OFF-FLAVORS IN FOODS &
BEVERAGES

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JAPANESE SOY SAUCE FLAVOR WITH EMPHASIS ON OFF-FLAVORS

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1. INTRODUCTION

Soy sauce is used as an all-purpose seansoning in countries of Asia such as Japan, China, Taiwan, Malaysia, Indonesia and others.

There are two catergories for soy sauce in the world. One is a fermented soy sauce, and the other is a chemical one. In fermented soy sauce, fermentation processes by useful microorganisms are included to produce the soy sauce. Fermented soy sauce is classified as Japanese-type soy sauce and Chinese-type. In Japanese-type soy sauce, wheat and soybeans are used for raw materials, and in Chinese-type, only soybeans are used. On the other hand, chemical soy sauce is made through hydrolysis of vegetable protein such as soybeans. The chemical soy sauce is now marketed mainly in European countries, U.S.A. and Canada. In U.S.A., Japanese-type soy sauce has been produced since 1972, and its production has been increased steadily.

The origin of soy sauce is considered to be China where only soybeans are used as a raw material. In the sixth century a vegetarian seansoning came with Buddhism from China to Japan. After that soy sauce underwent considerable development in Japan. One of the characteristics was to use almost equal amounts of wheat and soybeans to make soy sauce. This has brought a fine balance of the proper concentrations of the major flavor-enhancing proteins, amino acids, sugars and other components. Further, Japanese soy sauce which uses wheat and soybeans is one of the most flavorful soy sauces in the world.

Many books and papers on soy sauce and its flavor compounds are published.(1,2) Nearly 300 flavor components have been identified in Japanese fermented soy sauce by Japanese investigators since Tawara's study (3) in 1887. Among these compounds, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone, so-called HEMF, was found to be a character-impact component of Japanese soy sauce by Nunomura et al.(4,5) It has been proved to be produced during yeast fermentation by Sasaki et al.(6)

All processes of the production are related to the formation of flavor compounds. The heat treatment of raw materials, koji culturing by molds, lactic acid fermentation, yeast fermentation, aging of "moromi" mash and pasteurization. Among the processes, yeast fermentation in "moromi" mash contributes most for making flavors.

In late 1970's and 1980's, statistical approaches were attempted to explain which flavor components most affected the whole quality of soy sauce. Some of the studies had a problem with the quantitative analysis applied, so the statistical results lacked reliability. In scientific evaluation of food quality, an accurate and exact quantitative determination is one of the most important elements. A series of statistical studies by Mori et al.(7-12) has revealed very interesting results. They have found that there are three kinds of flavor compounds. The first is : the more a compound is contained, the better the quality of soy sauce. The second is : a compound has the optimum value in concentration. The last is : the more, the worse. The compounds which are included by the last category are called off-flavors in soy sauce. When the compounds with the optimum exist over the value in concentration in soy sauce, they may be also called off-flavors. Moreover, some compounds increase in their contents during storage of the final product. In some cases it causes undesirable flavor. They are also called off-flavors.

This chapter will describe the production procedure of Japanese-type soy sauce, flavor components made in the processes, methods of quantitaive analysis, organoleptic quality including off-flavors, and formation of off-flavors during storage.

2. PRODUCTION PROCEDURE OF JAPANESE-TYPE SOY SAUCE

The flow sheet of the production of Japanese soy sauce is shown in Fig.1. Its characteristic is to use wheat as one of the raw materials as described above. The process consists of five main steps : heat treatment of raw materials, koji making by culturing *Aspergillus oryzae* or *Aspergillus sojae*, moromi(mash) fermentation by *Pediococcus halophilus* and *Zygosaccharomyces rouxii* including aging of moromi, moromi pressing to get liquid layer or soy sauce, and refining including pasteurization. From the standpoint of making good flavor in soy sauce, koji culturing, moromi fermentation and pasteurization by heating are very important.

3. IDENTIFICATION OF FLAVOR COMPONENTS

3.1 Koji

Sasaki and Nunomura (13) identified 66 compounds as flavor components of koji by three methods : concentration of headspace gas aroma (top note aroma), carbon dioxide distillation of dichloromethane extract, and fractional extraction of dichloromethane extract. 1-Octen-3-ol, phenylacetaldehyde and phenylacetic acid are responsible for the overall characteristic flavor of koji. 1-Octen-3-ol is known well to possess a characteristic aroma of mushrooms such as *Armillaria Matsutake*. Phenylacetic acid presents a honey-like aroma. Both are produced by koji molds. 1-Octen-3-ol is also one of major constituents of a neutral fraction separated from a soy sauce flavor concentrate.(14) It indicates that metabolites by koji molds are related to a whole soy sauce aroma.

Formation of phenolic compounds has been studied by Japanese researchers. (15-18) This koji culturing is one of the most important steps for the formation of phenolic compounds. The heat treatment of wheat decomposes glycoside or lignin of wheat to make vanillin, ferulic

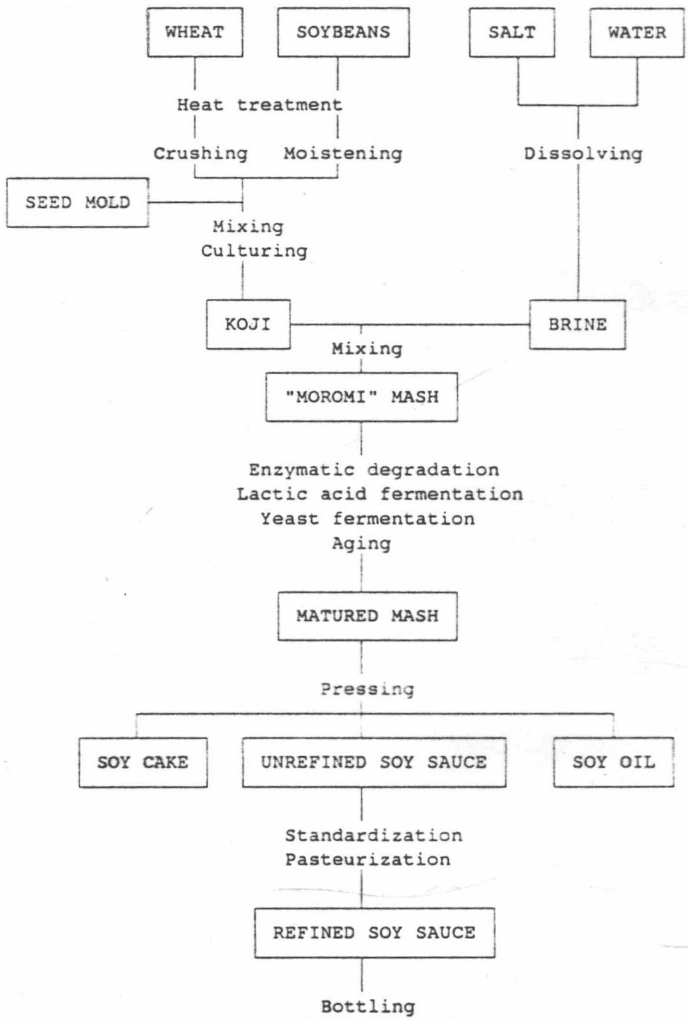


Fig. 1. Production Procedure of Japanese Soy Sauce

acid and vanillic acid. In the course of koji culturing, *Aspergillus* molds metabolite a part of ferulic acid to vanillin and vanillic acid, and *p*-coumaric acid to 4-hydroxybenzoic acid. The major constituent of the phenolic fraction was identified as ferulic acid. (19, 20) In moromi mash, ferulic acid and *p*-coumaric acid are changed to 4-ethylguaiacol and 4-ethylphenol by *Candida* (*Torulopsis*) yeasts which give an important character to soy sauce flavor. Thus, koji molds produce important precursors of soy sauce flavors.

In koji making, one important concern is that severe contamination by bacteria should be prevented. If koji suffers severe contamination by bacteria such as *Bacillus*, the bacteria produces ammonia, isovaleric acid and others which are taken over in the finished product. (1) They are considered to be off-flavors for soy sauce.

3.2 Moromi Mash and Soy Sauce Oil

In general, it takes around 6 months to complete the fermentation and aging of moromi in the recent production of soy sauce. Moromi of the early stage does not have soy sauce aroma so much, but it represents koji-like aroma. pH of moromi is almost neutral or weak acidic after mixing of koji and salt brine. The concentration of salt in moromi ranges from 16 to 19 %. This salt content prevents undesirable putrefactive bacteria from growing during the subsequent fermentation by lactic acid bacteria and yeasts and aging. Pure cultures of *Pediococcus halophilus* are added in the first stage to convert simple sugars to lactic acid and drop the pH to the optimum pH for the following yeast fermentation. In the next stage, pure cultured *Zygosaccharomyces rouxii* is added, and vigorous alcoholic fermentation occurrs. In some cases, pure cultured *Candida* (*Torulopsis*) yeasts are added along with *Z. rouxii* to change ferulic acid and *p*-coumaric acid to 4-ethylguaiacol and 4-ethylphenol and give a deep characteristic flavor to soy sauce as described above. Most flavor compounds are formed during these yeast fermentations. The pleasant aroma and flavor in the finished product are largely due to the activities of the both yeasts, *Z. rouxii* and *Candida* (*Torulopsis*).

Film-forming yeasts sometimes grow on the surface of moromi mash in the middle and final stages. n-Butyric acid produced by the film-forming yeasts gives an adverse odor to soy sauce. Since the film-forming yeasts produce the undesirable odor compounds under an aerobic condition, moromi mash should be regularly agitated to prevent them from growing under an aerobic condition. Compressed air is usually used for the agitation.

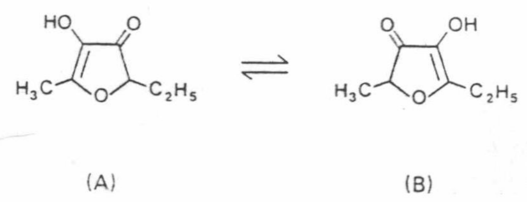
Nunomura et al.(21) identified 43 compounds from the flavor concentrate of matured moromi mash which was prepared by extraction with dichloromethane. The flavor fraction of soy sauce oil was also concentrated by means of ethanol vapor distillation under reduced pressure and 32 compounds were identified. Soy sauce oil is one of by-products after pressing of moromi mash. Both the flavor concentrates from moromi mash and soy sauce oil contained a great amount of many ethyl esters of fatty acids. These esters give the sweet aroma to moromi mash. Further, Nunomura et al.(21) found the ethyl esters of fatty acids as major constituents from a flavor concentrate of the press soy cake. These ethyl esters in moromi mash remain in the soy cake which is one of the by-products after pressing, and the lower-boiling compounds move to the liquid layer or soy sauce, including HEMF.

3.3 Finished Product of Japanese Soy Sauce

Nearly 300 flavor components have been found in Japanese soy sauce until now. They include hydrocarbons, alcohols, esters, aldehydes, acetals, ketones, acids, phenols, furans, lactones, furanones, pyrones, pyrazines, pyridines, miscellaneous nitrogen compounds, sulfur compounds, thiazoles, terpenes and others. The details are described in the reference written by Nunomura and Sasaki.(2)

Among them, phenolic compounds and caramel-like aroma compounds such as furanones and pyrones largely contribute to Japanese soy sauce flavor. The phenolic compounds were investigated by Yokotsuka et al.(16, 22) They indicated that 4-ethylguaiacol (4-ethyl-2-mehoxyphenol) had a very important effect on the quality of Japanese soy sauce. Asao and Yokotsuka (20) and Sasaki et al.(23) found that 4-ethylguaiacol and 4-ethylphenol were produced by *Candida* (*Torulopsis*) yeasts, not by *Zygosaccharomyces rouxii*. Mori et al.(8) verified that 4-ethylguaiacol had an important relation to the good aroma of soy sauce and that there was an optimum concentration of 4-ethylguaiacol for soy sauce as a result of their statistical study on basis of an accurate quantitative analysis.

HEMF was identified as a character-impact flavor compound of Japanese soy sauce.(4,5) The structure of HEMF is shown in Fig. 2. HEMF is formed by both *Zygosaccharomyces rouxii* and *Candida* (*Torulopsis*) yeasts.(23) Sasaki et al.(6) recently reported the biosyntheses of HEMF by yeasts. *Zygosaccharomyces* species have a tendency to produce more HEMF than *Candida*. In the report, it is shown that the pentose-phosphate cycle is necessary for yeasts to produce HEMF and that HEMF is probably changed from sedoheptulose 7-phosphate.



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

Fig. 2. Structure of HEMF, Character-Impact Compound of Japanese Soy Sauce.

Oxidative products of HEMF were studied by Nunomra et al.(2, 24) HEMF is very stable in soy sauce, but it is unstable in alkali or when it is exposed to the air. Under basic conditions, HEMF changes into the odorless compound 4,4,5-trihydroxy-2-ethyl(or methyl)-5-methyl(or ethyl)-3-tetrahydrofuranone (OX-HEMF). Autooxidation of HEMF gives α-keto acids such as 2-oxobutanoic acid and 2-oxopropanoic acid, aldehydes such as propanal and acetaldehyde, and carboxylic acids such as propanoic acid and acetic acid. α-keto acids release carbon dioxide by heating. The oxidative products of HEMF are summarized in Fig. 3.

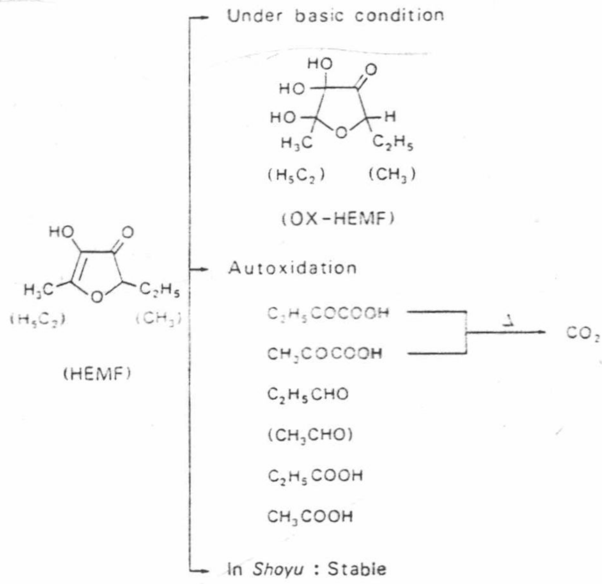


Fig. 3. Oxidative Products of HEMF.

4. QUANTITATIVE ANALYSIS OF FLAVOR COMPOUNDS

The accuracy of the quantitative analysis is essential to get reliable results in a statistical investigation of food quality, for example, the study of the relationship between gas chromatographic results and the organoleptic evaluation. In the field of soy sauce, Sasaki et al.(25) employed a unique quantitative method. Extraction of soy sauce with methyl acetate was the most suitable method for preparation of flavor extracts as a result of attempts of several other methods. 5 ml of soy sauce, 2 ml of methyl acetate and 1 g of sodium chloride were vigorously shaken in a capped test tube, and then the methyl acetate layer was separated from the aqueous layer by the centrifugation at 3,000 rpm for 10 min at 5°C. 1 ml of methyl acetate was added to the aqueous layer, and the subsequent operations were the same as the first. The addition of 1 ml of methyl acetate was repeated four times. The methyl acetate extract was subjected to gas chromatographic analysis after the addition of an internal standard. The coefficients of variation and the recovery of components were checked in full, and their figures were the best in comparison with the other methods tried. A part of the result is shown in Table 1.

Sasaki and Nunomura (26) further reported the quantitative analysis methods for top note or headspace gas of soy sauce. 1 ml of soy sauce was put into a 20 ml test tube which was tightly capped with a silicon septum. It was vigorously shaken for 10 min and then kept unshaken for 20 min. 10 ml of its headspace gas was taken by a gas-tight syringe from the test tube with compensation of the same volume of nitrogen gas by using another syringe. The headspace gas was analyzed by gas chromatography. The result of 10 times-analysis is presented in Table 2.

Nunomura and Sasaki (27) applied the extraction method with methyl acetate and the headspace gas method to determine sulfur-containing compounds by using gas chromatography with FPD detector. Dimethyl sulfide, 3-methylthiopropanal (methional), 3-methylthio-1-propanol (mehionol), ethylene sulfide, 1-propanethiol and dimethyl disulfide were determined accurately.

Table 3 shows the concentrations and odor units (28) of major components in the headspace gas of Japanese soy sauce.(26, 27) Isovaleraldehyde, ethanol, isobutyraldehyde and dimethyl sulfide seemed to contribute to the top note aroma of soy sauce.

The typical quantitative analysis result of Japanese soy sauce is shown in Table 4. It includes the result by another method which employs a direct injection of soy sauce into gas chromatograph. The method was developed by Sasaki and Nunomura.(25) Ethanol, lactic acid, glycerol, acetic acid, 4-hydroxy-5-methyl-3(2H)-furanone (HMMF), 2,3-butanediol, isovaleraldehyde and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF) are major flavor components in Japanese soy sauce.

5. FORMATION OF FLAVOR COMPONENTS IN PRODUCTION

5.1 During Fermentation in Moromi Mash

Most of the flavor compounds in soy sauce are produced during yeast fermentation as described above. Nunomura and Sasaki (29) kept track of each flavor component from the beginning of fermentation, and they found three type of compounds; the first one is the compound the concentration of which increases after addition of pure cultured yeasts, the second is the compound the concentration of which has the maximum at the yeast addition and then decreases, and the last one is the compound the concentration of which increases slowly after charging of moromi mash. The first group includes ethanol, isobutyl alcohol, n-butyl alcohol, isoamyl alcohol, ethyl lactate, methionol, 2-phenylethanol, 4-ethylguaiacol, HEMF and ethyl

TABLE 1

Coefficients of Variation (C.V.) and Recoveries of Extraction Method with Methyl Acetate

Compounds	C.V. (%) (n=10)	Recoveries of 10 ppm (%)
Isobutyl alcohol	1.39	102.0
n-Butyl alcohol	1.44	103.9
Isoamyl alcohol	1.39	108.1
Acetoin	2.23	96.2
Ethyl Lactate	1.29	102.3
Furfuryl alcohol	5.88	92.7
Methionol	1.86	88.4
2-Phenylethanol	3.37	97.3
4-Ethylguaiacol	1.48	97.9

TABLE 2

Quantitative Analysis Results of Headspace Gas Method of Japanese Soy Sauce

Compounds	Concentrations (ppm)	C.V.* (%) (n=10)
Methanol	9.45	4.43
Acetaldehyde	3.76	9.58
Ethanol	5605.18	3.50
Propionaldehyde	1.70	8.52
Acetone	2.09	3.75
Ethyl formate	1.66	3.02
Propanol	0.82	5.64
Isobutyraldehyde	6.38	3.16
Ethyl acetate	33.41	1.83
Isobutyl alcohol	3.79	1.75
n-Butyl alcohol	0.69	10.75
Isovaleraldehyde	8.17	2.88
2,3-Pentanedione	0.76	8.25
Isoamyl alcohol	2.36	9.38

* Coefficients of Variation

TABLE 3
Concentrations and Odor Units of Main Headspace Gas Constituents of Soy Sauce

Compounds	Concentrations (ppm)	Thresholds (ppm) (in water)	Odor units	Relative Odor units (%)
Ethanol	5605.18	0.183	30629.40	31.39
Ethyl acetate	33.41	0.6	55.68	0.06
Isovaleraldehyde	8.17	0.00015	54466.67	55.81
Isobutyraldehyde	6.38	0.0009	7088.89	7.26
Acetaldehyde	3.76	0.015	250.67	0.26
Propionaldehyde	1.70	0.0095	178.95	0.18
Dimethyl sulfide	1.5614	0.00033	4731.52	4.85
Dimethyl disulfide	0.0299	0.00015	186.88	0.19

TABLE 4
Typical Analysis Result of Flavor Constituents in Japanese Soy Sauce

Flavor components	Concentrations(ppm)
Ethanol	31,501.10
Lactic acid	14,346.57
Glycerol	10,208.95
Acetic acid	2,107.74
4-Hydroxy-5-methyl-3(2H)-furanone (HMMF)	256.36
2,3-Butanediol	238.59
Isovaleraldehyde	233.10
4-Hydroxy-2(or5)-ethyl-5(or2)-methyl-3(2H)-furanone (HEMF)	232.04
Methanol	62.37
Acetol	24.60
Ethyl lactate	24.29
2,6-Dimethoxyphenol	16.21
Ethyl acetate	15.13
Isobutyraldehyde	14.64
Methyl acetate	13.84
Isobutyl alcohol	11.95
Furfuryl alcohol	11.93
Isoamyl alcohol	10.01
Acetoin	9.78
n-Butyl alcohol	8.69
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF)	4.83
Acetaldehyde	4.63
2-Phenylethanol	4.28
n-Propyl alcohol	3.96
Acetone	3.88
Methionol	3.65
2-Acetylpyrrole	2.86
4-Ethylguaiacol	2.77
Ethyl formate	2.63
4-Butanolide	2.02
Methional	1.42
4-Ethylphenol	0.34
Dimethyl sulfide	0.04

acetate. The second includes 3-hydroxy-2-butanone (acetoin), acetaldehyde and acetone. The last includes furfuryl alcohol, isobutyraldehyde, isovaleraldehyde and HMMF. Fig. 4,5 and 6 show the change of the content of the representative in the respective group. HEMF of the first group, 3-hydroxy-2-butanone of the second one and furfuryl alcohol of the third were checked by the extraction method with methyl acetate.

After aging, the kinds and the amount of lower-boiling compounds in moromi mash are almost the same as those in final soy sauce except the components which increase during pasteurization or heating. The higher-boiling compounds, namely the ethyl esters of fatty acids remain in soy oil and soy cake after pressing which is a kind of filteration with cloth.

In a fermentation process of moromi mash, lower-molecular organic acids such as n-butyric acid and isovaleric acid cause a problem of off-flavor if the contents are high by the growth of film-forming yeasts.

5.2 During Heating as Pasteurization

Unrefined soy sauce is recently heated at more than 115 °C for several seconds for pasteurization after the soy sauce is standardized. Traditional pasteurization was performed at 60°C to 85°C, followed by cooling and holding. However, in some cases, higher temperatures, usually more than 115°C, are needed so that heat-tolerant bacterial spores are destroyed and removed. The bacterial spores are known to be derived from the contamination by *Bacillus* during koji culturing. (30) The main purposes of the heating are as follows: killing microbial

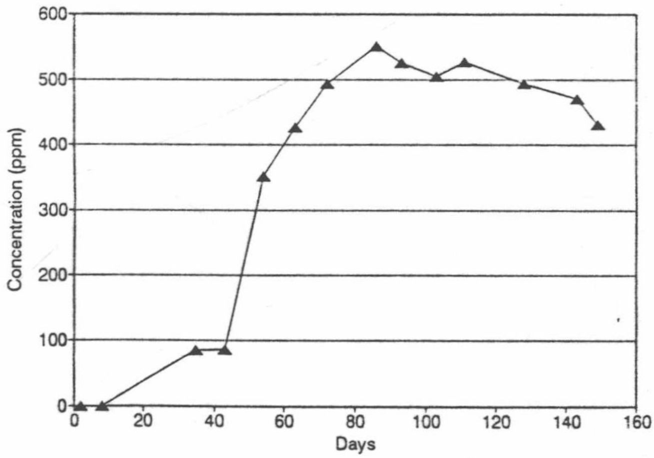


Fig. 4. Change of Content of HEMF during Moromi Fermentation.

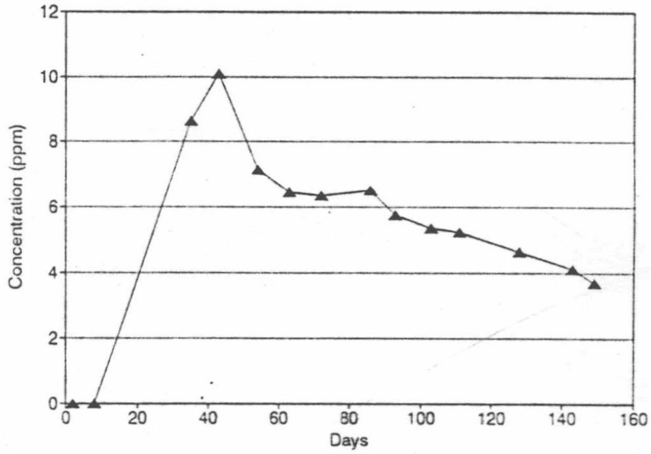


Fig. 5. Change of Content of 3-Hydroxy-2-butanone (Acetoin) during Moromi Fermentation.

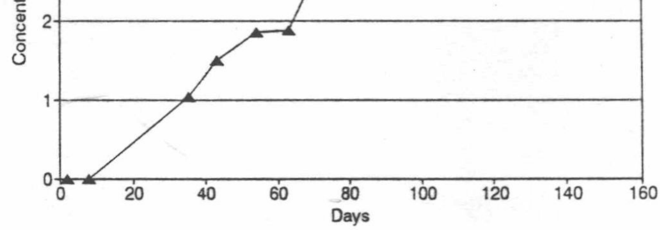


Fig. 6. Change of Content of Furfuryl alcohol during Moromi Fermentation.

cells such as molds, lactobacilli, yeasts and heat-resistant bacterial spores, denaturing microbial enzymes including phosphatases, removing heat-coagulable substances such as the native proteins of enzymes derived from koji molds, which are usually precipitated as sediment to the bottom of a holding tank, increasing the color intensity of soy sauce, and developing heated aroma which is called "fire aroma" in Japanese.

The heating causes the "browning reactions" including amino-carbonyl reaction and the Strecker degradation. The reactions affects not only the color increase of soy sauce but also the increase or decrease of the flavor. It was reported that the total contents of aldehydes (31), α-diketone compounds such as diacetyl, acetylpropionyl and acetylbutyryl (32), and free phenolic compounds (33) were increased during the heating of pasteurization. Nunomura et al. (34) found that the total quantity of volatile basic compounds in heated soy sauce was 1.5 times as large as that in unheated soy sauce, and reported the increase of concentrations and odor units of four major pyrazines during heating shown in Table 5 and 6. It is the result of thermal reactions regarding amino acids, proteins and sugars through the Maillard reaction and the Strecker degradation, although the latter is not always necessary. (38) An increase in the content of sulfur-containing compounds was also reported by Nunomura and Sasaki. (27) A part is indicated in Table 7.

Nunomura et al. (39) further studied the effects of heating on individual flavor components in detail in a scale of laboratories. The flavor components tested increase in the contents linearly with heating time and exponentially with centigrade temperatures. The relations of 2-methylpropanal (isobutyraldehyde) and 3-methylbutanal (isovaleraldehyde) to time and temperatures are illustrated in Fig. 7 and 8. Also, according to Nunomura et al., (39) furfuryl alcohol, propanal, 2-methylpropanal, 3-methylbutanal, dimethyl sulfide, ethylene sulfide, dimethyl disulfide, 3-(methylthio)propanal (methional), HMMF, HDMF, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, ethyl 2-hydroxypropionate (ethyl lactate), 2-phenylethyl acetate, 3-hydroxy-2-butanone (acetoin) and methyl 2-pyrrolyl ketone increase in the contents during heating, and on the other hand, methanol, 1-propanol, 2-phenylethanol and HEMF decrease in the contents. As described above, the degradation of amino acids, sugars, HEMF and other compounds is considered to result in the change.

6. ORGANOLEPTIC EVALUATION AND STATISTICAL ANALYSIS

Organoleptically preferable soy sauce generally has a good balance of taste, flavor and aroma. Sasaki (40) tested three popular brands of Japanese soy sauce on the market. The soy sauce tested was separated to the volatile flavor concentrate and the residue by distilling soy sauce under vacuum with cold traps of ice and sodium chloride, and dry ice, and then nine mixtures of combinations of three flavor concentrates and three residues were prepared to be subjected to a sensory evaluation by a ranking Hedonic method. According to this test, the preferred mixture always contained the flavor concentrate, not the residue, of the soy sauce which was selected as the best or the second in the evaluation among the original soy sauces

TABLE 5
The Increase of Concentrations of Major Pyrazines during Pasteurization

Compounds	Concentrations (mg/l)		Ratio of Concentrations
	Raw[a]	Heated	Heated/Raw
2-Methylpyrazine	0.024	0.075	3.1 times
Dimethylpyrazine	0.184	0.746	4.1 times
Ethyl-methylpyrazine	0.388	0.746	1.9 times
Trimethylpyrazine	0.040	0.050	1.25 times

[a] Raw means unheated.

TABLE 6
The Increase of Odor Units of Major Pyrazines during Pasteurization

Compounds	Odor Threshold[a] (ppm) (in water)	Odor Units[c]		Ratio of Odor Units
		Raw	Heated	Heated/Raw
2-Methylpyrazine	60 [35) b]	0.0004	0.0012	3.0 times
Dimethylpyrazine	1.0[36)]	0.184	0.746	4.1 times
Ethyl-methylpyrazine	0.1[35)]	3.88	7.46	1.9 times
Trimethylpyrazine	9 [37)]	0.0045	0.0056	1.25 times

[a] Published Data. The data of 2,5-dimethylpyrazine and 2-ethyl-5-methylpyrazine represented those of dimethyl- and ethyl-methylpyrazines respectively.
[b] 35) 36) 37) denote ref.Nos.
[c] Odor units were calculated on the basis of the concentrations in Table 5.

TABLE 7
The Change of Concentrations of Sulfur-containing Compounds during Pastuerization

Methods and Compounds	Concentrations (ppm)		Ratio of Concentrations
	Raw	Heated	Heated/Raw
Headspace Gas			
Dimethyl sulfide	0.3193	2.2581	7.07 times
Ethylene sulfide	0.0519	0.0647	1.25 times
Dimethyl disulfide	0.0237	0.0402	1.70 times
Extraction Method			
Dimethyl sulfide	0.0120	0.0385	3.21 times
Methional	1.3665	1.3489	0.99 times
Methionol	4.3053	4.4216	1.03 times

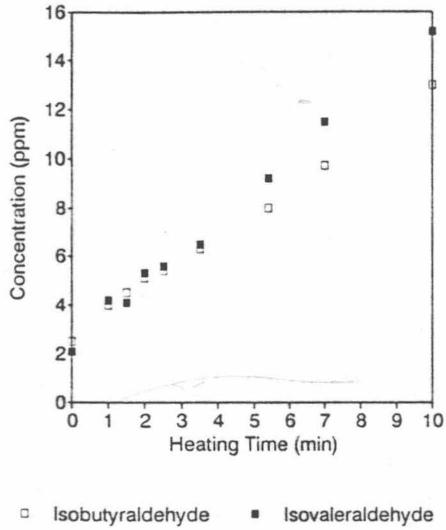


Fig. 7. The effects of Heating Time on Contents of 2-Methylpropanal (Isobutyraldehyde) and 3-Methylbutanal (Isovaleraldehyde) at 110°C.

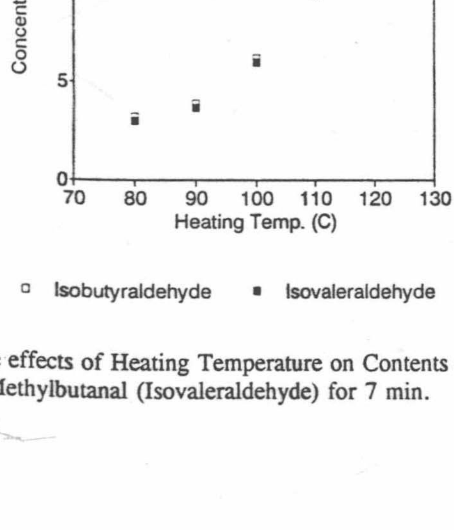


Fig. 8. The effects of Heating Temperature on Contents of 2-Methylpropanal (Isobutyraldehyde) and 3-Methylbutanal (Isovaleraldehyde) for 7 min.

before separation. The volatile flavor concentrates were found to be more important for organoleptic evaluations of soy sauce than the residues. This result means that finding relationship between a quantitative analysis results of volatile flavor concentrates and an organoleptic evaluation is to investigate a better quality of soy sauce. An accurate quantitative analysis is essential to get a reliable relation between the analysis result and the evaluation. For that, special attentions have been paid to the determination of flavor components in soy sauce as described above, and statistical approaches have been conducted to find the relation, using computer techniques.

Mori et al. (7-12) have studied the relationship between the flavor components of soy sauce and sensory evaluations with careful attention and in detail. The quantitative methods described above have been used for the determination of flavor compounds. An interval scale or a ratio scale has been applied as sensory measurement through the scaling procedure so that the scale could be used for a statistical analysis. In the statistical analysis, a linear regression analysis, a nonlinear regression analysis and a multiple regression analysis were employed for a calculation of a prediction model between a sensory evaluation and gas chromatographic data. A simplex design method was applied to an estimate of optimum concentrations of flavor components, and then principal component analysis was used for a reciprocal analysis among characteristics of flavors. They first investigated five different soy sauces in detail. The soy sauces were obtained from different types of tanks of moromi mash before blending on the way of production, and they were heated separatedly after standardization. An accurate quantitative analysis was performed for the headspace gas and the flavor concentrates, and the five soy sauces were subjected to organoleptic evaluations. In the sensory test, odor preference of the test samples was evaluated. The rank order test was used for measuring differences among the samples. Thurstone's Case-V scaling procedures was applied to the test data, and the scale values quantified by the Case-V were used for the statistical analysis. Eight functions (linear, linear variant, logarithmic, hyperbolic, power, exponential, square root and quadratic functions) were applied to combinations of data sets including the sum of concentrations of two components. A multiple regression analysis was used to distinguish the components with a statistically significant contribution, and Allen's PSS (prediction sum of squares) and cross validation procedure were applied to every combination of predictor variables to estimate the best multiple regression equation. As a result of this statistical analysis, three combinations of binary flavor components accounting for the variation of sensory data were found. The combination of 4-ethylguaiacol and methionol was closely associated with the blending effect (41), and this combination accounted for about 28 % of the variation of sensory data.(7) Next, an actual addition test of 4-ethylguaiacol and methionol into soy sauce was carried out in several combinations of both concentrations. The addition was done before heating, and, after pasteurization, a sensory evaluation and a quantitative analysis of flavor compounds were performed, followed by a statistical analysis including multiple regression analysis. The simplex design method was applied to the odor preference surface which was generated from the multiple regression equation, and the optimum concentrations of 4-ethylguaiacol and methionol were found to be 0.8 ppm and 3.8 ppm respectively.(8)

Moreover, Mori et al.(9) applied their techniques to 30 brands of soy sauces on Japanese market. As a result of a statistical analysis including principal component analysis and regression analysis, three stepwise prediction models were made up to explain the organoleptic evaluation for soy sauces. The first one was a condition of the odor component with a negative effect: the concentration of n-butyric acid < 3 ppm. The threshold of n-butyric acid was 2.7 ppm. The second one was a condition of the flavor component with a positive effect: the concentration of HEMF ≥ 50 ppm. HEMF was proved to be an essential component for Japanese soy sauce. The threshold of HEMF is known to be less than 0.04 ppb (42, 43). Both compounds seemed to affect an overall flavor of soy sauce directly. According to this result, n-butyric acid can be called an off-flavor component if its content is more than 3 ppm. The third one was a multiple regression model with explanatory variables of concentrations of ehtyl acetate, 4-ethylguaiacol, methionol, ethyl lactate and acetoin+isobutyric acid. Its contribution was 65 % (= R^2 x 100). This result suggests that it is almost impossible to estimate the odor preference of soy sauce by using a single multiple regression model.

7. CHANGES OF FLAVOR COMPONENTS DURING STORAGE

7.1 Evaporation of Aroma Components

It is known well that flavor deterioration occurrs quickly once a bottled soy sauce is opened. Sasaki and Nunomura (26) verified the loss of volatile compounds quantitatively by using the headspace gas method described above. 10 ml of soy sauce were put into small dishes at 22°C, and then the soy sauce was subjected to the gas chromatographic analysis after every 15 min, 30 min, 70 min and 150 min. The result is shown in Table 8, and illustrated in Fig. 9, where a percentage of the loss is calculated on the basis of a concentration at the starting point. Every volatile component in the headspace gas of soy sauce evaporates drastically. This result explains well the fact that the top note aroma disappears easily or decreases rapidly when soy sauce is kept opened. Also, it suggests that the remaining flavor compounds such as HEMF play an important part to keep good quality after opening the bottle.

7.2 Effect of Headspace during Storage

The color of soy sauce is very sensitive to oxidation. For the most part, the formation of color during production processes is the result of nonenzymatic, nonoxidative and heat-dependent chemical reactions. On the other hand, the color increase of soy sauce after bottling or after opening the bottle results from nonenzymatic and oxidative chemical reactions. The oxidative browning reactions generally occur between amino compounds and sugars.

The deterioration of aroma or flavor of soy sauce is also considered to happen during storage. According to Sasaki and Nunomura (44), there are three types of changes during storage to flavor compounds in cases when soy sauce has headspace in a container. Some flavor compounds decrease in concentrations, some increase, and some have no change. Two