Heat Destruction of Amino Acids in Soybean Products

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Several kinds of processed soybean food—Shoyu (fermented soy sauce), Miso (fermented soybean paste), Natto (fermented soybeans), Tofu (bean curd), Aburaage (fried bean curd), Kori-tofu (dried Tofu) and Kinako (roasted soybean flour)—have spread widely in every home as the traditional food in Japan.

Though each processed soybean food posses-ses its own peculiar flavor produced in the course of process, the common treatment for all processed soybean foods has the heating process for material soybean. This heating process caused adequate denaturation of soy-bean protein and raises the digestibility of the protein with the enzymes of microorganism used for the fermented soybean foods such as Shoyu, Miso and Natto.

The peculiar soybean flavor is eliminated from processed foods and the flavor and color of food are also developed by the heating process. The colored substances produced by this heating can prevent oxidation of the un-saturated fatty acid contained abundantly in soybeans during the fermentation process of Miso as an example.

The heating process also has many ad-ventages in the nutritional point of view, namely, elimination of the antinutritional fac-tors in soybeans and development of the digestibility of soybeans.

But overheating causes excessive denatu-ration of soybean protein and destruction of amino acids. This results in decreasing the digestibility of the protein with the enzymes in the fermentation process and decreases the quality and nutritional value of products. From these points of view, several problems on the change of amino acids shall be described hereunder.

Amino acid composition of soy-bean products

In the course of the process of Mame-miso, Natto, Tofu, Aburaage, Kori-tofu, and Kinako which are mainly made of soybeans, peculiar heating treatments are performed. Some variations can be recognized in glutamic acid, lysine, tryptophan and cystine in the com-parison of the amino acid composition of these products (Table 1)[3].

These variations might be caused by the different amino acid compositions of material soybeans and by the effects of processing, that is, heating, extraction of protein and fermen-tation.

The above-mentioned four kinds of processed soybean products turned out by different heat-ing processes respectively were investigated to see the change in amino acids by heating.

Mame-miso is made of soybeans which are soaked in water to absorb it sufficiently, heated at 106°C for three hours after heated at 100°C for one hour, and left in the process of "Tomegama" (the heated soyebans are left as they are during certain time after heating has been turned off.)

After heating, Tane-Koji (*Aspergillus oryzae*) is mixed well and inoculated for making Koji. Then the Koji is mixed with

Table 1. Amino acid composition of soybean products

g/16gN

Amino acid	Mamemiso	Natto	Tofu	Kori-tofu	Aburaage	Yuba	Kinako
Glycine	4.4	3.7	4.0	4.8	3.7	4.9	4.3
Alanine	4.8	4.1	4.5	4.8	4.9	4.5	5.0
Valine	5.8	5.4	5.0	5.7	5.0	5.8	5.5
Isoleucine	5.0	4.8	4.8	5.3	5.4	4.8	4.8
Leucine	8.5	7.5	6.9	7.6	7.9	7.6	8.0
Aspartic acid	11.0	10.2	10.8	11.4	11.1	11.3	11.0
Glutamic acid	15.5	19.8	13.9	17.2	15.2	17.6	17.1
Lysine	5.1	5.3	7.1	6.3	5.1	5.1	4.8
Arginine	6.1	6.3	6.7	6.6	6.7	6.4	6.3
Histidine	2.6	2.2	2.6	2.4	2.1	2.3	2.1
Phenylalanine	4.8	6.5	6.0	6.3	5.7	6.1	5.5
Tyrosine	2.5	3.4	3.1	3.7	3.1	3.5	2.9
Proline	7.0	6.9	6.9	7.9	7.1	7.5	6.9
Tryptophan	1.4	1.2	1.2	0.8	1.0	1.0	1.2
Methionine	1.0	1.0	1.2	1.0	1.2	1.2	1.1
Cystine	0.6	1.0	1.0	1.0	0.5	0.7	1.0
Serine	5.8	5.6	4.5	5.0	5.4	5.7	5.7
Threonine	3.5	3.8	3.4	3.7	3.8	3.9	3.8

Table 2. Change of amino acid during the process of Mame-miso

g/16gN

Amino acid	Material soybeans	Soaked soybeans	Heated soybeans*	Product
Glycine	4.4	4.3	4.1	4.0
Alanine	4.2	4.3	4.2	4.3
Valine	5.9	5.8	6.3	5.9
Isoleucine	5.9	6.1	5.9	5.9
Leucine	8.2	8.1	8.4	8.8
Aspartic acid	11.9	11.0	10.0	11.0
Glutamic acid	15.5	14.6	14.6	16.4
Lysine	6.8	6.9	5.8	5.4
Arginine	7.9	7.7	7.7	6.0
Histidine	3.1	3.2	3.1	3.3
Phenylalanine	5.0	4.9	4.9	4.8
Tyrosine	2.3	2.3	2.4	2.6
Proline	5.7	5.8	5.6	5.6
Tryptophan	1.2	1.3	1.2	1.2
Methionine	0.9	0.8	1.0	1.0
Cystine	1.0	1.0	0.7	0.7
Serine	5.5	5.5	5.7	5.6
Threonine	5.0	5.0	5.1	5.5

* Heating condition: without pressure for one hour, at 106°C for three hours, and "Tomegama"

salt solution and fermented about one year. Both lysine and cystine decreases were caused by these heatings (Table 2)[2].

Natto is made of soybeans which are soaked in water in the same way as Mame-miso and then are fermented with *Bacillus natto* after heating at a rather high temperature during a short time (120°C, 30 min.). No decrease of amino acids except arginine was brought about by this heating (Table 3)[5].

One of the soybean products processed without soaking into water is Kinako, which is processed by grinding after heating (roast) at 160°C for 10 minutes. This heating remarkably changed lysine (Table 1)[8].

The process of making Tofu is vastly different from that of the three products described above. That is, the soybeans are soaked and ground. Then soybean milk obtained by hot extraction .of the ground soybeans at 100°C for five minutes is precipitated with calcium sulfate. No amino acid is lost in this heating process (Table 4)[4]. Thus, the ways to lose amino acids during heating process were

Table 3. Change of amino acid during the process of Natto

g/16gN

Amino acid	Material soybeans	Heated soybeans*	Product
Glycine	3. 3	3. 0	3. 2
Alanine	4. 3	4. 1	4. 1
Valine	5. 3	5. 1	5. 2
Isoleucine	4 ·7	4. 8	4. 8
Leucine	7. 9	7. 9	7. 9
Aspartic acid	9. 9	9. 8	10. 0
Glutamic acid	16. 8	17. 1	17. 0
Lysine	6. 3	6. 2	6. 2
Arginine	6. 2	5. 1	4. 4
Histidine	3. 0	3. 0	2. 8
Phenylalanine	4. 5	4. 7	4. 8
Tyrosine	2. 6	2. 6	2. 6
Proline	7. 8	7. 6	7. 3
Tryptophan	0. 9	0. 9	1. 0
Methionine	0. 9	1. 0	1. 1
Cystine	1. 0	1. 0	1. 0
Serine	5. 8	5. 5	5. 9
Threonine	4. 3	4. 1	4. 2

* Heating condition: at 120°C for 30 minutes

Table 4. Change of amino acid during the process of Tofu

g/16gN

Amino acid	Material soybeans	Soaked soybeans	Heated soybeans*	Product
Glycine	4. 2	4. 2	4. 2	4. 2
Alanine	3. 7	3. 7	4. 0	5. 0
Valine	4. 8	4. 7	5. 0	5. 1
Isoleucine	4. 7	4. 7	4. 8	5. 0
Leucine	7. 5	7. 5	7. 6	7. 6
Aspartic acid	12. 2	12. 2	12. 8	12. 9
Glutamic acid	19. 6	19. 6	20. 0	19. 3
Lysine	7. 1	7. 3	7. 1	7. 5
Arginine	8. 5	8. 4	8. 4	8. 5
Histidine	2. 5	2. 5	2. 6	2. 6
Phenylalanine	4. 7	4. 7	4. 5	4. 7
Tyrosine	3. 1	3. 1	3. 5	3. 5
Proline	5. 6	5. 6	5. 6	5. 6
Tryptophan	1. 5	1. 4	1. 6	1. 4
Methionine	0. 8	0. 8	0. 9	0. 9
Cystine	1. 1	1. 1	1. 1	1. 1
Serine	6. 6	6. 6	6. 5	6. 4
Threonine	4. 7	4. 7	4. 9	4. 8

* Heating condition: at 100°C for 5 minutes

different by the products as in the above four examples.

The major affecting factors on the loss of amino acids by these heating processes may be the temperature, the time of heating, water (added water amount) and sugar contents of heating materials. And the affecting conditions of these factors are different according to the kind of amino acids.

Heat destruction of amino acids

1) *Influence of the temperature and time of heating*[6]

The amino acid destruction of defatted soybean flour heated with the same amount of water was investigated, shifting the temperature and time of heating process. Some parts of lysine, cystine, arginine, tryptophan and serine were destroyed while no change took place in other amino acids.

The destructive conditions of these amino acids are different from each other, viz., though lysine was scarcely destroyed at 100°C, 108°C and 115°C, it decreased suddenly at 126°C (Fig. 1). Cystine was hardly destroyed

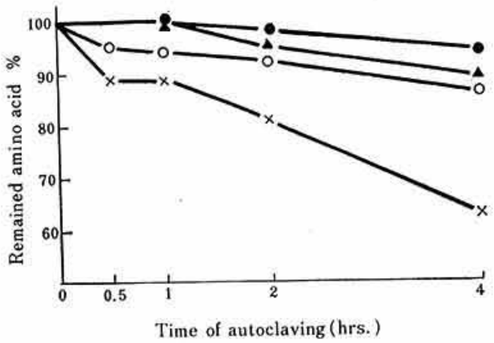

Fig. 1. The heat destruction of lysine in defatted soybean flour
—●— Heated at 100°C
—▲— Heated at 108°C
—○— Heated at 115°C
—×— Heated at 126°C

even by a long hour heating when the temperature was low but it showed a sudden increase of loss at the temperature above 100°C (Fig. 2).

The destructive condition of arginine showed

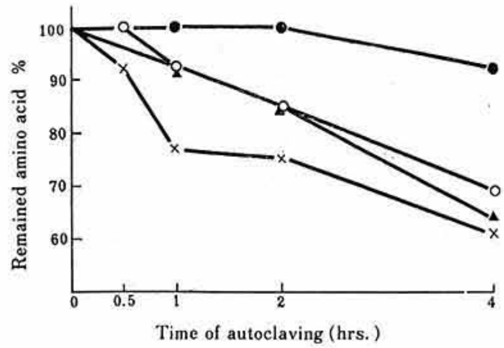

Fig. 2. The heat destruction of cystine in defatted soybean flour
—●— Heated at 100°C
—▲— Heated at 108°C
—○— Heated at 115°C
—×— Heated at 126°C

the intermediate aspect of that of lysine and cystine, and it was lost remarkably by heating at a high temperature of short duration. Tryptophan and serine were destroyed only by the long hour heating with high temperature.

The amino acid losses in the processed soybean products may be considered as follows from these results; lysine and cystine in Mame-miso were destroyed by the long hour heating with high temperature; as for Natto, the only one amino acid which was destroyed by the high temperature heating of short duration was arginine but as for Tofu, no amino acid was destroyed because of the low temperature heating of short duration.

Regarding the loss of arginine, it is known that amino acid is changed into ornithine and citrulline by the enzyme of the microorganism used for the fermentation processes of Shoyu, Miso, Natto and Tempeh.

2) *Influence of added water to material during heating*[7]

The influence of added water on the destruction of amino acids was examined, changing the amount of added water to defatted soybean flour. The loss of lysine was largely decreased by added water (Fig. 3), and arginine showed the same trend. Cystine was destroyed regardless of the added water (Fig. 4), and tryptophan and serine were also destroyed in the same way as cystine.

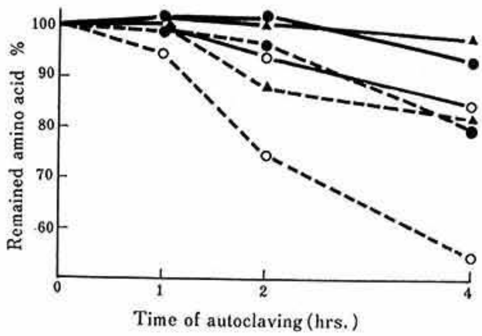

Fig. 3. The influence of water on the heat destruction of lysine in defatted soybean flour

○ Without water
● 3 times water added
▲ 6 times water added
— Heated at 115°C
··· Heated at 126°C

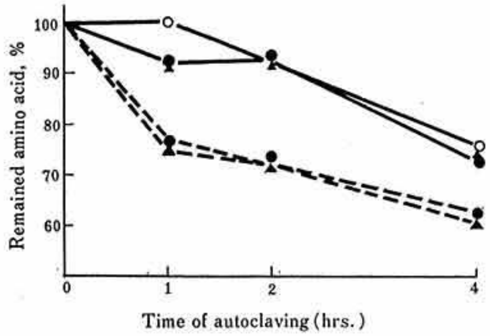

Fig. 4. The influence of water on the heat destruction of cystine in defatted soybean flour

○ Without water
● 3 times water added
▲ 6 times water added
— Heated at 115°C
··· Heated at 126°C

No decrease was seen in other amino acids notwithstanding the existence of added water. From these results, lysine and cystine in Aburaage and Tempeh are quickly destroyed and may be attributed to dehydration and heat by hot oil in frying[1].

It may be concluded that the decline of nutritive value caused by the destruction of the lysine should not be considered seriously because amino acid losses are prevented effectively by the water addition which is necessary with the general heating process of soybeans except roasting.

But cystine loss should be considered seriously from the nutritive standpoint because no effective measures to prevent it have been found yet as the sulfur-containing amino acids (methionine, cystine) are the limiting amino acids of soybeans.

3) Influence of added sugar to soybean protein during heating[10]

The influence of sugar was investigated, adding several kinds of sugar which exist in soybean protein; the heat destruction of lysine was affected by the existence of sugar and cystine was destroyed by heat independently to the existence of sugars.

The destruction of lysine can be prevented by increasing added water to the defatted soybean flour and soaking it into water for a long time to dilute or eliminate sugar.

But there is no means to prevent heat destruction of cystine; therefore, much attention should be paid in the heating process of soybeans so as not to increase the destruction of cystine because of the low methionine content in soybeans.

On the other hand, the heat destruction of amino acids is related to the flavor and color of soybean products. For example, the agreeable odor of aldehyde made by browning of the amino acids heated with reducing sugar is very important in the process of soybeans. And the odor of hydrogen sulfide, which has a bad smelling is an important composing factor of flavor being mixed adequately in foods. However, this is a difficult problem because nutritive value may decline by heating sulfur containing amino acids.

4) Heat destruction of available lysine[8]

Carpenter et al. proved that there is a high correlation between the free epsilon amino groups of lysine in the proteins and the growth response of chick, and they called the lysine as the "available lysine".

The amount of the available lysine in soybeans is about 70 per cent of the total lysine, and the loss of the former by heating was greater than that of the latter; the available lysine decreased even in the early stage of heating process while the total lysine showed no loss (Fig. 5). This means actual nutritive

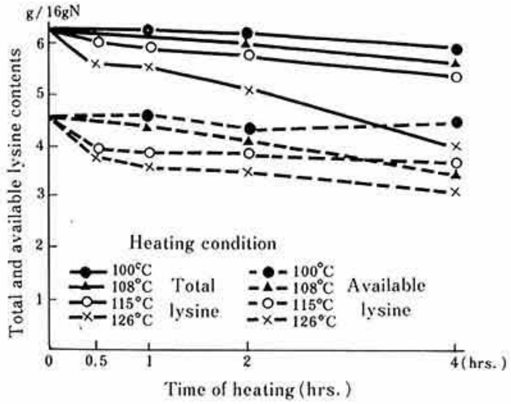

Fig. 5. Heat destruction of total and available lysine in defatted soybean flour

loss so much care must be taken for this point.

This trend was evident especially under the absence of added water and high temperature process of soybeans; for example, the available lysine in Kinako decreased much more than the change of total lysine.

Heat destruction of amino acids and decline of nutritive value[11]

The growth test of rats fed with the properly heated and with overheated soybean flour, which had been deprived of lysine, arginine, cystine and tryptophan, revealed digest difference of growth. As the result of the nutritive recovery test performed with the supplemental amino acids which correspond to that lost from soybean flour by overheating, the only one proved to be effective was cystine. Therefore, the loss of cystine is the most serious problem in the evaluation on the nutritive value of soybean products processed under high temperature treatment.

Heating and liberation of amino acids by enzyme[9]

The amounts of amino acids liberated by enzymes were different according to the difference of heat treatment of soybean flour; consequently the total amounts of liberated amino acids were also different (Fig. 6).

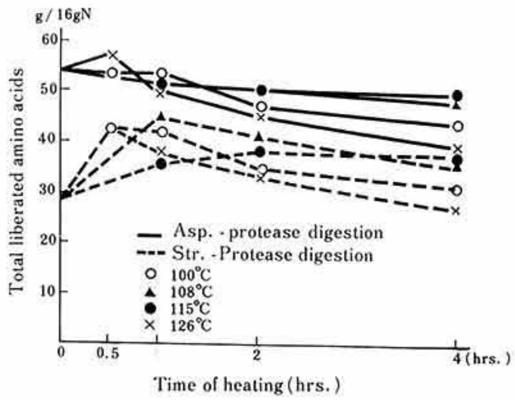

Fig. 6. Enzyme treatment and total liberated amono acies

This means that the condition of heating process renders a very important result to the quality of soybean products. For example, if the heat denaturation of soybean protein is not completed in the fermentation of Shoyu, the quality of products deteriorates with the precipitation of non denaturated protein.

On the other hand, overheating decreased the amino acid liberation by enzyme. As for the production of Mame-miso, it is important to derive good taste, color and flavor as much as possible from the material. For this purpose, excessive heating condition is used to produce Mame-Miso. For processing of fermented soybean products, therefore, heating establishment on suitable condition for each soybean product is necessary.

The heating process of soybeans and the change of amino acids were thus described mainly on the processed soybean food with the model experiments performed using soybeans and soybean products.

The suitable method for soybean process has been established by traditional experiences and the endeavor of many researchers, and it shall be improved to be favorable for the consumer and to produce new food.

The fundamental phenomena and mechanisms on the heating process of soybeans and on the change of amino acids derived from our experiments as described above are not only adaptable for the process of soybeans but also applicable for the process of other cereals like wheat or rice. It is hoped that this notion could be available for the establishment of food processing technics in the future and for accompanied evaluation of nutritive value.

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