

Partial translation of "Research Report of
Industry-Academic-Government Joint Research Development Project by
Fukuoka Industry, Science and Technology Foundation"

Development of Salt-Free Seasonings for Next Generation
Suitable for Prevention and Improvement of Hypertension

(For the 2005~2006 Fiscal Years)

Fukuoka IST

Chapter 1

Identification of Hypotensive Peptide in Salt-Free Seasonings and Clarification of Action Mechanism Thereof

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1-1 Identification of Hypotensive Peptide in Salt-Free Seasonings and Clarification of Action Mechanism Thereof

Salt-free soy sauce-like seasoning (hereinafter, it will be referred to as HFS) is a novel seasoning in the presence of ethanol where salt is substituted taking bacteriostatic action into consideration. Therefore, various peptides generated by fermentation of soybean protein are by-produced. Since it has been clinically proved that low-molecular peptides generally express a hypotensive action, an object of this investigation is to clarify the positive hypertension-preventing function by quick identification and clarification of physiological action of the by-produced peptides in addition to the functional characteristics as the absence of salt.

Chapter 2

Optimization of Peptide Productivity in Salt-Free Seasonings

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2-4 Research Results

2-4-1 Experimental Materials and Methods

Koji mold: As to the koji mold, K2-4 strain and HG strain of *Aspergillus oryzae* stored in the Soy Sauce Brewing Cooperative, Fukuoka Prefecture were used.

Soy sauce koji: Soy sauce koji was collected from the production line of the Soy Sauce Brewing Cooperative, Fukuoka Prefecture and used for the experiments. Thus, wheat was subjected to a roasting treatment by a fluidized roaster RX-2000 (KK Fujiwara Techno Art) or a super roaster (KK Nagata Jozo Kikai) and then crushed by a hammer mill to give crushed wheat. Defatted soybeans were steamed by a continuous steaming tank to give steamed soybeans. The resulting crushed wheat and steamed soybeans were mixed in the same amounts in terms of the wheat and the soybeans before the treatment, charged into a koji-manufacturing device of a big disk type (KK Nagata Jozo Kikai) and made into koji under aeration for about 40 hours to give soy sauce koji. Incidentally, the seed starters (koji mold spores) were previously dispersed into the cracked wheat before mixing with the steamed soy beans.

Steamed soy beans and soy beans treated with hot air: Treatment of the materials not being made into koji in conducting a reduced charging of koji was done as follows. Thus,

a wet heating treatment (steaming) at 1.0 atm for 60 minutes, 1.0 atm for 120 minutes, 1.6 tom for 30 minutes and 1.6 atm for 60 minutes was done by an autoclave (Yamato Kagaku KK) and that at 2.0 atm for 5 minutes and at 2.0 atm for 10 minutes was done by a continuous steaming tank. A heating treatment with hot air at 160°C for 30 minutes, 200°C for 10 minutes, 400°C for 2 minutes and 500°C for 2 minutes was done by a constant-temperature drier (Yamato Kagaku KK).

Test with small charging amount: Compounding of the materials in the test with small charging amount is shown in Table 2-1. The charging amount was made 1 L for the start and 1.2 L for water and the temperature of *moromi* was made 20°C. Incidentally, koji-reducing rates were made 30% and 50%.

Table 2-1

Compounding of Materials in Small Amount Charging Test

Koji-reducing rate (%)	Compounding of Materials (g)			Charged Water Amount (ml)	Alcohol Concn (%) in Charged Water	Wheat : Defatted Soy Bean
	Soy Sauce Koji	Wheat	Soy beans			
0	750	-	-	1,200	20	1:1
30	525	100	100	1,200	20	1:1
50	375	166	166	1,200	20	1:1

of Salt-Free Seasonings

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3-4-3 Analysis of Flavor Ingredients in Salt-Free Soy Sauce-like Seasonings

Analysis of flavor ingredients was conducted by the same method using the HFS prepared in this project.

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Table 3-10 shows the comparison of furanones. Among them, HEMF has been said to be most important as a compound contributing in a sweet smell of soy sauce. We think that compounds related to the above contribute in salt taste savor of soy sauce but, unfortunately, we were unable to make the evidence therefor clear. However, when the facts that HEMF concentration in soy sauce is as highest as not lower than 6000 µg/kg as compared with other flavor ingredients and that this compound was not detected in HFS are taken into consideration, the importance of HEMF is significant.

Table 3-10

Comparison of Main Furanones

Compound	Dark-Colored Soy Sauce (μg/L)	HFS (μg/L)
3-Methyl-2 (5H) -furanone	25	-
2 (5H) -Furanone	14	-
Furaneol	925	151
HEMF	6161	-

HEMF: 4-Hydroxy-2 (5) -ethyl-5 (2) -methyl-3 (2H) -furanone

To sum up the above, we conclude that the difference in the savor between dark-colored soy sauce and HFS will not be caused by alcohols, ketones, esters, volatile organic acids and pyrazines but will be caused by the decisive absence of methionol and HEMF. Particularly, the absence of HEMF in HFS is directly related to the absence of soy sauce savor of this product. On the other hand, since phenols are considerably present even in HFS, they are likely to play a role in giving firmness and punch to the savor of HFS.

3-4-4 Improvement in Savor by Fermentation Process

As mentioned in 3-4-3, HFS shows low concentrations of flavor ingredients and, unlike HEMF, it does not contain characteristic flavor ingredients of soy sauce and, accordingly, we attempted to improve the savor by modifying the brewing process of the salt-free seasonings. As shown in Table 3-11, koji was prepared by two strains of *oryzae* and *soya* as the koji mold and, after that, fermentation by *saké* yeast 901 or a fermentation process by soy sauce yeast S32 was additionally conducted. The fermented HFS prepared in each combination showed the savor characteristics as shown in Table 3-11. In

our organoleptic test, the fermented HFS prepared by the combination of *oryze* with *saké* yeast 901 in the sample No. 1 was found to show the best savor.

Table 3-11

Influence of Mold Starter and Yeast on the Odor of
Salt-Free Soy Sauce

	Mold Starter	Yeast	Organoleptic Evaluation
1	<i>Oryzae</i>	<i>saké</i> yeast 901	Soy sauce-like odor noted; no rough smell; strong punch noted; salty taste noted.
2	<i>Oryzae</i>	soy sauce yeast S32	A bit weaker than sample 1; slight rough smell noted.
3	<i>Soja</i>	<i>saké</i> yeast 901	Soy sauce-like odor noted although it is weak; specific mushroom-like odor noted; rough smell noted.
4	<i>Soja</i>	soy sauce yeast S32	Soy sauce-like odor noted although it is weak; specific mushroom-like odor noted; rough smell noted.

Table 3-12 shows the concentrations of main flavor ingredients in various fermented HFS. It was shown that characteristic flavor ingredients were produced by the microbes used therefor. For example, when koji was prepared using *soya*, characteristic 1-octen-3-ol (mushroom savor), 3-methylbutanoic acid (pungent odor) and maltol (sweet odor) were abundantly generated. When *oryzae* was used, production

of ethyl isonicotinate, 4-vinylguaiacol and coumaran increased. Even in the case of soy sauce yeast S32 and saké yeast 901 used in the fermentation process of *moromi*, clear difference was noted. Thus, when saké yeast was used, concentrations of 2-methylpropanol and 2-propylfuran became high. Organoleptically, a combination of *oryzae* with saké yeast 901 generates the best savor as shown in Table 3-11 but, in a flavor ingredient level, that was unable to be supported. In any combination of the microbes, production of HEMF being a characteristic flavor ingredient of soy sauce was very small.

Table 3-12
Main Flavor Ingredients in Salt-Free Soy Sauce
(Relation to the Microbes Used)

PN	Compound	<i>Soya</i> 901	<i>Soya</i> S-32	<i>Oryzae</i> 901	<i>Oryzae</i> S32
1	2-Methylpropanol	1.04	0.67	1.56	0.47
2	2-Propylfuran	4.84	1.53	14.1	1.52
3	3-Methylbutanol	8.90	7.35	9.92	5.53
4	(unknown)	2.34	0.25	5.79	0.17
5	Cyclohexanol (IS)	10	10	10	10
6	1-Octen-3-ol	0.89	1.28	0.05	0.05
7	3-Methylbutanoic acid	1.15	1.07	0.47	0.41
8	Methionol	0.54	0.44	0.39	0.46
9	Ethyl isonicotinate	0.49	0.56	0.91	1.54
10	(unknown)	2.26	0.16	0.40	0.08
11	Antioxidant	1.32	3.42	1.30	1.65
12	Phenethyl alcohol	3.38	4.51	5.09	4.90
13	Maltol	4.95	5.36	1.19	1.73

14	HEMF	0.58	1.79	0.53	1.59
15	5,6-Dimethyl-1,4-dioxin	1.67	3.82	0.84	1.34
16	4-Vinylguaiacol	4.41	7.10	8.86	15.3
17	2-Methoxy-6-methyl-4H-pyran-4-one	0.62	2.58	0.68	1.28
18	Coumaran	0.37	0.82	0.92	2.39

(mg/kg)

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3-6 Problems and Plans in Future

Since it was likely that, for brewing the soy sauce savor, a fermentation process under a high-concentration salt environment is essential, we wish to start in the study concerning a complete desalting treatment of the usual brewed soy sauce for the manufacture of salt-free fundamental seasoning in reduced salt, low salt or no salt having a soy sauce-like savor. We are also starting in the study at present for specifying the ingredients participating in the salty taste savor of the brewed soy sauce and for promoting the utilization of the flavor ingredient to salt-free seasonings.