Mem. Fac. Fish. Kagoshima Univ.,
Vol. 63, pp. 1~8 (2014)

A Proposal for Quality Assessment and Critical Control Point on Fresh Fishes Packed with Deoxidizer (MA storage)

脱酸素剤とともに包装された鮮魚（MA貯蔵）の品質評価と重点的監視項目の設定

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Keywords: K-value, Deoxidizer, Quality Assessment

Abstract

We investigated the effect of deoxidized atmosphere storage on *sashimi* qualities in order to propose the critical control point for the quality assessment of *sashimi*. The *sashimi* quality of bigeye-tuna and bonito with or without deoxidizer was evaluated by K-value, color deterioration, bacterial counts, VBN and polyamines. The quality of fishes packed with deoxidizer was guaranteed by monitoring the K-value, since the change of K-value is faster than the increase of discoloration, odor, polyamines, and microbial growth. In other words, K-value is utilized as the critical control point (CCP) for the quality assessment of *sashimi*. In addition, we could purchase the fresh *sashimi* labeled for the shelf life of date and time, since K-value was predictable provided the initial K-value and temperature history of the fish were given.

It is well known that fishes are easy to make the quality deteriorate until their consumption. The storage conditions have critical outcome in product quality. The quality as a "*sashimi*" (raw eating) grade has been evaluated by the muscle texture, K-value, color deterioration, and sometimes by bacterial counts, and then occurrence of volatile nitrogenous compounds or some amines. Among of all those evaluations, K-value is the most useful index of "*sashimi*" grade. High quality of "*sashimi*" is guaranteed to K-value of below around 20%.[1,2] On the other hand, the reddish muscle such as tuna and bonito is easily discolored with the accumulation of metmyoglobin at the early stage of storage.[3,4] The discolored muscle remarkably lowers the price of product, even if the fish has a sufficient condition as the "*sashimi*". Miki *et al.*[5] has reported that the discoloration of bonito muscle is faster than the decrease of K-value by the method of kinetic analyzing, and the index of muscle color is useful for the quality assessment of bonito "*sashimi*". The discoloration of reddish muscle is caused by the change of myoglobin into metmyoglobin, which is brought about by the exposure to oxygen. The vacuum packaging in distribution system prevents the muscle from autoxidation, and is generally intended for fillet and loin preservation. However, the application of vacuum packaging to raw fish leads to the disruptor of muscle cell or the generation of expressible drip.

Recently, many foods have been packed and distributed under the modified atmosphere. The deoxidized atmosphere (abbreviated to DA) condition enables the long shelf life of

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confectionery to control the aerobic microorganisms growth and lipid oxidation. In addition, Uchiyama *et al.*[6] have reported that the combined method of partially frozen storage and DA storage provides semi-dried fish and baked eel with the long shelf life. The bacterial growth, increase of volatile basic nitrogen and lipid oxidation were controlled when the fish products were stored under deoxidized atmosphere. Putting the deoxidizer (oxygen absorber) into a bag of air-tight-film easily makes the deoxidized atmosphere without expensive equipment.

Therefore, we investigated the effect of DA storage on *sashimi* qualities, and determined the critical control point for the quality assessment of *sashimi* which is preserved under deoxidized atmosphere. In addition, the investigation was carried out whether the priority-monitoring item of fish packed with deoxidizer was predicted by applying kinetics analysis.

Materials and Methods

Fish samples and storage test

Bigeye-tuna (*Thunnus obesus*) was obtained from Koyo-maru, training ship of National Fisheries University. Bonito was purchased from a fisheries cooperative of Yamaguchi Prefecture. The products of bonito loin had already been baked on the surface (*Katsuo Tataki*), vacuum-packed, and stored at −35℃. The samples were kept at -35℃ until use.

After the frozen samples were thawed, the blocks were cut into some slices of 1 cm thickness (about 20 g) under clean bench. The sliced muscle was put on the pasteurized aluminum cup and individually jammed into triplicate bag (films exclusively used for vacuum package) with or without deoxidizer (Ageless SS-400, Mitsubishi Gas Chem., Co. Ltd.). The storage conditions under deoxidized-atmosphere package and air-containing package were abbreviated as DAP and ACP, respectively. They were stored for given times at temperatures of 4, 10, 15, or 20℃. The concentration of remaining oxygen was confirmed by the color change of ageless-eye (Mitsubishi Gas Chem., Co. Ltd.). The pink color of ageless-eye indicated at least the below 0.1% of oxygen concentration (oxygen pressure $<$ 0.15 mmHg).

Chemical analysis (K-value, VBN, polyamines)

The K-value was measured by HPLC using ODS-II column (Shinshu Co. Ltd). ATP related compounds were eluted with acetonitoril and buffer (1: 100) containing 100 mM phosphate buffer and 50 mM triethylamine (pH 6.8), and the concentration of ATP related compounds (μ moles / g) was converted from the absorbance obtained at 260 nm by applying the manual described in Shimadzu HPCL application data book (June 30, 1996).

The Estimation of volatile basic nitrogen (VBN) was used by Conway Microdiffusion Cell[7] and the determination of 7 polyamines, tyramine, putrescine, cadaverine, histamine, agmatine, tryptamine and spermidine, was performed as described by Yamanaka[8].

Colorimetric analysis

Change in Hunter's color was determined by measuring the tristimulus values (L, a, and b) using a color difference meter (MC-200 Analyzer, Minolta Co. Ltd).

Bacterial counts

Microbial counts were measured on BPG agar medium (Peptone 0.5%, bonito extract 0.5%, glucose 0.1%, NaCl 2.5%, $MgSO_4 \cdot 7H_2O$ 0.25%, KCl 0.1%, pH 7.5), as described by Fujii.[9] These samples were incubated for 7 days at 20℃. Thirty colonies were randomly selected and purified. Each bacteria was stored on slant of the same media at 20 ℃. For the bacterial identification, morphology, Gram stain, catalase test, oxidation-fermentation of glucose (OF), and cytochrome oxidase test were performed.[10] The isolates were identified to the generic level according to the scheme of Cowan and Steel, also Bergey's manual.[11]

Prediction of quality changes from temperature histories

The rate of freshness-lowering in fish muscle was calculated by the equation as described in Miki *et al.*[5] In the formula, (100 - K-value) indicates the ratio of the remaining amount of ATP + ADP + AMP + IMP to the total amount of ATP related compounds.

The freshness-lowering of muscles after fluctuating temperatures was predicted by the equation from Charm[12] with some modification.[13] Samples of bigeye-tuna were used for prediction of K-values.

Results

Changes in K-value and color

When the muscles of bigeye-tuna and bonito were stored at different temperatures, the K-values of the deoxidized-

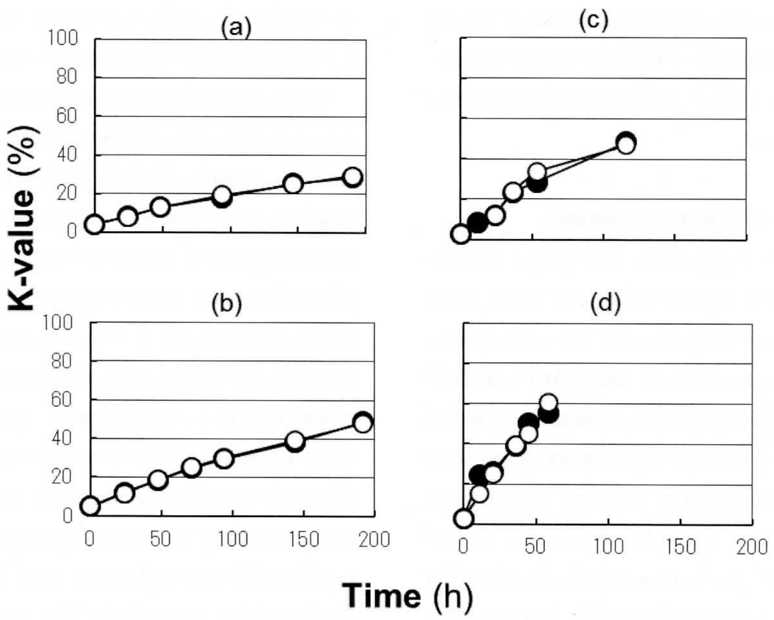

Fig. 1 Changes in K-values of big-eyed tuna (a, b) and bonito (c, d) during storage at 4℃ (a,c) and 10℃ (b, d) respectively.
●: sashimi stored under the air-containing package (ACP)
○: sashimi stored under the deoxidized-atmosphere package (DAP)

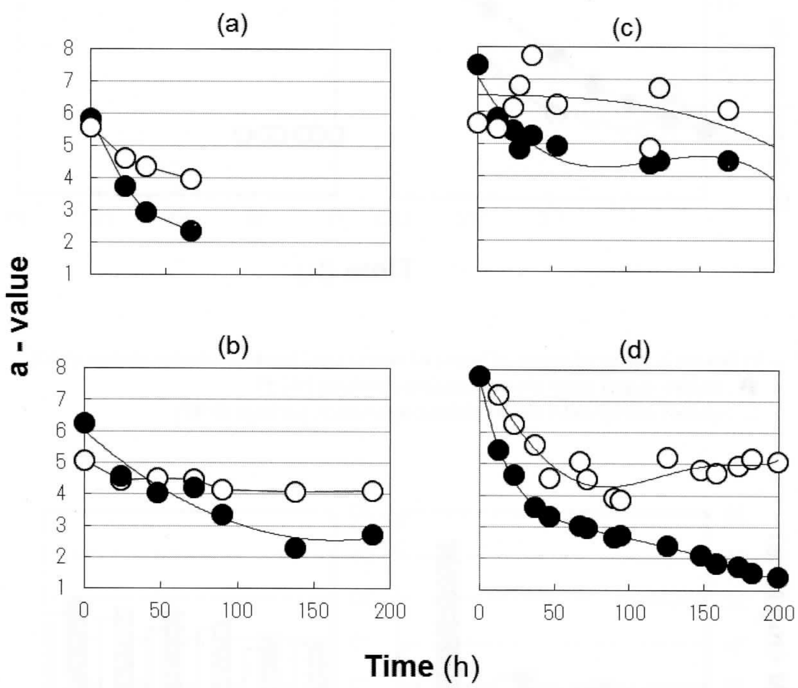

Fig. 2 Red color changes indicated in a-values of big-eyed tuna (a, b) and bonito (c, d) during storage at 4℃ (a,c) and 10℃ (b, d) respectively.
●: sashimi stored under the air-containing package (ACP)
○: sashimi stored under the deoxidized-atmosphere package (DAP)

atmosphere package (DAP) muscles quite correspond to those of the air-containing package (ACP) muscles (Fig. 1).

The changes in muscle color were analyzed as values of L, a, b mode by measuring the reflected color of sample surface, using Chroma Meters CR-200. Figure 2 indicated that the decrease of 'a'-value, a parameter for color stability of redness, was larger in ACP muscles than DAP muscles. The results meant that the preservation under DAP helped fish muscle to retain red color. In addition, the discoloration ratio of muscle was markedly decreased in bonito compared with bigeye-tuna. In organoleptic evaluation, muscle color became dark brown with the decrease of 'a'-value.

Changes in bacteria, VBN and polyamines

The bacterial counts on bigeye-tuna and bonito muscles stored at 6, 10 and 20℃ under ACP and DAP were investigated. No bacterial increases (< 100 cfu/g) were appeared in both muscles stored at 6℃ under ACP and DAP (Data not shown). When the bigeye-tuna muscles were stored for 144 h at 10℃ in ACP and DAP, the bacterial count were 10^6 and 10^4 levels, respectively (Fig. 3). Also, the bacterial counts of muscles, which were stored for 48 h at 20℃ in ACP and DAP, were 10^6 and 10^4 levels, respectively. On the other hand, the bacterial counts were not changed in bonito muscle stored at 10℃, since bonito loin had already been baked on the surface, and it seemed to be of hygienic treatment.

Micro-flora was investigated on ACP (10^6) and DAP (10^4) plates that the bigeye-tuna muscles were stored for 144 h at 10℃. As a result, all of the isolates had rod-shape and catalase positive. The bacteria, which indicated a characteristic of oxidase positive, oxidation of glucose, and Gram negative (*Pseudomonas* from a diagnostic table of Cowan and Steel), were 12 and 3.5% in the muscles of APC and DAP, respectively. The bacteria characterized by the physiological observation of oxidase negative, fermentation of glucose, and Gram negative (*Enterobacteriaceae*) were 46 and 57% in APC and DAP muscles, respectively. Furthermore, the bacteria that possessed the same characteristics except for Gram positive (*Corynebacterium*) were 51 and 39% in APC and DAP muscles, respectively. The preservation with deoxidizer

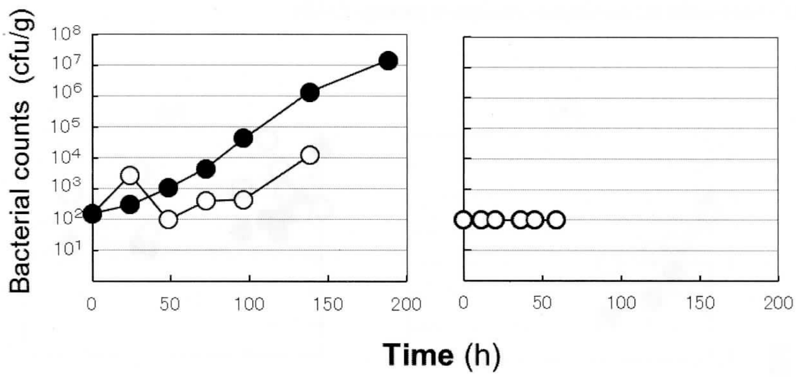

Fig. 3 Changes in bacterial counts of big-eyed tuna (a) and bonito (b) during storage at 10℃.
●: sashimi stored under the air-containing package (ACP)
○: sashimi stored under the deoxidized-atmosphere package (DAP)

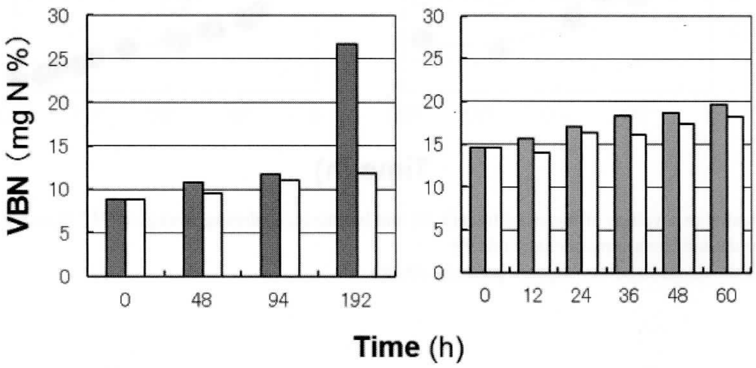

Fig. 4 Changes in VBN of big-eyed tuna (a) and bonito (b) during storage at 10℃.
Left bar: sashimi stored under the air-containing package (ACP)
Right bar: sashimi stored under the deoxidized-atmosphere package (DAP)

tended to make the ratio of *Enterobacteriaceae* increase, but controlled the total number of aerobic or facultative aerobic bacteria.

Figure 4 showed that the values of volatile base nitrogen (VBN) were slightly high in ACP fraction, when the bigeye-tuna and bonito muscles were stored at various temperatures of less than 10℃. In addition, VBN values were almost unchanged in both muscles with APC and DAP, and the bad odor was not felt. Polyamines (7 species) were not detected in APC and DAP, when the bigeye-tuna and bonito muscles were stored under the above conditions.

Prediction of the intensive monitoring item (K-value)

Rates of freshness-lowering of K-values were investigated for storage temperatures from 6 to 20℃. Figure 5 (a, b) showed that the plot of log (100 - K-value) versus time for muscle of bigeye-tuna yielded straight lines. These lines of freshness-lowering indicate first-order reactions. The slope of the straight lines gives the rate constant *kf* which was calculated by the least squares method. The rates of freshness-lowering increased with an increase in storage temperature. On the basis of the rate constant of freshness-lowering, Arrhenius' plots were prepared (Fig. 5 c). Arrhenius' plots for the bigeye-tuna had a linear regression in the range from 6 to 20℃. Table 1 indicated the kinetics parameters, *Ea* (energy of activation) and *A* (frequency factor) which were provided by the slopes and intercepts of Arrehenius' plot. We predicted the change in freshness for bigeye-tuna stored at fluctuating temperatures by applying the above kinetics parameters.

Temperature histories of the bigeye-tuna and those K-values are indicated in Table 2. The results show that experimental and calculated K-values approximately agreed, and that the freshness-lowering was predictable provided the initial K-value and the temperature history of the fish are given.

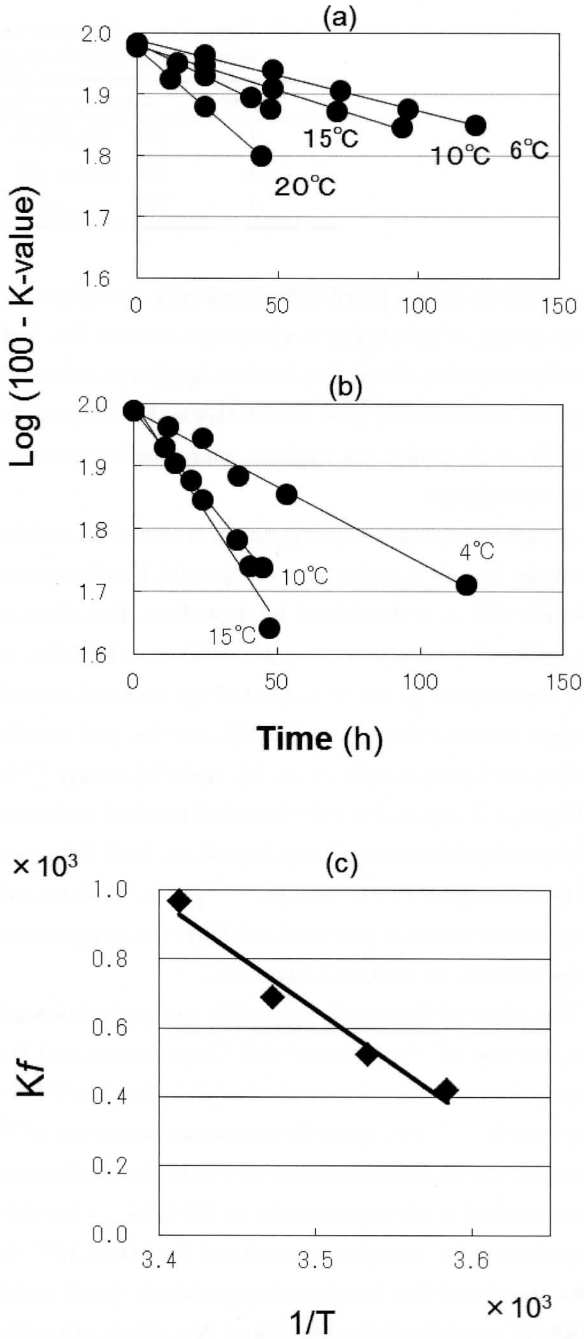

Fig. 5 Effects of temperatures on rate constant of freshness-lowing in big-eyed tuna (a) and bonito (b).
Arrhenius' plot (c) was obtained from the rate constant of big-eyed tuna.
K*f*: rate constant of freshness-lowing
T: absolute temperature

Discussion

In recent years, many fisheries factories become introduced a new hygienic system, hazard analysis and critical control point (HACCP). HACCP is an excellent system that takes

Table 1 Rate constant of freshness-lowing in the muscles of big-eyed tuna and bonito

Species	Rate constant Kf × 10^3 (/ h)				
	4℃	6℃	10℃	15℃	20℃
big-eyed tuna	---	2.632	3.331	4.887	9.283
bonito	5.583	---	14.742	16.123	

Table 2 Comparison of the experimental and calculated K-values in big-eyed tuna with history of different storage condition.

Run no.	Temp. and run time	Experimental	Calculated
1	20℃, 22 h and 4℃, 44 h	27.8	27.2
2	4℃, 4 h and 10℃, 45.5 h	24.8	22.8
3	10℃, 22 h, 4℃, 26 h and 20℃ 18 h	28.4	27.5

+precautions against possible food poisoning and injury, but high quality of the product is not always assured. The high quality assured to sliced fish involves hygiene in microbial aspects, tone of color, odor, and freshness. For keeping the high level of quality and hygiene, many positive methods have been studied.

A modified atmosphere (MA) storage is one of the positive methods. The air is replaced by nitrogen (N_2) and/or carbon dioxide (CO_2), or deoxidized by deoxidizer. The effect of N_2 and/or CO_2, is reported that the addition of 40% CO_2 or the replacement of 100% N_2 control the bacterial growth in jack mackerel fillets stored at 5℃, and the total aerobic counts are higher in order of air, N_2, and CO_2 storage.[14] In addition, it is reported that the microbial count of sea bream under the modified atmosphere is kept in low level for around 8 days during ice or 5℃ storage.[15, 16] On the contrary, the total aerobic counts in jack mackerel fillets (air storage) were approximately 10^7 level for 2 days at 5℃.[16]

The microbial flora on N_2 and/or CO_2 storage tends toward the decrease of *Pseudomonas* and Coryneforms, and the increase of Enterobacteriaceae and *Lactobacillus* sp. or *Vibrio-Aeromonas*.[14, 17] For anaerobic pathogens, Kimura *et al.*[18] reported that no apparent growth of *Clostridium perfringens* was resulted in the replacement of 100% N_2, when this organism on the agar plate was cultured for 24 h at 30℃. In this experiment that the sliced bigeye-tuna is stored under DAP, the same effects are observed on the controls of aerobic counts and changes in microbial flora. It is considered that the aerobic count in the bonito is not detected during storage, since the microbial is pasteurized and controlled by the baking of surface and vacuum packaging. We also presume that the growth of anaerobic pathogens is suppressed in the fishes stored under DAP. Furthermore, polyamines (7 species) as an allergic food poison were not detected in *sashimi* stored under the condition of APC and DAP in this experiment. The above results shows that the quality in hygienic aspects is reliable for *sashimi* stored under the combined condition of low temperature and DAP.

Discoloration of sliced fish is remarkably controlled by the preservation under deoxidized atmosphere as shown in Fig. 2. The MA storage is reported to control the discoloration in the sliced beef (CO_2 / O_2), and yellowtail or common mackerel (CO_2 and/or N_2), respectively.[19, 14, 20] Ueoka[20] also reports that the slow rate of oxygen absorbency may cause the discoloration of muscle, when the yellowtail is packed with gas exchanger under the low storage temperature. The autoxidation of myoglobin (Mb) is closely connected with the

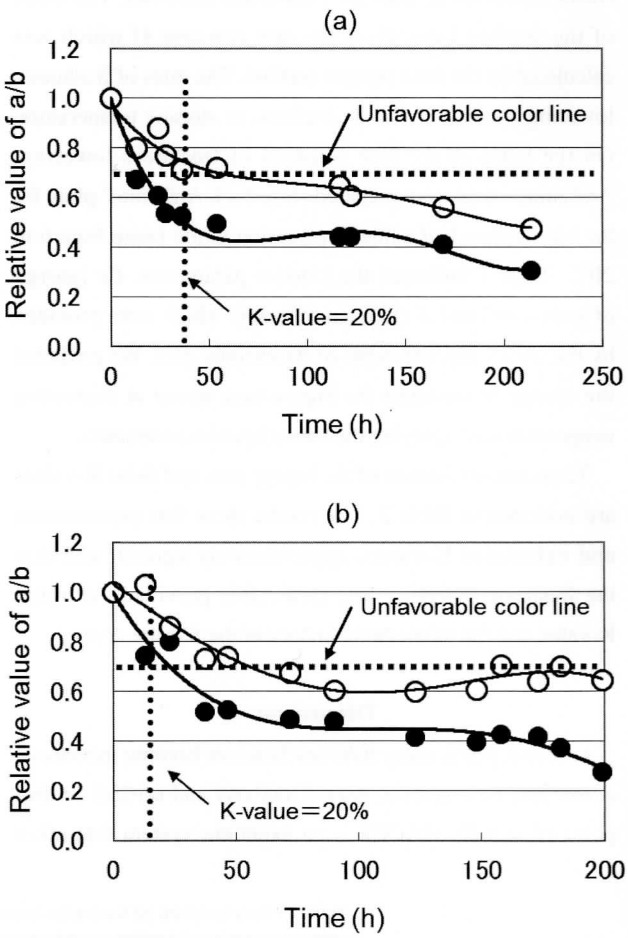

Fig. 6 Relation between the K-value and discoloration of sliced bonito muscle stored for various time at 4 (a) and 10℃ (b).
●: sashimi stored under the air-containing package (ACP)
○: sashimi stored under the deoxidized-atmosphere package (DAP)

oxygen pressure. Matsuura *et al.*[3] showed that Mb of tuna had maximum autoxidation values at an oxygen pressure of 3.3-4.0 mmHg. It is necessary for the deoxidizer that oxygen is effectively absorbed under the low temperature.

Further investigation was made on the relation between the K-value and discoloration when the sliced bonito muscle was stored for various time at 4 and 10℃ (Fig. 6). As to a discoloration using colorimetric analysis, we employed the relative value of 'a/b' corresponding to visual perception. Arai *et al.* show that an unfavorable color line provides below 70% of 'a/b' value from the viewpoint of organoleptic evaluation (5 persons).[21] In the sliced bonito under ACP (without deoxigenizer), the muscle color has already been unfavorable at the vertical line of K-value 20%. Therefore, the priority to quality assessment must give the discoloration rather than K-value under ACP. In the sliced bonito under DAP (with deoxigenizer), the K-value takes first priority to quality assessment, since the desirable muscle color has been kept by judging from the 'a/b' value and organoleptic evaluation.

We consider that the quality of fishes packed with deoxidizer is guaranteed by monitoring the K-value, since the change of K-value is faster than the increase of discoloration. In other words, K-value is utilized as the critical control point (CCP) from the above results of quality assessments such as the discoloration, odor, polyamines, and microbial growth.

The investigation has been performed on the prediction of K-value when the bigeye-tuna packed with deoxidizer is stored at fluctuating temperature. The change in K-value of bigeye-tuna leads to the rate constant for each temperature and subsequent the kinetics parameters.[5, 22] As a result, the simulated K-value indicates the approximate value for the actual K-value obtained from HPLC. Incidentally, Hara *et al.* explained that it was difficult to stimulate the K-value at the latter stage (K-value over 50-70%) of fish storage because of the bacterial increase.[23] The simulation of K-value as described in the previous papers could be practicable for many fishes stored under DAP.[5, 23] This knowledge may enable us to provide the shelf life of fresh *sashimi* stored under DAP, since shelf life can be estimated from K-value stimulated on the computer. We could purchase the fresh *sashimi* labeled for the shelf life of date and time at a convenience store. In addition, it will be necessary for another fishes to obtain each kinetics parameter of K-value, since the change in K-vale was different among fish species.

In conclusion, we consider that when the sliced fresh fishes are stored under the deoxidized atmosphere (DAP), K-value is employed as CCP for the quality assessment and is predictable provided the initial K-value and temperature history of the fish are given.

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