G. Charalambous (Ed.) SHELF LIFE STUDIES OF FOODS AND BEVERAGES
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The shelf life of soy sauce

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INTRODUCTION

In the world, soy sauce is produced by many manufacturers, and in many cases, soy sauce is used as a seasoning for foods. Soy sauce contains amino acids which give a significant taste to foods. It has also lots of flavor compounds which give accents to foods. These components add tasty and flavorful tone to foods, and enhance the flavors of foods. However, they can be the cause of deterioration after opening of a container or during storage under bad conditions.

Its raw materials are usually water, soybeans, wheat and salt. In European countries, the U.S.A. and Canada, the sauce has been made mainly through chemical processes, and the chemical soy sauce has been mainly marketed. The chemical soy sauce is a liquid amino acid produced by the processed decomposition of protein with strong acids such as hydrochloric acid. On the contrary, in Asian countries, fermented soy sauce is generally produced. 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. In the U.S.A., Japanese-type soy sauce has been produced since 1972. Its production has grown to get almost a half of market share in the U.S.A.

The origin of Japanese-type soy sauce is considered to be China where only soybeans are used as a raw material. In the sixth century a vegetarian seasoning came with Buddhism from China to Japan. After that soy sauce underwent considerable development in Japan. One of the characteristics was to employ 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.

Some studies on deterioration of quality, especially flavors and a color, have been reported in Japanese-type soy sauce. In this chapter, Japanese-type soy sauce will be described.

PRODUCTION PROCEDURE OF JAPANESE-TYPE SOY SAUCE

The flow scheme of the production is shown in Figure 1. Soybeans, wheat and salt are used as 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 halophillus 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 soy sauce in quality, heat treatment of raw materials, koji culturing, moromi fermentation and pasteurization by heating are very important. The final product has plentiful flavor and aroma and a dark reddish-brown color in comparison with other-types of soy sauce. This quality is one of the characteristics of Japanese-type brewed soy sauce. It is a result from utilization of wheat as one of raw materials and fermentation by yeast.

The finished product is further checked for quality control. A microbiological check, a chemical analysis and a sensory evaluation are usually performed after bottling and before shipping.

QUALITY OF SOY SAUCE

In Japan, the Japan Agricultural Standards (JAS) officially recognizes five main types of soy sauce by a production procedure, quality and others. Typical soy sauce of Japan is "koikuchi" which means dark in color. "Koikuchi"-type soy sauce occupies 83 % of a total production in Japan in 1990. The others are "usukuchi", "tamari", "shiro" and "saishikomi" in Japanese. Fermented soy sauce marketed in the U.S.A. is usually "koikuchi". Recently "tamari" also can be seen in the stores of the U.S.A., but it is not so often. "Tamari" is both thick and dark, and characterized by a high content in amino acids. It is made from soybeans and a small amount of wheat in a ratio of 10 : 1 or 10 : 2. It represents the Chinese-type and originates in China. Tables 1, 2, 3 and 4 show typical chemical analysis results for "koikuchi" and "tamari" types. The concentrations of aroma or flavor components in "koikuchi"-type are presented in Table 5.

In quality of soy sauce, not only a good balance of the chemical composition but also the flavors and color to bring about appetite are particularly important, to say nothing of microbiological safety. Fresh "koikuchi"-type soy sauce has a dark reddish-brown color without any off-flavors. The flavors and color are derived from the fermentation and pasteurization. However, they are delicate and changeable. In fact, they are very sensitive to exposure of the air, and remarkable changes by the exposure have been found to occur in the flavors and

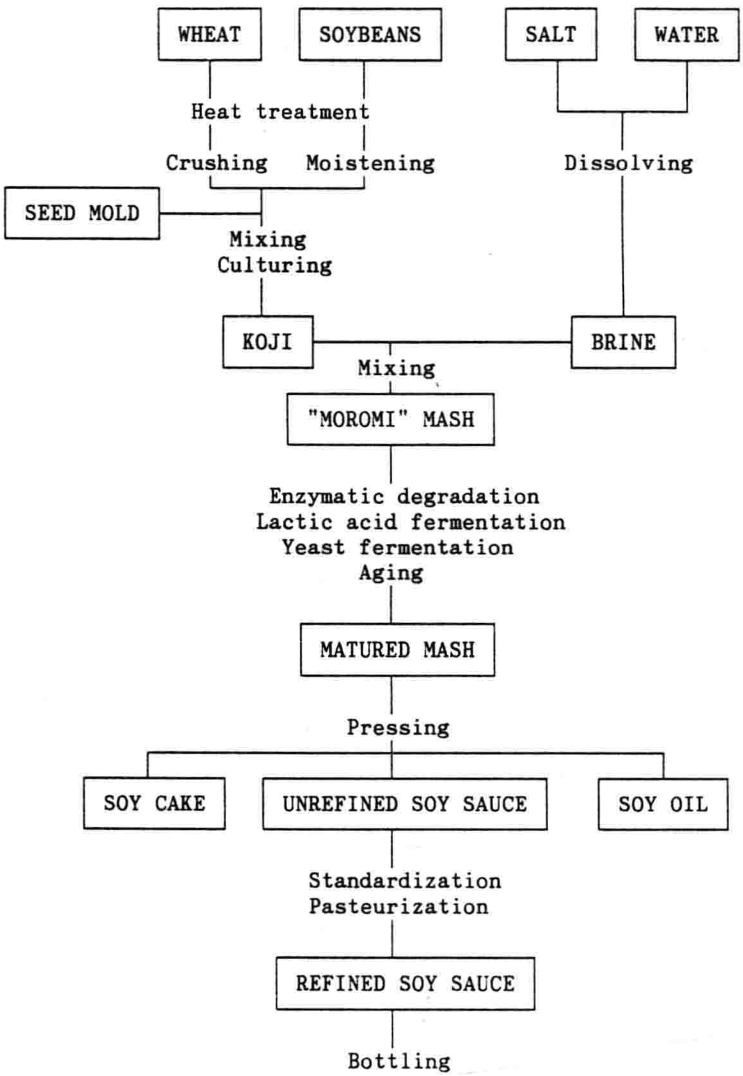

Figure 1. Production procedure of Japanese "koikuchi" soy sauce

Table 1
General analysis result of typical Japanese "koikuchi" and "tamari" soy sauce

		"Koikuchi"	"Tamari"
pH		4.79	4.90
Baume		21.30	26.08
NaCl	%(w/v)	16.20	17.60
Total nitrogen	%(w/v)	1.60	2.62
Ethanol	%(v/v)	2.71	3.41
Reducing sugars	%(w/v)	2.42	NA[a]
Ammonium nitrogen	%(w/v)	0.17	0.28

[a] : not available.

Table 2
Organic acid content of typical Japanese "koikuchi" and "tamari" soy sauce (mg/ml)

	"Koikuchi"	"Tamari"
Pyroglutamic acid	3.67	4.79
Lactic acid	7.59	9.01
Acetic acid	1.81	2.47
Formic acid	0.09	0.14
Citric acid	ND[a]	ND[a]
Succinic acid	0.26	0.30

[a] : not detected.

Table 3
Sugars of typical Japanese "koikuchi" and "tamari" soy sauce (mg/ml)

	"Koikuchi"	"Tamari"
Sucrose	ND[a]	ND[a]
Ribose	Trace	ND[a]
Mannose	0.23	1.02
Fructose	ND[a]	Trace
Arabinose	0.83	0.39
Galactose	2.26	3.80
Xylose	0.51	0.23
Glucose	9.42	12.55

[a] : not detected.

Table 4
Amino acid content of typical Japanese "koikuchi" and "tamari" soy sauce (mg/ml)

	"Koikuchi"	"Tamari"
Glycine	2.50	3.76
Alanine	7.05	7.71
Valine	4.82	6.73
Leucine	6.57	6.08
Isoleucine	4.24	4.80
Serine	4.40	6.71
Threonine	3.12	4.91
Cystine	ND[a]	0.28
Methionine	1.17	1.17
Phenylalanine	4.43	5.92
Tyrosine	1.23	2.28
Tryptophan	ND[a]	ND[a]
Aspartic acid	2.58	9.15
Glutamic acid	11.88	18.18
Ornithine	0.49	1.09
Lysine	4.09	6.44
Histidine	1.67	1.80
Arginine	4.34	5.41
Proline	4.07	6.08

[a] : not detected.

Table 5
Flavor constituents in typical Japanese "koikuchi" 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(or 5)-ethyl-5(or 2)-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
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

color.

FLAVOR COMPONENTS IN SOY SAUCE

Almost 300 flavor components have been found in Japanese "koikuchi" soy sauce. The details are described in the references written by N. Nunomura and M. Sasaki [1 and 2]. Among the compounds, phenolic compounds and caramel-like aroma compounds such as furanones and pyrones largely contribute to Japanese soy sauce flavor. One of the furanones, or 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 N. Nunomura et al. [3 and 4] HEMF has been proved to be formed by both Zygosaccharomyces rouxii and Candida (Torulopsis) yeasts [5]. M. 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. The phenolic compounds were investigated by T. Yokotsuka et al. [7 and 8] They indicated that 4-ethylguaiacol (4-ethyl-2-methoxyphenol) had a very important effect on the quality of Japanese soy sauce. Y. Asao and T. Yokotsuka [9] and M. Sasaki et al. [5] found that 4-ethylguaiacol and 4-ethylphenol were produced by Candida (Torulopsis) yeasts, not by Zygosaccharomyces rouxii. Mori et al. [10] 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.

Most of the flavor compounds in soy sauce are produced during yeast fermentation in moromi mash. N. Nunomura and M. Sasaki [11] kept track of each flavor components from the beginning of fermentation or the charging of moromi mash, and they found three types of compounds in a change of the concentration during the fermentation ; 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 a maximum at the yeast addition and then decrease, 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 acetate. The second includes 3-hydroxy-2-butanone (acetoin), acetaldehyde and acetone. The last includes furfuryl alcohol, isobutyraldehyde, isovaleraldehyde and 2-hydroxy-5-methyl-3(2H)-furanone (HMMF). 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, mainly the ethyl esters of fatty acids remain in soy oil and soy cake after pressing which is a kind of filtration with nylon cloth.

In most cases, 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 [12]. The main purposes of the heating are as follows : killing microbial 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 the amino-carbonyl reaction and the Strecker degradation. The reactions affect 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 [13], α-diketone compounds such as diacetyl, acetylpropionyl and acetylbutyryl [14], and free phenolic compounds [15] were increased during the heating of pasteurization. N. Nunomura et al. [16] 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 the odor units of four major pyrazines, namely 2-methylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine and trimethylpyrazine during heating. 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 [17]. An increase in the content of sulfur-containing compounds during heating was also reported by N. Nunomura and M. Sasaki [18]. According to N. Nunomura et al. [19] 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 cause the change.

Oxidative products of HEMF were studied by N. Nunomura et al. [1 and 20] HEMF is 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). Autoxidation 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.

COLOR OF SOY SAUCE

Fresh "koikuchi" type soy sauce has a dark reddish-brown color, and its appearance is clear liquid free from sediment and foreign material. It is known that this color changes easily to a darker and more blackish color by oxidation.

The flavors and color of soy sauce are very closely related, as both are affected by the aging in moromi mash and the pasteurization of raw soy sauce. During the brewing process, the development of soy sauce color is mainly derived from nonoxidative and nonenzymatic browning reactions. Enzymatic reactions between amino compounds and sugars are rare. After bottling of soy sauce, the color is relatively stable, but after opening, it darkens rather quickly due to the oxidative and nonenzymatic browning reactions. These reactions cause the organoleptic quality of soy sauce to be inferior.

In the soy sauce industry of Japan, the color standards are used. They are prepared from known chemical pigments so as to match the different colors of soy sauce. The standards consist of 56 degrees of color with the same visual distance. The standards are numbered from 1 to 56, and a smaller number means a darker color. The color standards with even numbers, namely 28 color standards, are actually used. The liquid with the color is packed in a vial and sealed. One vial has one color standard. The color degree of soy sauce is visually determined by comparison with the color standards in vials. This method using the color standards to assess the color of one soy sauce is very simple and convenient when the soy sauce has a single or consistent color tone. However, soy sauce possessing different color tones produced by oxidation during storage is difficult to be analyzed by this method.

An absorbance curve of color wave in a visible range has no peak, and the absorbance goes down from short wavelength to long wavelength as shown in Figure 5 which appears later. H. Motai et al. [21] and H. Motai [22] proposed an idea to explain the color of soy sauce. It is "delta-A". There is a linear relationship between wavelength and logarithm of absorbance (log E). Delta-A is figured out by the difference of log E in 100 nm of wavelength. For example, the difference, delta-A is calculated as follows,

$$\text{delta-A} = \log(E_{450}) - \log(E_{550})$$

A change of delta-A can explain a blacked and darken degree of soy sauce. With an increase of delta-A, a ratio of darker color to lighter color decreases relatively, and the soy sauce exhibits totally lighter and more reddish color. On the other hand, with a decrease of delta-A, a ratio of darker color to lighter color increases, and the soy sauce features darker and more blackish color in all.

M. Nakano [23] has tried to explain a color difference between two soy sauces using the color specification by CIE1976 (L^*, a^*, b^*) space expression. L^* means lightness of the Munsell colors, and a^* and b^* means red-green and yellow-blue degree of hue and chroma of the Munsell colors respectively. A distance of change in the color space in one soy sauce, delta-E^*, is calculated using L^*, a^* and b^*, and a distance in the space between two soy sauces, delta-e^*, is also figured out as follows,

$$\text{delta-}E^* = \{(\text{delta-}L^*)^2 + (\text{delta-}a^*)^2 + (\text{delta-}b^*)^2\}^{1/2}$$
$$\text{delta-}e^* = \{(L_1{}^* - L_2{}^*)^2 + (a_1{}^* - a_2{}^*)^2 + (b_1{}^* - b_2{}^*)^2\}^{1/2}$$

Delta-E^* can be employed to find stability of soy sauce by oxidation or heating, and delta-e^* is utilized to compare the color of two soy sauces. Table 6 shows relationship between delta-e^* and the human visual expression according to the National Bureau of Standard of the U. S. A. The color change or difference of two soy sauces can be represented with figures by calculating delta-E^* or delta-e^*.

Table 6
Visual expression and delta e^*

Expression	delta e^*
trace	0 - 0.5
slight	0.5 - 1.5
noticeable	1.5 - 3.0
appreciable	3.0 - 6.0
much	6.0 -12.0

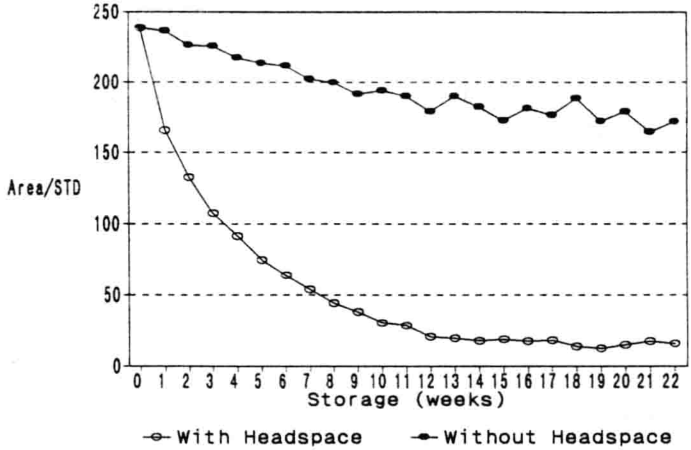

Figure 2. **The effect of headspace on change of HEMF in soy sauce during storage at 30°C.**

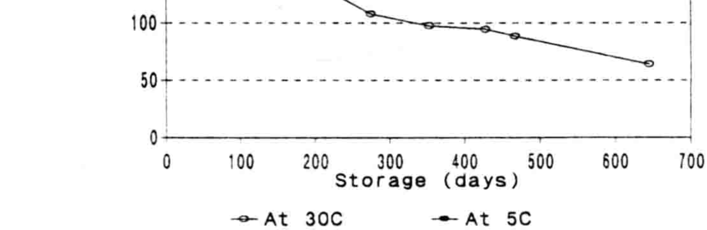

Figure 3. The effect of temperature on content of HEMF in soy sauce during storage without headspace.

CHANGE OF FLAVOR COMPONENTS DURING STORAGE

With headspace in a container, the deterioration of aroma or flavor happens during storage [2]. There are three types of changes during storage. Some flavor compounds decrease in concentrations, some increase, and some have no change. For instance, the contents of 3-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF) and 2-hydroxy-5-methyl-3(2H)-furanone (HMMF) decreased drastically, and those of isobutyric acid and isovaleric acid increased. These organic acids generally give undesirable odor for soy sauce. However, without headspace, the change was slow. The content of isoamyl alcohol had no change, and it was not affected by the existence of headspace. The existence of headspace in a soy sauce container causes the deterioration of the flavor because aroma compounds such as HEMF and HMMF are reduced in concentrations and off-flavors such as isobutyric acid and isovaleric acid increase. For example, Figure 2 shows the effect of headspace on the change of HEMF in soy sauce during storage at 30°C.

Such deterioration of the flavor compounds is affected by temperature. The higher the temperature, the larger the change except isoamyl alcohol and isovaleraldehyde [2]. Soy sauce was put without headspace in a 80 ml glass bottle capped tightly, and stored for 645 days at four different temperatures : 5°C, 20°C, 30°C and a natural temperature which was close to an outside temperature. At an elevated temperature and without headspace, HEMF and acetoin decreased in concentrations, and ethyl lactate increased. In case of isovaleraldehyde, the content at 30°C decreased more until 100 days than that at 5°C, but the content at 5°C still continued to go down constantly after 100 days. The formation of the aldehyde was considered to happen at the higher temperature after 100 days. On the other hand, isoamyl alcohol did not have a big change at both temperatures of 5°C and 30°C. Figure 3 indicates the effect of temperature on the content of HEMF in soy sauce during storage without headspace. The decrease of HEMF is the result from its degradation, and the decrease of isovaleraldehyde at the initial stage and the decrease of acetoin seem to result from the Aldol condensation. It is inferred that the formation of aldehydes such as isovaleraldehyde during storage results from degradation of amino acids, HEMF, HMMF and others. The flavor compounds such as HEMF with a positive effect to good quality decrease in concentrations during storage at a higher temperature even though there is no headspace.

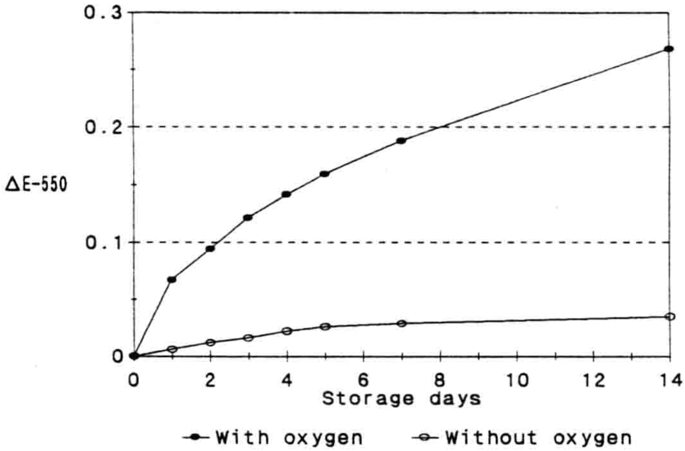

Figure 4. The effect of oxygen on change of the absorbance in soy sauce.

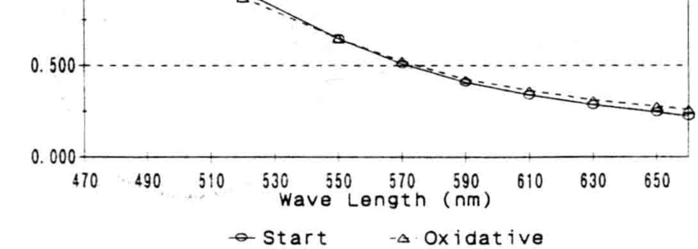

Figure 5. The change of the absorbance curve or color tone of soy sauce by oxidation.

COLOR CHANGE BY OXIDATION

Another noticeable change of Japanese "koikuchi"-type soy sauce during storage is its color.

The color of soy sauce is very sensitive to oxidation. Figure 4 shows the effect of oxygen on the browning [24 and 25]. Soy sauce was stored at 37°C, and the color change was checked. At the time when it was checked, soy sauce was diluted 20 times with water. When soy sauce is exposed to the air, the absorbance increases more than that without oxygen. Another change in the color of soy sauce occurs with oxygen. Figure 5 shows the change of the color tone. The absorbance curves of the fresh soy sauce and the soy sauce browned with oxygen are drawn. The drawing is adjusted so that both absorbances at 550 nm indicate the same value. Around shorter wavelength, the absorbance of fresh soy sauce is greater than that of the browned one, but in longer range, the absorbance of the browned one is greater. In human visual expression, the oxidative soy sauce or browned soy sauce has less tone in red zone and yellow zone and more tone in blue zone. Figures 4 and 5 indicate hyperchromic and bathochromic effects by oxidation respectively.

Another phenomenon is observed when a logarithm of the absorbance is calculated. The difference of the logarithms of absorbance between the difference of 100 nm of wavelength is consistent because there is a linear relationship between wavelength and logarithm of absorbance (log E) as described above. In other words, the difference in any log E between a difference of 100 nm of wavelength has always the same value for the color of one soy sauce. In nonenzymatic and oxidative browning during storage of soy sauce, delta-A decreases and the color tone becomes darker and more blackish as described above.

Thus, the deterioration of soy sauce is always accompanied with the change of flavors and color. The delta-A may be used as a parameter for the quality change of soy sauce because it is checked very easily.

SHELF LIFE TEST

In Japan a shelf life test was carried out to determine a period of the guarantee of soy sauce quality by the Japan Soy Sauce Research Institute [26 and 27]. Plastic containers are recently very popular for soy sauce in Japan. The material is usually made from polyethylene terephthalate. It is very thin, clear and transparent. Gas such as oxygen passes through the plastic barrier more easily than a glass bottle. Therefore, a shelf life was expected to be different between a glass bottle and a plastic container. The shelf life test was done using

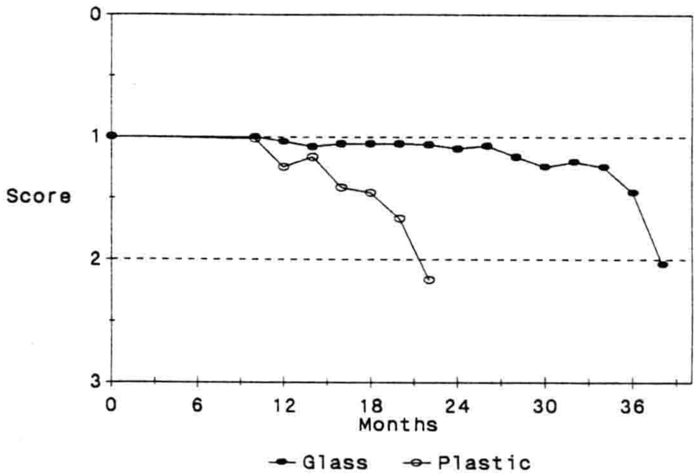

Figure 6. The change of the sensory score for soy sauce in a glass bottle and a plastic container stored at 20°C : score 1 means "good", and score 3 means "bad".

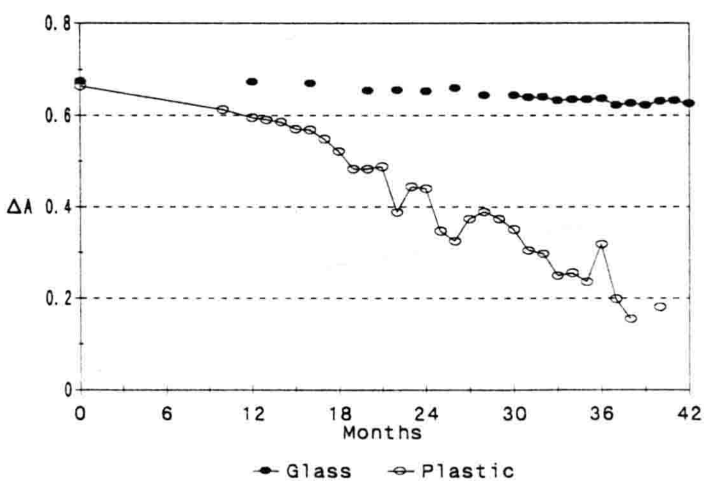

Figure 7. The change of delta-A for soy sauce in a glass bottle and a plastic container stored at 20°C.

both containers, namely 2 or 1.8 liter- glass bottles and 1 liter-plastic containers. In the test soy sauce was stored at 20°C in a glass bottle and a plastic container for over three years, and a sensory evaluation and color checks such as absorbance and delta-A were basically performed every other month.

Figures 6 and 7 show changes of the scores of sensory evaluation and delta-A respectively for "koikuchi"-type soy sauce. In a sensory evaluation, twelve panelists checked samples and the team for the evaluation consisted of representatives of several major manufacturers and the Japan Soy Sauce Research Institute. They checked the quality of samples such as color, odor, flavor and taste, and marked a score for one soy sauce stored. The score 1 was given to a "good" sample, 2 to a "questionable" one and 3 to a "bad" one. "Good" means the same quality as that of the starting sample in the storage test. An averaged score of the panelists was calculated for each sample.

Delta-A, a sum of absorbances at three wavelengths (420, 530 and 660 nm), Y%, x, y of the color specification by CIE1931 and a color degree by comparison with the color standards were measured for each soy sauce taken out at a scheduled interval during the storage test. These data were used as references to judge the shelf life. The shelf life is determined on basis of the result from the sensory evaluation. According to H. Tanaka [27], delta-A and the organoleptic score had the best relationship among the items measured. For instance, the coefficient of correlation between delta-A and the sensory score in a plastic container was 0.971. The change of delta-A in a glass bottle and a plastic container are plotted in Figure 7.

In conclusion the shelf life of "koikuchi"-type soy sauce was determined as 36 months for a glass bottle and 18 months for a plastic container by the Japan Soy Sauce Research Institute. The sensory score at the time was 1.36 and 1.48 for a glass bottle and a plastic one respectively.

SHELF LIFE OF SOY SAUCE

Fermented soy sauce is very sensitive to oxidation as described above. Its flavors and color become inferior quickly. The existence of headspace in a container causes the decrease of good flavor compounds such as HEMF and HMMF and the increase of off-flavors such as isobutyric acid and isovaleric acid. The storage temperature also affects the flavors, and the storage at a higher temperature results in the decrease of HEMF even without headspace in a container. The color becomes darker and more blackish with oxygen as a result of oxidative and nonenzymatic reactions. In the shelf life test, the quality of soy sauce in a glass bottle was proved to be kept better than that in a plastic container which has a property to pass oxygen through the plastic layer. Thus,