Colour and Quality Assessment of Tuna for *Sashimi*

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Abstract

The colour of tuna is the main criterion for quality assessment of sashimi. In Japan, the general rule is "The brighter the red, the better the quality". The colour of fresh meat is due to the occurrence of myoglobin in its different chemical states; in oxidized meat, the predominant chemical state is met-myolgobin. In this study, the main objective is to correlate the amount of myoglobin with the quality of yellowfin tuna (Thunnus albacares). The tuna samples in this study were subjected to 0°C storage and their colour and myoglobin values were monitored over 14 days. The value of met-myoglobin for 0-day is 10.0% and on the 14th day, the value increased significantly to 95.9%. For chemical tests, significant spoilage occurred during the time between the 9th and 12th day of storage when met-myoglobin values increased from 67.5% to 83.4%. For sensory evaluation, all panelists rejected the tuna samples as unsuitable for sashimi when the value of met-myoglobin increased to 70%.

Introduction

The main criterion for the acceptance of tuna meat for *sashimi* depends mainly on the appearance of its colour. For consumers in Japan, the general rule is "the brighter the red, the better the quality". The colour of meat is due mainly to the occurrence of reduced myoglobin (Red-Mb), oxy-myoglobin (Oxy-Mb) and met-myoglobin (Met-Mb). The colour for 100 % Red-Mb in meat is dark purplish red, for 100% Oxy-Mb in meat is bright red and for 100 % Met-Mb is brown. It is therefore necessary to analyze the amount of these three states of myoglobin for the determination of meat colour. The main objective for this study is to determine the correlation between the content of myoglobin and quality of yellowfin tuna by comparing results of colour with spoilage indices as well as sensory evaluation .

Definitions

R(λ) : Reflectance of a sample at an arbitrary wavelength;

ℜ(λ) : Reciprocal of reflectance of a sample at an arbitrary wavelength = 1/R(λ);

$_{blc}$(λ) : Reciprocal reflectance of an achromatic, matrix sample;

ℜ'(λ): The function equal to ℜ (λ) - ℜ$_{blc}$(λ);

K An absorption coefficient;

S : A light scattering coefficient;

r : Correlation coefficient; and

R² : Reliability of the trendline; R² = 1 when line or curve fits data points perfectly.

Theory

The fundamental property of this study is spectral reflectance which is proportional to the myoglobin content. According to Kubelka and Munk (1931), it is considered that ℜ (the reciprocal of reflectance) has the same numerical property as K/S. ℜ$_{blc}$ is the value of the achromatic meat matrix without any myoglobin and the ℜ spectrum of meat containing myoglobin is higher than the ℜ$_{blc}$ spectrum. With decreasing myoglobin content, the value of ℜ would finally approach the spectrum of ℜ$_{blc}$. The ℜ$_{blc}$ spectrum is the baseline for the myoglobin free meat so that the ℜ' corresponds to the spectrum of the solution. Thus the following equation is derived:

$$\Re' = \Re - \Re^{blc}$$

When meat is first cut, reduced myoglobin (Red-Mb) content is the most dominant of all three forms. This is due to the high Myoglobin Reducing Activity (MRA) present in all fresh tuna. When the meat is exposed to gaseous oxygen, the oxygen molecule would attach itself to the myoglobin, resulting in the formation of oxy-myoglobin (Oxy-Mb) leaving the meat bright red. On exposure to air or oxidizing agent, the myoglobin present in the meat will be oxidized into met-myoglobin (Met-Mb), giving the undesirable brown colour. This is due to the oxidation of Fe(II) ion to Fe(III) ion in the myoglobin molecule.

The characteristics of the spectra for all 3 forms are unique to each, as shown in Fig. 1. For the case of Red-Mb, the 1/R spectra peaked at 480 nm and a valley occurs at 560 nm. In this spectra, the reduced form was characterized by the slope between 480 nm and 520 nm. Thus the following function is determined:

$$P_r = R'_{480} / R'_{520} \text{ ——————(1)}$$

where P_r is the parameter that is inversely proportional to reduced-myoglobin content. In this region of the spectra, P_r is at its minimum for Red-Mb and maximum for the other two forms.

The P_o value for the Oxy-Mb form is directly proportional to its content. In the spectra, the R slope is positive between 560 nm and 570 nm while the R slope for the other two forms are negative. Thus the following equation is derived :

$$P_o = R'_{570} / R'_{560} \text{ ——————(2)}$$

The decreased P_o value will be accompanied by a decrease in the oxy-myoglobin content, finally reaching a minimum.

The spectral characteristic of met-myoglobin is significant at 640 nm as the direction of the curvature is opposite to the other two forms. This is represented by the following equation :

$$P_m = (R'_{640} - R'_{660}) / (R'_{600} - R'_{660}) \text{ ——(3)}$$

When P_m value was a maximum for met-myoglobin and it was a minimum value for the other two forms (Table 1). This function was confirmed to be the best according to Professor Izumimoto (1992). As in the case of oxy-myoglobin, a decrease in P_m will result in an increase in met-myoglobin content and a decrease in the oxy-myoglobin content.

All P_o, P_r and P_m functions are dependent on the oxy-, reduced- and met-myoglobin forms respectively but they are independent of each other. For the case of oxy-myoglobin form, when the P_o value indicates 100% content, the lower values occurring in both reduced- and met-myoglobin forms indicate the absence of the pigment. This also applies to P_r of reduced-myoglobin and P_m of the met-myoglobin form (Table 1).

The required parameters for yellowfin tuna were determined prior to this experiment and the calibration data is stored in the Myoglobin Analyzer Programme of the Qbasic software. The parameters determined are shown in Table 1. From these values, it is possible to plot the calibration curve for the three forms of myoglobin. The programme will then be able to automatically calculate and show the value of the myoglobin of the yellowfin tuna on the computer screen once reflectance measurement is taken.

Materials and Methods

A 30 kg deheaded and degutted yellowfin tuna (*Thunnus albacares*), was purchased from a fish supplier located at Pandan Loop. The fish was caught in the Indian Ocean and stored in ice on board the fishing vessel for 14 days prior to arrival at Singapore. The fish was collected one day after its arrival. The fish was iced and transported to Marine Fisheries Research Department in an insulated box. On arrival, the fish was cut into rectangular blocks, each piece with a size of 5 x 9 x 0.5 cm. The tuna blocks were then immediately stored in a 0°C refrigerator over a period of 14 days. The samples were also divided into 3 groups for colour and myoglobin studies, chemistry, and sensory evaluation. The chemical parameters included pH, moisture, K-value, volatile basic nitrogen (VB-N) and trimethylamine nitrogen (TMA-N). Before each tuna sample was taken for chemical and sensory evaluation tests, colour and myoglobin contents were first measured and and then each sample was photographed.

1. Colour and myoglobin

The colour and myoglobin determinations were possible with the use of a program called Myoglobin Analyzer Program designed by Mr. Yoshishige Mori on the Qbasic software. A Minolta 508-d reflectance spectrophotometer was attached to the computer. The colour measurement was done throughout 14 days on 5 pieces of tuna each labelled A to E. The *L*, *a* and *b* values which indicates the whiteness, redness and the blueness respectively of the tuna meat was measured together with the myoglobin values. The spectral readings obtained for each of the tuna loin were taken from three separate points of measurements on the meat surface. At the instant when spectral reflectance values were recorded by the meter, the software programme immediately converted the reflectance readings into reduced myoglobin, oxy-myoglobin and met-myoglobin percentage values. These readings were taken everyday except for Sundays and public holidays.

2. pH

The pH values of the tuna samples stored at 0°C were monitored over a period of 14 days. The method used was according to Lim (1992).

3. Moisture

Moisture was determined according to Ng M.C. (1992).

4. Volatile basic nitrogen (VB-N) and trimethylamine nitrogen (TMA-N)

VB-N and TMA-N tests were conducted to find out how bacterial freshness as indicated by VB-N and TMA-N can be correlated to the colour changes of the tuna. For both analyses, the Conway's microdiffusion method used was as described by Yamagata and Low (1992).

5. K-value

This test was conducted to find out how enzymatic freshness can be correlated with the colour of the tuna. Upon its death, ATP and related compounds are broken down by endogenous enzymes. K-value thus measures the extent of this breakdown (Equation 4). The method used for this determination is the ion-exchange chromatography as described by Ng C.S. (1992).

$$K(\%) = \frac{[HxR]+[Hx]}{[ATP]+[ADP]+[AMP]+[IMP]+[HxR]+[Hx]} \times 100 \quad \text{——(4)}$$

where ATP = adenosine triphosphate; ADP = adenosine diphosphate; IMP = inosine monophosphate; HxR = inosine or hypoxanthine riboside; Hx = hypoxanthine

6. Sensory Evaluation

This study was conducted over a period of 12 days and 10 untrained sensory panelists from the Department were invited to test the tuna samples for their visual appearance, mainly colour, taste, smell and texture. The tuna samples were kept chilled in an ice box prior to sensory evaluation. This test was terminated when all of the panelists had rejected the tuna samples as *sashimi*.

Results and Discussion

1. Colour and myoglobin

The 5 tuna samples were monitored until their met-myoglobin content was almost 100%. Fig. 2 shows the change in myoglobin, which includes the three chemical states, with time. The results showed that the changes correlated very well ($r = 0.9867$). It was found statistically that the overall met-myoglobin increase with storage days was significant ($P<0.05$).

Fig. 3 shows that redness was significantly correlated with oxy-myoglobin ($r = 0.942$). However, the change in met-myoglobin content had a smaller correlation coefficient with redness ($r = 0.8912$) compared to oxy-myoglobin. The results for the relationship between redness of tuna using the *a* values and storage period is shown in Fig. 4. It was noted that the tuna samples achieved maximum redness between 2-5 days of storage. The increase in redness between 0 to 2 days is due to the increase in oxy-myoglobin in the tuna samples. As the formation of met-myoglobin increases, the redness decreases because of the decrease in oxy-myoglobin. Thus the samples would turn more brownish towards the end of the curve. On the 14th day, there was a slight increase in the redness which was consistent with the increase in reduced myoglobin originating from the reducing enzyme from *Lactobacillus*.

As seen in Fig. 2, reduced myoglobin value for the 0-day sample was low, indicating that the initial freshness of the sample was not that of high quality tuna. For very fresh, high quality tuna, the reduced myoglobin value should normally be about 90-100% on the cut surface. This may be due to improper handling on board after the catch which resulted in some oxidation occurring before the fish was even cut open.

2. pH

The overall increase in pH (Table 2) was significant ($P<0.05$) and the changes in pH was highly correlated with the changes in met-myoglobin ($r = 0.842$). During the initial stage of storage, the pH remained quite stable ranging from 5.97 to 6.08 until on the 12th day of storage when the value was increased to pH 6.28. This increase was accompanied by the increase in met-myoglobin content from 67.5% to 83.4% and spoilage with regards to a significant ($P<0.05$) increase in volatile basic nitrogen from 35.60 to 54.16 mg/100g on the 9th and 12th day of storage respectively (Table 2).

3. Moisture

The change in moisture during the storage period ranged from 74.12% to 75.31% (Table 2). Although the change throughout the storage period was significantly ($P<0.05$) different, there was apparently little correlation ($r = 0.4033$) with the met-myoglobin content.

4. VB-N and TMA-N

The overall increase of VB-N over the 14 days was significant ($P<0.05$, Table 2). There was a significant increase in VB-N from 9 to 12 days of storage which was accompanied by a significant increase in met-myoglobin from 67.5% to 83.38%. The values did not vary much during the beginning and at the end of the storage period. Fig. 6 shows that the change in met-myoglobin was highly correlated (r = 0.9075) with the changes in VB-N.

The range of values of TMA-N during the 14 days of 0°C storage was between 0 to 2.79 mg/ 100g. The results of these values did not correlate directly with the increase in met-myoglobin (correlation coefficient, r = 0.7023) as TMA-N contents differed when sampling was done in different parts of the fish muscle.

The rate of increase in TMA during spoilage of fish depended on storage temperature and was quite pronounced at room temperature, but absent or negligible at sub-zero temperatures. At low temperatures such as refrigeration above 0°C, TMA formation slows down noticeably (Ishida *et al.*, 1976). Thus, the values obtained were not very high in this experiment and were irregular. This was actually consistent with the findings of Horie and Seine (1956) when they compared TMA formation between ordinary muscle of white meat fish to that of the dark meat fish. They concluded that the formation was greater and more regular in the former than the latter. They also reported that white meat fish contained larger amounts of trimethylamine oxide (TMAO), the precursor of TMA, about 35-60 mg / 100g, whereas dark meat fish contained smaller amounts of about 2-10 mg/100g.

One other reason for the irregularity of the TMA-N values may be due to the fact that the rate of spoilage varies with meat from different parts of the fish. It was reported that for yellowfin tuna, the TMA formation was faster in the tail as compared to the head and the middle portion; the TMAO content was higher in the tail end muscle than in muscles near the head (Koizumi *et al.*, 1967).

6. K-value

The K-value of yellowfin tuna muscle increased significantly ($P<0.05$) during storage (Table 2). The K-value changed significantly ($P<0.01$) when met-myoglobin ranged from 9.17% to 34% and it became slightly stabilized when met-myoglobin values ranged between 34% to 67.5%. The values then increased significantly ($P<0.05$) between the 9th and 14th day of storage (Fig. 7). The changes in K-value was highly correlated (r = 0.91119) with the changes in met-myoglobin content. From the value of 31.59% of the 0-day sample, it was obvious that enzymatic spoilage has already occurred before the fish was sampled. According to Japanese standards, the acceptable K-value for fresh fish for *sashimi* should be below 20%. However, the 0-day iced sample at the laboratory was already 15 days after harvesting.

7. Sensory Evaluation

The sensory evaluation tests on *sashimi* tuna samples, stored at 0°C, were conducted over a 12 day period of 0°C storage. The results are shown from Figs. 8-11 and the table which summarizes the corresponding myoglobin contents and their acceptability is in Table 3.

All panelists rejected tuna as *sashimi* when met-myoglobin values reached the value of 70% on the 12th day. This was consistent with the significant increase in pH, VB-N and K-value of the samples stored from 9 to 12 days of storage when met-myoglobin increased from 67.5% to 83.4%.

Fig. 8 shows the change in colour of the tuna samples as judged by the sensory panelists. The trend towards the right is from the bright red to brown. It was noted that some tuna samples have become progressively brown from the 7th day of storage onwards.

Fig. 9 shows the trend of the overall appearance of the tuna samples. From the figure, it was also noted that the optimum appearance of the tuna is on the 2nd day and that some of the panelists had already rejected the samples as suitable for *sashimi* from the 7th day onwards.

Fig. 10 shows the trend of the odour of tuna as the storage days progressed. Many of the panelists find the odour non-offensive during the first few tests. It was from the 7th day onwards that they found the samples fishy which led to rejections of the tuna as *sashimi*.

Fig. 11 shows the trend in texture over the number of storage days. The texture of the samples did not change significantly over the storage period and most of the comments are "slightly soft" to "soft". Although all panelists rejected the samples as *sashimi* on the 12th day, the results from the texture tests show that texture is not one of the factors contributing to the rejection.

From 0-day to 7-day storage, most of the panelists commented that the tuna samples were bland and did not have much flavour but there were no strange offensive flavours as well. It was after the 9th day that they found the taste to be more fishy and repulsive.

Conclusion

The results of this study showed that the software program Myoglobin Analyzer designed by Mr. Yoshishige Mori gave met-myoglobin values which correlated very well with the changes in K-value, VB-N and sensory evaluation results (r = 0.9119, r = 0.9075 and r = 0.9742) for the yellowfin tuna during iced storage. The changes in met-myoglobin was fairly well correlated with the changes in redness, a-value (r = -0.8912). This shows that it is possible to manage the quality assessment of *sashimi* tuna in the field with a more objective method using the Minolta 508d Spectrophotometer and the Myoglobin Analyzer software Program.

Majority of the sensory panelists rejected the tuna for *sashimi* consumption on the 7th day of iced storage based on the colour, appearance and odour. At this point, the K-value was 49.11%, VB-N was 36.06 mg/100g, TMA-N was 2.32mg/100g and the met-myoglobin content ranged between 34.47% for the chemistry sample and 40.63% for the sensory sample.

When all the sensory panelists rejected the tuna samples on the 12th day of iced storage at the laboratory (27 days after catch), the K-value was 52.32%, VB-N was 54.16 mg/100g, pH was 6.28 and the met-myoglobin ranged between 71% for sensory sample and 83.38% for the chemistry sample. Thus, once the met-myoglobin content is greater than 70%, the tuna meat was not acceptable for *sashimi.*

The chemical data showed that the sample upon arrival at the laboratory was not of premium quality as the K-value was already 31.59%. Thus, the authors recommend that further work should be carried out with better quality yellowfin tuna. In future studies, attempts will be made to try and match the met-myoglobin content with the colour of the tuna meat to produce a colour chart for field use.

Acknowledgment

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Ng C.S. 1992. Determination of K-value. In: Laboratory Manual on Analytical Methods and Procedures for Fish and Fish Products. 2nd Ed. K.Miwa and S. J. Low (Ed.), p. A-3.1 - A-3.2. MFRD, SEAFDEC, Singapore.

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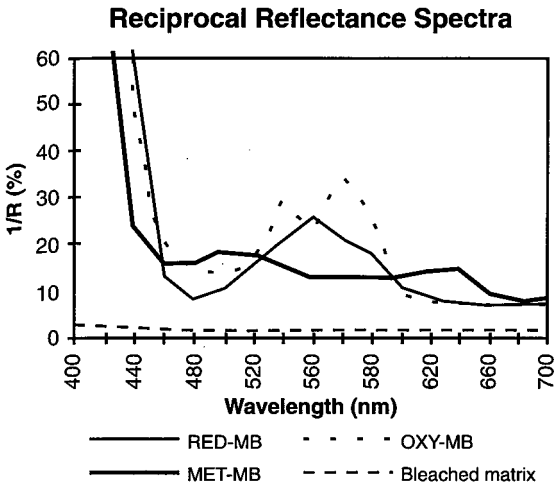

Note : These spectra were obtained from a previous experiment to set the basic parameters for the different states of myoglobin in yellowfin tuna for use in the computation of the software program.

Fig. 1. Reciprocal reflectance () spectra of yellowfin tuna.

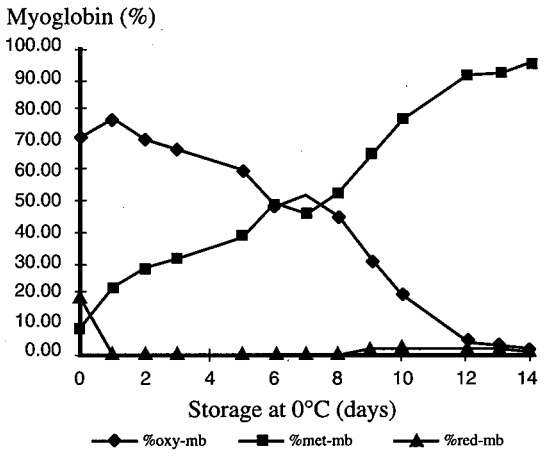

Fig. 2. Changes in oxy-, red- and met-myoglibin (%) content of yellowfin tuna during storage (0°C).

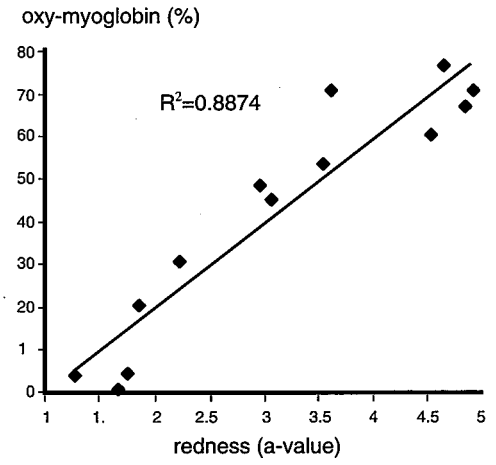

Fig. 3. Relationship between redness (a-value) and oxy-myoglobin content of yellowfin tuna.

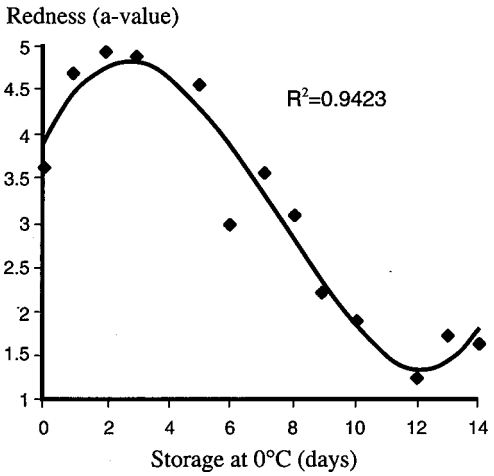

Fig. 4. Changes in the redness (a-value) of yellowfin tuna with storage time (0°C).

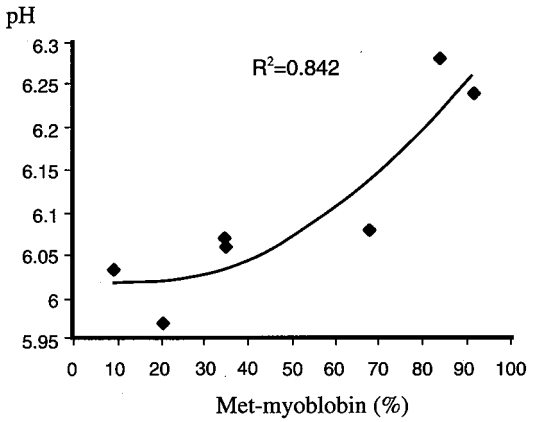

Fig. 5. Relationship between changes in pH and met-myoglobin content of yellowfin tuna.

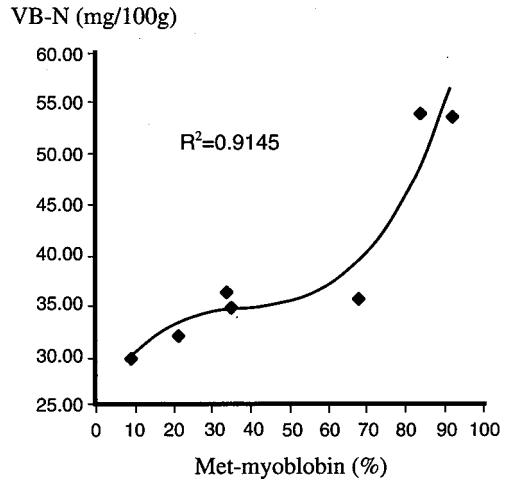

Fig. 6. Relationship between changes in VB-N and met-myoglobin content of yellowfin tuna.

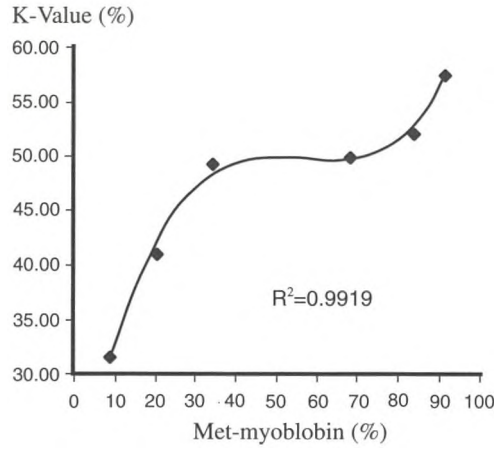

Fig. 7. Relationship between changes in K-value and met-myoglobin content in yellowfin tuna.

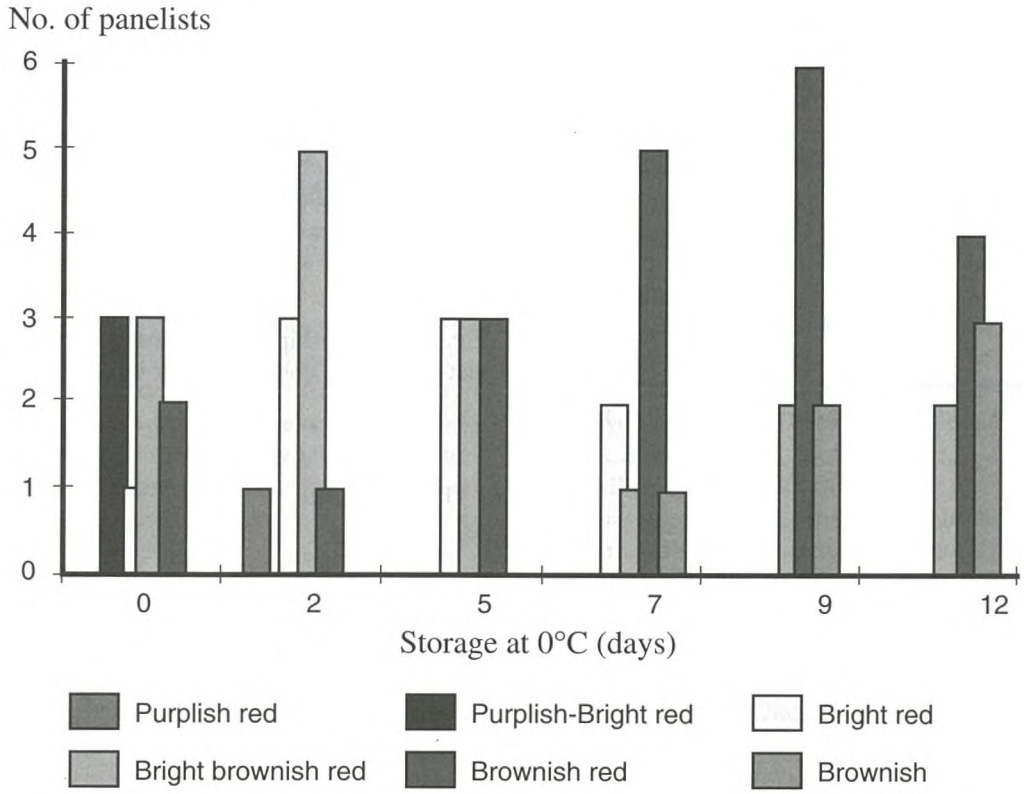

Fig. 8. Sensory panelists' evaluation of the changes in colour of *sashimi* yellowfin tuna during storage at 0°C.

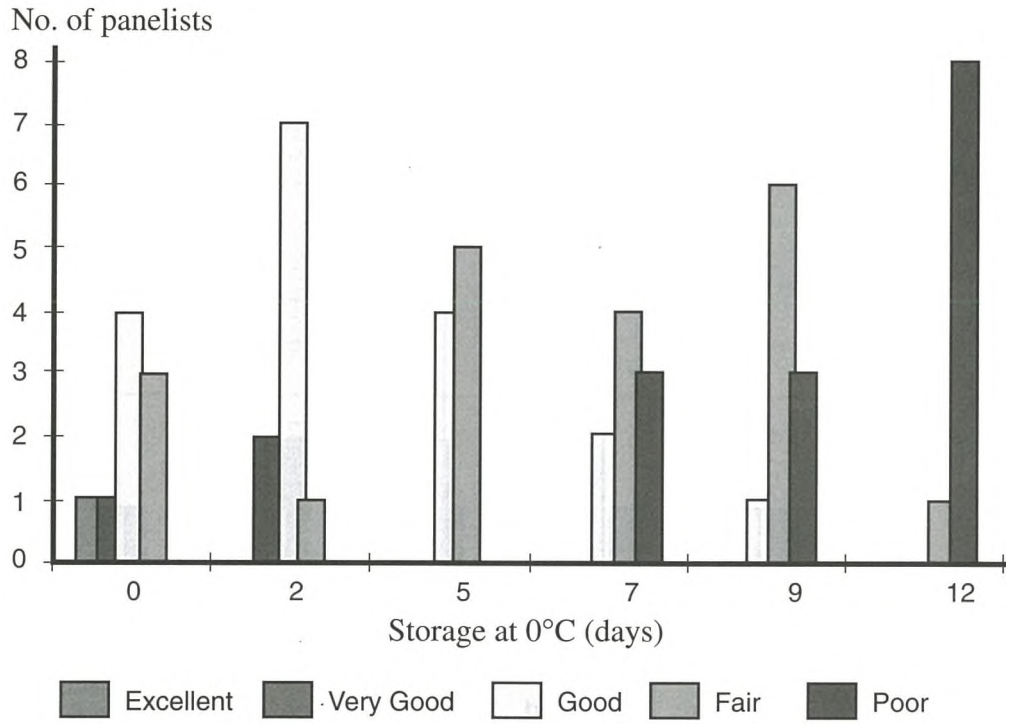

Fig. 9. Sensory panelists' evaluation of changes in appearance of yellowfin tuna during storage at 0°C.

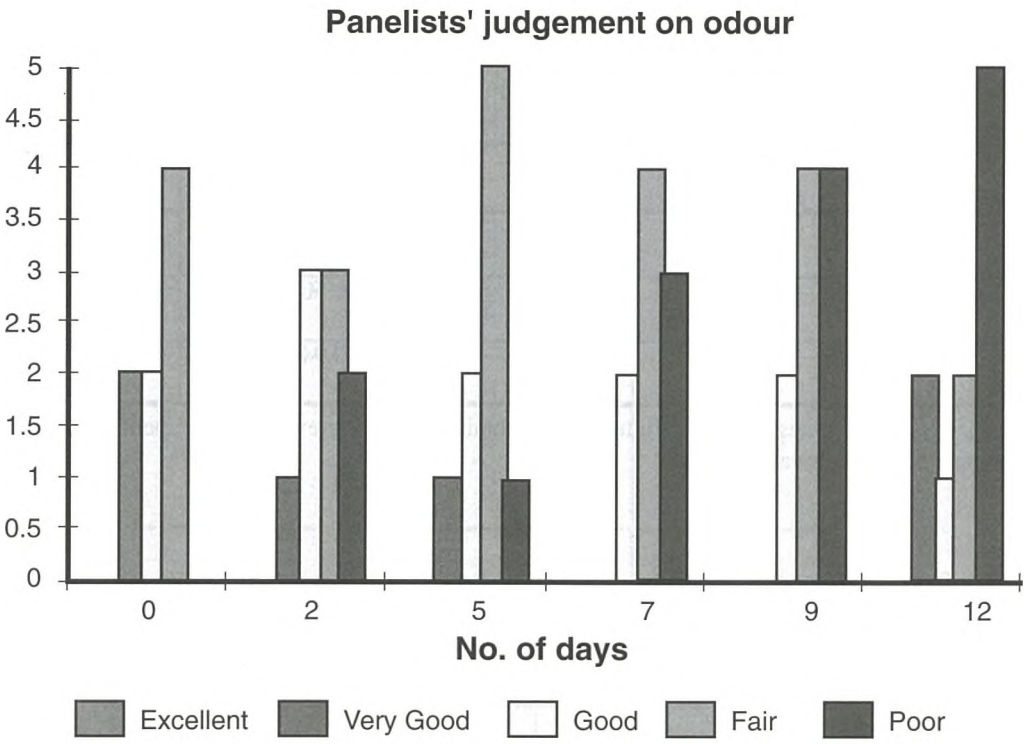

Fig. 10. Sensory panelists' evaluation of changes in odour of yellowfin tuna during storage at 0°C.

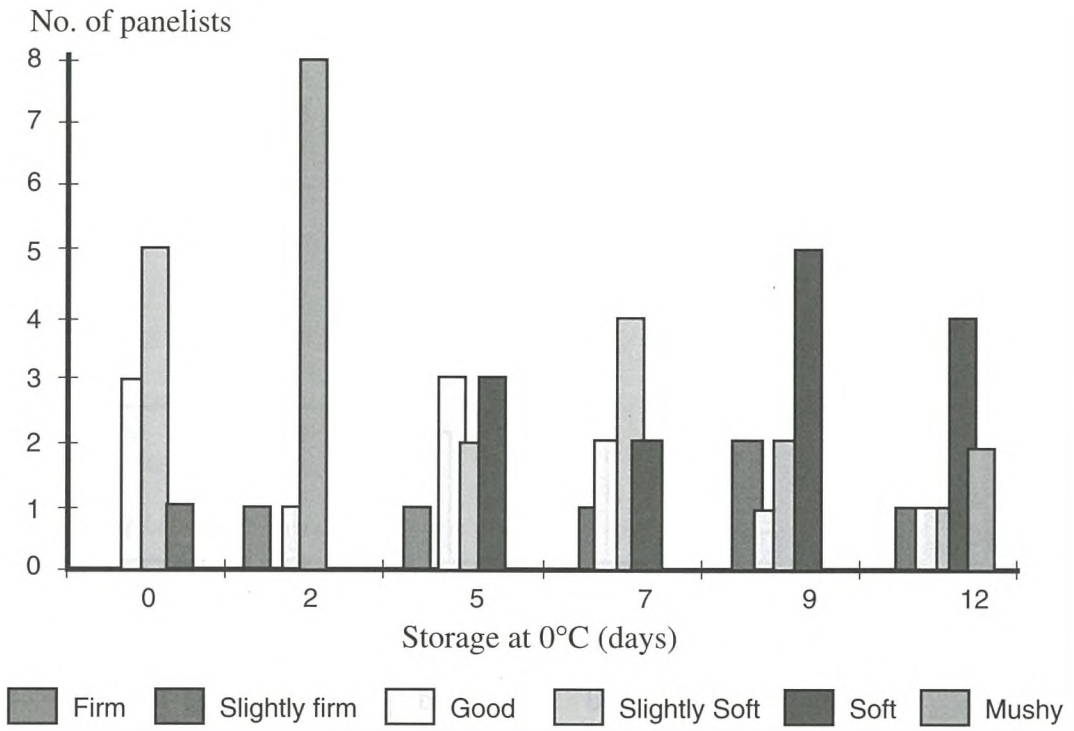

Fig. 11. Sensory panelists' evaluation of the changes in texture of raw yellowfin tuna stored at 0°C.

Table 1. Parameters (P_r, P_o and P_m) for the three states of myoglobin.

Form	P_r	P_o	P_m
100% red-Mb	0.597	0.9453	0.1756
100% oxy-Mb	0.8697	1.5075	0.1756
100% met-Mb	0.8697	0.9453	1.8248

Note : The P_r, P_o and P_m values of yellowfin tuna were obtained from a previous experiment conducted to set the basic parameters for the software program.

Table 2. Chemical test results with corresponding myoglobin and redness values

Storage days	Red-Mb %	Oxy-Mb %	Met-Mb %	Redness (a*)	pH	Moisture %	K-value %	VB-N (mg/100g)	TMA-N (mg/100g)
0	19.71±7.70*	71.13±7.98	9.17±3.80	3.61±0.50	6.03±0.006	74.58±0.18	31.59±0.42	29.69±0.98	0.50±0.48
2	0.00±0.00	79.03±8.02	20.97±8.02	4.30±0.53	5.97±0.02	74.12±0.16	40.86±3.01	31.97±0.78	0.00±0.00
5	0.00±0.00	65.07±2.22	34.93±2.22	3.90±1.46	6.06±0.01	74.35±0.08	42.30±1.07	34.70±1.22	0.40±0.35
7	0.00±0.00	65.53±3.65	34.47±3.65	4.21±0.35	6.07±0.01	74.94±0.06	49.11±2.98	36.06±1.84	2.32±0.32
9	11.03±4.84	21.43±11.93	67.50±9.81	2.65±0.62	6.08±0.00	75.31±0.06	49.90±0.83	35.60±1.74	2.57±1.15
12	10.83±3.26	5.78±3.14	83.38±2.05	1.66±0.39	6.28±0.006	74.26±0.16	52.32±1.10	54.16±1.61	1.32±0.18
14	6.30±12.13	2.60±6.79	91.13±11.44	0.66±0.55	6.24±0.01	75.09±0.10	57.48±0.91	53.84±2.03	2.79±0.76

* Mean ± Standard deviation

Table 3. Sensory evaluation of yellowfin tuna for *sashimi* (total number of panelists=10).

Day	Reduced-Mb %	Oxy-Mb %	Met-Mb %	Number of panelists who accept samples as *sashimi*
0	19.70	71.13	9.17	10
2	0.00	73.46	26.44	6
5	0.00	65.73	34.27	6
7	0.00	59.37	40.63	6
9	0.40	49.50	50.10	4
12	8.25	20.75	71.00	0