan. However, the irregular occurence of mycotoxin in Koean soypaste and soysauce and the possible disappearence of mycotoxin in Meju during the ripening of Meju-brine mixture need further investigation.

General Conclulsion

The fermentation of soybean resulting in Korean soysauce and soypaste is undoubtedly a process of the Biological Ennoblement, as coined by Platt[15], with respect to the flavour enhancement of soybean as well as food preservation. Soysauce and soypastes with their meat-like taste and flavour enhance the palatability of the staple foods. However, it is difficult to conclude whether this process is beneficial or deliterious to the utilization of the nutrients of soybean. The fermentation appears to increase some of the vitamin contents of soybean. On the other hand, it would appear to be necessary to consume some of the amino acids in soybean in order to produce the flavour substances and brown colour of soysauce and soypaste. Since soybean is an important protein source, the estimation on the beneficial utilization should be based on the effect of the processing method on the protein utilization. An important aim of this study has been how to achieve good quality of soysauce and soypaste with a minimum cost of

amino acids for the production of flavour and colour substances using technologies available to the individual Korean households.

The most apparent factor deciding the soybean protein utilization during the making of Korean soysauce and soypaste appears to be the type of Meju. Ill-prepared Meju can waste a large amount of soyprotein that may be decomposed to ammonia, which degenerates the taste of the products. The Improved Meju used in the present study produced good quality products with relatively low loss of amino acids.

The length of the ripening of the Meju-brine mixture is another important factor. Ripening of the Meju-brine mixture for as long as 8 months appears to be unfavorable, since a drastic degeneration of the amino acid pattern of soybean was observed between the third and the eighth month of ripening. It would appear that ripening for 3 months is adequate to yield good quality products.

For the estimation of protein retention during the process, the measurement of only the changes in crude protein content can give misleading figures. The content of total amino acid in soybean can be reduced by 15~20% during fermentation without any significant reduction in the content of the crude protein, since most of the decomposed amino nitrogen is present in the products as ammonia and other N-compounds.

The determination of total lysine content of the fermented products by the ordinary column chromatographic method can also result in a large error. A large amount of ornithine is produced as a microbial metabolite during the fermentation, particularly during the ripening process. This ornithine is included to the lysine content with the ordinary chromotographic method with the result that the lysine content of soybean erroneously appears to have increased during fermentation.

From the results of the lysine availability of the products, determined by the chemical method, it seems that the damages to lysine by the inactivation is not greater than the destruction during the process. The ripening process can actually increase the lysine availability of fermented Meju.

It can be concluded from the results of the rat feeding experiments that the making of Korean soysauce and soypaste does not increase the protein quality of soybean but can reduce it seriously when the processing method is not adequat. Furthermore, there is danger of the occurence of mycotoxin or other unknown antinutritional factors in Meju, which probably disappear during the subsequent ripening process in the brine.

In order to eliminate the danger of mycotoxin from the Korean fermented soybean foods, it is desirable to produce Meju industrially by controlled fermentation. Improved Meju used in the present study showed a good potential for this purpose. The wider distribution of industrially produced Improved Meju for Meju-brine ripening at the household level would appear to be a desirable trend in Korea.

This will provide relatively inexpensive fermented soybean foods to the people and also reduce the loss of amino acid during processing.

요 약

삶은 콩, 두부, 비지 및 메주장 제품들을 실험실에서 제조하여 이들 식품의 백미식이에 대한 단백질 보충효과를 비교 관찰하였다.

식이의 총단백질의 20%에 상당하는 대두식품을 증숙된 쌀에 첨가하여 시험식이를 만들고 총 9일간의 백쥐사양시험으로 이들 식이의 단백질 소화율, 생물가(BV), NPU, PER 및 부분도체질소가를 측정하였다.

이들 시험결과에 의하면 일반적으로 비발효 대두제품 즉 삶은 콩, 두부, 비지는 백미식이의 단백가를 상당히 증진시키나, 메주 발효식품은 이를 별로 증진시키지 못하며 오히려 어떤 경우에는 저하시킴을 나타내었다. 또한 재래식 가정메주를 첨가한 식이에서 영양장애요소의 존재 가능성이 검측되었는데 이 재래식 메주의 생장저해효과는 6개월간의 메주장 숙성기간중에 소실되는 것으로 나타났다.

끝으로 본논문의 Part 1 부터 Part 4 까지의 전반에 걸친 결론을 기재하였다.

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